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Effects of a Combination of Elderberry and Reishi Extracts on the Duration and Severity of Respiratory Tract Infections in Elderly Subjects: A Randomized Controlled Trial

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Abstract: Elderly people are particularly vulnerable to respiratory tract infections, so natural strategies to ameliorate the duration and severity of these infections are of great interest in this population. The objective of this study is to evaluate the efficacy of the consumption of a combination of elderberry and reishi extracts on the incidence, severity, and duration of respiratory tract infections in a group of healthy elderly volunteers. A randomized, double-blind, placebo-controlled pilot study was performed during the winter season. A group of 60 nursing home residents ≥ 65 years of age was randomly assigned to receive a combination of 1.5 g of elderberry +0.5 g of reishi or a placebo daily for 14 weeks. Data about the health conditions of the volunteers were evaluated and recorded by a medical doctor every 2 weeks. The incidence of respiratory infections was similar in both groups. However, volunteers in the extract group presented a significantly lower duration of common cold events (2.5 vs. 4.8 days, $p = 0.033$) and a significantly lower probability of having a high severity influenza-like illness event ($p = 0.039$). Moreover, the incidence of sleep disturbances was significantly lower in the extract group ($p = 0.049$). Therefore, the administration of a combination of elderberry and reishi extracts to the elderly population during the winter season might be used as a natural strategy to reduce the duration and severity of respiratory tract infections.

Keywords: elderberry; reishi; respiratory tract infections; common cold; influenza-like illness; respiratory infection symptoms; randomized controlled trial

1. Introduction

Respiratory tract infections, defined as any of a number of infectious diseases involving the respiratory tract, are the most common infections in humans of all ages, leading to a significant rate of mortality and morbidity worldwide [1]. According to the World Health Organization (WHO), approximately 290,000 to 650,000 deaths annually are caused by influenza virus infection alone [2]. Ominously, in the last 20 years, novel coronaviruses causing acute respiratory syndromes, such as SARS-CoV, MERS-CoV, and, currently, SARS-CoV-2, have emerged and triggered several global pandemics with high case fatality rates [3].

Among all populations, elderly people are at increased risk of acquiring respiratory infections and of experiencing a more severe disease course following infection due to coexisting chronic diseases, functional impairment, malnutrition, polypharmacy and immunosenescence [4,5]. The number of hospital admissions for respiratory infections is much higher among people aged ≥ 75 years, and it has been shown that the average length of hospital stay increases with age [1,6]. Moreover, these risks are even greater in older adults who reside in long-term care facilities [7] due to the close proximity of residents in combination with advanced age, multimorbidity and frailty [8]. Therefore, strategies that prevent respiratory infections, ameliorate their severity, or shorten the average duration of the disease will have the greatest benefit in the elderly population, especially older adults residing in long-term care facilities.

Natural substances and their derivatives have a long history of use for protection against infection and enhancement of the immune response [9]. Indeed, over the years, scientific evidence demonstrated the immunomodulatory and antiviral properties of several of these botanical and fungal extracts used in ancient medicine [9,10]. In this regard, elderberry (the whole fruit of *Sambucus nigra* L. containing multiple bioactive compounds) extract, which has been traditionally used to address cold and flu symptoms [11], has been shown in two recent meta-analyses to reduce overall symptom duration and severity when it is supplemented at the onset of upper respiratory infections in children and adults [12,13]. Moreover, preclinical studies have shown that the acidic polysaccharide-rich fraction contained in elderberry extract presented antimicrobial and antiviral effects, specifically against influenza viruses [14]. On the other hand, reishi (*Ganoderma lucidum*), the oldest known mushroom in ancient Chinese medicine [15], has been largely used to promote health and treat a large number of ailments [16]. Reishi has been reported to have a number of pharmacological effects, including immunomodulatory, anti-inflammatory, analgesic, antibacterial, and antiviral properties [17–20], which has been mainly attributed to its content in *G. lucidum* polysaccharide (GLPS) [17].

In addition, it has been suggested that the combination of different plant extracts may have a synergistic effect on the protection and treatment of respiratory infections [10]. On this subject, a drink containing *Echinacea purpurea* and *Sambucus nigra* extracts was demonstrated to be as effective as oseltamivir in the treatment of influenza virus infection in patients with early symptomatology [21]. Considering the individual properties of elderberry and reishi extracts, the combination of both compounds may have a promising effect on the protection against respiratory infection in the elderly population.

Therefore, the objective of the present study is to evaluate the efficacy of daily consumption of a combination of elderberry and reishi extracts on the incidence, severity and duration of respiratory tract infections in a group of healthy elderly volunteers living in a nursing home.

2. Materials and Methods

2.1. Study Design and Subjects

A randomized, double-blinded, placebo-controlled pilot trial was performed. The study was started in December 2019 and ended in March 2020. Volunteers were recruited from the nursing home “Residencia de Mayores Claret”, which is located in Granada (Spain). The inclusion criteria were men and women older than 65 years of age who resided in a nursing home with medical service. The exclusion criteria were the presence of any disease or disorder that may affect the development and results of the study, namely, swallowing disorders, chronic pulmonary disease, need for enteral or parenteral nutrition, or allergy to some ingredient of the study product and use of any supplement or medication that could influence the outcomes of the trial. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Regional Ethical Committee (Granada, Spain). Informed consent was obtained from all subjects. The trial was registered with the US Library of Medicine (<http://www.clinicaltrials.gov>) under the number NCT04386408.

The volunteers were randomly assigned to one of two groups: the individuals in the control group consumed a sachet containing a placebo (maltodextrin), whereas the individuals in the extract group consumed a sachet containing the combination of elderberry and reishi. The study products were provided as oral suspension sachets. The subjects were given treatments for 14 weeks, and they consumed one sachet per day (consuming the extract supplement or placebo) diluted in water at lunchtime. Participating subjects were instructed to not deviate from their regular habits during the 14 weeks of intervention. Neither the researchers nor the subjects knew which treatment sequence the subjects had been assigned to; the researchers were unblinded only at the end of the study. All volunteers were vaccinated against the flu during the same week, according to the vaccine campaign of 2019/2020.

2.2. Study Products

Each extract sachet (Elderpro™, Biosearch S.A., Granada, Spain) contained 1.5 g of elderberry (*Sambucus nigra* L.) dried fruit juice standardized to 0.15% anthocyanosides and 0.5 g of reishi (*Ganoderma lucidum*) body aqueous extract standardized to 35% polysaccharides plus excipients (aspartame, sucralose and flavorings). Each placebo sachet contained 1.955 g of maltodextrin, plus the same excipients as extract sachet. Additionally, cochineal carmine and caramel dye were added to the placebo to ensure that the appearance of the placebo powder was identical to that of the extract powder. The powder mix of the extract and the placebo were provided in identical sachets with a code number that referred to the volunteer code according to the randomization. Elderberry extract was obtained through atomization of the concentrated elderberry juice obtained from the whole fruit by an aqueous method, whereas reishi extract was obtained from the fruity body of *G. lucidum* through the hydroalcoholic method. The content of anthocyanosides and polysaccharides in the extracts were analyzed by spectrophotometry. Elderberry and reishi extracts were provided by Biosearch Life (Granada, Spain), and sachets were prepared by HC Clover PS in Madrid (Spain).

2.3. Study Outcomes and Data Collection

The study's primary outcome was the incidence of respiratory infections, mainly influenza-like illness (ILI) and common cold, during the intervention. The ILI diagnosis was based on the case definition used by the European Centre for Disease Prevention and Control [22] as follows: sudden onset of symptoms with one or more respiratory symptoms (cough, sore throat and/or nasal congestion) plus one or more systemic symptoms (fever, headache, myalgia and/or malaise). Common cold was defined as sudden onset of one or more respiratory symptoms (cough, sore throat and/or nasal congestion) with no systemic symptoms [23]. The total number of respiratory infections was the sum of common cold and ILI episodes.

Secondary outcomes included the determination of the severity and duration of the respiratory infections and incidence of symptoms related to them. The severity of the respiratory infections was determined according to the number of symptoms (previously defined) presenting simultaneously. Common cold was considered mild severity if subjects presented 1 symptom and high severity if they presented ≥ 2 symptoms simultaneously, whereas ILI was considered mild severity if subjects presented < 4 symptoms simultaneously and high severity if subjects presented ≥ 4 symptoms simultaneously. The consumption of analgesics and antibiotics during the follow-up period was also recorded. Finally, some safety parameters, such as nausea, lack of appetite, sleep disturbances, and changes in weight and blood pressure, were recorded during the intervention.

All data about the health conditions of the volunteers and the consumption of medical treatments were evaluated and recorded by a medical doctor every 2 weeks in the case report form corresponding to each volunteer. Weight was determined using a Tanita BC-418 Body Composition Analyzer (Tanita, Tokyo, Japan) at baseline and at the end of the intervention. Systolic and diastolic blood pressures (BP) were measured with a validated digital automated blood pressure monitor (Medisana Healthcare, Barcelona, Spain). Compliance was assessed at the end of the intervention by comparing the number of

sachets provided and the number returned. Adverse events, defined as any unfavorable, unintended effect, were recorded at the follow-up visits (at 2, 4, 6, 8, 10, 12 and 14 weeks).

2.4. Statistical Analysis

The normality of the distribution was tested for all measured variables by normal probability plots and the Shapiro–Wilk test. Data are presented as the mean (standard deviation) for continuous variables and as n (%) for categorical variables.

For comparisons between groups at the beginning of the study (extract vs. control), continuous variables were analyzed with Student’s t-test or the nonparametric Kruskal–Wallis method, as appropriate, and categorical variables were analyzed with chi-square tests.

The occurrence of infections and symptoms were described using the incidence ratio (IR) and incidence rate ratio (IRR) with the 95% CI and *p*-value for the IRR. A Poisson regression model was applied to adjust the number of events by sex, age, and smoking habits, and additionally, by use of sleeping medication in the sleep disturbance parameter. Differences in the duration of respiratory infections between groups and changes in weight and blood pressure between baseline and the end of the intervention were determined by univariate model analysis adjusted by age, sex, and smoking habits and by BMI and hypertension medication in systolic and diastolic blood pressure variables.

A general alpha level of 0.05 was used as the cutoff point for statistical significance. Statistical analysis was carried out using SPSS software version 27.0 for Windows (SPSS, Chicago, IL, USA).

3. Results

3.1. Study Data, Compliance and Baseline Characteristics of the Subjects

A total of 60 older adults were recruited and randomly distributed into two groups: the control group (n = 30) and the extract group (n = 30). Before completion of the 14-week intervention period, 6 volunteers in the control group and 1 in the extract group discontinued the intervention and dropped out of the study for the reasons detailed in the study flow chart (Figure 1). No differences among the groups were detected between the number and the causes of withdrawal. The compliance rate was confirmed to be very high (≈100%). No adverse events resulting from the intake of either type of treatment were reported. Data were analyzed for all the subjects included in the study who had attended at least one of the follow-up visits (analysis per intention to treat, ITT).

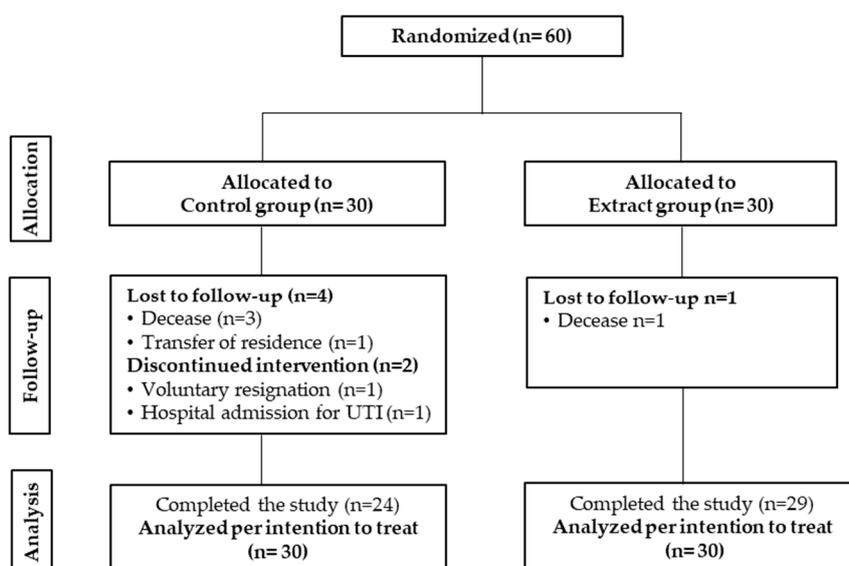


Figure 1. Diagram of the Consolidated Standards of Reporting Trials.

The baseline characteristics of the 60 older adults included in the statistical analyses are presented in Table 1. Most of the volunteers (71.7%) were older than 80 years, 20% were between 70 and 80 years old, and 8.3% were between 65 and 69 years old. No significant differences were detected between subjects in the study groups in the baseline characteristics.

Table 1. Baseline characteristics of the subjects participating in the study.

	Control Group (n = 30)	Extract Group (n = 30)	<i>p</i> between Groups
Age (years)	82.7 ± 9.2	85.9 ± 7.8	0.155
Sex			0.739
Men	5 (16.7%)	6 (20%)	
Women	25 (83.3%)	24 (60%)	
Weight (kg)	68.1 ± 13.9	63.6 ± 10.9	0.176
BMI (kg/m ²)	26.1 ± 4.6	25.4 ± 3.8	0.478
Smoking habit			0.109
Current smoker	4 (13.3%)	2 (6.7%)	
Former smoker	1 (3.3%)	6 (20%)	
No smokers	25 (83.3%)	22 (73.3%)	
Physical activity			0.519
Very low	23 (76.7%)	25 (83.3%)	
Low	7 (23.3%)	5 (16.7%)	
Systolic BP (mm Hg)	126.2 ± 14.7	130.3 ± 10.4	0.218
Diastolic BP (mm Hg)	70.7 ± 10.2	71.8 ± 9.4	0.667
Sleeping medication	10 (33.3%)	8 (26.7%)	0.389
Hypertension medication	19 (63.3%)	18 (60.0%)	0.518

Values are mean ± SD for continuous variables and n (%) for categorical variables. *p* indicates differences between groups.

3.2. Respiratory Tract Infections Incidence and Duration and Severity of Related Symptoms

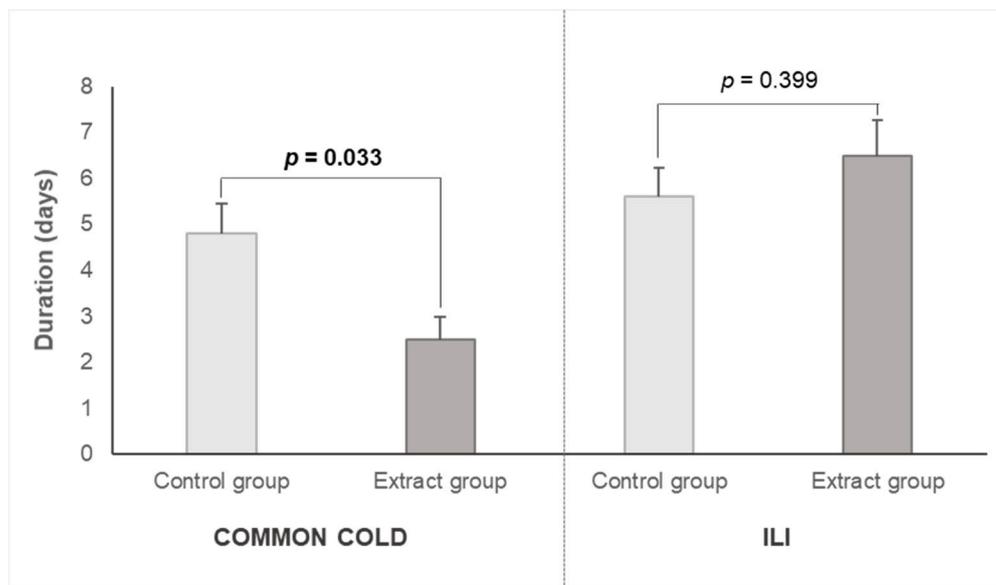
Table 2 shows the incidence of respiratory infections during the intervention period (14 weeks). The incidence of total respiratory infections and common colds was 9.4% and 25.0% lower in the extract group than in the control group, but the differences were not significant ($p = 0.728$ and $p = 0.508$, respectively), whereas the incidence of ILI was very similar in both groups ($p = 0.978$).

Table 2. Incidence of respiratory tract infections.

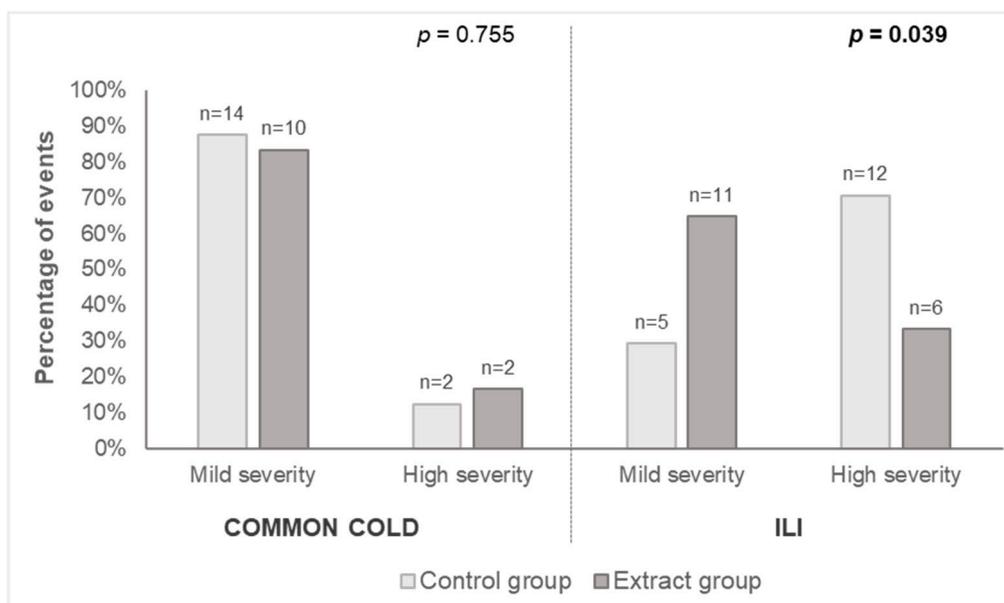
	Control Group IR (SD)	Extract Group IR (SD)	IR Ratio (95% CI)	IR Decrease, %	<i>p</i> Value ^c
Common cold	0.321 (0.188)	0.240 (0.135)	0.750 (0.320–1.759)	25.0	0.508
ILI ^a	0.494 (0.188)	0.523 (0.174)	1.059 (0.514–2.182)	−0.06	0.978
Respiratory infections ^b	0.921 (0.273)	0.834 (0.224)	0.906 (0.523–1.570)	9.4	0.725

^a Incidence of influenza-like illness; ^b Incidence of Common cold + ILI; ^c Poisson regression model adjusted by sex, age and smoking habits.

However, the duration and severity of the respiratory infections seemed to be lower in the group that received the extract than in the control group. Common cold events mean duration were significantly lower in the extract group than in the control group ($2.5 ± 1.0$ vs. $4.8 ± 1.4$ days, $p = 0.033$), although we found no differences in ILI events ($6.5 ± 3.1$ vs. $5.7 ± 2.1$ days, $p = 0.399$) (Figure 2a). When common cold and ILI events were classified as mild or high severity according to the number of simultaneous symptoms, we observed that subjects in the extract group presented a significantly lower probability of having a high severity ILI event [OR (95% CI) 0.227 (0.054–0.961), $p = 0.039$], although this was not significant in common cold events [OR (95% CI) 0.714 (0.086–5.959), $p = 0.755$] (Figure 2b).



(a)



(b)

Figure 2. Duration and severity of respiratory tract infections. (a) Duration in days of common cold and influenza-like illness (ILI) events according to intervention groups. Values are represented as mean \pm standard error of mean (SEM) (light grey bars for control group and dark grey bars for extract group). *p*-value indicates differences between groups for the duration of common cold or ILI events (univariate model adjusted by sex, age and smoking habits). (b) Percentage of events (common cold or ILI) that course with mild or high severity according to intervention groups (light grey bars for control group and dark grey bars for extract group). The number above each bar indicates the number of events in that group. *p*-value indicates differences in severity of common cold or ILI events between groups (chi-square test). Statistically significant values ($p < 0.05$) are given in bold.

Finally, the incidence of sore throat was significantly lower in the extract group, presenting the volunteers that consumed the extract an incidence ratio decreased by 69.8% compared to the control group., ($p = 0.043$, Table 3). With respect to other symptoms related to respiratory infections,

their incidence was, in general, lower in the extract group than in the control group, except for the symptom tiredness and malaise, which had a higher incidence in the extract group, but differences were not statistically significant.

Table 3. Incidence of symptoms related to respiratory infections.

	Control Group IR (SD)	Extract Group IR (SD)	IR Ratio (95% CI)	IR Decrease, %	p-Value ^d
Cough	0.558 (0.202)	0.552 (0.177)	0.989 (0.495–1.976)	1.1	0.976
Nasal congestion	0.478 (0.200)	0.357 (0.141)	0.747 (0.334–1.6719)	25.3	0.478
Sore throat	0.356 (0.211)	0.108 (0.073)	0.302 (0.095–0.963)	69.8	0.043
Fever	0.244 (0.112)	0.128 (0.717)	0.528 (0.52–1.834)	47.2	0.315
Headache	0.128 (0.937)	0.102 (0.070)	0.798 (0.266–2.396)	21.2	0.688
Muscle/Bone Pain	0.146 (0.92)	0.138 (0.794)	0.941 (0.327–2.713)	5.9	0.911
Tiredness/Malaise	0.180 (0.101)	0.342 (0.155)	1.897 (0.755–4.770)	−89.7	0.173
Local Respiratory Symptoms ^a	1.466 (0.352)	1.031 (0.239)	0.703 (0.440–1.124)	29.7	0.141
Systemic Symptoms ^b	0.754 (0.233)	0.656 (.189)	0.870 (0.500–1.511)	23	0.62
Total Symptoms ^c	2.185 (0.410)	1.808 (0.316)	0.828 (0.584–1.174)	27.2	0.289

^a Sum of local respiratory symptoms (sore throat, cough and/or nasal congestion); ^b Sum of systemic symptoms (fever, headache, muscle/bone pain and/or tiredness/malaise); ^c Sum of all symptoms associated with respiratory infections (sore throat, cough, nasal congestion fever, headache, muscle/bone pain and/or tiredness/malaise); ^d Poisson regression model adjusted by sex, age, and smoking habits. Statistically significant values ($p < 0.05$) are given in bold.

The administration of antibiotics and analgesics to treat respiratory infections during the intervention was similar in both groups [IRR (95% CI): 0.993 (0.388–2.545) and 1.034 (0.411–2.980), respectively].

3.3. Safety Parameters

Regarding safety parameters (Table 4), we found no significant differences between groups in nausea or lack of appetite ($p = 0.484$ and $p = 0.873$, respectively) or in changes in body weight between baseline and the end of the intervention in both groups ($p = 0.842$ for the control group and $p = 0.981$ for the extract group). Systolic and diastolic BP tended to decrease in the extract group, although no significant differences were observed between baseline and the end of the intervention ($p = 0.079$ for systolic BP and $p = 0.092$ for diastolic BP). No changes were observed in the control group for these parameters ($p = 0.633$ for systolic BP $p = 0.494$ for diastolic BP). Interestingly, the incidence of sleep disturbances was significantly lower, 58.6%, in the extract group than in the control group [IR ratio (95% CI) 0.414 (0.172–0.983), $p = 0.049$].

Table 4. Safety parameters.

	Control Group		Extract Group	
Nausea IR (SD) ^a	0.100 (0.057)		0.166 (0.074)	
Lack of appetite IR (SD) ^a	0.666 (0.149)		0.633 (0.145)	
Sleeping disturbance IR (SD) ^a	0.899 (0.325)		0.372 (0.152) *	
	Baseline	End of intervention	Baseline	End of intervention
Weight (kg) ^b	68.4 ± 14.2	67.3 ± 13.4	64.2 ± 10.6	63.8 ± 9.5
Systolic BP (mm Hg) ^b	126.8 ± 15	127.8 ± 10.7	130.6 ± 10.4	126.5 ± 9.1
Diastolic BP (mm Hg) ^b	70.5 ± 10.7	72 ± 8.1	72 ± 9.5	68.3 ± 8.7

^a Poisson regression model adjusted by sex, age, and smoking habits; and additionally, by use of sleeping medication in the sleeping disturbance parameter. Asterisk (*) indicate $p < 0.05$ between control and extract groups. ^b Values are mean ± SD. Univariate models adjusted by sex, age and smoking in the weight variable; and additionally by BMI and hypertension medication in systolic and diastolic BP variables were used to evaluate differences between baseline and the end of the intervention at each group.

4. Discussion

The present study shows that daily consumption of a combination of elderberry and reishi extracts during the winter season is effective in reducing the duration and severity of respiratory

infections in elderly people. Several clinical studies have shown the beneficial immunological effect of elderberry [12,13] and reishi [24], but to our knowledge, this is the first study that evaluated the combined effect of both extracts on the incidence, severity, and duration of respiratory infections in older adults, a population group at increased risk of this type of infection and a worse prognosis of the disease [5].

Elderberry (*Sambucus nigra* L fruit) has been indicated in several clinical trials to relieve the symptoms of the common cold and influenza when ingested at the onset of infection in children and adults [21,25–27]. Additionally, the effect of elderberry has been tested for prevention of respiratory symptoms in situations of high risk of infection, such as intercontinental flights, showing a reduction in the duration and severity of common cold events the travelers developed [28]. The therapeutic effects of elderberry have been attributed to its content of bioactive compounds, such as anthocyanins and other polyphenols, through several mechanisms [29,30]. First, elderberry extract has been reported to enhance the immune response by increasing both inflammatory and anti-inflammatory cytokines [31,32]. Second, it was observed that elderberry preparations exert antiviral activity, specifically against human influenza (H1N1) virus. It has been shown that several elderberry compounds directly bind the H1N1 virus particles, resulting in the inability of the particles to enter host cells and in a decreased viral load and spread [33,34]. Regarding the reishi (*Ganoderma lucidum*), it has been observed in vitro and in animal studies that the triterpenoids contained in the reishi exert anti-influenza activity by neuraminidase inhibition [35,36]. Moreover, a recent clinical study reported that a yogurt enriched with β -glucans from reishi increased the absolute count of peripheral blood total lymphocytes (CD3⁺, CD4⁺, and CD8⁺ T cells) compared to a nonenriched yogurt in a group of healthy children [24]. Therefore, the results achieved in this randomized clinical trial could be related to the observed immunomodulatory and antiviral activities of elderberry and reishi. Moreover, this study adds scientific evidence to support the traditional use of these extracts to relieve respiratory infection symptoms.

In addition, a decline in immune function in older people has been attributed, at least in part, to an increase in oxidative stress [37]. Therefore, increases in dietary antioxidants, such as anthocyanosides of elderberry and other phenolic compounds present in elderberry and reishi extracts, may contribute to improving immune function in the elderly. Indeed, both elderberry and reishi extracts have been confirmed to exert antioxidant properties during antiradical activity assays in vitro [38,39]. Additionally, one study showed a significant increase in the total antioxidant capacity and total thiol concentration in the serum of a group of healthy subjects who consumed an elderberry infusion for 30 days with similar content of anthocyanins to our extract [40]. Although we did not analyze any antioxidant capacity biomarkers in our volunteers, we cannot discard a possible relationship between the reduction in the duration and severity of respiratory infections and the possible reduction in oxidative stress, as observed by other authors. In a crossover study performed in older adults, regular consumption of gold kiwifruit increased plasma antioxidants and reduced the duration and severity of some symptoms associated with respiratory infections [41]. However, further studies should be performed to test this hypothesis.

Interestingly, the older adults who took the combination of extracts reported fewer sleep problems than those who took the placebo. This effect could be attributed to reishi, which has been traditionally used for insomnia treatment [16]. Indeed, a randomized controlled trial described that the polysaccharide extract of *Ganoderma lucidum* improved the insomnia severity scores in patients with neurasthenia [42]. Moreover, *Ganoderma lucidum* extract has been shown to increase total sleep time and nonrapid eye movement sleep time in animal studies [43,44], with a probable mechanism linked to the modulation of cytokines such as tumor necrosis factor- α [44].

One strength of the present study was the design as a double-blind randomized controlled trial controlled by placebo. This methodology allowed us to avoid bias related to confounding factors (through a control group), selection bias (through randomization), and interpretation bias (through double blinding). However, a limitation of the study was the small sample size, limited by the fact that

this is a pilot study. Additionally, the diagnosis of flu was based on a confluence of symptoms rather than viral detection. Moreover, further studies in other population groups should be performed.

5. Conclusions

In conclusion, the administration of a combination of elderberry and reishi extracts to the elderly population during the winter season reduces the duration and severity of respiratory infections. The study subjects did not suffer from any adverse effects and even presented a beneficial effect on night sleep. Therefore, the use of this combination of extracts from elderberry and reishi may be a natural, suitable, and safe strategy to short and ameliorate respiratory tract infections symptomatology in older adults, a particularly vulnerable population.

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