

# Inhibition of Several Strains of Influenza Virus *in Vitro* and Reduction of Symptoms by an Elderberry Extract (*Sambucus nigra* L.) during an Outbreak of Influenza B Panama

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## ABSTRACT

A standardized elderberry extract, **Sambucol®** (SAM), reduced hemagglutination and inhibited replication of human influenza viruses type A/Shangdong 9/93 (H3N2), A/Beijing 32/92 (H3N2), A/Texas 36/91 (H1N1), A/Singapore 6/86 (H1N1), type B/Panama 45/90, B/Yamagata 16/88, B/Ann Arbor 1/86, and of animal strains from Northern European swine and turkeys, A/Sw/Ger 2/81, A/Tur/Ger 3/91, and A/Sw/Ger 8533/91 in Madin-Darby canine kidney cells. A **placebo-controlled, double blind study** was carried out on a group of individuals living in an agricultural community (kibbutz) during an outbreak of influenza B/Panama in 1993. Fever, feeling of improvement, and complete cure were recorded during 6 days. Sera obtained in the acute and convalescent phases were tested for the presence of antibodies to influenza A, B, respiratory syncytial, and adenoviruses. Convalescent phase serologies showed higher mean and mean geometric hemagglutination inhibition (HI) titers to influenza B in the group treated with SAM than in the control group. **A significant improvement of the symptoms, including fever, was seen in 93.3% of the cases in the SAM-treated group within 2 days, whereas in the control group 91.7% of the patients showed an improvement within 6 days ( $p < 0.001$ ). A complete cure was achieved within 2 to 3 days in nearly 90% of the SAM-treated group and within at least 6 days in the placebo group ( $p < 0.001$ ).** No satisfactory medication to cure influenza type A and B is available. Considering the efficacy of the extract *in vitro* on all strains of influenza virus tested, the clinical results, its low cost, and absence of side-effects, this preparation could offer a possibility for **safe treatment for influenza A and B.**

## INTRODUCTION

Influenza virus A or B causes an acute, febrile illness that occurs in outbreaks of varying severity almost every winter.

Amantadine and rimantadine were shown to be mainly effective in the prevention of influenza A (Younkin et al., 1983; Reuman et al., 1989; Brady et al., 1990). They inhibit influenza B *in vitro* at such high concentration that can-

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not be achieved in patients (Douglas, 1990). Besides the high cost of these products they elicit side effects, especially in elderly people (Stange et al., 1991). Moreover, it has been reported that mutations in the influenza M2 membrane protein confer resistance to amantadine (Grambas et al., 1992). Rimantadine-resistant influenza A strains appeared during therapeutic use of this product as early as 2 days after starting treatment (Hayden et al., 1989, 1991). This could lead to rapid selection and transmission of drug-resistant influenza A viruses. Ribavirin is effective against type A and B viruses, but only when given in aerosol. This mode of administration is difficult in influenza patients suffering from respiratory diseases and is an expensive and cumbersome mode of therapy (Gilbert et al., 1986).

The black elder had been used in the folk medicine for its properties against influenza. Therapeutic indications of the elder flowers are influenzal colds and sinusitis (British Herbal Pharmacopoeia, 1983). Antiviral activity of the infusion of three plants including the elder has been reported against influenza and herpes (Serkedjieva et al., 1990).

A standardized extract, Sambucol® (SAM), is a preparation based on the berries of the black elder, used as herbal remedy against influenza virus infections. It contains a high amount of three flavonoids (Bronnum-Hansen and Hansen, 1983). The flavonoids are naturally occurring plant substances. Numerous reports have been published on the antiviral activity of polyphenols such as the flavonoids, flavonols, and flavones. Antiviral activity against herpes virus type 1, respiratory syncytial, parainfluenza, and influenza viruses was demonstrated using several plant extracts containing flavonoids or purified flavonoids (Amoros et al., 1992; Serkedjieva et al., 1992; Nagai et al., 1990; Mahmood et al., 1993).

The aim of this study was to test this extract for its antiviral properties under *in vitro* conditions in cell cultures infected by several human strains of type A and B and animal influenza viruses. In addition, its ability to reduce the duration of the illness caused by influenza viruses was tested in a double-blind clinical placebo-controlled, randomized study carried out in a

group of normally healthy population that was not previously vaccinated against flu.

## MATERIALS AND METHODS

### *In vitro* tests

**Cells.** Madin–Darby canine kidney (MDCK) cells were grown in RPMI 1640 medium containing 10% inactivated fetal calf serum (FCS), penicillin G (100 units/ml), and streptomycin (100 µg/ml). The cells were maintained in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C. For assays, 2 × 10<sup>5</sup> cells per well were plated in 24-well plastic culture plates (Nunc, Roskilde, Denmark) and used when confluent monolayers were formed.

**Influenza viruses.** A/Shangdong 9/93 (H3N2), A/Texas 36/91 (H1N1), A/Beijing 32/92 (H3N2), A/Singapore 6/86 (H1N1), B/Panama 45/90, B/Yamagata 16/88, and B/Ann Arbor 1/86 were obtained from Dr. J.M. Wood (National Institute of Biological Standards and Control, Potter Bar, Hertfordshire, UK).

H1N1 strains from northern European pigs and turkeys, A/Sw/Ger 2/81, A/Tur/Ger 3/91, and A/Sw/Ger 8533/91 were obtained from Prof. C. Scholtissek (Institute of Virology, University of Giessen, Germany). The viruses were grown in allantoic sacs of 10-day-old embryonated eggs for 48 h at 34°C. The allantoic fluid was harvested, clarified at 2000 rpm 10 min, and the supernatant was stored in small portions at –70°C.

The viruses were titrated on MDCK cultures in the absence of trypsin to receive a limited number of virus replication cycles (Tobita et al., 1975). The final dilution of the virus that gave a complete cytopathic effect (CPE) was used to test the protective effect of Sambucol® as well as higher concentrations in some cases. The number of TCID<sub>50</sub> inhibited by the elderberry extract was calculated from the titer in the MDCK.

**Black elderberry extract.** Sambucol® (Razei Bar Ltd, Jerusalem) is a syrup containing elderberry juice, raspberry extract, glucose, citric acid, and honey. For the *in vitro* studies,

Sambucol D®, a formulation without glucose and honey, was used. Flavonoids are measured by their absorbance at 516 nm (not less than 0.60). The extract diluted in phosphate-buffered saline (PBS) at 1:8 has a pH of 4.9. Therefore, the virus controls were performed at the same pH. Dilutions lower than 1:8 were not tested for studies in tissue culture because of their low pH. In the hemagglutination reduction test, the extract could be used at a dilution of 1:4 as well.

*Hemagglutination test of the viruses.* The hemagglutinin titration was effected using modified standard procedures. For this purpose, 0.1 ml of 2-fold dilutions of each of the viruses suspensions in PBS was mixed with 0.1 ml of a 1% sheep red blood cell (SRBC) suspension.

*Hemagglutination reduction using SAM.* Virus suspensions [8 hemagglutination units (HAU) in 0.1 ml] were incubated with an equal volume of 2-fold dilutions of SAM at room temperature for 1 h or overnight at 4°C. After incubation, 50 µl of a 2% SRBC suspension was added. In other experiments, equal volumes of virus suspensions (32–64 HAU) and SAM (final dilution 1:8) were incubated overnight at 4°C. An SRBC suspension was added to 2-fold dilutions (0.1 ml) of each virus incubated as above with SAM. Reduction of the hemagglutination titer was assessed by comparison with controls.

*Inhibition of infectivity.* Titration of the viruses: Confluent monolayers of MDCK cells were infected with influenza viruses at different multiplicity of infection, in 0.2 ml PBS (pH 7.4). Following 30 min adsorption, 1 ml serum-free RPMI medium was added and the cultures were further incubated at least for 48 h or until complete lysis was observed in the virus control wells. The final dilution that gave a complete lysis was determined.

*Inhibition assay:* The viruses [at a final concentration producing 100% CPE (2 TCID<sub>50</sub>) and in some cases at higher dilutions] were incubated at room temperature with various concentrations of SAM 15 min before infection of the cells. The experiments for each virus were performed on triplicate samples and were repeated four times. The number of TCID<sub>50</sub> in-

hibited by SAM was calculated from the titer determined as above. Evidence of cytopathic effect was shown by staining the plates. The plates were washed with PBS to eliminate the dead cells and stained with Giemsa solution after fixation in cold methanol.

#### *Clinical study design*

A double-blind study on 40 individuals living in an agricultural community (kibbutz) in Southern Israel and visiting the dispensary was carried out. Before inclusion in the study, a description of the objectives, procedures, and benefits of participation was given to each patient, and a written informed consent was obtained from him or her. Bottles identical in appearance containing experimental medication or placebo were assigned numbers from a predetermined list kept in a sealed envelope, which resulted in random distribution. On the first visit to the dispensary patients received one bottle with the next number in sequence.

*Study group.* Patients who were admitted to the study had at least three of the following symptoms of less than 24 h duration: fever >38°C, myalgia, nasal discharge, and cough. In the presence of streptococcus A (tested with Biosign strep. A, Princetown Biomeditech Corp., Princeton, NJ), patients with a sore throat were excluded from the study. None of the patients had been vaccinated against influenza.

*Treatment.* Children received two, and adults four tablespoons of either SAM or its placebo daily for 3 days.

Follow-up of the patients was performed by recording over a period of 6 days the presence of the following symptoms: fever, rhinitis with flow (thick, liquid, frequent, rare), headache, pharyngitis, cough, malaise, fatigue, and myalgia. Feelings of improvement or complete cure were also noted.

*Serological studies.* Samples of sera were obtained from the patients on their first visit to the dispensary and in the convalescent phase. The sera were tested for the presence of antibodies to influenza A and B by two independent tests. Antibodies to RSV and adenoviruses were tested by complement fixation test.

*Complement fixation test (CFT).* A micro-method technique of CFT was used as described by Taylor et al. (1970). Antigens were extracted as follows: RSV and adenovirus antigens from human kidney infected cells and influenza A and B from chorioallantoic membranes of 10-day-old embryonated eggs inoculated with influenza A and B.

Antibody titers were determined as the highest dilution giving maximum 50% hemolysis. A 4-fold and over increase in antibody titer between the first and the second sample was indicative of active infection.

*Hemagglutination Inhibition Test (HI).* HI is a subtype-specific serological test. Antibodies were evaluated using a known concentration of hemagglutinin and a chicken red blood cell suspension. The following influenza antigens were provided by the WHO collaborating Influenza Center, London: A/Taiwan/1/86, A/Beijing/353/89, B/Victoria/2/87, and B/Panama/45/90. Sera were treated to remove nonspecific inhibitors by receptor destroying enzyme (provided by the WHO collaborating Influenza Center, London) and by heat (56°C, 30 min). The test was performed by microtiter method using four units of antigen. The HI titer of each serum was the highest dilution causing a complete inhibition of agglutination.

*Statistical Analysis.* The Fisher exact test was used to test for a difference between the treated group and the control group. An odds ratio was used as a summary measure.

## RESULTS

### *Inhibition of virus hemagglutination*

Short incubation (1 h) of 8 HAU of influenza virus with SAM at the dilution of 1:4 inhibited hemagglutination for A/Beijing 32/92 (H3N2), A/Singapore 6/86 (H1N1), B/Panama 45/90, and B/Yamagata 16/88. Higher dilutions of SAM (1:8 to 1:16) inhibited hemagglutination when the duration of the incubation with the extract was increased to 16 h.

In other experiments, the viruses were incubated overnight with SAM at the final dilution of 1:8. Hemagglutination titer of the viruses

was reduced 4-fold for A/Beijing, 16-fold for A/Singapore, and 8-fold for B/Panama and B/Yamagata strains.

The hemagglutination titer of the viruses was not affected when using SRBC previously incubated for 16 h with SAM.

### *Antiinfluenza virus activity of the elderberry extract in cell cultures*

The effect of SAM on replication of influenza viruses was studied on human influenza viruses type A/Shangdong 9/93 (H3N2), A/Texas 36/91 (H1N1), A/Beijing 32/92 (H3N2), A/Singapore 6/86 (H1N1), type B/Panama 45/90, B/Yamagata 16/88, B/Ann Arbor 1/86, and on new animal strains from northern European swine and turkeys, A/Sw/Ger 2/81, A/Tur/Ger 3/91, and A/Sw/Ger 8533/91. The inhibition of replication of these strains was observed when the virus inoculum was left in contact with the elderberry extract before infecting the cell cultures. This inhibition was dose-dependent. SAM completely inhibited viral CPE at the dilution of 1:8 [final dilution during incubation with the virus was 1:16 and approximately 1% (1:96) in the culture medium]. SAM at initial dilution of 1:16 (final concentration in culture medium 0.5%) could only partially inhibit the cytopathic effect produced by the viruses at the same concentration. The number of TCID<sub>50</sub> inhibited by SAM is shown for each strain in Table 1.

No changes were observed in cell controls in the presence of SAM, undiluted and at different dilutions in the same conditions of the experiment.

### *Clinical study*

Before the beginning of the study, SAM was tested for the absence of side-effects on 35 healthy individuals from Jerusalem who received 4 tablespoons daily for 3 days. No side-effects were recorded.

The symptoms of the patients that were observed during the first visit to the dispensary are summarized in Table 2. Headache, myalgia, fever, malaise, fatigue, and rhinitis were uniform complaints and, more rarely, cough.

In the treatment group 5 out of 20 patients

TABLE 1. INHIBITION OF INFECTIVITY OF INFLUENZA VIRUS STRAINS BY SAMBUCOL® (SAM)

Strain	Number of TCID <sub>50</sub> of virus inhibited dilution of SAM	
	1:8	1:16
A/Beijing 32/92 H3N2	40	40
A/Shangdong 9/93 H3N2	40	ND
A/Singapore 6/86 H1N1	4	ND
A/Texas 36/91 H1N1	2	ND
B/Panama 45/90	2	ND
B/Yamagata 16/88	20	10
B/Ann Arbor 1/86	7	3.5
A/Sw/Ger 2/81 H1N1	8	4
A/Tur/Ger 3/91 H1N1	2	1
A/Sw//Ger 8533/91 H1N1	8	2

and in the control group 8 out of 20 patients were excluded from the study due to inconsistency of the treatment or visits to the physician, medication with antibiotics due to streptococcal infection and tonsillitis, or because they were negative in all virological tests. The details of the 27 patients are given in Table 3. The age of the patients was 5 to 50 years (mean 23.5) in the group treated with SAM and 7–56 years (mean 19.4, median 12) in the placebo group. The male:female ratio was 9:6 in the SAM group and 9:3 in the placebo group.

In the group treated with SAM, convalescent-phase serologies showed a mean HI antibody titer of 201 to influenza B Panama/45/90 in 13 out of 15 cases and at least a 4-fold titer rise in the complement fixation test (Figs. 1 and 2). One patient was found positive to RSV and another one positive to both RSV and adenovirus. In the placebo group, convalescent-phase serologies showed a mean HI antibody titer of 171 to influenza B/Panama/45/90 in 10

TABLE 2. DISTRIBUTION OF SYMPTOMS AMONG 27 PATIENTS INCLUDED IN THE STUDY

Symptoms	% of cases
Headache	100
Fever	96.3
Fatigue	88.9
Rhinitis	85.2
Pharyngitis	74.0
Myalgia	51.8
Cough	18.5

TABLE 3. AGE, SEX, AND VIRAL INFECTIONS IN THE TREATED AND CONTROL GROUP

	Sambucol®	Placebo
Total in group	15	12
Males:females	9:6	9:3
Age (years)	5–50	7–56
Influenza B	13	10
Influenza A	—	2
RSV	1	—
Adeno/RSV	1	—

out of 12 cases (one of them was also positive to RSV). Although the differences between the antibody titers in the two groups were not significant, a clear trend in favor of the group treated with the extract was noted. The geometric mean HI titers were 154.88 in the SAM treated group and 109.06 in the control group. Two patients had serologic evidence of influenza A together with antibodies to influenza B.

Persistence of the symptoms was checked for 6 days after the onset of the treatment. The fever persisted for 4 days in the treatment group, and at least 6 days in the placebo group (Fig. 3). Mean numbers of days with fever were  $2.36 \pm 0.9$  in the SAM group and  $3.33 \pm 1.5$  in the placebo group. The odds for having less than 5 days of fever in the treated group were 21.3 times the odds in the control ( $p < 0.01$ ).

Improvement of the symptoms was recorded daily (Fig. 4). In the group treated with SAM, improvement was noted in 20% of the cases one

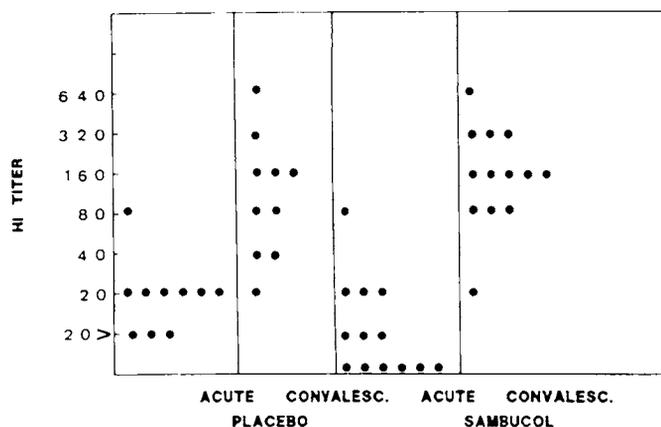


FIG. 1. Influenza B/Panama/45/90 antibody titers in acute and convalescent phase sera measured by the hemagglutination-inhibition test (HI).



by influenza viruses, suggesting that the extract inhibits the hemagglutinin of the viruses by binding to the virus itself and does not interfere with the glycoconjugate receptor on the erythrocytes. Moreover, the replication of human influenza viruses type A and B, including the strains (Shangdong, Singapore, Panama) of the vaccine proposed for the winter 94/95 as well of new strains of animal influenza viruses from turkey and swine, could be prevented in cell cultures by previous incubation of the virus inoculum with SAM. To avoid any nonspecific interaction due to its low pH, SAM was used in most of the experiments at the dilution of 1:8. It could be assumed that SAM at higher concentrations would have been an even more potent inhibitor. To remove any doubt on the efficacy of the pure elderberry extract itself, a number of experiments were performed with this extract alone, e.g., without the presence of additives such as citric acid. Influenza A and B viruses express two envelope glycoproteins: hemagglutinin and neuraminidase. The hemagglutinin is known to mediate the attachment of the virus to the host cells via sialic acid residue in glycoconjugate receptors and the subsequent fusion of viral and host cell membranes (Wieley and Skehel, 1987). The sialidase catalyzes cleavage of terminal sialic acid residue from the sialoconjugate receptors (Gottschalk et al., 1972). It can be assumed that the inhibitory effect on influenza virus in tissue culture is mediated by inactivation of viral glycoproteins, which prevent the initial stage of reproduction. The inhibition of the hemagglutinin was clearly demonstrated. The presence of flavonoids (Bronnum-Hansen and Hansen, 1983) in SAM could be responsible for blocking of the virus sialidase since flavonoids are known to have potent antiinfluenza activity. Further experiments are underway to test this hypothesis and preliminary results indicated that SAM may partially block the sialidase of influenza viruses.

Influenza vaccine is useful for prophylaxis of influenza virus infection, but antigenicity of influenza viruses is often alterable by antigenic shift and antigenic drift on their two antigens, hemagglutinin and neuraminidase. In this study we have shown that SAM inhibits the hemagglutinin of all strains of influenza

viruses tested. Moreover, fresh pandemic may come from an influenza virus that infects animals but also infects humans under favorable conditions. Data have accumulated that indicate that genetic reassortment occurs *in vivo* in mixed infections in swine and turkeys. They may also arise from a hybridization of an animal strain and a human strain. Mutant type A swine flu viruses may have been responsible for the widespread epidemics in 1918 and 1957 (Scholtissek et al., 1978; Hinshaw et al., 1978; Kilbourne et al., 1971; Webster et al., 1973). SAM was shown to inhibit strains isolated from turkeys and swine that under favorable circumstances could produce such pandemic epidemics to which people lack immunity. The eventuality of a new pandemic has been raised lately and seems quite likely (Hannoun, 1994).

The antiviral properties of SAM were further tested in a double-blind, placebo-controlled study. The comparison of 15 patients who received SAM with 12 patients who received a placebo showed that in the treatment group a significant improvement of the symptoms of flu, including fever, was seen in 93.3% of the cases within 2 days. In the control group 91.7% of the patients showed an improvement within 6 days. A complete cure was achieved within 2 to 3 days in nearly 90% of the group treated with SAM and within at least 6 days in the placebo group ( $p < 0.001$ ).

Most of the patients showed laboratory documented influenza B infection. In the control group, the two patients who showed evidence for influenza A had also antibodies to influenza B. This could be the result of a heterotypic antibody response to influenza A as reported in 25% persons infected with influenza B (WHO, 1991–1992). Convalescent-phase serologies showed higher mean and geometric mean HI antibody titer to influenza B in the elderberry extract group. Administration of SAM seems to enhance the immune response, whereas amantadine suppresses the serologic response to influenza A (Reuman et al., 1989).

These preliminary results should be confirmed by a study on a larger number of patients, which should also include more influenza A-infected individuals. Although the laboratory data documented the diagnosis of influenza, it would be of interest to isolate the

virus in the nasal and throat secretions of the patients. Treatment with SAM could result in a decrease of transmissibility of influenza viruses, resulting in fewer secondary cases of infection in communities such as the homes for the elderly, army camps, and university residences.

Vaccination with influenza B induces a poor antibody response, particularly in elderly patients, since influenza B is less immunogenic (Peters et al., 1988). In the absence of any proper medication against influenza B virus, and considering the efficacy of the Sambucol® against all strains tested and its absence of side effects, this preparation could offer a possibility for a safe treatment for influenza, and especially in the eventuality of a new pandemic.

### ACKNOWLEDGMENTS

We thank Mrs. M. Horowitz, head nurse of kibbutz Kfar Aza, and her staff for their help in the organization of this study. We also thank the members of the kibbutz for their collaboration.

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# Randomized Study of the Efficacy and Safety of Oral Elderberry Extract in the Treatment of Influenza A and B Virus Infections

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Elderberry has been used in folk medicine for centuries to treat influenza, colds and sinusitis, and has been reported to have antiviral activity against influenza and herpes simplex. We investigated the efficacy and safety of oral elderberry syrup for treating influenza A and B infections. Sixty patients (aged 18 – 54 years) suffering from influenza-like symptoms for 48 h or less were enrolled in this randomized, double-blind, placebo-controlled study during the influenza season of 1999 – 2000

in Norway. Patients received 15 ml of elderberry or placebo syrup four times a day for 5 days, and recorded their symptoms using a visual analogue scale. Symptoms were relieved on average 4 days earlier and use of rescue medication was significantly less in those receiving elderberry extract compared with placebo. Elderberry extract seems to offer an efficient, safe and cost-effective treatment for influenza. These findings need to be confirmed in a larger study.

**KEY WORDS:** BLACK ELDERBERRY; SAMBUCOL®; INFLUENZA A AND B; CLINICAL EFFICACY; TOLERABILITY; CONTROLLED STUDY

## Introduction

The influenza virus is an orthomyxovirus and causes an acute respiratory tract disease. Influenza is typically characterized by abrupt onset of fever, headache, myalgia, sore throat and non-productive cough. The illness is usually self-limiting, with relief of symptoms occurring within 5 – 7 days. Nevertheless, it is an important disease due to its ease of communicability, short incubation time, rapid rate of viral mutation, related morbidity, resultant loss of productivity,

and the possibility of severe complications. Influenza can be fatal, particularly in the very young, the elderly and in immunocompromised patients.

Most widespread epidemics are caused by influenza virus type A. Between 1972 and 1995, the estimated worldwide influenza-associated deaths ranged from approximately 25 to > 150 per 10 000 in those aged ≥ 65 years; > 90% of the deaths attributed to pneumonia and influenza occurred in this age group.<sup>1</sup>

Vaccination with inactivated viruses and

chemoprophylaxis or therapy with influenza-specific antiviral drugs such as amantadine, rimantadine, zanamivir and oseltamivir are widely used. Vaccinating those at high risk of influenza-related complications before the influenza season each year is the most effective and most commonly used ways of reducing the impact of influenza.

Amantadine and rimantadine interfere with the replication cycle of type A (but not type B) influenza viruses. When administered prophylactically to healthy adults or children, both drugs are effective in preventing the illness in approximately 70 – 90% of influenza type A infections. When administered within 48 h of illness onset, amantadine and rimantadine can reduce the severity and shorten the duration of an influenza A infection. Zanamivir and oseltamivir belong to a new class of antiviral agents known as neuraminidase inhibitors, and their efficacy for influenza A and B treatment is under evaluation. Although effective in decreasing symptoms, none of these agents prevents pneumonia or hospitalization secondary to influenza.<sup>2</sup>

The black elder (*Sambucus nigra* L.) has been used in folk medicine for centuries to treat influenza, colds and sinusitis.<sup>3</sup> Antiviral activity of three plants, including the elder, has been reported against influenza and herpes.<sup>4</sup> The berries of black elder contain high levels of flavonoids,<sup>5</sup> which are naturally occurring plant substances. Several plant extracts containing flavonoids or purified flavonoids have been shown to have antiviral activity against herpes simplex virus type 1, respiratory syncytial virus, and the parainfluenza and influenza viruses.<sup>6–9</sup> The main flavonoids found in elderberries are the anthocyanins cyanidin 3-glucoside and cyanidin 3-sambubioside.<sup>10,11</sup> It has recently been shown that these substances

are detectable in plasma after oral intake of elderberry extract.<sup>12</sup>

Elderberry extracts are commercially available as nutritional supplements for humans, and are used extensively in many countries. Standardized elderberry extract has been shown to reduce haemagglutination and inhibit replication of influenza A and B viruses *in vitro*,<sup>13</sup> and be effective in treating influenza B/Panama.<sup>13</sup> The prophylactic and symptom-dependent treatment of influenza-like symptoms using a commercial elderberry extract was also demonstrated in a colony of chimpanzees in the Jerusalem Zoo, Israel.<sup>14</sup>

We aimed to investigate the efficacy and tolerability of a standardized elderberry extract for treating influenza A and B infections in humans.

## Patients and methods

### THE STUDY

This multicentre (four sites) randomized, placebo-controlled study was conducted according to the revised Declaration of Helsinki and with the approval of the regional ethical committee. The study was conducted during a period when influenza was known to be present in the community. The decision regarding when to begin the study was based on surveillance data received from the clinical and viral spotting system of the National Laboratory of Health, Norway.

Individual, computer-generated randomization envelopes were kept sealed throughout the study. The randomization code was only broken once all the data had been collected.

### PATIENTS

During autumn and winter 1999 – 2000, 80 candidates presenting at an investigator's office with respiratory influenza symptoms (classified as 487 in the International

Classification of Primary Care) were screened for inclusion. Those with verified influenza were enrolled in the study. All subjects had a fever  $\geq 38.0^{\circ}\text{C}$  and at least one respiratory influenza symptom. Exclusion criteria included those who were pregnant or breastfeeding, those with suspected bacterial infections, recent antiviral therapy, recent participation in another clinical trial, anti-influenza vaccination and treatment for chronic diseases. The subjects were all healthy individuals, with the exception of the current episode of influenza, and did not belong to high-risk groups. Written informed consent was obtained from each patient before enrolment.

### INFLUENZA VIRUS ISOLATION

Nasal wash, nasopharyngeal aspirate or swabs from patients were tested in local laboratories using routine methods for antigen detection.<sup>15,16</sup> Influenza virus infection was confirmed by a four-fold increase between the acute and the convalescent haemagglutination-inhibition antibody titre and/or a positive influenza culture at any time-point between days 1 and 7.

### TREATMENT

A standardized elderberry extract (Sambucol<sup>®</sup>, Razei Bar, Jerusalem, Israel) was used. The syrup formulation contained 38% of the standardized extract plus small amounts of raspberry extract, glucose, citric acid and honey. Standardization of the flavonoid content was maintained by ensuring the absorbance at 516 nm was above 0.60. The extract is produced according to good manufacturing practice, and both its production and the production facilities are certified by the Israeli Health Authorities.

The syrup was supplied in amber bottles containing 120 ml. To make the study blind,

the placebo syrup had an identical appearance and taste and was supplied in the same type of bottles. The placebo syrup did not contain the elderberry extract, but was otherwise identical. Both syrups were produced and supplied by Razei Bar Ltd (Jerusalem, Israel).

The subjects were randomly assigned to two groups, and received either 15 ml elderberry or placebo syrup four times a day, during meals, for 5 days. The first dose of medication was given within 48 h of the onset of the influenza-like symptoms.

### CONCOMITANT MEDICATION

Patients were allowed to take concomitant medications during the study in the form of the antipyretic/analgesic agent paracetamol (Paracet<sup>®</sup>, Weifa, Oslo, Norway; 500 mg tablets) and/or a dose-metered nasal spray (Otrivin<sup>®</sup>, Novartis, Basel, Switzerland; 1 mg/ml) to relieve the influenza symptoms (rescue medications) if treatment with Sambucol<sup>®</sup> or placebo did not help. These medications were provided free of charge and were marketed drugs. In cases of known allergy to the rescue medications, alternatives were provided (acetylsalicylic pain killers instead of paracetamol and salt water spray instead of Otrivin<sup>®</sup>). Patients recorded the date, time and dose of any concomitant medication taken, as well as the name of the drug used.

All unused test syrups and concomitant medications were returned at the end of the study so that they could be checked against the diary card for compliance.

### EFFICACY EVALUATION

The treatment efficacy was evaluated by assessing the symptoms and overall well-being (global evaluation). The symptoms assessed were: aches and pains, degree of coughing, frequency of coughing, quality of

sleep, mucus discharge in the respiratory tract and nasal congestion. These symptoms were assessed at baseline to investigate if the two groups were clinically comparable at the start of the study. The visual analogue scales (VAS) used at baseline had the endpoints 0 'no problems' and 10 'pronounced problems'.

Patients scored their symptoms on diary cards at baseline, four times a day during treatment and twice daily for 5 days after the treatment had finished. The VAS endpoints during and after treatment were 'no improvement' (at 0 cm) and 'pronounced improvement' (at 10 cm) and were independent of the baseline scores.

For all VAS assessments, patients marked their assessment of the symptom on a line separating the points 0 and 10. The distance from the zero point to the mark was used for the statistical evaluation.

## STATISTICAL ANALYSIS

Variables assumed to be continuous were expressed as mean values, with 95% confidence intervals constructed using Student's *t*-distribution method. The standard deviation and total range were used as indices of distribution. Both inter- and intra-group analyses were carried out using two-tailed tests with a significance level of 5%.

The continuously distributed variables were analysed using analysis of variance models with repeated measurements, for comparisons both between and within groups. SAS® (version 6.0) software (Statistical Analysis System, SAS Institute, Cary, NC, USA) was used for all the statistical analyses.

## Results

Recruitment of patients took place from week 50 of 1999 until week 6 of 2000. This period was the time of main activity of an influenza epidemic in Norway.<sup>17</sup>

Sixty patients (aged between 18 and 54 years) were enrolled in the study. Their demographic characteristics, infecting virus and symptoms on enrolment are shown in Table 1. At the beginning of the study, no significant differences were observed between the active treatment group (those receiving elderberry syrup;  $n = 30$ ) and the placebo group ( $n = 30$ ) with regard to demographic characteristics, smoking status, clinical symptoms, problems related to sleeping and normal activity or absenteeism from work. The mean duration of the illness before receiving the first dose was 27.2 h.

The baseline (day 1) VAS scores for the different parameters examined are listed in Table 2. There were no significant differences between the groups.

Visual analogue scale (efficacy) scores on the follow-up days (days 2 – 10) for the various symptoms studied are listed in Table 3. There was a significant difference ( $P < 0.001$ ) in the development of the mean scores. In the elderberry group, most of the scores were near to 'pronounced improvement' (0 = no improvement and 10 = pronounced improvement) after 3 – 4 days, while the placebo group reached this level after 7 – 8 days. Patients from both groups were fully recovered after day 8.

The global evaluation scores for the elderberry group showed a pronounced improvement (VAS score nearer to 10) after a mean ( $\pm$  SD) of  $3.1 \pm 1.3$  days, while a similar score was obtained after  $7.1 \pm 2.5$  days in the placebo group (Fig. 1, Table 3). This difference was significant ( $P < 0.001$ ).

None of the patients reported any adverse reactions related to the medication. One participant receiving elderberry syrup disliked the taste. As sedation is a main side-effect of most anti-influenza medications, the participants were specifically asked if they had any problem with sedation during the study period; none of the patients

reported having such problems.

Use of rescue medication is shown in Table 4. Usage was significantly less ( $P < 0.001$ ) in the elderberry group compared with the placebo group.

A positive correlation ( $P < 0.01$ ) was found between the amount of unused medication and the information provided by each patient on the number of days the preparation was used. This indicated that all patients fulfilled the compliance criteria by taking  $> 80\%$  of the recommended dose (15 ml four times a day).

## Discussion

The efficacy of elderberry syrup has previously been investigated in a placebo-controlled, double-blind clinical study during an outbreak

of influenza B/Panama.<sup>13</sup> A complete cure was achieved within 2–3 days in nearly 90% of the elderberry-treated group compared with at least 6 days in the placebo group ( $P < 0.001$ ). The results of our study show that elderberry syrup is also effective against influenza A virus infections. Both studies show that the duration of the illness can be reduced by 3–4 days with elderberry syrup compared with placebo. The prophylactic and curative effects of this syrup have been demonstrated in a study performed in a chimpanzee colony,<sup>14</sup> in which the appearance of symptoms was reduced by two-thirds. To our knowledge, no placebo-controlled, double-blind studies have been done with other natural remedies against the influenza viruses.

The main flavonoids present in elderberries

**TABLE 1:**  
Baseline characteristics of the 60 patients with influenza symptoms who were randomized to the receive elderberry or placebo syrup in this study

	Placebo group	Elderberry group
Influenza type		
A	28	26
B	2	4
Age (mean $\pm$ SD)	29.4 $\pm$ 2.8 years	30.6 $\pm$ 2.9 years
Gender		
Female	15	12
Male	15	18
Fever	30	30
Smokers	1	4
Non-productive dry cough	7	5
Cough with mucus from the chest	23	25
Cough so severe that it is associated with a feeling of vomiting	18	15
Duration of cough (mean $\pm$ SD)	1.3 $\pm$ 0.5 days	1.5 $\pm$ 0.7 days
Problems with sleeping	25	20
Awoken several times during night	15	20
Able to work (not on sick leave)	7	10
Time absent from work (mean $\pm$ SD)	1.3 $\pm$ 0.5 days	1.2 $\pm$ 0.6 days

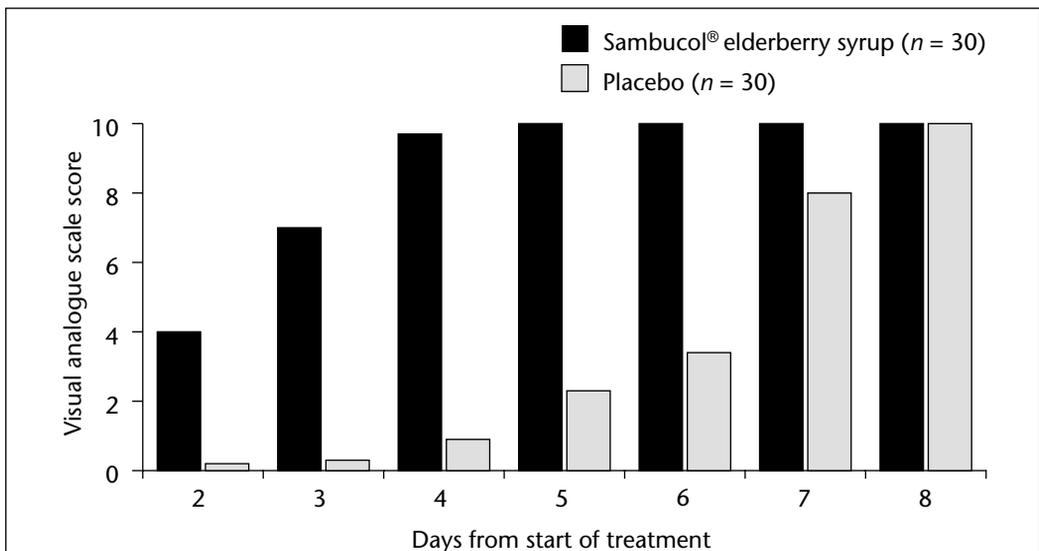
**TABLE 2:**  
 Baseline (day 1) visual analogue scores for influenza symptoms and global evaluation as assessed by the 60 patients with influenza in this study

	Placebo group	Elderberry group
Aches and pains	7.5 ± 1.5	7.0 ± 1.4
Frequency of coughing	6.9 ± 1.2	7.3 ± 1.6
Quality of sleep	5.2 ± 1.4	6.0 ± 1.5
Mucus discharge in the respiratory tract	7.6 ± 1.5	7.0 ± 1.6
Nasal congestion	7.7 ± 1.3	7.4 ± 1.4
Global evaluation	7.0 ± 1.5	7.2 ± 1.5

Values given are the mean ± SD score (cm). A score of 0 cm indicates no problems and a score of 10 cm indicates pronounced problems (i.e., the higher the score, the more severe the symptoms).

are the anthocyanins cyanidin 3-glucoside and cyanidin 3-sambubioside,<sup>10,11</sup> and are detectable in plasma after oral intake of elderberry extract.<sup>12</sup> A possible mechanism of action of elderberry extract in the treatment of influenza is that the flavonoids stimulate the immune system by enhancing production of

cytokines by monocytes.<sup>18</sup> In addition, elderberry has been shown to inhibit the haemagglutination of the influenza virus and thus prevent the adhesion of the virus to the cell receptors.<sup>13</sup> Anthocyanins also have an anti-inflammatory effect comparable to that of acetylsalicylic acid;<sup>19</sup> this could explain the



**FIGURE 1:** The development of self-evaluation scores in global well-being in the 60 patients with influenza who received either elderberry syrup or placebo (15 ml, four times daily with meals, for 5 days)

**TABLE 3:** Visual analogue scores for symptoms and global evaluation on days 2 – 10 as assessed by the 60 patients with influenza in this study

	Day																		
	2		3		4		5		6		7		8		9		10		
	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E	P	E	
Aches and pains	0.2	2.2	0.3	6.5	0.5	9.8	2.0	10.0	2.6	9.9	8.4	9.8	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Frequency of coughing	0.0	2.5	0.2	7.0	0.6	8.7	2.5	9.8	3.2	9.8	8.1	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Quality of sleep	0.2	3.0	0.4	7.0	1.0	9.5	2.7	9.7	3.4	10.0	7.8	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mucus discharge in the respiratory tract	0.3	3.5	0.5	6.2	1.0	9.2	2.5	9.5	3.1	9.8	7.5	9.7	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Nasal congestion	0.1	4.0	0.2	6.7	0.6	9.2	2.4	9.4	3.5	9.8	8.6	9.8	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Global evaluation	0.2	4.0	0.3	7.0	0.9	9.7	2.3	10.0	3.4	10.0	8.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

P, placebo group; E, elderberry group. Values given are the mean score (cm). A score of 0 cm indicates pronounced problems and a score of 10 cm indicates no problems (i.e., the higher the score the greater improvement in symptoms).

**TABLE 4:**  
Number of patients with influenza using rescue medication in the placebo and elderberry syrup-treated groups

Type of medication	Placebo group	Elderberry group
Nasal spray	21	5
Painkiller	26	7

pronounced effect on aches, pain and fever seen in the group treated with elderberry syrup.

Vaccination is effective for prophylaxis and in reducing the impact of influenza, but only about 60% of people aged 65 years and above, and less than 30% of people aged less than 65 years, are vaccinated annually (worldwide figures). Some elderly individuals and immunocompromised people do not respond optimally to the vaccine, and the vaccine may not always include the strain of virus circulating within a given community.<sup>1</sup>

It is not known whether amantadine and rimantadine prevent the complications of type A influenza infections among people at high risk. Use of these drugs is limited due to their side-effects and the frequent incidence (approximately 30%) of drug resistance. No data are available to determine the efficacy of rimantadine among children, so it is currently approved for prophylaxis but not treatment of influenza in children.

Zanamivir has been shown to reduce the duration of influenza A and B infections by 1–2.5 days. The route of administration is by inhalation via a Diskhaler® (GlaxoSmithKline, Middlesex, UK) and the drug is designed for patients aged 12 years and above.<sup>20,21</sup> Oseltamivir may reduce the duration of illness by 1.5 days.<sup>22</sup>

In contrast to these antiviral drugs, elderberries can be administered to the whole

population, including infants and children. It should, however, be stressed that a wide number of elderberry preparations are available on the market, in the form of both syrups and capsules. The extract tested in this study was standardized with respect to the content of flavonoids and was produced in accordance with good manufacturing practice. A number of the other preparations available lack or have a very low flavonoid content. We believe that adequate amounts, as well as the composition, of flavonoids present in the extract are essential for the therapeutic effect of elderberry syrup as reported in our study.

In view of its *in vitro* and *in vivo* efficacy on influenza A and B viruses, elderberry extract offers an efficient, safe and cost-effective supplement to the present armamentarium of medications for the prophylaxis and treatment of influenza. It should be stressed that our study involved only adult influenza patients who were otherwise healthy, and did not include any high-risk patients. Further studies are required to confirm these results in larger numbers of patients and to investigate the effect of elderberry syrup in other patient groups.

## Acknowledgement

The study was sponsored by Razei Bar, Jerusalem, Israel.

- Received for publication 12 September 2003 • Accepted subject to revision 23 September 2003
- Revised accepted 3 November 2003

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