Osmanthus fragrans extracts for preventing noise induced hearing loss in brewery workers: A randomized, double-blind, controlled study

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ABSTRACT

This studies whether the water extract of Osmanthus fragrans can prevent noise-induced hearing loss (NIHL) after exposure to a noisy environment. Twenty-four healthy factory workers in southern Taiwan were recruited. The hearing threshold was determined before and after each dosing period. Measurements of oxygen radical absorbance capacity (ORAC), glutathione (GSH), oxidized glutathione disulfide (GSSG), GSH/GSSG ratio, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in plasma as well as audiometric measurements were made before and after the extract was ingested for 270 days. The results demonstrate that consuming single Osmanthus fragrans extract (SOFE) during continuous exposure to noise levels that exceed 85 dB for 270 days reduced temporary hearing loss (TTS) from 6.4 ± 2.3 dB HL to 2.8 ± 1.6 dB HL. The values of ORAC, GSH, GSH/GSSG ratio and GPx activity were significantly increased whereas those of GSSG and SOD were significantly reduced by the ingestion of Osmanthus fragrans extract. Furthermore, the complex Osmanthus fragrans extract (COFE) group exhibited a perceptible improvement in hearing threshold and antioxidant activity. The results herein showed that the correlation coefficients of ORAC, GSH, GSH/GSSG ratio, GPx, GSSG and SOD with the TTS at 4000Hz exceeded 0.90. After taking Osmanthus fragrans extract (OFE), workers at a factory with a high-noise environment exhibited increased antioxidant capacity, which ultimately mitigated TTS.

Keywords: Antioxidant activity, Noise-induced hearing loss (NIHL), *Osmanthus fragrans,* Pure-tone audiometry (PTA), Temporary threshold shifts (TTS)

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INTRODUCTION

Noise is the most common cause of occupation-related hearing loss. Noise-induced hearing loss (NIHL) affects more than 10% of the adult population in industrialized countries [1]. NIHL is a sensory deficit that typically presents as an audiometric "notch" at frequencies of over 4000Hz (4k notch) [2]. A working environment with noise that exceeds 85 dB should be categorized as highly hazardous workplace [3,4]. Eight hours of exposure to noise that exceeds 85 dB in the workplace can adversely affect the health of employees [5]. Damaging noise usually causes a transient blunting of hearing acuity, increasing the subject's audibility threshold for a period of hours. Repeated exposure to noise in these temporary threshold shifts (TTS) may eventually lead to a permanent threshold shift (PTS)[2].

Relevant literature has verified that noise is an important environmental factor in causing hearing loss as it damages tissues in the inner ear mechanically and metabolically [6] Biochemical and histological evidence suggests that exposure to noise alters the responses of cochlear tissues to oxidative stress and increases the level of free radicals in

the cytosol [7,8]. As highly toxic molecules, reactive oxygen species (ROS) adversely affect the organizational structure of the inner ear cells and mitochondrial bodies, even damaging the cochlea. This mechanism causes the up-regulation of oxidative stress response chemicals, owing to noise-induced hearing damage. Antioxidant therapy may therefore prevent NIHL[9].

As the main auditory organ of the inner ear, the cochlea contains two major classes of active antioxidant enzymes. The first class of enzymes, which includes glutathione S-transferase enzymes, GPx, and glutathione reductase, is involved in GSH metabolism. The second class of enzymes is responsible for the cleavage of superoxide anions and the antioxidant activities of hydrogen peroxide (and includes catalase and SOD, for example) [9]. According to a previous investigation, α-tocopherol [10-12], water-soluble coenzyme Q10 [13], GSH [14], N-acetylcysteine [15], and D-methionine [16] can retard the oxidative stress signals that are caused by hair cell death after exposure to high noise [17-19].

Osmanthus fragrans is a genus of approximately 30 species of flowering plants of the family Oleaceae. It is used not only as an ornamental plant, but also as an additive in food, tea, and other beverages because of its strong fragrance. The flower of Osmanthus fragrans has been demonstrated to exhibit strong antioxidant activity [20,21] This study investigates the ability of *Osmanthus fragrans* to alleviate hearing impairment after exposure to high noise.

EXPERIMENTAL SECTION

Preparation of OFE
Dried flowers of *Osmanthus fragrans* were purchased from Hung Chao Co., Ltd., Taipei, Taiwan, in 2009. The samples were authenticated by Dr. Mo-shin Tang, Department of Pharmaceutical Sciences and Technology, Chung Hwa University of Medical Technology. The *Osmanthus fragrans* (10 kg) were milled, suspended in 150 L of distilled water, and boiled for 60 min at 100°C. After filtration with filter paper, the solution was spray dried into a powder and filled in capsule by the Standard Chem. & Pharm. Co., Ltd., Tainan, Taiwan.

Determination of total phenolic content in *Osmanthus fragrans*
Following the method described by Yen and Hung [22], the sample solution in methanol (0.1 mL, 1 mg/mL) was well mixed with 2% Na_2CO_3 (2 mL). After 3 min, 50% Folin-Ciocalteaure agent (0.1 mL) was added. The mixture was allowed to stand at room temperature (RT) for 30 min with intermittent mixing. The absorbance at 750 nm was recorded. A standard curve using gallic acid was prepared. The total phenolic content was expressed as gallic acid equivalents (mg of GAE per g extract).

Determination of total flavonoid content in *Osmanthus fragrans*
Following the methods described by Woisky and Salatino [23] and also by Chang *et al*.,[24] 0.5 mL of sample solution was mixed with 1.5 mL of 95% EtOH, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M KOAc, and 2.8 mL of distilled water. The mixture was allowed to stand at RT for 30 min, and the absorbance was measured at 415 nm. The amount of sample solution was substituted by the same amount of a quercetin solution (0–200 μg/mL) as a standard. The amount of 10% aluminum chloride was substituted by the same amount of distilled water to serve as a blank. The total flavonoid content was calculated from the plot of absorbance against quercetin concentration using linear regression analysis and expressed as quercetin equivalents (μg of QE per g extract).

DPPH free radical scavenging assay
DPPH is a stable free radical with a purple color that is reduced by antioxidants to a colorless compound. We employed DPPH in an assay modified from the method of Shimada *et al*.[25] MeOH (3.8 mL), sample solution in methanol (0.2 mL, 1 mg/mL), and 1 mM DPPH solution (1.0 mL) were mixed well and left to stand in the dark at RT for 30 min. The final concentration of the sample was 40 μg/mL. The absorbance at 517 nm was measured. The sample in methanol was used as a blank, while DPPH radical in methanol solution was used as a control. The DPPH radical scavenging activity was calculated according to the following equation:

% of DPPH radical scavenging activity = $[1 - (A_{sample} - A_{blank})/A_{control}] \times 100$, where A is the absorbance at 517 nm. The concentration providing 50% inhibition (IC_{50}) DPPH radical scavenging activity calculated from the plot of inhibition percentage against sample concentration by linear regression analysis.

Subject selection
This randomized, double-blind, parallel-group study was the sample and placebo preparations were packaged in capsules. All doctors, research staff, and volunteers involved remained unaware of the actual product administered during the entire study period. This study received approval from the Institutional Review Board (IRB) of Chung Hwa University of Medical Technology. The brewery was a noisy workplace, because the measured noise exposure

exceeds 90 dB. The volunteers were considered eligible for study participation if they worked in an area where exposure levels equaled or exceeded 85 dB. Potential volunteers were selected based on a dietary questionnaire regarding their dietary habits, to enroll individuals with similar eating habits, especially fruit and vegetable consumption. Subjects with low fruit and vegetable intake and on hypocaloric, vegetarian or vegan diet were excluded from this study. Subjects reporting the use of medication or dietary supplement, and subjects with a history of major illness were excluded as well. Twenty-four volunteers were randomly divided into 3 groups: (1) placebo (n=10), served with capsules containing only corn starch; (2) single *Osmanthus fragrans* extract (SOFE, n=14), served with capsules containing 200 mg of *Osmanthus fragrans* extract; and (3) complex *Osmanthus fragrans* extract (COFE, n=15), served with capsules containing 200 mg of *Osmanthus fragrans* extract, 25 mg of grape seed extract and 50 mg of astaxanthin extract. The astaxanthin extract is a strong antioxidants and grape seed extract purchased from Standard Chem. & Pharm. Co., Ltd., Tainan, Taiwan.

The test supplements were taken one capsule per day at breakfast. The volunteers were instructed to fast for at least 8 hr before blood collection on the first, 90th, 180th and 270th day. 10 mL of fasting blood were collected in heparinized tubes and centrifuged at 3500 rpm for 10 min, for the determination of ORAC, GSH, GSSG, SOD and GPx.

Questionnaire
A structured questionnaire was administered to solicit information about demographic characteristics, work history, health habits (smoking and alcohol drinking), medical conditions, noise exposure, as well as use of medications, dietary supplements and hearing protection devices.

Noise exposure measurement
Personal noise exposure was evaluated from 7 a.m. to 3 p.m. during the work shift using a noise dose meter. Frequency characteristics of noise were then measured using a real-time noise analyzer with one-third octave bands. A half-inch, free-field condenser microphone with a frequency range of 31.5 Hz to 16 kHz was also used. During the work shift, the participants did not use hearing protection devices.

Pure-tone audiometry
These workers were reminded to avoid explosive noise exposure for at least 12 hr before receiving these audiological assessments. Each subject received pre-shift audiometry from 6:30 to 7:30 in the morning of the test day. Post-shift audiometry was performed immediately after the work shift. It was uniform across subjects.

An audiologist obtained pure-tone audiometry (PTA) for all subjects in a sound-attenuating chamber with a background noise level of ≤ 25 dB, which complies with the criteria of International Organization for Standardization (ISO) 8253-1. For each ear, the test frequencies included 250, 500, 1000, 2000, 3000, 4000, and 8000 Hz.

The hearing level (HL) for high frequencies (HF) by PTA was defined as the average of HLs at 4000 Hz. All four hearing assessments by PTA were completed for each formulation period on the frist day pre- and post-shift, and the 270th day pre- and post-shift. Finally, the amount of TTS was calculated by subtracting the pre-shift hearing threshold from the post-shift hearing threshold at each frequency.

Plasma antioxidant capacity assessment
Determination of plasma ORAC
As a modification of the protocols outlined by Chung *et al*. [26] 100 μL of 0.1 μM β-phycoerythrin and 85 μL of 75 mM AAPH were added to 15 μL of plasma. The fluorescence was measured immediately (excitation emission 480 nm, 520 nm) using the FLOUstar (FLUOstar OPTIMA, BMG Labtech, Inc., USA). The fluorescence was recorded at 5 min intervals for 120 min, until the fluorescence of last reading declined to less than 5% of the first reading. To calculate the area under the curve (S), the following equation was used:

$$S = (0.5 + f_5/f_0 + f_{10}/f_0 + f_{15}/f_0 \ldots\ldots + f_{25}/f_0)*5$$

Where fn is fluorescence measurement at time n.

ORAC values are expressed using Trolox equivalents:

$$\text{ORAC value (μM)} = 20 * k * (S_{sample} - S_{blank}) / (S_{trolox} - S_{blank})$$

Where k is the sample dilution factor.

Determination of GSH, GSSG and GSH/GSSG ratio
This GSH method was modified from the procedure reported by Sedlak and Lindsay, [27] 150 µL of each sample were added to 450 µL 5% trichloroacetic acid solution then subjected to centrifugation at 10000 rpm for 10 min. 30 µL of supernatant were injected into 96 well plates. In each well 140 µL of 0.4 M Tris buffer and 10 µL of 0.01 M DTNB were added and incubated for 5 min. The absorbance value was measured using the ELISA reader (VERSA, Molecular derices, LLC, U.S.A), and the GSH content in the plasma was subsequently calculated.

The GSSG level of plasma was measured with a commercial kit (NWLSS™th, Northwest Life Science Specialties, LLC, Vancouver). Using GSSG (0-10 µM) as the standard. The diluted sample solution or standard (200 µL) was mixed with 200 µL of 5% MPA, centrifuge at 7700 rpm for 2 min. Then, 200 µL of the supernatant was taken and mixed with 10 µL of 1N NaOH and 10 µL of 4-vinylpyridine. The mixture was allowed to stand at room temperature for 1 hr. Fifty microliter of the reaction mixture was mixed with 50 µL of the reagent (DTNB in phosphate buffer) and 50 µL of GR Enzyme (Glutathione reductase in phosphate buffer with EDTA, pH 7.6 with protein stabilizer) in an incubation microplate for 5 min at room temperature, followed by the addition of 50 µL NADPH (β-nicotinamide adenine dinucleotide phosphate in buffer with stabilizer). The reaction mixture was then incubated at room temperature, and the absorbance at 405 nm was determined at 15 sec intervals for 15 min using an ELISA reader (VERSA, Molecular derices, LLC, U.S.A). The concentration was expressed as GSSG (µM) in plasma. For each plasma sample, GSSG/GSH ratio was calculated.

Determination of plasma GPx activity
The GPx activity of plasma was measured with a commercial kit (Cayman, Cayman Chemical Company, U.S.A). 20 µL of plasma were added to 100 µL of assay buffer (50 mM Tris-HCl, pH 7.6, containing 5 mM EDTA) and 50 µL of co-substrate mixture (containing a lyophilized powder of NADPH, GSH, and glutathione reductase by adding 2 mL of HPLC-grade water to each vial and vortex). The reaction was initiated by the addition of 20 µL of cumene hydroperoxide into the mix. Then, the reaction mixture was incubated at 37 °C, and the absorbance at 340 nm was determined every minute for 5 min using a ELISA reader (VERSA, Molecular derices, LLC, U.S.A).The measurement values were incorporated into the following formula:

$$\Delta A_{340}/min = (A_{340,\ time\ 2} - A_{340,\ time\ 1})/(time\ 2 - time\ 1)$$

$$GPx\ activity\ (\mu M/min) = (\Delta A_{340}/min/0.00373\ \mu M^{-1}) / (0.19\ mL/0.02\ mL) \times sample\ dilution$$

Determination of plasma SOD activity
This SOD method was modified from the procedure reported by Magnani *et al*.[28] Briefly, 50 µL of pyrogallol and 50 µL of plasma (Tris buffer for the blank) were added to a test tube. Additionally 3 mL of Tris-buffer were added to the test tube. The contents were mixed gently then the tube was placed in a spectrophotometer (Uv/Vis, V-630 bio, JASCO International Co., Ltd. Japan). The readings at 0-sec mark and at 30-sec mark under 325 nm were taken to calculate the change in absorbance value. The absorbance change of the blank (*A*max), and of the sample (*A*1) were used to calculate the % inhibition:

$$Inhibiting\ superoxide\ anion\ activity\ (\%) = [(A\text{max} - A1)/A\text{max}] \times 100$$

Statistical analysis
Each experiment (plasma antioxidant capacity assessment: ORAC, GSH, GSSG, GSH/GSSG ratio, GSHPx, SOD) was conducted in triplicates. Two-tailed unpaired student's t-test is used to compare the differences between groups and a $p < 0.05$ indicates statistical significance.

RESULTS

Demographic and personal characteristics
Thirty-nine workers were involved in this study (39/44, 88.6%). The workers had been employed in a factory for an average of 18.5 years. Most of them were middle-aged (44-59 years old). During the days in which TTS measurements were made, the average daily noise exposure (time-weighted average of 8 hr, TWA-8 hours) ranged from 88.6 to 93.3 dB, as determined using personal noise monitors. Some investigations have mentioned that smoking is a risk factor in the development of NIHL.[29-31] The risk of NIHL has been shown to increase with the number of pack-years of smoking.[31] Although this study included seventeen (43.1%) smoking subjects and fifteen (38.5%) alcohol-drinking subjects, Table 1 reveals that smoking tobacco and drinking alcohol had no significant effect on TTS level. We speculate that the amounts of tobacco smoked and alcohol drunk may have been too low to have significantly affected changes in the hearing threshold.

Table 1. Demographic and personal characteristics

	Placebo group	SOFE group	COFE group
Quantitative variable	Mean(SD) (n=10)	Mean(SD) (n=14)	Mean(SD) (n=15)
Age	52.8(2.7)	54.5(3.0)	51.0(3.7)
Employment (year)	18.4(3.7)	20.7(2.6)	18.5(6.9)
Personal noise exposure (TWA-8 hours) (dB)	90.1(1.1)	90.7(1.8)	91.1(2.1)
Pre-shift HF PTAa (dB HL)			
Frist	26.3 (2.2)	32.5 (3.2)	30.0 (3.1)
90 th	26.9 (3.6)	31.7 (2.8)	29.6 (2.5)*
180th	25.6 (3.0)	29.2 (3.0)	27.5 (3.4)*
270th	27.5 (5.3)	28.9 (3.5)*	25.7 (3.0)**
HF TTSb (dB HL)			
Frist	5.8 (3.1)	6.4 (2.3)	6.4 (1.8)
90 th	5.0 (0.0)	4.6 (3.7)	5.4 (2.8)
180th	4.2 (1.2)	4.3 (2.6)	4.6 (1.6)*
270th	6.4 (3.3)	2.8 (1.6)*	2.1 (2.5)**
Categorical variable	Number (%)	HF TTS(dB HL)	p value
Drink alcohol			
Yes	17(43.1)	7.5(2.3)	0.3
No	22(56.4)	6.2(2.6)	
Smoking tobacco			
Yes	15(38.5)	7.3(1.9)	0.36
No	24(61.5)	6.6(1.5)	

[a] The average of hearing threshold levels of the right and the left ears at 4000 Hz by pure-tone audiometry (PTA). [b] The average of hearing threshold levels of the right and the left ears at 4000 Hz by temporary threshold shift (TTS). * Significant difference between the frist and 90th, 180th, 270th at $p < 0.05$. ** Significant difference between the frist and 90th, 180th, 270th at $p < 0.01$.

Table 2. Effect of antioxidant status in plasma after consuming *Osmanthus*

	Placebo group Mean (SD) (n=10)	SOFE group Mean (SD) (n=14)	COFE group Mean (SD) (n=15)
ORAC (mM Trolox equivalent)			
Frist	3.29(0.35)	3.41(0.23)	3.32(0.19)
90th	3.01(0.36)	3.62(0.34)	4.04(0.22)*
180th	2.89(0.37)	3.90(0.24)**	4.48(0.30)**
270th	2.75(0.35)	4.09(0.19)**	4.59(0.24)***
GSH (μM)			
Frist	6.33(0.12)	5.98(0.43)	6.49(0.19)
90th	6.38(0.14)	6.48(0.55)	7.56(0.16)***
180th	5.94(0.20)	7.14(0.24)**	7.80(0.10)***
270th	5.59(0.07)*	7.51(0.21)***	8.13(0.19)***
GSSG (μM)			
Frist	0.15(0.01)	0.16(0.02)	0.18(0.03)
90th	0.16(0.03)	0.14(0.01)	0.15(0.02)*
180th	0.15(0.03)	0.13(0.01)*	0.13(0.01)**
270th	0.18(0.01)**	0.12(0.02)**	0.12(0.02)**
GSH/GSSG ratio			
Frist	42.15(2.33)	39.03(1.21)	37.19(1.18)
90th	39.80(2.11)	45.82(1.46)*	52.53(0.99)**
180th	35.07(1.42)	55.92(1.35)**	59.68(1.64)**
270th	31.20(1.50)**	61.16(1.58)***	69.63(1.40)***
GPx (μM/min)			
Frist	0.43(0.05)	0.23(0.08)	0.34 (0.13)
90th	0.42(0.01)	0.27(0.09)	0.38(0.13)*
180th	0.41(0.05)	0.29(0.09)**	0.39(0.10)*
270th	0.40(0.08)	0.32(0.12)**	0.40(0.14)**
SOD (U/L)			
Frist	5.92(2.50)	4.72(0.26)	4.73(0.68)
90th	5.79(2.36)	4.56(0.37)	4.56(0.78)
180th	6.31(2.52)	4.38(0.14)*	3.89(0.09)*
270th	5.48(2.40)	4.26(0.16)**	3.59(1.01)**

* Significant difference between the frist and 90th, 180th, 270th at $p < 0.05$. ** Significant difference between the frist and 90th, 180th, 270th at $p < 0.01$. *** Significant difference between the frist and 90th, 180th, 270th at $p < 0.001$.

**Audiometry and temporary threshold shift**

Figure 1 displays an audiogram that reveals the hearing thresholds at various frequencies from 250 to 8000 Hz for different treatment groups on the first day and the 270th day of treatment. According to Figs. 1a and 1b, for the

placebo group, the hearing threshold increased from 28.8 dB HL to 30.0 dB HL for the right ear and from 23.6 dB HL to 25.0 dB HL for the left ear. Therefore, the placebo group exhibited increases of 1.2 dB HL and 1.4 dB HL in the hearing thresholds of the right and left ears by the end of the trial. In the SOFE group, the hearing threshold was improved from 29.0 dB HL to 28.6 dB HL for the right ear and from 34.2 dB HL to 29.3 dB HL for the left ear (Figs. 1c and 1d), representing a recovery of hearing of 0.4 dB HL for the right ear and 4.9 dB HL for the left ear after 270 days in the same working environment.

Table 3. Correlation coefficient of 4000Hz temporary hearing loss and antioxidant capacity

		ORAC	GSH	GSSG	GSH/GSSG	GPx	SOD
Temporary Hearing loss at 4000 Hz	Placebo	-0.57	-0.43	0.67	-0.54	0.77	0.71
	SOFE	-0.97	-0.95	0.81	-0.9	-0.97	0.95
	COFE	-0.95	-0.91	0.98	-0.95	-0.94	0.93

Figure 1. Hearing threshold before the work shift PTA of placebo group at frist day (a) and 270th day (b), SOFE group at frist day (c) and 270th day (d), and COFE group at frist day (e) and 270th day (f)
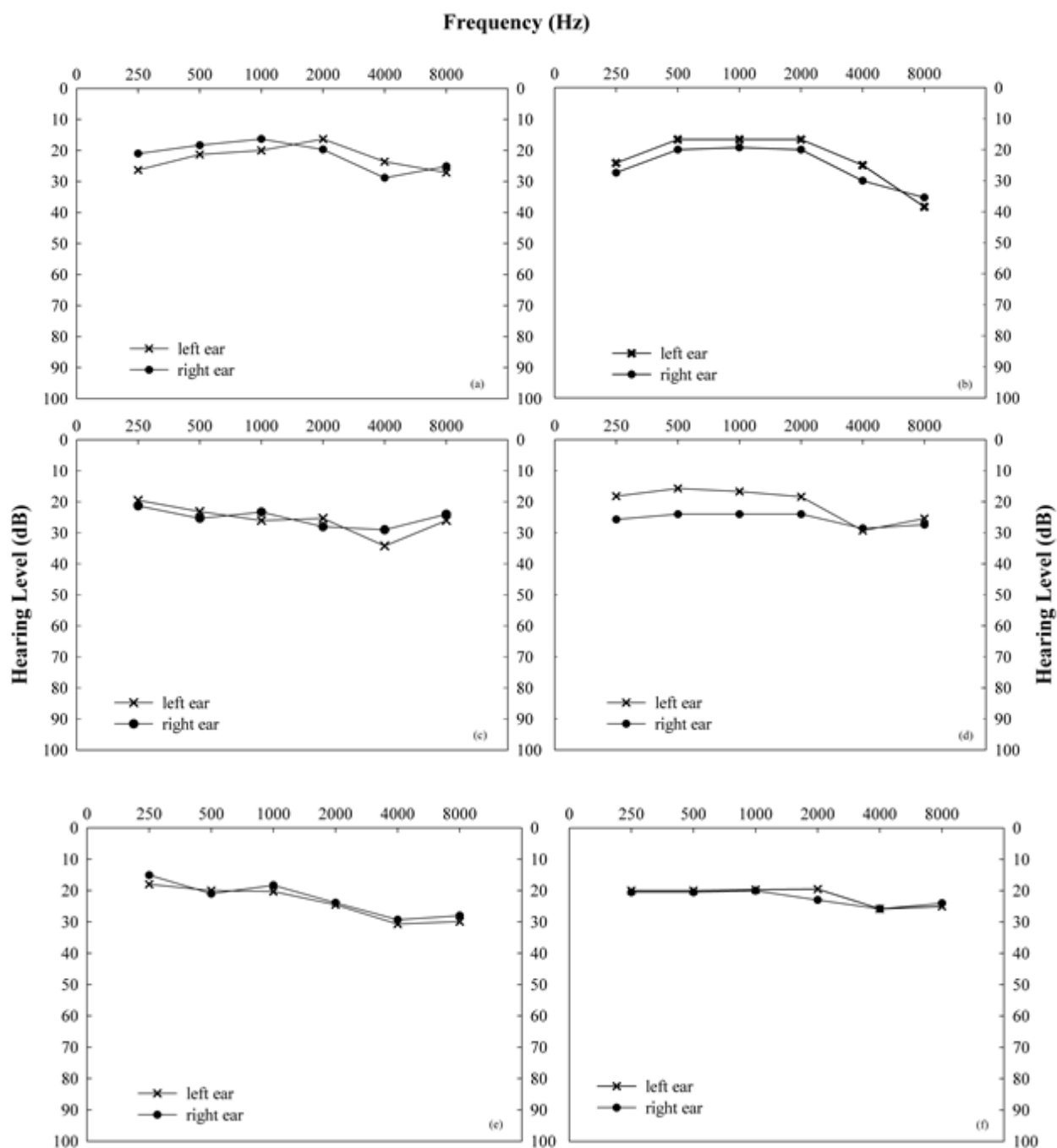
The COFE group also exhibited an improvement in the threshold from 29.3 dB HL to 25.8 dB HL for the right ear and from 30.7 dB HL to 25.8 dB HL for the left ear, representing recoveries of 3.5 and 4.9 dB HL, respectively (Figs. 1e and 1f). A clear notch was observed at 4000 Hz.

In Table 1, the PTA and TTS at 4000 Hz for the different groups were presented as averages for the left and right ears. For the placebo group, the pre-shift hearing threshold on the first day was close to that on the 270th day, and the difference was insignificant. For the SOFE and COFE groups, the pre-shift HF PTA declined gradually from 32.5 ± 3.2 on the first to 28.9 ± 3.5 dB HL on the 270th day and from 30.0 ± 3.1 on the first day to 25.7 ± 3.0 dB HL on the 270th day, respectively. The HF TTS level in the SOFE group on the first day was 6.4 ± 2.3 and that on the 270th day was 2.8 ± 1.6 dB HL; the corresponding levels in the COFE group were 6.4 ± 1.8 and 2.1 ± 2.5 dB HL, respectively. The significant decreases in both the HF PTA and HF TTS values indicate that the OFE significantly reduced noise-induced temporary hearing threshold shift.

Total phenolic and flavonoid contents and DPPH scavenging effects of *Osmanthus fragrans*
The total phenolic content was 340.68 ± 19.47 mg GAE/g extract, while the total flavonoid content was 49.97 ± 4.40 mg QE/g extract. The DPPH IC_{50} of the water extract was 15 μg/mL, which was less than that of the methanol extract (12.8 μg/mL) and trolox (4.9 μg/mL) [21]. As previously, *Osmanthus fragrans* is rich in phenolics and flavonoids, and exhibits strong antioxidative activity.[21]

Antioxidant capacity of plasma
An increase in antioxidant capacity can be described as an increase in ORAC, GSH level, GSH/GSSG ratio and GPx activity or a decrease in GSSG level. Table 2 presents the ORAC, GSH, GSSG, GSH/GSSG ratio, GPx activity, and SOD values on the (first, 90th, 180th, and 270th days. After the subjects in the SOFE group had consumed OFE for 270 days, their plasma ORAC values had increased by $18.5 \pm 13.7\%$; their GSH levels had increased by $25.9 \pm 12.0\%$; their GSH/GSSG ratios had increased by $54.4 \pm 25.8\%$ and their GPx values had increased by $33.6 \pm 17.7\%$. Their GSSG and SOD values had declined by $27.2 \pm 21.6\%$ and $9.7 \pm 7.8\%$, respectively. However, in the COFE group, the ORAC and GSH values began to increase significantly from the 90th day. By day 270, ORAC, GSH level, the GSH/GSSG ratio and GPx had increased by $32.6 \pm 15.4\%$, $30.3 \pm 6.4\%$, $88.2 \pm 45.8\%$ and $34.9 \pm 22.3\%$, respectively, whereas their GSSG and SOD had decreased by $48.2 \pm 33.1\%$ and $15.2 \pm 5.5\%$, respectively. The GSH/GSSG ratio was an indicator of antioxidant status [28,29] Since *Osmanthus fragrans* increased the GSH/GSSG ratio by significantly increasing GSH and reducing GSSG, the results herein confirm that *Osmanthus fragrans* enhances the antioxidative activity of plasma.

Correlation of temporary hearing loss at 4000Hz with antioxidative capacity
The physiological effects of consuming OFE, an antioxidant supplement, for 270 days on ORAC, GSH level, GSSG level, GSH/GSSG ratio, GPx activity, and SOD activity were quantified. Interestingly, a definitive correlation was observed between temporary hearing loss at 4000 Hz and antioxidant activity ($p < 0.05$). As shown in Table 3, negative correlations were observed between TTS and plasma ORAC, GSH, GSH/GSSG ratio and GPx, with $r = -0.90$ to -0.97 for the SOFE group and $r = -0.91$ to -0.95 for the COFE group. In both groups, GSSG and SOD are positively correlated with TTS ($r > 0.81$). The placebo group exhibited negative correlations between TTS and plasma ORAC, GSH and GSH/GSSG ratio with $r = -0.43$ to -0.57. The antioxidant capacity is an important factor in importantly affects NIHL. Consequently, an increase in antioxidant capacity by OFE reduces the likelihood of hearing loss at 4000Hz.

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DISCUSSION

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This study demonstrated that continuous exposure to noise levels of over 85 dB HL for 270 days resulted in TTS values at 4000 Hz of 6.4 ± 3.3 dB HL. The issue of "PTS" was not evaluated in this study. This finding reveals that noise progressively worsens the hearing of workers. In previous tests, the antioxidative capacity of *Osmanthus fragrans* was found to be second only to that of green tea; Additionally, *Osmanthus fragrans* can scavenge free radicals [21].

Treatment with OFE significantly strengthens the antioxidative capacity of plasma, and has been associated increases in ORAC, GSH, GSH/GSSG ratio and GPx and reductions of GSSG and SOD. These effects reduce the TTS level at 4000 Hz. This study confirms the strong correlations between TTS and antioxidative capacity, suggesting that the antioxidative state may affect susceptibility to hearing loss.

Relevant literature has established that noise reduces GSH levels, increases GSSG levels in the inner ear, and induces ROS-related cell injury [34,35]. Several studies have indicated that GSH reduces hearing loss in animals that are exposed to noise [14,19,25-38]. Kaygusuz *et al.*[39] found that the blood of workers under high-noise

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conditions exhibits increased GPx activity, and that this physiological change is a natural antioxidant defense mechanism. The antioxidizing enzyme that is found in the mammalian cochlea GSTM1 appears to be capable of protecting hair follicle cells from noise and aging. Therefore, antioxidants can obviously withstand NIHL[14, 35-38]. In this experiment, GSH and GPx was related to TTS at 4000 Hz with negative coefficients of −0.91 to −0.95 and −0.94 to −0.97, respectively. The results in this study that taking OFE may reduce hearing damage by increasing the GSH level and GPx activity and reducing the GSSG level, helping to counterbalance the generation of superoxide anions, reactive oxygen species, and malondialdehyde by the cochlea in noisy environments [39,40].

This study demonstrated that *Osmanthus fragrans* increased antioxidant levels in the human body, which were negatively correlated (−0.95 to −0.97) with TTS at 4000Hz in individuals who work in noisy environments after they took OFE for 270 days. This result arose from the ability of antioxidants to neutralize ROS radicals, reducing TTS at 4000 Hz. Accordingly, *Osmanthus fragrans* not only is an excellent antioxidant in the human body, but also mitigates hearing impairment.

CONCLUSION

After 270 days of taking with OFE, workers at factory with a high noise level exhibited increased antioxidant capacity and, consequently, significantly reduced activity of reactive oxygen species in their bodies. These effects ultimately mitigated temporary hearing loss at 4000 Hz. Hence, this study finds that *Osmanthus fragrans* is an excellent source of antioxidants and can protect individuals from hearing loss.

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