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## An Evaluation of *Echinacea angustifolia* in Experimental Rhinovirus Infections

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### ABSTRACT

#### BACKGROUND

Echinacea has been widely used as an herbal remedy for the common cold, but efficacy studies have produced conflicting results, and there are a variety of echinacea products on the market with different phytochemical compositions. We evaluated the effect of chemically defined extracts from *Echinacea angustifolia* roots on rhinovirus infection.

#### METHODS

Three preparations of echinacea, with distinct phytochemical profiles, were produced by extraction from *E. angustifolia* roots with supercritical carbon dioxide, 60 percent ethanol, or 20 percent ethanol. A total of 437 volunteers were randomly assigned to receive either prophylaxis (beginning seven days before the virus challenge) or treatment (beginning at the time of the challenge) either with one of these preparations or with placebo. The results for 399 volunteers who were challenged with rhinovirus type 39 and observed in a sequestered setting for five days were included in the data analysis.

#### RESULTS

There were no statistically significant effects of the three echinacea extracts on rates of infection or severity of symptoms. Similarly, there were no significant effects of treatment on the volume of nasal secretions, on polymorphonuclear leukocyte or interleukin-8 concentrations in nasal-lavage specimens, or on quantitative-virus titer.

#### CONCLUSIONS

The results of this study indicate that extracts of *E. angustifolia* root, either alone or in combination, do not have clinically significant effects on infection with a rhinovirus or on the clinical illness that results from it.

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**T**HE COMMON COLD IS A BENIGN AND self-limited illness most commonly caused by the rhinoviruses. Although the importance of the common cold derives primarily from its frequency and from the enormous socioeconomic impact it has, it is clear that the common cold in general and rhinovirus infection in particular are associated with significant medical consequences.<sup>1-8</sup> There are no specific antiviral treatments for rhinovirus infection. Perhaps because of the lack of specific therapies, concern about the risks relative to the benefits of treatments for symptoms, and the relatively benign nature of the common cold, there is wide interest in the use of alternative medicines for the treatment of this illness.

*Echinacea angustifolia* roots were originally used by North American Indians to treat a variety of infections and wounds. In the late 1800s, these echinacea preparations became popular as remedies for the common cold. There has been renewed interest in echinacea in the United States since the passage of the Dietary Supplement Health and Education Act in 1994 liberalized the regulation of herbal medicines. There are three species of echinacea, with different phytochemical characteristics, that are used for medicinal purposes. The phytochemical composition of echinacea preparations may also vary owing to differences in the part of the plant used, the method used to extract the material in the preparation, and even the geographic location and time of year that the plant is harvested.<sup>9</sup> In spite of the variability among echinacea preparations, only recently have there been attempts to standardize and characterize the material used in clinical studies.

The experimental model for colds caused by rhinoviruses is a well-established, carefully controlled model for the study of the pathogenesis and treatment of the common cold.<sup>10,11</sup> The purpose of our study was to use the experimental model and carefully defined preparations of echinacea to evaluate systematically the effect of different echinacea constituents on rhinovirus infection and common-cold symptoms.

## METHODS

### VOLUNTEERS

Healthy young adult volunteers were recruited for this study from the University of Virginia community. Volunteers susceptible to rhinovirus type 39, as evidenced by a serum-neutralizing antibody titer

of 1:4 or less, were invited to participate. Written informed consent, in a form approved by the Human Investigations Committee of the University of Virginia, was obtained from all volunteers before study participation, and subjects were compensated for participating.

### STUDY MEDICATION

The echinacea preparations for the study were developed from a single lot of *E. angustifolia* root. The root material was extracted with either supercritical carbon dioxide, 60 percent ethanol, or 20 percent ethanol to produce three different preparations. The placebo for the study contained a mixture of alcoholic beverages, denatonium benzoate (250 ppm), and tap water. The treatments were given three times each day as a 1.5-ml tincture containing the equivalent of 300 mg of echinacea root. The 437 volunteers were randomly assigned in blocks to receive one of the seven treatment regimens (described below) to ensure that the regimens would be equally distributed over the course of the study. The participants and all study staff at the University of Virginia were blinded to the group assignments until all data had been collected and transmitted to the study statistician.

### CONDUCT OF THE STUDY

Six cohorts of volunteers were studied between May 2002 and March 2004. The numbers of subjects in the first five cohorts ranged from 69 to 84; 45 volunteers were enrolled in the sixth cohort. The study was divided into a prophylaxis phase (day -7 until virus challenge on day 0) and a treatment phase (virus challenge to day 5). Within each cohort, there were seven possible treatment assignments, with carbon dioxide extract, 60 percent extract, or 20 percent extract given during both phases or with placebo given during the prophylaxis phase and the carbon dioxide extract, 60 percent extract, or 20 percent extract given during the treatment phase. The control group received placebo throughout both phases of the study period. Volunteers took their assigned study medication as outpatients on days -7 to 0. On day 0, all asymptomatic volunteers were challenged with rhinovirus type 39 and then isolated in individual hotel rooms for the remainder of the study. Between virus challenge and the morning of day 5 of the study, the symptom scores of the subjects were evaluated every morning and evening, and a nasal lavage

was performed each morning after symptom scoring was completed. Approximately three weeks after the virus challenge, all volunteers returned to the study site to have blood collected for testing for antibody to rhinovirus type 39.

#### ASSESSMENT OF COMPLIANCE

A known volume of study medication was provided during the prophylaxis phase, and compliance was assessed through the measurement of the volume of medication returned. The study staff dispensed and observed the consumption of all medications during the treatment phase.

#### ASSESSMENT OF BLINDING

The adequacy of the study's blinding procedures was assessed according to the subjects' responses when asked which study medication they believed they were taking ("active," "placebo," or "don't know"). This question was asked at the end of the prophylaxis phase just before virus challenge and again after administration of the third dose of study medication in the treatment phase of the trial.

#### CHALLENGE VIRUS

The challenge virus used for this study was rhinovirus type 39. This virus has been tested for safety according to consensus guidelines.<sup>12</sup> All of the subjects were inoculated with approximately 100 50 percent tissue-culture infectious doses of virus.

#### VIRUS ISOLATION AND SEROLOGY

Nasal-lavage specimens collected on day 0 before virus challenge were cultured in HEp-2, rhesus-monkey-kidney, A549, and fibroblast cells for the detection of unsuspected viral infections. Nasal-lavage specimens collected on study days 1 to 5 were cultured for rhinovirus by standard methods, as previously described.<sup>13</sup> Serum specimens were tested for neutralizing antibody to rhinovirus type 39 by a standard microtiter assay.<sup>14</sup> Volunteers in whom rhinovirus type 39 was isolated from at least one postinoculation specimen, in whom serum-neutralizing antibody to rhinovirus type 39 was increased by a factor of four, or both, were considered infected with the study virus. Viral titers in the original nasal-wash specimens were determined from specimens stored at  $-80^{\circ}\text{C}$  by culturing serial dilutions, in which virus was serially diluted by a factor of 10, in microtiter plates of MRC-5 cells, as previously described.<sup>13</sup>

#### ASSESSMENT OF SYMPTOMS

Symptom scores were recorded by members of the study staff. Volunteers were asked to rate their symptoms of sneezing, rhinorrhea, nasal obstruction, sore throat, cough, headache, malaise, and chilliness on a scale of 0 to 4; the numbers corresponded to a symptom severity of absent, mild, moderate, severe, or very severe. Scoring of symptoms was done before virus challenge and then every morning and evening on study days 1 to 5. Each morning, symptom scores were recorded before the nasal-wash procedure. The daily symptom score for each symptom on study days 1 to 5 was defined as the higher of the two scores reported for the symptom on each day. Volunteers who had a symptom score of at least 6 for the five days after challenge and either at least three days of rhinorrhea or the subjective impression that they had a cold were defined as having a clinical cold. The nasal-secretion weight per 24-hour period was also determined by a previously described method<sup>13</sup> for each volunteer on study days 0 to 5.

#### ASSESSMENT OF INFLAMMATION

The interleukin-8 concentration was measured in nasal-lavage specimens with the use of a commercially available enzyme-linked immunosorbent assay, as previously described.<sup>15</sup> For determination of the polymorphonuclear leukocyte concentration, an aliquot of nasal-lavage specimen was stained with acridine orange, and then the polymorphonuclear leukocytes, identified by size and nuclear morphology, were counted in a hemacytometer.

#### PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of the extracts to detect the alkamides was performed with the use of mass spectroscopy. A photometric method was used for the analysis of the polysaccharides.<sup>16</sup> A complete description of the methods used for the phytochemical analysis is provided in the Supplementary Appendix (available with the full text of this article at [www.nejm.org](http://www.nejm.org)).

#### STATISTICAL ANALYSIS

On the basis of previous data for rhinovirus type 39,<sup>13</sup> it was assumed that placebo-treated subjects would have an infection rate of 85 percent and a mean ( $\pm$ SD) total symptom score of  $17.7 \pm 13.5$ . With these assumptions, in a sample size of 50 subjects per active treatment group, with 100 subjects in the placebo group, a 20 percent reduction in the

infection rate or a 35 percent reduction in the total symptom score would be detected, with a two-sided type I error of 0.05 and a power of 80 percent.

The evaluation of efficacy in the experimental model assumed that subjects were equally susceptible to the study virus and that all were infected with the same pathogen. Subjects who were found retrospectively to have acute antibody titers greater than 1:4 or in whom a viral respiratory pathogen was isolated from the nasal-lavage specimen on day 0 were excluded, by protocol, from the data analysis.

The primary end point for the prophylaxis phase of the study (i.e., treatment with echinacea from study day -7 through study day 5) was the comparison of the proportion of volunteers who became infected with rhinovirus in each group with the proportion infected in the placebo group. This was performed as six pairwise comparisons with the use of the chi-square analysis. This analysis was supplemented with a multiple logistic-regression analysis that included as covariates the baseline antibody titer, the baseline interleukin-8 concentration in the nasal-lavage specimen, age, sex, and race. The primary end point for the subjects given echinacea as treatment (i.e., those volunteers treated with echinacea only from virus challenge to study day 5) was the comparison of the total symptom score for the infected subjects in each treatment group with the total symptom score for the infected subjects in the placebo group. This comparison was made by analysis of variance, supplemented with a multiple logistic-regression analysis, as described above.

Planned secondary analyses included evaluation of the different study groups with regard to effects on the incidence of clinical colds, effects on quantitative virus titer in nasal secretions, effects on individual symptoms, and effects on polymorphonuclear leukocytes and interleukin-8 as markers of intranasal inflammation.

All reported P values are two-sided and unadjusted for multiple tests. An interim safety analysis was performed after each cohort was discharged from the study; no interim efficacy analysis was carried out.

## RESULTS

### PHYTOCHEMICAL ANALYSIS OF EXTRACTS

Analyses revealed that the supercritical carbon dioxide extract contained no polysaccharides but did contain 73.8 percent alkamides; the 60 percent

ethanol extract contained 48.9 percent polysaccharides and 2.3 percent alkamides; and the 20 percent ethanol extract contained 42.1 percent polysaccharides and only 0.1 percent alkamides. Assays for caffeic acid derivatives revealed that the 60 percent ethanol extract contained 0.16 mg per milliliter of cynarine; however, echinacoside was not detectable in any extract.<sup>17</sup> Details of the phytochemical results are provided in the Supplementary Appendix. Repeated analyses of the study treatments over the course of the study ensured that the composition of the treatments remained constant.

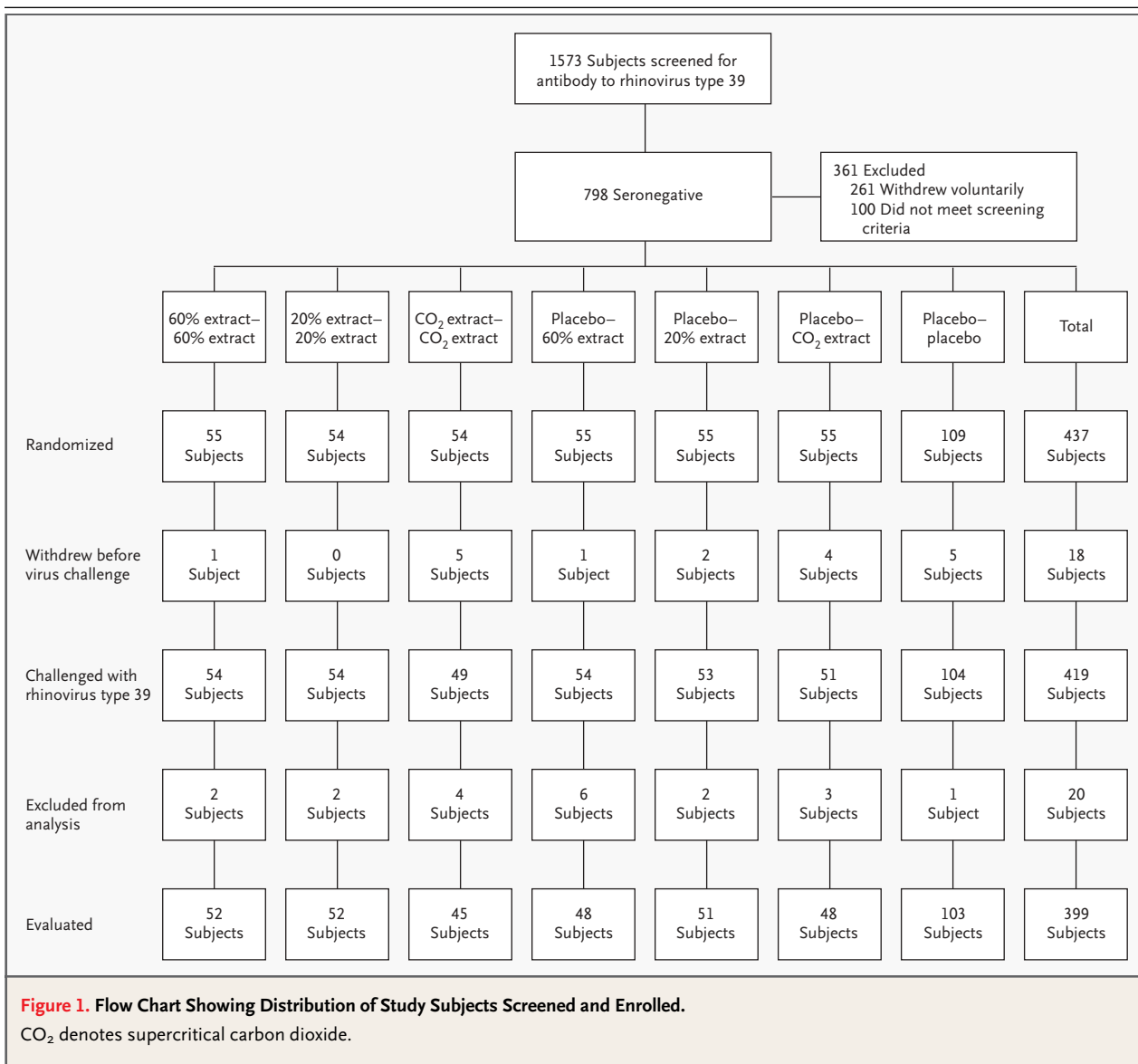
### STUDY SUBJECTS

Four hundred thirty-seven volunteers were randomly assigned to receive study medication (Fig. 1). Eighteen subjects who had undergone randomization were not challenged with study virus because of voluntary withdrawal from the study (8 subjects) or because an illness developed before virus challenge (10 subjects). Of the 419 subjects who were challenged with virus, 2 withdrew from the study before data were collected, and in 18 there was either an antibody titer to the challenge virus of greater than 1:4 in the serum sample collected on day 0 or a viral respiratory pathogen isolated from the nasal-lavage specimen collected before the virus challenge. These 20 volunteers were excluded by the study protocol from the data analysis.

The mean ( $\pm$ SD) age of the 399 subjects was  $20.8 \pm 3.3$  years. Of these, 240 subjects (60 percent) were female; 1 (0.3 percent) was self-classified as American Indian, 37 (9.3 percent) were self-classified as Asian, 42 (11 percent) as black, 316 (79 percent) as white, and 3 (0.8 percent) as being of mixed race. The random assignment of subjects to the treatment groups resulted in a balanced distribution with regard to age and sex, with the exception of the treatment group that received the 60 percent extract, in which women were overrepresented (75 percent,  $P=0.02$ ) as compared with the placebo group.

### COMPLIANCE AND BLINDING

Evaluation of compliance during the prophylaxis phase of the study revealed that more than 90 percent of the subjects took at least 80 percent of their medication. During the treatment phase, there were two missed doses of study medication as a result of doses held because of side effects. Evaluation of blinding revealed that the proportion of volunteers who believed they were taking the active med-



ication during the treatment phase of the study ranged from 21 of 51 (41 percent) to 24 of 48 (50 percent) in the active-treatment groups; in the placebo group, 37 of 103 volunteers (36 percent) believed they were receiving the active medication (P=0.63 according to the chi-square analysis for all groups).

**EFFECT OF ECHINACEA ON INFECTION**

Prophylaxis with these echinacea preparations had no significant effect on rhinovirus infection (Table 1), and there was no effect on the infection rate in the groups that received echinacea only in the treatment phase of the study. The quantitative virus titer

also was not affected by either prophylaxis or treatment with these echinacea preparations.

**EFFECT OF ECHINACEA ON SYMPTOMS**

Treatment with these echinacea preparations had no significant effect on symptoms associated with rhinovirus, whether assessed by the total symptom score or by the proportion of subjects with clinical colds (Table 1). There was also no effect of either prophylaxis or treatment on the course of the illness (Fig. 2). Evaluation of nasal-secretion weights as an objective measure of severity also revealed no beneficial effect. During the prophylaxis phase, nasal-secretion weights (mean ±SD)

**Table 1. Effect of Various Extracts of *E. angustifolia* Root on Rhinovirus Infection and Common-Cold Illnesses.\***

Treatment Day -7 to 0	Treatment Day 0 to 5	No. of Subjects	No. Infected (%)†	95% CI for Difference in Infection Rate vs. Placebo‡	P Value for Difference in Infection Rate vs. Placebo	No. of Clinical Colds in Infected Subjects (%)	Mean Total Symptom Score§
CO <sub>2</sub> extract	CO <sub>2</sub> extract	45	40 (89)	-0.07 to 0.15	0.57	25 (62)	15.45±2.34
60% extract	60% extract	52	42 (81)	-0.09 to 0.17	0.46	24 (57)	13.21±1.91
20% extract	20% extract	52	48 (92)	-0.03 to 0.17	0.22	24 (50)	12.06±1.74
Placebo	CO <sub>2</sub> extract	48	43 (90)	-0.06 to 0.16	0.48	27 (63)	14.60±1.70
Placebo	60% extract	48	44 (92)	-0.03 to 0.17	0.28	33 (75)	19.20±2.28
Placebo	20% extract	51	44 (86)	-0.11 to 0.13	0.89	28 (64)	15.64±1.97
Placebo	Placebo	103	88 (85)	Reference group	—	58 (66)	15.05±1.43

\* Plus-minus values are means ±SE. CI denotes confidence interval, and CO<sub>2</sub> supercritical carbon dioxide.

† The P value for homogeneity for the infection rates is 0.58.

‡ Negative numbers indicate a higher infection rate for placebo, and positive numbers a higher rate for echinacea treatment.

§ The total mean symptom score is the sum of symptom scores on days 1 to 5. Higher scores indicate more severe symptoms.

were 17.2±22.5 g in the subjects who received carbon dioxide extract, 17.6±19.7 g in those who received 60 percent ethanol extract, and 19.3±29.4 g in those who received 20 percent ethanol extract. The nasal-secretion weights in subjects who received placebo during the prophylaxis phase and carbon dioxide extract, 60 percent extract, and 20 percent extract during the treatment phase were 20.3±23.3 g, 33.1±32.2 g, and 15.5±14.0 g, respectively. The nasal-secretion weight in subjects who were treated with placebo in both study phases was 21.4±27.9 g. A comparison of the severity of individual symptoms revealed no significant effect of the echinacea preparations on any of the symptoms assessed (data not shown).

#### EFFECT OF ECHINACEA ON INFLAMMATORY MARKERS

The effect of echinacea on rhinovirus-induced inflammation was assessed by measurement of interleukin-8 and polymorphonuclear-leukocyte concentrations in nasal-lavage specimens. Neither prophylaxis nor treatment with the echinacea preparations had a significant effect on either the interleukin-8 response or the polymorphonuclear-leukocyte response to rhinovirus infection. As expected, there was a significant correlation between the interleukin-8 and polymorphonuclear-leukocyte responses and symptom severity.

#### ADVERSE EVENTS

During the prophylaxis phase of the study, 19 of the 437 randomized subjects reported adverse events.

Adverse events judged to be possibly related to the study medication were reported by 4 of 163 (2 percent) of the subjects receiving an echinacea preparation and by 5 of 274 (2 percent) receiving placebo. During the treatment phase of the study, 15 of 315 (5 percent) of the subjects receiving an echinacea preparation reported an adverse event that possibly was related to the treatment, as compared with 4 of 104 (4 percent) of the placebo-treated subjects. Gastrointestinal side effects were the most common events, reported by 12 subjects in the echinacea groups and 4 subjects in the placebo group.

#### DISCUSSION

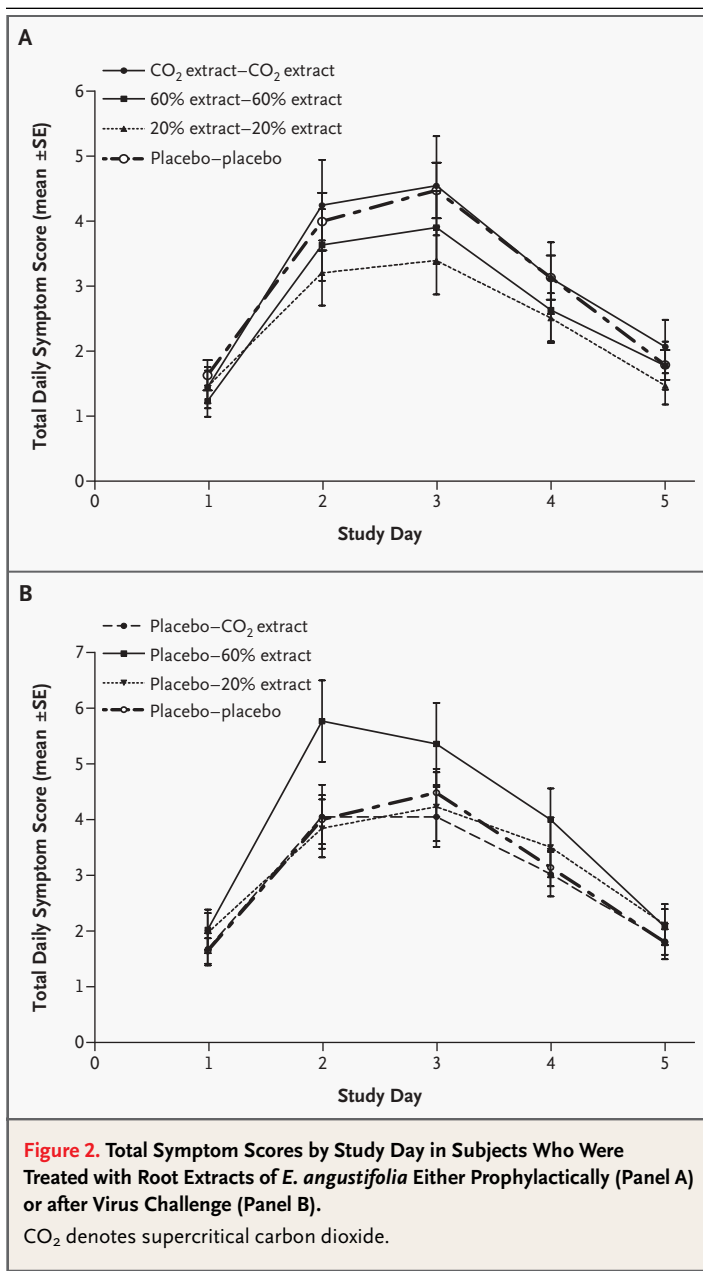
The effect of echinacea on common-cold illnesses has been assessed in a number of clinical studies in which various echinacea preparations and study designs were used. Despite the large number of studies, recent systematic reviews have concluded that the effectiveness of echinacea remains unproved.<sup>18-20</sup> One of these reviews specifically cited the need for studies that involve chemically well-defined preparations, clearly defined illnesses, and early intervention.<sup>20</sup> The purpose of our study was to evaluate the effect of root extracts of *E. angustifolia* on rhinovirus infection and illness in a manner that would permit the systematic evaluation of the contribution of different echinacea constituents to any observed treatment effect. The alkaloids, polysaccharides, and caffeic acid derivatives present in extracts of echinacea have demonstrated biologic activity both in vitro and in vivo (reviewed in the Sup-

plementary Appendix and in Bauer<sup>9</sup> and Barrett<sup>19</sup>). It has been proposed that these constituents, either alone or in combination, are the active ingredients in commercially available echinacea preparations. The methods used for extraction of the plant material in our study produced extracts with different concentrations of these putative active constituents. None of the extracts had a detectable effect on rhinovirus infection or illness.

Echinacea products available in the United States are made from the roots, the whole plant, or aerial parts of *E. angustifolia*, *E. pallida*, or *E. purpurea*. These products may be formulated as powdered plant material, alcoholic tinctures, tea preparations, or pressed juice of the aerial parts. Variations in species, plant parts, extraction procedures, and manufacturing processes may affect the chemical constituents or ratios of selected constituents in the final preparation.<sup>9</sup> Specific chemical constituents have various biologic effects (see the Supplementary Appendix), but there have been no previous studies that have compared the clinical effects of different echinacea preparations. For this study, we chose to use experimental extracts of *E. angustifolia*, the species originally used by the Native Americans of the American Midwest and recently endorsed by the World Health Organization for treatment of the common cold.<sup>21</sup>

The experimental model for rhinovirus infection was used for this study. Previous experience with this model for the evaluation of conventional antiviral therapies and treatments for symptoms of the common cold has demonstrated that the experimental model accurately predicts the effectiveness of treatments in the natural setting.<sup>22,23</sup> The reduced variability afforded by the model appears to increase the apparent effect size for effective treatments.<sup>24</sup>

The results of this study demonstrate that, as tested, the putative active constituents of *E. angustifolia* do not have clinically significant effects on rhinovirus infection or illness. There are several considerations for the generalizability of the results of our study, which attempted to correlate the phytochemical composition of echinacea extracts with clinical efficacy. The potential sources of variation in different echinacea preparations include plant species, the method of extraction, the part of the plant that is used, and perhaps even the location and season of cultivation. The polysaccharides and alkamides of echinacea have biologic activity and are generally perceived as the active components



of these treatments. It is conceivable, however, that other chemical constituents or combinations of constituents that were not tested in this study have important biologic effects. It is also possible, although unlikely, that echinacea is effective for the treatment of respiratory pathogens other than rhinovirus. Given the great variety of echinacea preparations, it will be difficult to provide conclusive evidence that echinacea has no role in the treatment of the common cold. Our study, however, adds to the accumulating evidence that suggests that the bur-

den of proof should lie with those who advocate this treatment.

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