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Effects of erinacine A-enriched *Hericium erinaceus* on elderly hearing-impaired patients: A double-blind, randomized, placebo-controlled clinical trial☆

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ABSTRACT

We aimed to investigate the effects and mechanisms of *Hericium erinaceus* (HE) on hearing degeneration. By a double-blind, randomized, clinical trial, all 80 hearing-impaired patients aged 50–79 were randomly divided into the two groups, which received HE mycelia (2000 mg/day) and placebo boluses for eight months, respectively. A student's *t*-test was used to evaluate the differences of the average pure tone hearing threshold of all, low, and high frequencies (PTA-all, PTA-low, and PTA-high); speech recognition threshold (SRT); speech discrimination score (SDS); and serum concentrations of brain-derived neurotrophic factor (BDNF) and neurotrophic growth factor (NGF) between the two study groups. Subgroup analysis by age showed that PTA-high-Right, PTA-all-Left, PTA-high-Left, SRT-Right, SRT-Left, and NGF were significantly different between the two groups among patients aged ≧ 65. HE mycelia treatment could ameliorate hearing loss, especially for high frequencies and speech recognition, and increase NGF in patients aged ≧ 65.

1. Introduction

Age-related hearing impairment (ARHI) is the most prevalent sensory disorder among senior citizens (Heine & Browning, 2002). According to the statistics from the National Institute on Deafness and Other Communication Disorders in United States, one in three people aged 65–74 suffer in hearing loss, and nearly half of people aged over 75 have hearing impairment (Bulğurcu, Uçak, Yönem, Erkul, & Çekin, 2020). People with hearing loss experience difficulties in verbal communication, which affects their psychological situation and reduces quality of life (Dalton, Cruickshanks, Klein, Klein, Wiley et al., 2003; Uchida, Sugiura, Nishita, Saji, Sone et al., 2019). Additionally, affected people may feel frustrated, socially isolated, and even suffer from depression and cognitive declines such as dementia and Alzheimer' disease (Behrman, Chouliaras, & Ebmeier, 2014; Panza, Solfrizzi, & Logroscino, 2015).

Several studies have demonstrated that the etiology of hearing impairment includes genetic defects and environmental influences such as noise, ototoxic overexposure, obesity, obstructive sleep apnea, hypertension, diabetes mellitus, and dyslipidemia (Hwang, Chen, Hsu, &

Abbreviations: BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; PTA-all, average pure tone hearing thresholds of all tested frequencies; PTA-low, average pure tone hearing thresholds of 0.25, 0.5, 1 kHz; PTA-high, average pure tone hearing thresholds of 2, 4, 8 kHz; SRT, speech recognition threshold; SDS, speech discrimination scores.

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Liu, 2011; Hwang, Wu, Hsu, Liu, & Yang, 2009; Lin, Wu, Hsu, Hwang, & Liu, 2011; Van Eyken, Van Camp, & Van Laer, 2007). The potential mechanisms for the progression of ARHI in the cochlea were investigated. Imbalance in the generation of reactive oxygen species (ROS) induced by noise, drugs, and aging leads to chronic inflammation and oxidative damage of hair cells in the organ of Corti, stria vascularis, and spiral ganglion neurons (SGNs) (Fujimoto & Yamasoba, 2014; Hwang, Chen, Hsu, Yang, & Liu, 2012). Moreover, reduction of cochlear blood flow (CoBF) associated with aging may damage the stria vascularis and hair cells due to overproduction of ROS and ischemia (Lee, 2013; Wangemann, 2002). A decline in brain-derived neurotrophic factor (BDNF) with age also contributes to impairment of the vestibular ganglion and SGNs (Schimmang, Tan, Müller, Zimmermann, Rohbock et al., 2003), while nerve growth factor (NGF) has been reported to attenuate the degeneration of SGNs after aminoglycoside ototoxicity (Schindler, Gladstone, Scott, Hradek, Williams et al., 1995). Therefore, substances can both serve as free-radical scavengers and facilitate the genesis of neurotrophic factors are suspected to be able to provide therapeutic treatment for ARHI.

Hericium erinaceus (HE) is a culinary mushroom which is widely consumed without toxic effects and can be used as salads, soups, and risottos in Asia countries (Khan, Tania, Liu, & Rahman, 2013). Besides, it has been employed in traditional Chinese medicine in East Asia. This mushroom exhibits antioxidant, anti-inflammatory, neuroprotective, and hepatoprotective activities (Thongbai, Rapior, Hyde, Wittstein, & Stadler, 2015; Valu, Soare, Ducu, Moga, Negrea et al., 2021; Valu, Soare, Sutan, Ducu, Moga et al., 2020; Zhang, Lv, Pan, Pandey, He et al., 2012). HE contains miscellaneous bioactive compounds, including polysaccharides, proteins, and terpenoids. It was investigated that three diterpenoids named erinacine A, B, C were strong stimulators of NGF (Kawagishi, Shimada, Shirai, Okamoto, Ojima et al., 1994). In particular, erinacine A derived from the mycelia is reported to enhance the expression of NGF in the central nervous system of rats (Shimbo, Kawagishi, & Yokogoshi, 2005). Besides, erinacine A was effective in improving degenerative diseases in the central nervous systems such as Alzheimer's and Parkinson's diseases (Kuo, Lu, Shen, Tung, Hsieh et al., 2016; Li, Chang, Lin, Chen, Lu et al., 2020). Furthermore, there was a research into the effects of HE on the auditory function on aged senescence-accelerated prone 8 (SAMP8) mice, which demonstrated that oral administration of HE mycelia prevented cochlear impairment in SAMP8 mice by elevating the level of neurotrophic expression in SGNs, inhibiting caspase-independent apoptosis signaling, and eliminating damage of outer hair cells (OHCs) (Hwang, Chen, Lee, Chiang, Wang et al., 2020). However, despite the promising effects on mice, the efficacy of HE mycelia on elderly people with hearing impairment is still unclear. Therefore, in this study, a clinical trial was performed with elderly patients who have hearing loss to confirm the benefits and related mechanisms of HE.

2. Materials and methods

2.1. Study design

This study was a double-blind, placebo-controlled, randomized clinical trial on subjects with ARHI. The study was registered on ClinicalTrials.gov under number NCT03632512. The study protocol was approved by the ethics committee of the participating hospital (No: B10604025-2).

2.2. Subjects

The study recruited 80 patients between 50 and 79 years old with symmetric sensorineural hearing loss (defined as divergence of the average pure tone hearing thresholds at all frequencies (PTA) for both ears $<$ 15 dB HL). The patients were recruited from Dalin Tzu Chi Hospital, Chiayi, Taiwan. Written informed consent was obtained from all participants before enrollment in this clinical trial. The exclusion criteria included subjects with normal hearing, severe or very severe hearing loss (PTA $>$ 70 dB HL for the better ear), hearing impairment before 30 years of age, conductive hearing impairment (air bone gap $>$10 dB hearing level (dBHL)), no tinnitus or non-subjective sensorineural tinnitus, history of high ambient noise exposure, pure tone hearing threshold at 4 kHz of 20 dB greater than that at 8 kHz, abnormal liver and kidney function, history of alcohol or drug abuse, moderate cognitive impairment or higher, and inability to cooperate or understand the details of this clinical trial (Hwang, Chen, Hsu, Yang, & Liu, 2012; Hwang, Chan, Hsu, Liu, & Chen, 2012). The patients were randomly assigned to HE and control groups, in which participants received 8 boluses of HE mycelia or placebo honey every day for eight months, respectively.

2.3. Sample preparation

Study samples included HE mycelia and placebo honey boluses, which were both pro-vided by Biotechnology Center, Grape King Bio Ltd. (Taoyuan, Taiwan). HE mycelia was cultured in submerged liquid medium (0.25% yeast extract, 4.5% glucose, 0.5% soybean powder, 0.25% peptone, and 0.05% MgSO4) with an initial pH of 4.5 at 26℃ for 5 days on a rotary shaker (120 rpm), and later scaled up in 20-ton fermenters (Li, Chen, Lee, Chen, Tsai et al., 2014). Each 500 mg HE mycelia honey bolus incorporated 250 mg of freeze-dried mycelia powder and 250 mg of honey. For the placebo counterpart, the mycelia powder was replaced with maize starch, and there was still 250 mg of honey. According to the dosage used in mice (Hwang, Chen, Lee, Chiang, Wang et al., 2020), eight honey boluses (2000 mg HE mycelia) were taken per day in both the control and HE groups, and patients in the HE group consumed 10 mg of erinacine A from the mycelia honey boluses daily. The residue of study samples for patients was tracked to evaluate the compliance of the participants.

2.4. Procedures

Basic clinical characteristic assessments (age, sex, body weight, waist circumference, medi-cal history, life habits, and dietary habits), audiometric examinations, and blood test analyses were conducted at the start and the end (the 8th month) of this study. Pure tone audiometry was used to measure hearing thresholds at 0.25, 0.5, 1, 2, 4, and 8 kHz for each ear of the patients. The average pure tone hearing thresholds were computed for all tested frequencies (PTA-all), low frequencies of 0.25, 0.5, and 1 kHz (PTA-low), and high frequencies of 2, 4, and 8 kHz (PTA-high) (Hwang, Wu, Hsu, Liu, & Yang, 2009). The speech recognition threshold (SRT) and speech discrimination scores (SDS) were also acquired by one senior audiologist (Hamid & Brookler, 2006).

A hematological analysis was done at Dalin Tzu Chi Hospital to measure the white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HB), hematocrit, mean corpuscu-lar hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), blood platelet (PLT), red blood cell volume distribution width (RDW), and mean platelet volume (MPV). A biochemistry analysis was also done to measure glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), creatinine (CRE), estimated glomerular filtration rate (eGFR), and gamma-glutamyltransferase (GGT). In addition, serum BDNF and NGF were measured by enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, USA).

2.5. Statistical analysis

Pearson's chi-squared test or a 2 $\times$ 2 Fisher exact test was used to assess the distributions of categorical variables between groups. A student's *t*-test was used to evaluate the difference of each variable or changes of each outcome from the start and the end of the study between

the two study groups. Subgroup analysis by age (<65 and ≧ 65 years) was also performed by the same methods. Statistical analyses were conducted with SPSS version 25 (SPSS Inc., Chicago, IL). The value of statistical significance was set at $p < 0.05$.

3. Results

3.1. Patient characteristics

The 80 patients enrolled in this study were randomly distributed to the control group (n = 39) and the HE group (n = 41). There were 21 subjects who were lost to follow-up during the intervention period because of the high number of capsules to take daily, disbelief in the therapeutic effects of *H. erinaceus*, non-compliance, stroke, and injuries. Therefore, of the 59 remaining participants that completed the trial, 27 were in the control group, and 32 were in the HE group (Fig. 1).

The age and body morphology of both groups are presented in Table 1. The mean age of both groups was 62.3 years. The mean body mass indexes (BMIs) of subjects in the control and HE groups were 24.1 and 23.9, while their mean waist circumferences were 32.8 and 33.3, respectively. There were no significant differences between the two groups regarding BMI and waist circumference at baseline ($p > 0.05$).

The basic clinical characteristics of participants in the two groups at the start and end of the trial are demonstrated in Table 1. The data about gender, medical history, and dietary habits of the subjects were evaluated in both groups. There were no significant differences between clinical features of patients and the two treatment groups ($p > 0.05$) according to the chi-squared test and 2 × 2 fisher exact test, which indicated that this randomized control trial is reliable.

3.2. Safety assessments

The hematology and biochemistry values at the start and end of the study are shown in Table 2. The values at the start and end were all within normal ranges according to the standards of Dalin Tzu Chi Hospital (https://hualien.tzuchi.com.tw/tcopo/). No statistically significant differences were noted between groups except for the values of GPT at the start of the study ($p < 0.05$).

3.3. Changes of concentration of serum neurotrophic factors in groups

The changes in concentration of serum neurotrophic factors were obtained from subtracting values tested at the start from those tested at the end, and the results of the two groups are shown in Table 3. BDNF levels tended to increase from the start of the study to the end in both groups, but the changes were not significantly different in both groups ($p > 0.05$). In contrast, NGF tended to decrease from the start of the study to the end in both groups, and the changes were not significantly different in both groups ($p > 0.05$).

3.4. Changes of audiometric data in two groups

The changes of audiometric data were obtained from subtracting values tested at the start from those tested at the end in both groups and are shown in Table 4. The changes of PTA-all, PTA-low, and PTA-high of the right ear and left ear were not significantly different in both groups ($p > 0.05$). Also, the changes of SRT or SDS of each ear were not significantly different in both groups.

3.5. Subgroup analyses

A subgroup analysis was done by age (<65 and ≧ 65 years) for changes in PTA, SRT, SDS, BDNF, and NGF in the two groups, as shown in Table 5. There were no significant differences between the two groups for all tested outcomes in patients age < 65 years ($p > 0.05$). However, PTA-high-Right ($p < 0.05$), PTA-all-Left ($p < 0.05$), PTA-high-Left ($p <$

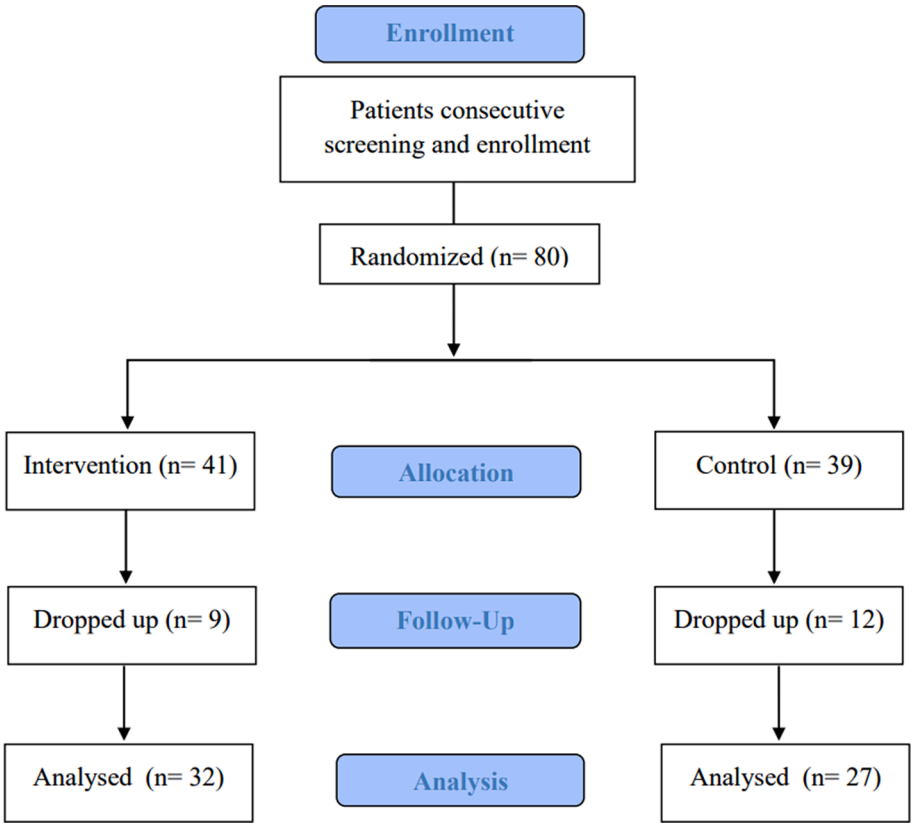

Fig. 1. CONSORT flow diagram.

Table 1
Age, body morphology, and basic clinical characteristics of patients with hearing impairment.

Variables	CG (n = 27) Mean (SEM)	HEG (n = 32)
Age (years)	62.3 (1.2)	62.3 (1.3)
BMI (kg/m2)	24.1 (0.7)	23.9 (0.5)
Waist (inches)	32.8 (0.7)	33.3 (0.7)
	Number (%)	
Sex	12 (20.3%)	15 (25.4%)
		17 (28.8%)
Male	15 (25.4%)	15 (25.4%)
Female	12 (20.3%)	17 (28.8%)
Disease		
Heart disease	1 (1.7%)	3 (5.1%)
Hypertension	6 (10.2%)	7 (11.9%)
DM	6 (10.2%)	4 (6.8%)
Hyperlipidemia	3 (5.1%)	6 (10.2%)
Hepatitis	1 (1.7%)	1 (1.7%)
Liver cirrhosis	–	–
Stroke	–	1 (1.7%)
Cancer	1 (1.7%)	2 (3.4%)
Anxiety	5 (8.5%)	–
Depression	1 (1.7%)	2 (3.4%)
Head trauma	4 (6.8%)	2 (3.4%)
MW	3 (5.1%)	–
Menopause	11 (18.6%)	15 (25.4%)
Stuffy nose	5 (8.5%)	3 (5.1%)
Asthma	1 (1.7%)	–
Snoring	10 (16.9%)	11 (18.6%)
Sleep apnea	6 (10.2%)	3 (5.1%)
Life habits		
Smoking	3 (5.1%)	4 (6.8%)
Alcohol	3 (5.1%)	4 (6.8%)
Areca	–	1 (1.7%)
Noise	5 (8.5%)	4 (6.8%)
Dietary habits		
Coffee	12 (20.3%)	13 (22.0%)
Tea	15 (25.4%)	16 (27.1%)
Soymilk	17 (28.8%)	15 (25.4%)
Vegetarian	11 (18.6%)	12 (20.3%)
SB	–	–
Vitamins	11 (18.6%)	12 (20.3%)
Healthy food	12 (20.3%)	16 (27.1%)
Hormones	5 (8.5%)	4 (6.8%)

Abbreviation: BMI, body mass index; DM, diabetes mellitus; MW, Muscle weakness; SB, stimulating beverage; CG, Control group; HEG, *Hericium erinaceus* group.
The data are presented as means (standard error of the mean, SEM), and numbers (percentage, %).
P values were acquired by Pearson's chi-squared test if the variable was categorical.

Table 2
Hematology and biochemistry values at the start and end of the study.

Variables	Start Mean (SEM)		End Mean (SEM)		Laboratory reference intervals #
	CG (n = 27)	HEG (n = 32)	CG (n = 27)	HEG (n = 32)	
WBC (103/uL)	6.3 (0.3)	5.9 (0.2)	6.1 (0.3)	5.9 (0.3)	3.59–9.64
RBC (106/uL)	4.7 (0.1)	4.6 (0.1)	4.7 (0.1)	4.6 (0.1)	4.5–5.9
Hb (g/dL)	14.2 (0.3)	13.7 (0.2)	14.1 (0.3)	13.8 (0.2)	13.5–17.5
Ht (%)	42.5 (0.7)	41.3 (0.6)	42.7 (0.7)	41.9 (0.5)	41–53
MCV (fL)	90.3 (0.8)	90.3 (1.1)	90.5 (0.8)	90.9 (1.2)	85.6–102.5
MCH (pg)	30.2 (0.3)	30.0 (0.5)	29.9 (0.3)	29.9 (0.5)	28.2–34.4
MCHC (g/dL)	33.5 (0.2)	33.2 (0.2)	33.0 (0.2)	32.9 (0.2)	31.8–34.8
PLT (103/uL)	212.0 (11.1)	217.7 (7.6)	206.1 (11.0)	221.5 (7.9)	148–339
RDW (%)	12.9 (0.1)	12.9 (0.1)	13.0 (0.1)	13.0 (0.1)	11.9–14.5
MPV (fL)	10.0 (0.1)	10.3 (0.2)	10.1 (0.1)	10.3 (0.2)	9.4–12.6
GOT (U/L)	20.5 (1.3)	20.7 (0.9)	20.1 (1.1)	20.1 (0.8)	15–37
GPT (U/L)	33.2 (2.7)	26.2 (1.4)*	26.4 (2.6)	23.2 (1.6)	16–63
BUN (mg/dL)	16.3 (1.2)	14.9 (0.9)	15.6 (1.0)	14.4 (0.8)	7–18
CRE (mg/dL)	0.9 (0)	0.9 (0)	0.9 (0)	0.8 (0)	0.7–1.3
eGFR (ml/min/1.73 m2)	88.5 (3.5)	85.2 (2.7)	85.6 (2.7)	90.8 (3.8)	90–120
GGT (U/L)	32.3 (8.1)	19.3 (2.1)	27.7 (5.8)	18.7 (1.7)	15–85

Abbreviations: GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; BUN, blood urea nitrogen; CRE, creatinine; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl transferase; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, blood platelet; RDW, red blood cell volume distribution width; MPV, mean platelet volume; CG, Control group; HEG, *Hericium erinaceus* group.
The data are presented as means (standard error of the mean, SEM).
P values were acquired by Student's *t*-test if the variable were continuous, and the level of significance was set at $p < 0.05$*.
Laboratory reference intervals of blood tests were obtained from the website of Dalin Tzu Chi Hospital. (https://hualien.tzuchi.com.tw/tcopo/).

Table 3
Changes of concentration of serum neurotrophic factors in two groups.

Variables	Changes of concentration # Mean (SEM)	
	CG	HEG
BDNF (pg/mL)	129.3 (73.9)	144.4 (65.0)
NGF (pg/mL)	−157.7 (108.6)	−63.1 (97.8)

Abbreviation: BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; CG, Control group; HEG, *Hericium erinaceus* group.
P values were acquired by student's *t*-test if the variable was continuous.
The data were obtained from subtracting values tested at the start from those tested at the end.

0.05), SRT-Right ($p < 0.05$), SRT-Left ($p < 0.005$), and NGF ($p < 0.05$) were significantly different between the two groups in patients ≧ 65 years old. In other words, HE mycelia treatment could prevent hearing loss, especially for high frequencies and speech recognition, as well as increase the serum concentration of NGF in hearing-impaired patients age ≧ 65 years old, but not in patients of < 65 years old.

4. Discussion

This double-blind, randomized, placebo-controlled clinical trial showed that treatment with HE mycelia could ameliorate hearing loss, especially for high frequencies and speech recognition, as well as increase the serum concentration of NGF in hearing-impaired patients ≧ 65 years old, but not < 65 years old. SDS and BDNF tended to increase but did not reach significant differences between the HE and control groups in patients over 65 years old. Excessive ROS damage in the cochlear hair cells, stria vascularis, and SGNs are the most well-known mechanisms for ARHI (Fetoni, Picciotti, Paludetti, & Troiani, 2011; Jiang, Talaska, Schacht, & Sha, 2007). Compared to wild-type mice, knockout mice for copper/zinc superoxide dismutase (SOD1) and cellular glutathione peroxidase (GPx1) genes were more vulnerable to cochlear injury (Ohlemiller, McFadden, Ding, Flood, Reaume et al., 1999; Ohlemiller, McFadden, Ding, Lear, & Ho, 2000). Hence, it is

Table 4
Changes of audiometric data in two groups.

Variables	Changes # Mean (SEM)	
	CG	HEG
PTA-all-Right (dBHL)	0.0 (0.7)	−0.3 (0.6)
PTA-low-Right (dBHL)	0.8 (1.1)	−0.6 (0.6)
PTA-high-Right (dBHL)	2.0 (1.1)	0.0 (1.0)
PTA-all-Left (dBHL)	0.0 (2.0)	−1.3 (1.1)
PTA-low-Left (dBHL)	−0.7 (1.9)	−2.1 (1.4)
PTA-high-Left (dBHL)	−0.2 (1.6)	−1.3 (1.1)
SRT-Right (dBHL)	5.7 (2.3)	2.3 (1.2)
SRT-Left (dBHL)	3.3 (1.9)	0.5 (1.9)
SDS-Right (%)	−2.8 (1.9)	0.0 (1.4)
SDS-Left (%)	0.6 (4.2)	2.4 (2.2)

Abbreviations: PTA-all, average pure tone hearing thresholds of all tested frequencies; PTA-low, average pure tone hearing thresholds of 0.25, 0.5, 1 kHz; PTA-high, average pure tone hearing thresholds of 2, 4, 8 kHz; SRT, speech recognition threshold; SDS, speech discrimination scores; CG, Control group; HEG, *Hericium erinaceus* group.
P values were acquired by student's *t*-test if the variable was continuous.
The data were obtained from subtracting values tested at the start from those tested at the end.

Table 5
Subgroup analysis for changes of PTA, SRT, SDS, BDNF and NGF in the two groups.

Age	Variables	Changes # Mean (SEM)	
		CG	HEG
< 65	**PTA-all-Right** (dBHL)	−0.6 (1.0)	−0.2 (0.8)
	PTA-low-Right (dBHL)	0.1 (1.5)	−0.9 (0.8)
	PTA-high-Right (dBHL)	0.5 (1.4)	0.6 (1.6)
	PTA-all-Left (dBHL)	−1.9 (3.0)	−2.3 (1.8)
	PTA-low-Left (dBHL)	−1.9 (2.7)	−3.4 (2.3)
	PTA-high-Left (dBHL)	−2.0 (2.2)	−1.0 (1.8)
	SRT-Right (dBHL)	3.8 (3.3)	2.8 (1.4)
	SRT-Left (dBHL)	−0.6 (2.1)	0.6 (3.2)
	SDS-Right (%)	−3.0 (2.8)	1.3 (1.1)
	SDS-Left (%)	5.9 (5.5)	4.9 (3.3)
	BDNF (pg/mL)	245.6 (63.2)	142.5 (93.7)
	NGF (pg/mL)	6.1 (89.1)	−148.1 (153.8)
≧65	**PTA-all-Right** (dBHL)	1.1 (1.1)	−0.3 (1.0)
	PTA-low-Right (dBHL)	1.9 (1.7)	−0.3 (1.1)
	PTA-high-Right (dBHL)	4.6 (1.5)	−0.8 (1.3)*
	PTA-all-Left (dBHL)	3.2 (1.1)	−0.1 (1.1)*
	PTA-low-Left (dBHL)	1.5 (2.7)	−0.4 (1.2)
	PTA-high-Left (dBHL)	2.9 (1.9)	−1.7 (1.0)*
	SRT-Right (dBHL)	9.0 (3.0)	1.8 (2.0)*
	SRT-Left (dBHL)	10.0 (2.6)	0.4 (1.6)**
	SDS-Right (%)	−2.4 (2.5)	−1.7 (2.8)
	SDS-Left (%)	−8.0 (5.9)	−0.9 (2.5)
	BDNF (pg/mL)	−56.7 (150.1)	147.1 (89.1)
	NGF (pg/mL)	−419.7 (227.1)	54.5 (93.1)*

Abbreviations: PTA-all, average pure tone hearing thresholds of all tested frequencies; PTA-low, average pure tone hearing thresholds of 0.25, 0.5, 1 kHz; PTA-high, average pure tone hearing thresholds of 2, 4, 8 kHz; SRT, speech recognition threshold; SDS, speech discrimination scores; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; CG, Control group; HEG, *Hericium erinaceus* group.
P values were acquired by student's *t* test if the variable was continuous with significantly differences at $p < 0.05$*, $p < 0.005$**.
The data were obtained by subtracting values tested at the start from those tested at the end.

reasonable to expect that treatment using antioxidant substances or enzymes could provide a therapeutic effect on ARHI.

Erinacine A derived from HE mycelia has antioxidative activity. Erinacine A-enriched HE mycelia reduces levels of thiobarbituric acid reactive substance (TBARS) and increase the activities of antioxidant enzymes in a dose-dependent manner in SAMP8 mice (Li, Lee, Chen, Chou, Wang et al., 2019). Furthermore, erinacine A has therapeutic activities in cognitive diseases. In an MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease, consumption of HE mycelia decreased neuronal cell cytotoxicity and apoptosis induced by oxidative and endoplasmic reticulum stress (Kuo, Lu, Shen, Tung, Hsieh et al., 2016). Treatment with erinacine A-enriched HE mycelia for 49 weeks delayed deterioration of Alzheimer's disease (increase in CASI, MMSE, and IADL) by increasing BDNF, superoxide dismutase (SOD), apolipoprotein E4 (APOE4), and alpha1-antichymotrypsin (α-ACT) (Li, Chang, Lin, Chen, Lu et al., 2020).

Neurotrophic factors such as BDNF and NGF are crucial for neuronal survival, but their concentrations decrease with age and are related to many neurodegenerative diseases (Obara & Nakahata, 2002; Xia, Ikeda, Xia, & Ikenoue, 2000). Reduction of BDNF levels was observed in the auditory nervous systems of aged animals (Rüttiger, Panford-Walsh, Schimmang, Tan, Zimmermann et al., 2007). NGF could exert its neuroprotective effect by activating tyrosine kinase receptor A (TrkA) and inhibiting apoptosis pathways in the cochlea (Lee, Hong, Her, Castañeda, Moon et al., 2015).

Treatment with BDNF or NGF for two weeks prevented SGN loss in deaf guinea pigs (Gillespie, Clark, & Marzella, 2004). Erinacine A from HE mycelia was demonstrated to promote NGF levels in the locus coeruleus and hippocampus of rats and to facilitate the secretion of NGF in mouse astroglial cells (Ma, Shen, Yu, Ruan, Wu et al., 2010; Shimbo, Kawagishi, & Yokogoshi, 2005). Erinacine A can cross the blood–brain barrier to the brains of rats through passive diffusion and thus induce NGF synthesis (Tsai, Wu, Hu, Li, Lin et al., 2021). Moreover, HE mycelia could trigger BDNF pathways and interfere with NF-κB signals, thereby preventing neural impairment and the onset of depression (Chiu, Chyau, Chen, Lee, Chen et al., 2018).

In the evaluation of neurotrophic factors of this study (Table 3), the changes of concentrations of serum BDNF and NGF in the HE group were higher than those in the control group. This means that the HE group showed an increasing tendency in BDNF levels and an attenuated rate of decline of NGF in comparison to the control group. Based on Table 5, the concentration of serum BDNF in the HE group increased, while NGF levels were significantly higher than those in the control group in the subgroup analysis of patients over 65 years old. However, the opposite results occurred in patients < 65 years old, which demonstrates that HE could promote the concentrations of BDNF and NGF in patients over 65 years old better than in younger patients.

Neurotrophic factors and antioxidant activities can exert a synergistic effect for the prevention of neurodegenerative diseases. BDNF along with the free radical scavenger N-tert-butyl-(2-sulfophenyl)-nitrone (S-PBN) increased the survival rate of axotomy-induced retinal ganglion cells compared to those treated with BDNF alone (Klöcker, Cellerino, & Bähr, 1998). This mechanism was similar to that of HE administration, which can stimulate BDNF synthesis to protect against neuronal damage (Chiu, Chyau, Chen, Lee, Chen et al., 2018). Furthermore, extract of *Asparagus cochinchinensis* root stimulated NGF generation and reduced oxidative stress in a Tg2576 model with phenotypes of Alzheimer's disease (Lee, Kim, Sung, Yun, Kim et al., 2018). For the same reason, erinacine A-enriched HE mycelia ameliorated Alzheimer's disease by increasing the ratio of NGF/NGF precursor (Tzeng, Chen, Lee, Chen, Lu et al., 2016). In addition, HE exerts antioxidant activities to alleviate cognitive deficits by suppression of inducing nitric oxidase synthase (iNOS) activity (Lee, Chen, Teng, Shen, Hsieh et al., 2014; Zhou, Zhang, Ying, Chen, Chen et al., 2017).

HE mycelia exert both antioxidative and neuroprotective activities. The effect of HE on cochlear cell apoptosis was investigated in SAMP8 mice. HE mycelia could promote NGF expression in SGNs, prevent loss of OHCs, and reduce levels of apoptosis inducing factor (AIF) and poly (ADP-ribose) polymerase-1 (PAR-1) in the middle turn of the cochlea, brainstem, and temporal lobes (Hwang, Chen, Lee, Chiang, Wang et al., 2020). Therefore, HE could be a promising candidate for the treatment

of ARHI. In Table 4, the values of PTA of the HE group decreased over the 8-month study period, indicating that hearing thresholds were prone to decline due to intervention with HE mycelia. As for SRT, although the values of both the HE and control groups were increased, the rate of increase in the hearing level of the HE group was less than that in the control group. This demonstrates that receiving HE mycelia attenuated the deterioration of hearing. The values of SDS of the HE group were elevated over the study period, which suggested that the accuracy of word recognition was improved in this group. According to Table 5, in the subgroup analysis of patients over 65 years old, the changes in PTA and SRT in the HE group were smaller than those in the control group, while the change of SDS in the HE group was higher than that in the control group. These results were similar to those obtained in patients aged 50–79 years.

In this clinical trial, supplementation with HE mycelia did not have a significant protective role in auditory function and NGF concentration in patients of age $<$ 65 years. It is possible that the speed of hearing degeneration was slower in patients younger than 65 years old than in older patients (Lee, Matthews, Dubno, & Mills, 2005). However, the case number and statistical power of this clinical trial were too small to obtain a positive result in patients $<$ 65 years old.

Some limitation of this clinical trial was present. First, the case number was not large enough to show a positive result in subjects of younger than 65 years old. Also, it might account for the negative for SDS and BDNF in all subjects. Second, the period of this trial was only 8 months. The negative results in this trial might become positive in a longer treatment period. Third, no comparison with a reference drug was performed. So, we could not know whether the HE was better than other agents in the prevention of ARHI.

5. Conclusions

This double-blind, randomized, placebo-controlled clinical trial showed that treatment with HE mycelia could improve hearing loss, especially for high frequencies and speech recognition, as well as significantly increase the serum concentration of NGF. This effect was observed in hearing-impaired patients of age $\geqq$ 65 years, but not younger patients. However, the SDS and BDNF were not significantly affected by HE mycelia treatment.

Ethics statement

I have read and adhered to the Publishing Ethics. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Research Ethics Committee of Dalin Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (protocol code: B10604025−2 and approved on 2019/06/06). The study protocol was also registered with ClinicalTrials.gov under the number NCT03632512. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All data are contained in the article or available upon reasonable request.

Author contributions

JH Hwang supervised the work. CC Chen, YC Chan and JH Hwang conceptualized the experiments. YZ Liu carried out the experimental analysis. TC Lin wrote the manuscript. WP Chen and LY Lee critically analyzed and reviewed the manuscript. CC Chen and JH Hwang attributed to the revision of manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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