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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.[1]

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.[2]

The black elder (*Sambucus nigra* L.) has been used in folk medicine for centuries to treat influenza, colds and sinusitis.[3] Antiviral activity of three plants, including the elder, has been reported against influenza and herpes.[4] The berries of black elder contain high levels of flavonoids,[5] 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.[6 – 9] The main flavonoids found in elderberries are the anthocyanins cyanidin 3-glucoside and cyanidin 3-sambubioside.[10,11] It has recently been shown that these substances are detectable in plasma after oral intake of elderberry extract.[12]

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*,[13] and be effective in treating influenza B/Panama.[13] 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.[14]

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 ≥ 38.0 °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.[15,16] 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®, 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®, Weifa, Oslo, Norway; 500 mg tablets) and/or a dose-metered nasal spray (Otrivin®, Novartis, Basel, Switzerland; 1 mg/ml) to relieve the influenza symptoms (rescue medications) if treatment with Sambucol® 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®). 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.[17]

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 (± SD) of 3.1 ± 1.3 days, while a similar score was obtained after 7.1 ± 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.[13] 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,[14] 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 ± SD)	29.4 ± 2.8 years	30.6 ± 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 ± SD)	1.3 ± 0.5 days	1.5 ± 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 ± SD)	1.3 ± 0.5 days	1.2 ± 0.6 days

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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,[10,11] and are detectable in plasma after oral intake of elderberry extract.[12] 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.[18] 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.[13] Anthocyanins also have an anti-inflammatory effect comparable to that of acetylsalicylic acid;[19] this could explain the

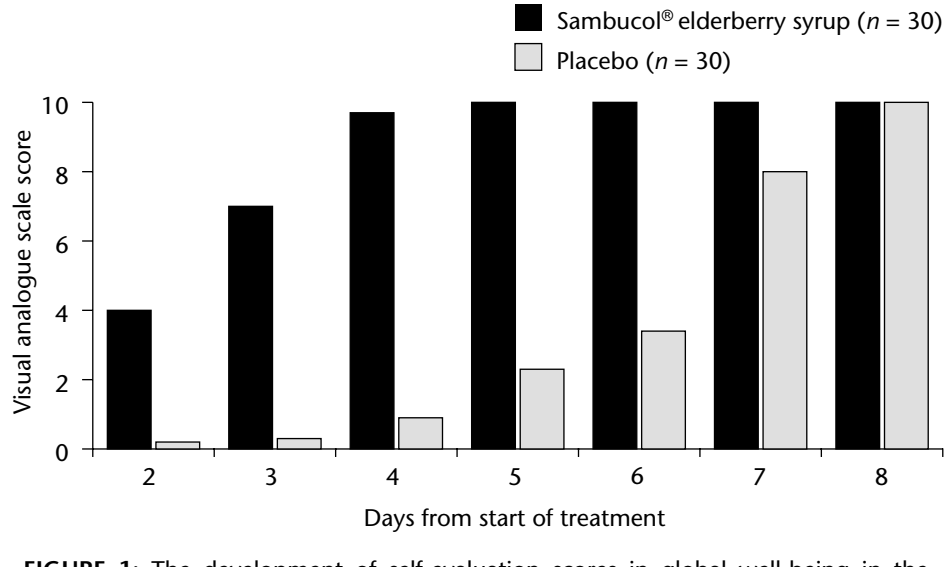

FIGURE 1: The development of self-evaluation scores in global well-being in the 60 patients with influenza who received either elderberry syrup or placebo (15 ml, four times daily with meals, for 5 days)

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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		
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		
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		
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		
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		
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		

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.[1]

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.[20,21] Oseltamivir may reduce the duration of illness by 1.5 days.[22]

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.

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