OJPK™

Online Journal of Pharmacology and PharmacoKinetics ©

Volume 5: 32-43, 2009

Pilot Clinical Study on a Proprietary Elderberry Extract: Efficacy in Addressing Influenza Symptoms

Fan-kun Kong, PhD.

Trial conducted by Medical Personnel at Shanghai Construction Technical College, China, on behalf of HerbalScience Singapore Pte. Ltd., Singapore.

ABSTRACT

Kong F, Pilot Clinical Study on a Proprietary Elderberry Extract: Efficacy in Addressing Influenza Symptoms, Online J Pharmacol Pharmacokin 5:32-43, 2009. Elderberry (*Sambucus nigra* L.) has a long history of being used in treating colds and influenza. A proprietary standardized elderberry extract has been formulated into a slow-dissolve lozenge. A pilot, randomized, double-blind, placebo-controlled clinical trial was conducted during the spring flu season of 2009 to evaluate the efficacy of the extract in relieving flu-like symptoms. Sixty-four patients with three or more flu-like symptoms (fever, headache, muscle aches, coughing, nasal mucus discharge, and nasal congestion) for less than 24 hours were enrolled in the study. The patients were randomized into two groups and given 4 doses of 175 mg of the proprietary elderberry extract per dose (n=32) or a placebo (n=32) daily for two days. The severity of symptoms was self-monitored by the patients and scored on a Visual Analogue Scale (VAS), with "0" equal to no problem and "10" equal to a pronounced problem. The extract treated group showed significant improvement in most of the symptoms except 24 hours after the onset of the treatment, whereas the placebo group showed no improvement or an increase in severity of the symptoms at the same time point. By 48 hours, 9 patients (28%) in the extract treated group were void of all symptoms, 19 patients (60%) showed relief from some symptoms, and had only one or two mild symptoms (VAS=1). The remaining 4 patients also showed symptoms improvement but to a lesser degree. In contrast, complete recovery was not achieved by a single patient in the placebo group. Only 5 patients (16%) showed improvement in one or two

symptoms. For most patients in this group, the symptoms remained the same or even worsened over the 48-hour monitoring period. No adverse effects were observed in either group indicating that the proprietary elderberry extract is safe and highly effective in treating flu-like symptoms.

Key words: proprietary elderberry extract, clinical trial, influenza, flu-like symptoms

INTRODUCTION

Influenza is an acute respiratory illness caused by infection with influenza type A or B viruses (Nicholson *et al*. 1998) with symptoms of fever, sore throat, chills, fatigue, cough, headache, and muscle aches. The disease has high morbidity rates for people of all ages and particularly high mortality rates for children, adults over 60 years old, patients with chronic illnesses, and pregnant women (Fields *et al.* 2001; Thompson *et al.* 2003). On average, 226,000 people are hospitalized every year because of influenza and 36,000, mostly elderly, die yearly in the United States alone (Fields *et al.* 2001; Thompson *et al.* 2003). Influenza A viruses continuously mutate, resulting in new type A virus strains that pose significant pandemic threats to populations lacking natural immunity. In 2003, the outbreak of a highly pathogenic strain of avian influenza (H5N1) infection caused 423 human cases and 258 deaths in 15 countries, mainly in Asia (WHO, 2009). The most recent H1N1 swine flu outbreak in Mexico has spread to more than 100 countries with over ninety-four thousand cases and 429 deaths reported thus far (WHO, July 2009; Trifonov *et al*. 2009; Vivek *et al*. 2009).

Mass vaccination of the human population is considered the most effective approach to prevent an influenza pandemic (Subbarao *et al.* 2006). In the event of an influenza outbreak, antiviral drugs are recommended for reducing the severe morbidity and mortality. There are currently four influenza antiviral drugs approved for use in the United States. The M2 ion channel-blocking drugs amantadine and rimantadine inhibit the influenza virus by interfering with the M2 ion-channel protein once inside the host cells (Wang *et al.* 1993). Unfortunately, influenza A resistance to amantadine and rimantadine has been increasing since these drugs were introduced to the market (Belshe *et al.* 1989; Hayden 1994). The neuraminidase inhibitors oseltamivir and zanamivir interfere with the release of the influenza virion from infected host cells preventing the spread of infection (von Itzstein *et al.* 1993). Since pandemic influenza viruses are generally not sensitive to the M2 blocker drugs, the neuraminidase inhibitor drugs are likely to be the primary drugs used in a pandemic. However, neuraminidase inhibitors show limited efficacy in the treatment of H5N1 avian influenza infections (Le *et al* 2005; Jefferson *et al.* 2006) and increasing reports indicate that annually circulating flu viruses have also developed significant resistance to the neuraminidase inhibitors (Moscona 2005; Jefferson *et al.* 2006).

Elderberry (*Sambucus nigra* L.), as a folk medicine, has a long history of being used in treating colds and flu (Roxas and Jurenka 2007). Two independent clinical trials demonstrated that a syrup made from elderberry extract was effective in treating influenza A and B virus infection (Zakay-Rones *et al.* 1995; 2004). Both studies showed that patients diagnosed with influenza and receiving an elderberry syrup treatment had significantly shorter durations of flu-like symptoms. To further investigate the anti-viral properties of elderberry extracts and their efficacy in relieving

symptoms of influenza, proprietary extraction technologies were used to produce a dose-reliable, elderberry extract with *in vitro* anti-viral activity (Fink *et al.* 2009; Roschek Jr. *et al.* 2009). The proprietary elderberry extract was formulated as a slow-dissolve lozenge. In the present study, a randomized, double-blind, placebo-controlled pilot clinical trial was conducted to evaluate the efficacy of the proprietary elderberry extract for treatment of flu-like symptoms.

MATERIALS AND METHODS

This short-term, randomized, double-blind, placebo-controlled clinical trial was conducted by medical personnel at Shanghai Construction Technical College during the spring flu season of 2009 (March-April).

Participants: Volunteers (age ranged 16 to 60 years) presenting flu symptoms for less than 24 hours, and otherwise healthy individuals were included in the clinical study. The admitted participants had at least three of the following symptoms: fever, headache, muscle aches, coughing, mucus discharge and nasal congestion. Patients who suffered from chronic diseases, were suspected of having a bacterial infection, participated in another clinical trial, or recently received flu medication, antiviral therapy, or influenza vaccination were excluded from the study. Pregnant and breastfeeding women were also excluded from the study.

Randomization: Patients were assigned a 'patient' number based on computer-generated randomization. The information was kept sealed throughout the study. The randomization code remained unbroken until all of the data had been collected.

Treatment: A proprietary elderberry extract formulated as a slow-dissolve lozenge was used in the study. Each lozenge contained 175 mg of the proprietary elderberry extract plus non-active ingredients (maltodextrin, dextrose, fructose, silica, citric acid, natural flavors, cyclodextrin and magnesium stearate). A placebo lozenge that was identical in appearance, taste, and composition except that there was no elderberry extract included, was supplied in similar packaging. The bottles were labeled with only numbers and the contents were blinded to the investigator, doctor and patients. Both the proprietary elderberry extract and placebo lozenges were supplied by HerbalScience Singapore Pte. Ltd. Enrolled patients were randomly assigned to two groups and received either the proprietary elderberry extract lozenges (treatment) or placebo lozenges (control). Patients were asked to take 4 lozenges a day for two days, one before each meal and one before bed. The first dose of medication was administered immediately after the investigator made the decision to enroll the patient into the study. All enrolled patients provided written informed consent before the trial.

Evaluation: The improvement of flu-like symptoms was assessed to evaluate the efficacy of the proprietary elderberry extract. Symptoms monitored were fever, headache, muscle aches, cough, mucus discharge from the respiratory tract, and nasal congestion. Patients were asked to self-assess and score their symptom improvements on the Visual Analogue Scales (VAS) four times a day during the 2-day treatment. These symptoms were assessed at the onset of treatment (baseline) to investigate if the two groups were clinically comparable at the start of the

study. The VAS used in the study had the endpoints 0 = no problems and 10 = pronounced problems. The before and after treatment VAS scores were used for statistical analysis.

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 ranges 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 analyzed using the analysis of variance model with repeated measurements in order to compare both between and within groups.

RESULTS

A total of 64 patients with flu symptoms were enrolled in the study and randomized into two groups. There were no obvious differences in demographic characteristics between the groups ($p>0.05$) (Table 1). At their first visit, the majority of patients from both groups complained of headache, muscle aches, and nasal congestion, while fewer patients had fever, cough and nasal mucus discharge, all indicative symptoms of an influenza infection (Table 2). The symptoms were evaluated before receiving the first dose of treatment. The mean VAS scores of most symptoms showed no significant differences between the two groups ($p>0.05$), only the mean VAS score of fever revealed statistical difference ($p=0.0256$) (Table 3A).

The patients were administered either the proprietary elderberry extract (n=32) or placebo (n=32) lozenges 4 times daily for 2 days. Symptom improvement was self-assessed and scored on the VAS cards each time when the proprietary elderberry extract lozenge or placebo lozenge was administered. The mean VAS scores of pre- and post-treatment in each group were analyzed, and intergroup comparisons were conducted as well to evaluate the effectiveness of the treatment.

Table 1. Demographic characteristics of the included patients.

	Proprietary Elderberry Extract Group	**Placebo Group**
Total number	32	32
Male	17	17
Female	15	15
Age range	20-55 years	27-59 years
Mean age	40 years	40.1 years

Fever: 15 out of 32 (46.9%) patients in the proprietary elderberry extract group and 9 out 32 (28.1%) patients in the placebo group had fever at the onset of the study (Table 2). The temperatures ranged between 37.3 to 38.8 ºC. Following the first 24 hours of treatment, the proprietary elderberry extract group showed significant reduction in fever as evidenced by a decrease in the mean VAS score from 2.67 ±1.80 to 0.47 ±0.64 ($p<0.0001$) (Figure 1) and 60% of the fever patients returned to normal temperature (Figure 2). All patients with fever in the

elderberry group returned to normal temperature within 48 hours (Figure 2). In the placebo group, the majority of the patients failed to show any improvement in fever within the 48-hour treatment period, and only 2 patients (22%) in this group returned to normal temperature (Figure 2).

Headache: All patients in both groups reported headaches at the onset of the study (Table 2). Through 24 hours of treatment, the proprietary elderberry extract group showed a significant reduction in headache symptom. The mean VAS score decreased from 4.47 ±2.14 to 1.53 ±1.41 ($p<0.0001$) (Figure 1). By 48 hours, the mean VAS score for the proprietary elderberry extract group was close to zero (0.28 ±0.63) (Figure 1) and 78% of patients in this group were free of headaches (Figure 2) while the remaining 22% reported only mild headaches (VAS=1). In contrast, headaches became more severe in the placebo group where the mean VAS score increased from 3.78 ±1.66 to 5.25 ±1.34 ($p<0.0001$) over the 48-hour treatment period (Figure 1). No improvement in headache was reported by any single individual subject in the placebo group.

Table 2. Distribution of symptoms in treatment and control groups at the beginning of the study.

Symptoms	Proprietary Elderberry Extract Group %	Placebo Group %
Head ache	100	100
Nasal congestion	100	87.5
Muscle aches	96.9	93.8
Coughing	50	50
Mucus discharge	50	34.3
Fever	46.9	28

Muscle aches: Over 90% of the patients in both groups reported muscle aches (Table 2). The mean VAS score in the proprietary elderberry extract group decreased from 2.87 ±2.13 to 1.19 ±1.05 ($p=0.0002$) within 24 hours (Figure 1), indicating a significant improvement in symptoms. By 48 hours, 87% of the patients had completely recovered from muscle aches (Figure 2), and the mean VAS score reached 0.16 ±0.45 (Figure 1). The placebo group reported a worsening of muscle aches as the mean VAS score increased from 2.13 ±2.10 to 3.47 ±1.50 ($p=0.0013$) at 48 hours (Figure 1).

Nasal congestion: All patients in the proprietary elderberry extract group and 87.5% of patients in the placebo group reported nasal congestion when enrolled in the study (Table 2). By 24 hours into the treatment, the proprietary elderberry extract group showed significant improvement in symptoms. The mean VAS score for this group decreased from 4.03 ±2.10 to 1.47 ±1.14 ($p<0.0001$) (Figure 1). By 48 hours, the mean VAS score dropped to 0.56 ±0.62 (Figure 1) and 50% of the patients were symptom free (Figure 2). In contrast, nasal congestion in the placebo group worsened in most individuals at 48 hours. The mean VAS score in this group increased from 3.30 ±1.71 to 4.26 ±1.81 ($p=0.049$) (Figure 1). Only 2 out of 30 patients (7%) in the placebo group showed alleviation of nasal congestion.

Table 3. Comparison of the VAS scores of proprietary elderberry extract and placebo treated groups at the onset (A), 24 hours (B) and 48 hours (C) of treatment.

A.

Symptoms	Proprietary Elderberry Extract Group	Placebo Group	p value
	mean±SD	mean±SD	
Headache	4.47±2.14	3.78±1.66	0.1561
Nasal congestion	4.03±2.10	3.30±1.71	0.1508
Muscle aches	2.87±2.13	2.13±2.10	0.1777
Coughing	2.07±2.19	2.19±1.47	0.8571
Mucus discharge	1.94±1.61	2.36±2.01	0.5473
Fever	2.67±1.80	1.11±0.93	0.0256

B.

Symptoms	Proprietary Elderberry Extract Group	Placebo Group	p value
	mean±SD	mean±SD	
Headache	1.53±1.41	5.25±1.34	< 0.0001
Nasal congestion	1.47±1.14	4.19±2.02	< 0.0001
Muscle aches	1.19±0.77	3.47±1.50	< 0.0001
Coughing	1.87±1.07	2.69±1.62	0.1556
Mucus discharge	1.38±1.09	2.27±2.05	0.1513
Fever	0.48±0.64	2.56±1.24	< 0.0001

C.

Symptoms	Proprietary Elderberry Extract Group	Placebo Group	p value
	mean±SD	mean±SD	
Headache	0.28±0.63	5.69±1.35	<0.0001
Nasal congestion	0.56±0.62	4.26±1.81	<0.0001
Muscle aches	0.16±0.45	3.80±1.69	<0.0001
Coughing	1.00±0.92	3.69±1.25	<0.0001
Mucus discharge	0.50±0.52	3.18±1.78	<0.0001
Fever	0	2.67±2.24	<0.0001

A score of 0 indicates no problems, and a score of 10 indicates pronounced problem.

Nasal mucus discharge: Nasal mucus discharge was a less common and less severe symptom among patients in both study groups. Only 50% of patients in the proprietary elderberry extract group and 34.3% of patients in the placebo group reported nasal mucus discharge (Table 2). Although patients in the proprietary elderberry extract group showed some improvement over the 24-hour treatment (Figure 1), the improvement was not significant (p=0.26). By 48 hours of treatment, the proprietary elderberry extract group showed significant symptom improvement with the mean VAS score decreasing from 1.94 ±1.61 to 0.50 ±0.52 (p=0.0019) (Figure 1), 8 out of 16 patients (50%) reporting no symptoms and the remaining 50% reporting only mild symptoms (VAS=1). In the placebo group, only 1 out 16 (6%) reported symptom improvement, whereas the remaining 15 patients showed no symptom improvement.

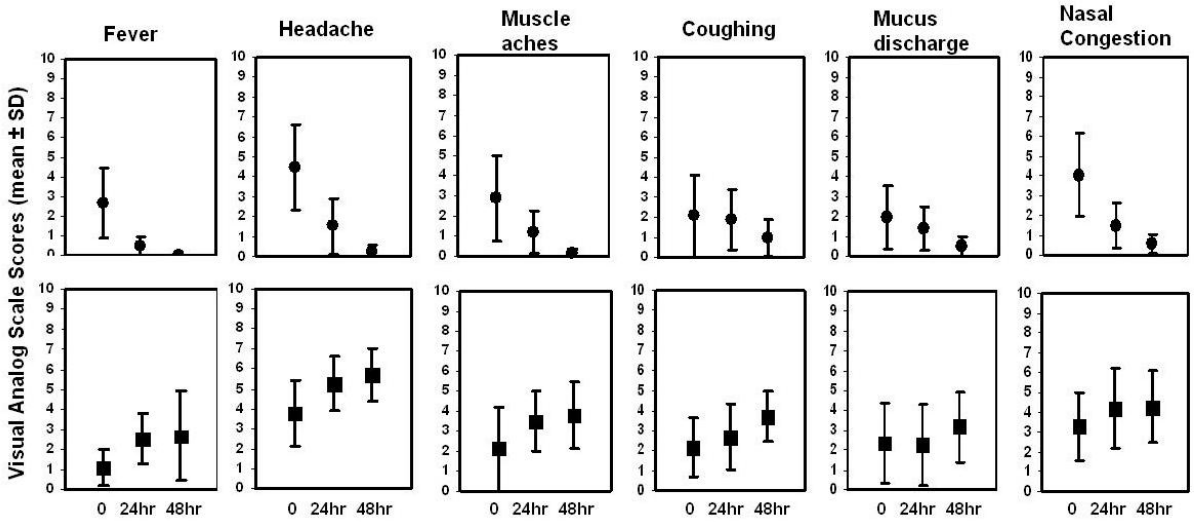

Figure 1. The development of Visual Analog Scale scores in the proprietary elderberry extract treated group (●) and the placebo group (■).The data is presented as mean ± SD, 0=no problem, 10=pronounced problem.

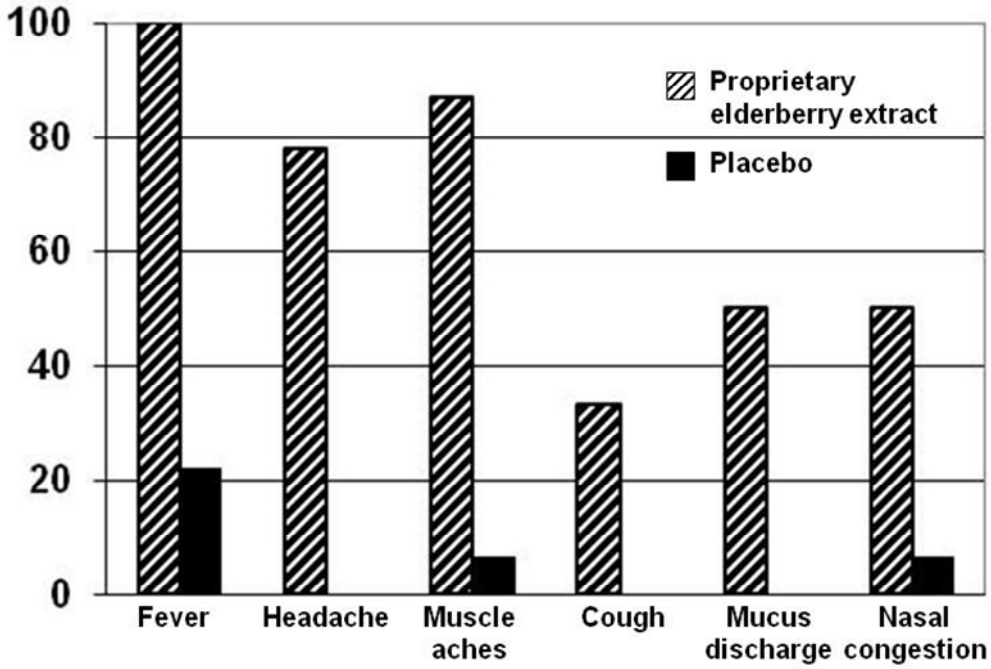

Figure 2. Percentage of patients recovered from influenza symptoms in the proprietary elderberry extract and placebo groups after 48 hours of treatment.

Coughing: Fifty percent of the patients in both groups reported coughing when enrolled into the study (Table 2). In the proprietary elderberry extract group, coughing persisted longer than the other symptoms. No significant improvement was recorded for this group over the 24-hour treatment period (Figure 1). By 48 hours, 5 out of 16 patients (31%) were relieved from coughing and 6 patients (37%) showed symptom improvement (VAS=1). Although the mean VAS score decreased from 2.07 ±2.19 to 1.00 ±0.926 (Figure 1), this decrease was not statistically significant ($p=0.093$). However, intergroup comparison (Table 3C) revealed that coughing was also significantly improved ($p<0.0001$) in the proprietary elderberry extract group. In the placebo group, 14 out of 16 (87%) patients showed worsening symptoms and the remaining 2 patients (13%) showed slight symptom improvement. The mean VAS score in the placebo group increased from 2.19 ±1.47 to 3.69 ±1.25 ($p=0.0041$) (Figure 1).

Adverse effects: No adverse reactions related to the treatment were reported by either group.

DISCUSSION

The results of this pilot, placebo-controlled, double-blinded clinical trial clearly show that administration of the proprietary elderberry extract can rapidly relieve influenza-like symptoms. Both intra-group and inter-group analysis was conducted to evaluate the efficacy of the proprietary elderberry extract. While the placebo group demonstrated no symptoms improvement, the proprietary elderberry extract treated group showed significant improvement of influenza-like symptoms within 24 hours of the onset of treatment. The severity of systemic

symptoms (fever, headache and muscle aches) and nasal symptoms (nasal congestion) were all significantly reduced. Coughing and nasal mucus discharge did not show significant improvement within 24 hours but significant improvement was achieved within 48 hours. At 48 hours of treatment, nearly 90% of the proprietary elderberry extract treated patients were either symptom free or had only mild symptoms (VAS = 1). Previously, elderberry syrup was shown to reduce the duration of flu symptoms by 3-4 days (Zakay-Rones *et al.* 1995; Zakay-Rones *et al.* 2004). In comparison, a reduction of only 2-2.5 days was reported for the neuraminidase inhibitor drugs oseltamivir and zanamivir treatment (Monto *et al.* 1999; Makela *et al.* 2000; Nicholson *et al.* 2000). These results suggest that the elderberry extract has similar or even superior efficacy than the currently used anti-viral drugs in improving symptoms and shortening the duration of influenza. Whether treatment with the proprietary elderberry extract can reduce viral shedding is unclear and additional studies including a larger patient population is needed to further confirm its anti-viral efficacy.

In the clinical study here, the proprietary elderberry extract was shown to be safe as no patients receiving the proprietary elderberry extract reported any adverse events including nausea and vomiting, which are two adverse-events common in anti-viral treatments (Nicholson *et al.* 2000). The safety profile of the proprietary elderberry extract provides supporting evidence for extending the efficacy study into children and elderly influenza patients, who are more vulnerable to influenza infections and who can develop complications more readily.

The anti-viral mechanisms of the proprietary elderberry extract are currently under investigation. Influenza virus infection is initiated by the binding of hemagglutinin (HA), a viral carbohydrate-binding membrane protein, to sialoglycoproteins or sialoglycolipids on host cell surface receptors (Webster *et al.* 1992). The neuraminidase enzyme is essential for the influenza virus replication cycle as it cleaves the terminal sialic-acid residues from glycoproteins required for the release of virions from host cells (Webster *et al.* 1992). Previous studies have proposed that flavonoids in elderberry may bind to neuraminidase and inhibit its activity though no direct evidence exists (Nagai *et al.* 1990; Zakay-Rones *et al.* 1995). In a more recent study, Direct Analysis in Real Time (DART) Time-of-Flight (TOF) mass spectrometry (Cody *et al.* 2005) was used to identify two specific flavonoids in the proprietary elderberry extract that bind to the surface of the H1N1 influenza virus and inhibit viral infection *in vitro* by interfering with host cell receptor recognition and/or blocking receptor binding (Roschek Jr. *et al.* 2009).

Facing the emerging threats of H5N1 avian influenza or H1N1 swine influenza pandemics, a large stockpile of anti-viral drugs and therapies is needed to prepare for a possible outbreak. The M2 blocking drugs amantadine and rimantadine have no therapeutic effects for pandemic influenza due to the current high level of resistance (Belshe *et al.* 1989; Hayden 1994; Beigel 2005). The neuraminidase inhibitor drugs oseltamivir and zanamivir are the only current drugs available for use in the event of a pandemic outbreak, and they too suffer from a very high level of resistance (Le *et al*. 2005; Moscona 2005). The limited efficacy of neuraminidase inhibitors in treating H5N1 avian influenza infection (Jefferson *et al.* 2006) raises concerns for our readiness in controlling a pandemic outbreak. Alternative approaches to anti-viral drugs, such as dietary supplements, to treat and/or prevent flu are certainly needed, and particularly under pandemic

conditions that cross many geographic, cultural and economic boundaries. Botanicals have a long history of use for upper respiratory disorders, and elderberry extracts have been shown to have efficacy *in vitro* as well as in human clinical trials. The current study supports previous clinical findings, and indicates that the proprietary elderberry extract used here is very effective in mitigating flu-like symptoms. Additionally, the flavonoids in the elderberry extract that bind to the H1N1 human influenza virus (Roschek Jr. *et al.* 2009) also bind to the H5N1 avian influenza virus (B. Roschek and R. Alberte, unpublished data) indicating that the proprietary elderberry extract has the potential to be effective against the H5N1 avian influenza virus and warrants further investigation of its clinical efficacy on pandemic influenza infections.

Conclusion

In conclusion, the proprietary elderberry extract used here is effective in controlling influenza symptoms and is complementary to current anti-viral agents. The safety and ease of administration warrant further investigation of its clinical efficacy in children, elderly and other high-risk patients of the proprietary elderberry extracts.

Acknowledgements

The author thanks Mr. Zhang Donghui and Ms. Huang Hong for administrative support of this clinical study. The author also thanks Dr. Xu Qiaozhi for assistance in interviewing and evaluating patients. This work was sponsored by HerbalScience Singapore Pte. Ltd. and the author admits no competing interests.

REFERENCES

Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO. May 6, 2009. [cited 2009 June 17].
http://www.who.int/csr/disease/avian_influenza/country/cases_table_2009_05_06/en/index.html

Epidemic and Pandemic Alert and Response (EPR): World Health Organization. Influenza A(H1N1). Update 58. July 6, 2009. [cited 2009 july 23].
http://www.who.int/csr/don/2009_07_06/en/index.html

Beigel J H. (2005) Avian Influenza A (H5N1) Infection in Humans. New England Journal of Medicine 353:1374-1386.

Belshe R B, Burk B, Newman F, Cerruti R L and Sim I S. (1989) Resistance of influenza A virus to amantadine and rimantadine: results of one decade of surveillance. Journal of Infectious Diseases 159:430-435.

Cody R B, Laramee J A and Durst H D. (2005) Versatile new ion source for the analysis of materials in open air under ambient conditions. Analytical Chemistry 77:2297-2302.

Fields B N, Knipe D M and Howley P M (2001) Fields Virology: in Orthomyxoviruses, 1533-1580. Lippincott, Williams & Wilkins, Philadelphia

Fink R, Roschek Jr B and Alberte R S. (2009) HIV-1 entry inhibitors with a new mode-of-action Antiviral Chemistry and Chemotherapy In Press.

Hayden F G. (1994) Amantadine and rimantadine resistance in influenza A viruses. Current Opinion in Infectious Diseases 7:674-677.

Jefferson T, Demicheli V, Rivetti D, Jones M, Di Pietrantonj C and Rivetti A. (2006) Antivirals for influenza in healthy adults: systematic review. Lancet 367:303-313.

Le Q M, Kiso M, Someya K, Sakai Y T, Nguyen T H, Nguyen K H, Pham N D, Ngyen H H, Yamada S, Muramoto Y, Horimoto T, Takada A, Goto H, Suzuki T, Suzuki Y and Kawaoka Y. (2005) Avian flu: isolation of drug-resistant H5N1 virus. Nature 437:1108.

Makela M J, Pauksens K, Rostila T, Fleming D M, Man C Y, Keene O N and Webster A. (2000) Clinical efficacy and safety of the orally inhaled neuraminidase inhibitor zanamivir in the treatment of influenza: a randomized, double-blind, placebo-controlled European study. Journal of Infection 40:42-48.

Monto A S, Fleming D M, Henry D, de Groot R, Makela M, Klein T, Elliott M, Keene O N and Man C Y. (1999) Efficacy and safety of the neuraminidase inhibitor zanamivirin the treatment of influenza A and B virus infections. Journal of Infectious Diseases 180:254-261.

Moscona A. (2005) Oseltamivir resistance—disabling our influenza defenses. New England Journal of Medicine 353:2633–2636.

Nagai T, Miyaichi Y, Tomimori T, Suzuki Y and Yamada H. (1990) Inhibition of influenza virus sialidase and anti-influenza virus activity by plant flavonoids. Chemical & Pharmaceutical Bulletin 38:1329-1332.

Nicholson K G, Webster R G, Hay A J, eds. (1998) Textbook of influenza. Oxford: Blackwell Science.

Nicholson K G, Aoki F Y, Osterhaus A D, Trottier S, Carewicz O, Mercier C H, Rode A, Kinnersley N and Ward P. (2000) Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet 355:1845-1850.

Roschek Jr. B, Fink R C, McMichael M D, Li D and Alberte R S. (2009) Elderberry flavonoids bind to and prevent H1N1 Infection *in vitro*. Phytochemistry In Press:

Roxas M and Jurenka J. (2007) Colds and influenza: a review of diagnosis and conventional, botanical, and nutritional considerations. Alternative Medicine Review 12:25-48.

Subbarao K, Murphy B R and Fauci A S. (2006) Development of effective vaccines against pandemic influenza. Immunity 24:5-9.

Thompson W W, Shay D K, Weintraub E, Brammer L, Cox N, Anderson L J and Fukuda K. (2003) Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA: Journal of the American Medical Association 289:179-186.

Trifonov, V., Khiabanian, H. and Rabadan, R.(2009) Geographic dependence, surveillance, and origins of the 2009 influenza A (H1N1) virus. New England Journal of Medicine. DOI: 10.1056/nejmp0904572

Vivek S., et al. (2009) Triple-Reassortant Swine Influenza A (H1) in Humans in the United States, 2005–2009. New England Journal of Medicine 360. DOI: 10.1056/NEJMoa0903812.

von Itzstein M, Wu W-Y, Kok G B, Pegg M S, Dyason J C, Jin B, Phan T V, Smythe M L, White H F, Oliver S W, Colman P M, Varghese J N, Ryan D M, Woods J M, Bethell R C, Hotham V J, Cameron J M and Penn C R. (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature 363:418-423.

Wang C, Takeuchi K, Pinto L H and Lamb R A. (1993) Ion channel activity of influenza A virus M2 protein: characterization of the amantadine block. Journal of Virology 67:5585-5594.

Webster R G, Bean W J, Gorman O T, Chambers T M and Kawaoka Y. (1992) Evolution and ecology of influenza A viruses. Microbiological Reviews 56:152-179.

Zakay-Rones Z, Varsano N, Zlotnik M, Manor O, Regev L, Schlesinger M and Mumcuoglu M. (1995) 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. Journal of Alternative and Complementary Medicine 1:361-369.

Zakay-Rones Z, Thom E, Wollan T and Wadstein J. (2004) Randomized study of the efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections. Journal of International Medical Research 32:132-140.