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Original article

Effect of polyphenols from water chestnut pericarp on hair-related quality of life in healthy middle-aged, and older subjects: A randomized, placebo-controlled, double-blinded, parallel-group, comparison study

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Abstract

***Purposes*:** This study aimed to investigate the impact of continuous intake of polyphenols from water chestnut pericarp on thinning hair and the decline in hair-related quality of life (QOL) in healthy middle-aged and older Japanese subjects. The study employed a randomized, placebo-controlled, double-blinded, parallel-group design.

***Methods*:** The participants in this 24-week study were healthy Japanese males and females aged 40 to 64 years. They were randomly assigned to either the *Trapa bispinosa* Roxb. pericarp polyphenol (TBPP)-containing food group (containing 25 mg of polyphenols from water chestnut pericarp) or a placebo food group. The primary outcome was the hair-related quality of life assessed by the Skinex29 score. Secondary outcomes included a visual analogue scale (VAS) questionnaire, assessment of hair and scalp condition, evaluation of scalp moisturization, and measurement of advanced glycation endproducts (AGEs) in blood plasma.

***Results*:** The TBPP group showed significant improvement in the composite score of Skindex 29, VAS questionnaire regarding hair volume, hair diameter, and the amount of pentosidine (one of AGEs) in blood plasma compared to the placebo group. Moreover, the TBPP group exhibited a significant decrease in the amount of carbonylated protein compared to pre-ingestion. No adverse events were reported during the TBPP-containing food intake period.

***Conclusion*:** Polyphenols derived from water chestnut pericarp prove to be an effective food material that enhances health by addressing thinning hair and improving QOL in middle-aged and older males and females.

***KEY WORDS*:** *Trapa bispinosa* Roxb. pericarp polyphenol (TBPP), thinning hair, quality of life (QOL), glycative stress, clinical study

Introduction

One inevitable aspect of human aging is the transformation in physical appearance. Although alterations in appearance themselves may not directly impact physical health, they can contribute to a self-negative emotional state known as body dissatisfaction [1)]. Elevated body dissatisfaction stands as a potential precursor to mental disorders, including eating disorders and dysmorphophobia [2,3)]. Conversely, positive changes in appearance have been linked to enhanced mental health, exemplified in instances where makeup application instills self-confidence and satisfaction, particularly observed in Japanese women [4)]. In alignment with the World Health Organization's (WHO) definition of health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", it becomes evident that preserving and enhancing mental health necessitates attention to age-related changes in appearance.

Within the Japanese population, thinning hair emerges as a notable factor influencing an individual's impression. Studies on impression formation among Japanese adults [5)] indicate that both individuals with thinning hair and those around them harbor negative stereotypes towards thinning hair, leading to negative emotions and potential impacts on interpersonal relationships. Thinning hair, being a conspicuous marker qof aging, has been associated with a decline in quality of life (QQL), for example, in patients with androgenetic alopecia (AGA) [6)]. This suggests that the more severe the hair thinning,q and the longer the AGA period of a patient, the lower their mental QOL, and the more likely they are to become emotionally depressed and passive in social activities. Based on the aforementioned, it is thought that

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thinning hair impairs health as defined by the WHO due to the decline in mental QOL and passivity in social activities.

Hair, originating and growing from specialized tissues known as hair follicles in the skin, undergoes a cyclic process called the hair cycle, consisting of the anagen, catagen, and telogen phases. Disruptions in the hair cycle, resulting in thinning hair, can be caused by various factors[7], one of which is glycation reaction, a known inhibitor of hair follicle formation and growth. Glycation reaction produces advanced glycation endproducts (AGEs) through the interaction of sugars and aldehydes with proteins and amino acids. Accumulation of glycative stress, involving AGEs and their intermediates, induces cellular and tissue stress, leading to inflammation and contributing to age-associated changes in appearance[8]. According to a previous report, in mice, N^{ε}-(carboxymethyl)lysine (CML), a type of AGEs, delays hair follicle formation, and at the same time, morphologically weakens hair, that is, worsens the condition of cuticles, making hair thin[9]. Based on these facts, it is thought that reducing glycative stress *in vivo* contributes to the prevention and improvement of thinning hair.

This study centers on water chestnut (*Trapa bispinosa* Roxb.) pericarp extract as a potential means to mitigate glycative stress in humans. *Trapa bispinosa* Roxb., an annual aquatic plant belonging to the Trapa genus of Lythraceae family, is commonly consumed, bears fruits in autumn, and its pericarp is dried and used in the preparation of healthy tea and herbal medicine[10]. Previous *in vitro*[11] and human clinical trials[12] demonstrated that *Trapa bispinosa* Roxb. pericarp polyphenol (TBPP), obtained through hot water extraction, possesses anti-glycation effects. Components of TBPP, such as ellagic acid and gallic acid, have also exhibited anti-glycation properties[13]. Moreover, studies on hair removal mouse models have confirmed the positive impact of consuming *Trapa bispinosa* Roxb. pericarp extract on hair growth and cuticle improvement[14].

Based on this background, TBPP emerges as a potential candidate for improving thinning hair through its anti-glycation effect, with anticipated positive effects on QOL that has diminished due to thinning hair. Therefore, this study recruited healthy middle-aged and older Japanese men and women experiencing concerns about thinning hair, conducting a 24-week randomized, placebo-controlled, double-blinded, parallel group comparison study on TBPP intake with the objective of enhancing QOL impacted by thinning hair. This report presents the findings of our investigation.

Methods

1. Study design

In this randomized, placebo-controlled, double-blinded, parallel group comparison study, two groups were assessed: the TBPP group, consuming TBPP-containing food, and the placebo group, consuming a placebo food. This study was conducted with approval from the Shiba Palace Clinic Ethics Review Committee (Chairman: Dr. Motonori Sano, Tokyo, Japan) granted on August 9, 2022, in compliance with the "Declaration of Helsinki (amended at the 2013 Fortaleza General Assembly)" and the "Ethical Guidelines for Medical and Biological Research Involving Human Subjects (2021 Ministry of Education / Ministry of Health, Labor and Welfare / Ministry of Economy, Trade and Industry Public Notice No.1)," and with the written consent of the subjects who had been provided with a full explanation of the objective and methods of this study, in an effort to protect the safety and human rights of the subjects (study implementation period: October 20, 2022–April 27, 2023). In addition, the information of this study was registered to the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN ID: UMIN000049039).

2. Test food setting/intervention

The water chestnut pericarp extract used, manufactured by Hayashikane Sangyo Co., Ltd. (Shimonoseki, Yamaguchi, Japan), was incorporated into TBPP-containing food. The TBPP-containing food included 100 mg of water chestnut pericarp extract (containing over 25 mg of TBPP), corn starch, and calcium stearate in hard capsules (Sankyo Co., Ltd., Fuji, Shizuoka, Japan). The placebo food, with no water chestnut pericarp extract, contained starch decomposition products and caramel coloring that would not affect the hair and scalp, in identical-looking capsules with corn starch and calcium stearate. All test foods were designed to be indistinguishable in sensory aspects and appearance, and differed minimally in terms of content, such as energy and sodium. The composition of each test food is shown in ***Table 1***.

Each group consumed one capsule of TBPP-containing food or placebo food daily before breakfast with water or lukewarm water. The intake period was set as 24 weeks, and observations were conducted three times, namely, before test intake, 12 weeks after intake, and 24 weeks after intake.

3. Participants

The subjects of this study were healthy Japanese men and women aged 40 to 64, experiencing thinning hair compared to their 20s. Inclusion criteria included consent to a haircut of the targeted area by a professional, commitment to maintaining their hair styling, hair dyeing, and hair washing methods, and agreement to have approximately 10-15 strands of hair cut and collected from near the root at the top of the head by a professional.

Exclusion criteria covered allergies to test food components, thinning hair attributed to diseases, previous alopecia diagnoses, such as male or female pattern baldness, current alopecia diagnoses (male pattern baldness, alopecia areata, infection, scarring, drugs, accompanying symptoms of systemic diseases, trichotillomania, and physiological hair loss), previous experience of hair transplantation, or individuals scheduled for hair transplantation during the study period, regular steroid use for hay fever or allergic rhinitis, difficulty maintaining lifestyle habits during the study period, difficulty keeping a journal, pregnancy, breastfeeding, or plans to become pregnant or breastfeed during the study period, use or application of drugs that may affect examination results, consumption of health foods that may affect the study results on a daily basis, skin allergy symptoms and skin sensitivity, regular visits to a dermatology clinic, participation in other clinical trials, use of hair wash products, such as shampoos, claiming to have

Table 1. Composition of test foods.

Test food	Ingredients	Combination ratio (per capsule 234 mg)
TBPP containing food	• water chestnut pericarp extract	42.73 %
	• Corn starch	35.74 %
	• Edible hard capsule	20.94 %
	• Calcium stearate	0.59 %
Placebo food	• Colorant (caramel color)	34.19 %
	• Starch degradation products	26.92 %
	• Edible hard capsule	20.94 %
	• Corn starch	17.09 %
	• Calcium stearate	0.86 %

TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol.

hair growth effects, underlying diseases [diabetes mellitus under medication treatment or diabetes with complications, chronic respiratory disease, chronic heart disease (including hypertension), chronic kidney disease, chronic liver disease (excluding fatty liver and chronic hepatitis), chronic blood disease (excluding iron deficiency anemia), and neurological disease and neuromuscular disease associated with immune abnormality, chromosomal abnormality, severe psychosomatic disorder (the state where severe physical disability and severe intellectual disability overlap)], and drug treatment or outpatient treatment.

Recruitment of participant applicants was done by Souken Co., Ltd. (Tokyo). Before the start of the study, informed consent was sought from each participant applicant individually, and their consent to participate in this study was obtained following full explanation that participating in this study was on a voluntary basis, and that they would not suffer any disadvantage even if they did not consent.

4. Selection, randomization, and blinding

Based on the data and preliminary study results of our previous study on usefulness, the required number of subjects was calculated, and the target number of subjects was 24 for each group, accumulating to a total of 48 subjects.

A third party allocator not directly involved in the implementation of this study randomly (simple randomization method) allocated the subjects using a random number table generated using a computer so that age and gender would be equal in each group. In addition, until the number of subjects to be analyzed after study completion was fixed, the allocator did not disclose the allocation results to anyone.

5. Assessment items

For efficacy assessment, the primary outcome was the measurement results of Skindex29, a hair-related QOL questionnaire. In addition, to obtain secondary outcomes, a visual analogue scale (VAS) questionnaire with uniquely set wordings, assessment of hair and scalp conditions, assessment of scalp moisturization, and measurement of AGE amount in blood were performed.

1) QOL questionnaire

Skindex29 was used as a method to assess scalp/hair-specific health-related QOL. In this study, it was conducted in the form of an online questionnaire after changing parts stated as “skin/skin condition” in each question to “scalp/alopecia” in accordance with instructions in the manual. Before answering, the subjects were informed that “the following questions will ask to what extent alopecia bothered you in the past week,” and each of the 29 questions were to be answered by the scores of 1: “never,” 2: “almost never,” 3: “sometimes,” 4: “often,” and 5: “always.” The higher the scores, the more severe the concerns about scalp/alopecia.

2) VAS questionnaire

The VAS method was employed to gauge concerns related to hair conditions. The questions were set as “the degree of concern” on 19 items including “hair loss,” “visibility of parting and scalp,” “low hair volume,” “hair thinness,” “lack of elasticity and firmness,” “dryness,” “spreading,” “waviness,” “lack of shine,” “poor root growth,” “poor combing and difficulty in setting,” “scalp stickiness,” “scalp odor,” “scalp acne,” “scalp dryness,” “scalp hardness,” “dandruff,” “split ends and hair breakage,” and “grey hair.” Participants provided responses moving a cursor on a scale from “I am not concerned” (0 points) to “I am concerned” (100 points).

3) Measurement of hair and scalp conditions

Digital photographs were taken of three areas: the top, back, and front of the head.

Additionally, the number and diameter of actively growing hair were assessed through a haircut of the targeted area and phototrichogram analysis. The haircut of the targeted area was conducted approximately 3 cm to the right of the head midline and covered an approximate area of 1 cm × 1 cm, starting from the intersection of the line (horizontal line) connecting the head midline and the top of the left and right ear helices. The corresponding area was trimmed using

clippers, leaving 1 cm of hair. The haircut of the targeted area area was photographed using a multi-distance scope GOKO EV-6HD (Goko Imaging Devices Co., Ltd., Kawasaki, Kanagawa, Japan). The number of hairs and hair diameter in this area were assessed through image analysis.

To evaluate hair loss, the number of hairs shed during hair wash was counted.

A professional hairdresser conducted the hair wash using a shampoo without hair growth or treatment effects. After rinsing for more than 3 min with warm water (approximately 40 °C), the number of hairs that had fallen out during the wash was assessed.

As an additional measure to assess skin aging and oxidative stress, carbonylated proteins were quantified with reference to the method of Iwai *et al.*[15], The stratum corneum of the forehead was collected by tape stripping using cellophane tape. The collected stratum corneum was fixed to a glass slide, stained with 0.1 M 2-morpholinoethane sulfonic acid-Na (pH 5.5) buffer solution containing 20 μM FTCZ (fluorescence-5-thiosemicarbazide) for carbonyl group staining. Fluorescence images were taken under a fluorescence microscope, and the amount of carbonylated proteins in the stained images was calculated as fluorescence area using ImageJ (Wayne Rasband, National Institutes of Health, Bethesda, MA, USA).

4) Assessment of scalp moisturization

To gauge scalp moisturization and barrier function, measurements were taken for skin moisture content and water transpiration amount. The Corneometer CM825 (Integral Corporation, Tokyo) served as the measuring device for skin moisture content, while the portable TEWL meter VapoMeter (Keystone Scientific K.K., Tokyo) was employed to quantify the skin water transpiration amount. Both assessments were conducted on the forehead hairline to ensure a representative measurement of the targeted area.

5) Blood test

To assess glycative stress experienced by the subjects and the anti-glycation effect of the test foods, changes in blood AGEs during the study period were examined. Specifically, two types of AGEs, pentosidine and CML, were analyzed from the subjects' blood plasma using the ELISA method. This measurement process was conducted by Fushimi Pharmaceutical Co., Ltd. (Marugame, Kagawa, Japan).

6. Statistical analysis

1) Subjects for statistical analysis

In considering subjects for analysis, certain criteria for exclusion were established. These criteria encompassed various scenarios: firstly, subjects experiencing delays in observation date extending beyond one week; secondly, those found to have significantly violated instructions pertaining to subject management during the study period; thirdly, instances where major concerns arose regarding data reliability due to issues in examinations; furthermore, subjects with non-use days (when fixed daily usage was not met) surpassing 15% of scheduled use days were also taken into consideration for exclusion. Additionally, participants found to have violated participation criteria and met exclusion criteria were identified for potential exclusion. The criteria extended to subjects with measurement values distinctly determined as abnormal. Finally, any subjects presenting other clear reasons deemed appropriate for withdrawal were included in the criteria for potential exclusion.

Subjects meeting any of the aforementioned criteria, underwent review during a case review meeting. Subsequently, unless special reasons warranted an exception, these subjects would be excluded from the pool of subjects for analysis.

2) Efficacy assessment

All tests followed a two-tailed approach, with a significance level set at less than 5%. Temporal comparisons, in relation to pre-intake values within each group, utilized Dunnett's test to assess each actual value for both primary and secondary outcomes, taking into account the multiplicity of tests. Furthermore, to examine intergroup significant differences for each value, the difference values for both primary and secondary outcomes, in comparison to pre-intake values within each group, were verified and assessed using an unpaired *t*-test.

Results

1. Subject background

A total of 115 healthy Japanese men and women (54 and 61, respectively), aged 40 to 64, who were conscious of thinning hair compared to their 20s, voluntarily participated in this study following a detailed explanation of its content. After screening, 59 subjects (25 men and 34 women) met the eligibility criteria and were enrolled in the study. The participants were then divided into the TBPP group (29 subjects: 12 men and 17 women) and the placebo group (30 subjects: 13 men and 17 women). Throughout the study period, three subjects from the placebo group withdrew for personal reasons, leaving 29 subjects (12 men and 17 women) in the TBPP group and 27 subjects (11 men and 16 women) in the placebo group who successfully completed all predetermined schedules. Upon deliberation in the case review meeting regarding data handling for each group, it was determined that three subjects (two subjects in the TBPP group and a subject in the placebo group) were confirmed to have taken medications or supplements that could affect the results of the study, nine subjects (five subjects in the TBPP group and four subjects in the placebo group) had significant abnormalities in the measured values after assignment, six subjects (four subjects in the TBPP group, two subjects in the placebo group) had a medical history that met the exclusion criteria contrary to the participation criteria after the study was confirmed, and eight subjects (three subjects in the TBPP group and five subjects in the placebo group) had difficulty maintaining lifestyle habits due to their own circumstances. These subjects met the exclusion criteria for analysis subjects and were judged to be unsuitable for evaluation of efficacy. Consequently, the number of subjects eligible for analysis amounted to 15 (eight men and seven women) in the TBPP group, and 15 (six men and nine women) in the placebo group. The flow of these subjects are shown in ***Fig. 1***.

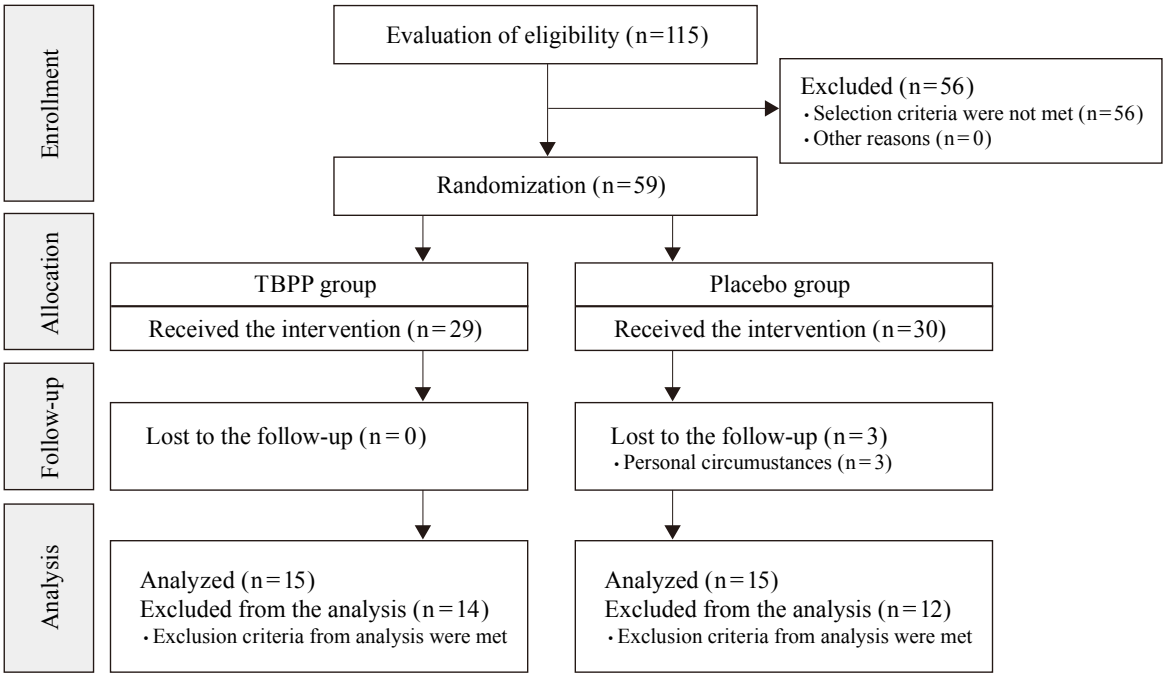

Fig. 1. **Flow of participant's progress in a clinical study**
TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol.

2. Efficacy assessment

2.1 Skindex29

Table 2 presents the actual measurement values of Skindex29 scores, the amount of change compared to before intake, and the results of statistical analysis. Notably, in the TBPP group, the amount of change in total scores 24 weeks after intake was significantly lower compared to the placebo group. Temporal comparisons of actual measurement values to before intake, revealed significant reductions in the three subscales of emotions, symptoms, and functions, as well as total scores, at both 12 and 24 weeks after intake in the TBPP group. Conversely, in the placebo group, temporal comparisons to before intake showed no significant fluctuation in scores.

2.2 VAS questionnaire

Table3 illustrates the actual measurement values of the VAS questionnaire, the amount of change compared to before intake, and the results of statistical analysis. Notably, in the TBPP group, the amount of change for Q3 at 24 weeks after intake was significantly lower compared to the placebo group. Temporal comparisons of actual measurement values to before intake revealed that Q12 at 12 weeks after intake, and Q3, 5, and 7 at 24 weeks after intake, showed significantly lower values compared to before intake exclusively in the TBPP group. Additionally, only in the placebo group, Q1 and 11 at 12 weeks after intake, and Q9 at 24 weeks after intake, displayed significantly lower values compared to before intake. Furthermore, in both the TBPP group and the placebo group, Q1 and 2 at 24 weeks after intake showed significantly lower scores compared to before intake.

2.3 Measurement of hair and scalp conditions

Fig. 2 showcases cases where an improvement in hair was observed on the entire head and the targeted area of haircut in the TBPP group.

Furthermore, ***Table 4-a*** provides actual measurement values of the number of hairs and hair diameter obtained by phototrichogram from haircut of the targeted area, the amount of change compared to before intake, and statistical analysis results. Significantly higher changes were observed in the TBPP group compared to the placebo group for hair diameter at 24 weeks after intake. Temporal comparisons of the actual measurement values of the number of hairs compared to before intake revealed significantly higher values at 12 weeks after intake than before intake exclusively in the TBPP group. Additionally, both the TBPP group and the placebo group exhibited a significantly larger number of hairs at 24 weeks after intake compared to before intake. The temporal comparison of actual measurement values of hair diameter compared to before intake showed significantly larger hair diameter at both 12 and 24 weeks after intake than before intake in both the TBPP group and the placebo group.

Table 4-b presents actual measurement values of the number of fallen hairs collected from hair wash, the amount of change compared to before intake, and statistical analysis results. According to the analysis results, no significant difference was observed in temporal comparisons for both the TBPP group and the placebo group, and no significant difference was confirmed in the intergroup comparison.

Additionally, ***Table 4-c*** illustrates the quantified values of carbonylated protein collected by tape stripping, the

amount of change compared to before intake, and statistical analysis results. No significant difference was found between the groups in the amount of change in quantified values from week 0. The temporal comparison of quantified values to before intake revealed significantly lower values at 24 weeks after intake only in the TBPP group.

2.4 Scalp moisturization

Table 5 presents actual measurement values of skin moisture content and skin water transpiration amount, the amount of change compared to before intake, and statistical analysis results. No significant difference was found between the groups in the amount of change from before intake. The temporal comparison of actual measurement values of skin moisture content compared to before intake showed significantly lower values at 12 and 24 weeks after intake in the TBPP group. Additionally, the temporal comparison of actual measurement values of skin water transpiration amount compared to before intake revealed significantly higher values at 12 weeks after intake in both the TBPP group and the placebo group.

2.5 Blood test

Table 2 presents actual measurement values of pentosidine and CML, which are plasma AGEs, the amount of change compared to before intake, and statistical analysis results. The amount of change in plasma pentosidine amount 24 weeks after intake was significantly lower in the TBPP group compared to the placebo group. In the temporal comparison of actual measurement values to before intake, plasma CML amount was significantly lower 12 weeks and 24 weeks after intake in the placebo group.

3. Adverse event

No adverse event with a cause-and-effect relationship with the test foods in this study occurred in the TBPP group and the placebo group.

Discussion

For Japanese individuals, age-associated changes in appearance pose risks for mental disorders due to body dissatisfaction[1-3]. Thinning hair, a notable age-associated change in appearance with established negative preconceptions in both the person with thinning hair and the people around them[5], contributes to a decline in mental QOL and influences socialization frequency[7,16]. Additionally, the use of thinning hair treatment drugs, such as minoxidil and finasteride, has been associated with improved QOL alongside addressing thinning hair concerns[17,18]. This suggests that addressing thinning hair concerns can positively impact QOL. Considering the importance of maintaining good mental and social states, aligning with the WHO's definition of health, this study focused on individuals concerned about thinning hair. It aimed to assess the effects of oral TBPP intake on the decline in QOL associated with thinning hair. The study employed a randomized, placebo-controlled, double-blinded, parallel group comparison design with healthy middle-aged and older Japanese men and women aged 40–64 as the subjects.

Table 2. Skindex29.

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Emotion	0 w	15	33.8 ± 23.0	15	24.3 ± 17.9	15	–	15	–
	12 w	15	23.7 ± 21.6 **	15	18.7 ± 16.8	15	-10.2 ± 15.2	15	-5.7 ± 14.8
	24 w	15	21.3 ± 22.5 ***	15	18.8 ± 15.9	15	-12.5 ± 10.6	15	-5.5 ± 11.0
Symptom	0 w	15	16.7 ± 11.3	15	12.6 ± 10.4	15	–	15	–
	12 w	15	10.5 ± 8.7 ***	15	12.9 ± 9.7	15	-6.2 ± 6.7	15	0.2 ± 11.1
	24 w	15	11.7 ± 9.9 **	15	13.3 ± 12.3	15	-5.0 ± 5.2	15	0.7 ± 12.2
Function	0 w	15	21.2 ± 19.8	15	14.9 ± 16.8	15	–	15	–
	12 w	15	15.3 ± 15.3 *	15	12.6 ± 15.2	15	-6.0 ± 10.2	15	-2.2 ± 9.5
	24 w	15	13.6 ± 16.2 **	15	12.6 ± 17.2	15	-7.6 ± 7.0	15	-2.2 ± 13.0
Total score	0 w	15	24.5 ± 17.1	15	17.6 ± 12.4	15	–	15	–
	12 w	15	17.0 ± 13.8 **	15	14.8 ± 12.8	15	-7.5 ± 9.8	15	-2.8 ± 8.8
	24 w	15	15.8 ± 15.5 ***	15	14.9 ± 13.7	15	-8.7 ± 6.0	15	-2.6 ± 9.5 †

*p < 0.05, **p < 0.01, ***p < 0.001, significantly different from 0 w by Dunnett-test. †p < 0.05, significantly different between groups by independent t-test. TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol; SD, standard deviation.

Table 3. VAS questionare.

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Q1. Hair loss	0 w	15	78.4 ± 23.1	15	78.7 ± 16.2	15	–	15	–
	12 w	15	59.3 ± 33.4	15	61.5 ± 25.2 *	15	-19.1 ± 28.7	15	-17.3 ± 24.8
	24 w	15	48.7 ± 25.2 **	15	56.5 ± 23.9 **	15	-29.7 ± 28.7	15	-22.3 ± 23.5
Q2. Visibility of parting and scalp	0 w	15	78.0 ± 24.4	15	81.3 ± 12.3	15	–	15	–
	12 w	15	67.9 ± 25.6	15	69.1 ± 19.0	15	-10.1 ± 23.8	15	-12.1 ± 19.9
	24 w	15	51.3 ± 25.7 **	15	64.1 ± 18.9 **	15	-26.7 ± 24.7	15	-17.1 ± 24.3
Q3. Low hair volume	0 w	15	71.5 ± 24.3	15	69.4 ± 22.6	15	–	15	–
	12 w	15	60.7 ± 32.8	15	60.5 ± 22.9	15	-10.8 ± 24.6	15	-8.9 ± 14.8
	24 w	15	47.7 ± 25.3 **	15	63.3 ± 23.1	15	-23.9 ± 26.4	15	-6.1 ± 13.9 †
Q4. Hair thinness	0 w	15	74.0 ± 22.6	15	61.1 ± 24.2	15	–	15	–
	12 w	15	62.8 ± 25.2	15	57.7 ± 21.1	15	-11.2 ± 17.8	15	-3.5 ± 10.9
	24 w	15	54.1 ± 23.5 **	15	53.3 ± 24.1	15	-19.9 ± 23.3	15	-7.9 ± 25.0
Q5. Lack of elasticity and firmness	0 w	15	73.3 ± 23.1	15	69.4 ± 25.1	15	–	15	–
	12 w	15	68.7 ± 21.3	15	67.5 ± 26.7	15	-4.6 ± 11.0	15	-1.9 ± 21.4
	24 w	15	56.6 ± 21.1 **	15	61.7 ± 25.5	15	-16.7 ± 18.7	15	-7.7 ± 17.2
Q6. Hair dryness	0 w	15	65.8 ± 26.9	15	75.1 ± 22.3	15	–	15	–
	12 w	15	67.9 ± 22.4	15	66.7 ± 27.3	15	2.1 ± 17.0	15	-8.4 ± 28.6
	24 w	15	55.6 ± 22.0	15	63.4 ± 24.8	15	-10.2 ± 19.0	15	-11.7 ± 20.1
Q7. Hair spread	0 w	15	65.2 ± 25.1	15	69.5 ± 21.8	15	–	15	–
	12 w	15	62.9 ± 24.4	15	63.8 ± 27.0	15	-2.3 ± 12.4	15	-5.7 ± 25.1
	24 w	15	54.3 ± 21.3 *	15	62.7 ± 17.8	15	-10.9 ± 16.4	15	-6.7 ± 14.4
Q8. Hair waviness	0 w	15	60.1 ± 31.6	15	74.3 ± 25.4	15	–	15	–
	12 w	15	54.1 ± 34.4	15	62.3 ± 32.1	15	-5.9 ± 24.9	15	-11.9 ± 21.2
	24 w	15	50.2 ± 26.8	15	65.1 ± 22.6	15	-9.9 ± 22.0	15	-9.1 ± 19.6
Q9. Lack of shine in hair	0 w	15	63.1 ± 25.5	15	72.6 ± 32.2	15	–	15	–
	12 w	15	60.5 ± 30.6	15	69.3 ± 28.1	15	-2.6 ± 17.9	15	-3.3 ± 13.3
	24 w	15	54.0 ± 25.8	15	62.7 ± 26.1 *	15	-9.1 ± 22.7	15	-9.9 ± 15.3
Q10. Poor hair root growth	0 w	15	62.2 ± 24.8	15	66.1 ± 26.9	15	–	15	–
	12 w	15	71.8 ± 27.9	15	67.5 ± 26.6	15	9.6 ± 28.3	15	1.3 ± 20.3
	24 w	15	49.3 ± 25.4	15	55.7 ± 23.5	15	-12.9 ± 16.4	15	-10.4 ± 28.4
Q11. Poor combing and difficulty in setting	0 w	15	56.8 ± 26.3	15	73.3 ± 17.5	15	–	15	–
	12 w	15	58.7 ± 27.4	15	54.5 ± 27.2 *	15	1.9 ± 27.3	15	-18.9 ± 30.2
	24 w	15	48.8 ± 22.6	15	61.4 ± 19.3	15	-8.0 ± 21.1	15	-11.9 ± 23.9
Q12. Scalp stickiness	0 w	15	53.8 ± 24.4	15	49.8 ± 23.7	15	–	15	–
	12 w	15	43.1 ± 22.8 *	15	50.1 ± 28.9	15	-10.7 ± 12.4	15	0.3 ± 33.9
	24 w	15	48.1 ± 24.3	15	49.5 ± 27.4	15	-5.7 ± 21.8	15	-0.3 ± 34.3
Q13. Scalp odor	0 w	15	56.5 ± 30.0	15	46.4 ± 29.3	15	–	15	–
	12 w	15	52.5 ± 32.7	15	46.7 ± 31.4	15	-4.0 ± 19.6	15	0.3 ± 29.5
	24 w	15	49.7 ± 25.1	15	43.6 ± 27.9	15	-6.9 ± 16.1	15	-2.8 ± 28.6
Q14. Scalp acne	0 w	15	22.3 ± 21.4	15	42.3 ± 29.0	15	–	15	–
	12 w	15	28.7 ± 23.7	15	32.7 ± 26.0	15	6.4 ± 17.0	15	-9.7 ± 26.9
	24 w	15	30.9 ± 24.7	15	34.7 ± 22.1	15	8.6 ± 22.1	15	-7.6 ± 23.4
Q15. Scalp drying	0 w	15	48.7 ± 25.9	15	61.5 ± 21.7	15	–	15	–
	12 w	15	48.8 ± 20.7	15	58.9 ± 25.1	15	0.1 ± 29.6	15	-2.6 ± 23.8
	24 w	15	41.4 ± 25.4	15	49.7 ± 24.8	15	-7.3 ± 18.7	15	-11.9 ± 24.6

Q16. Scalp hardness	0 w	15	50.2 ± 28.9	15	58.1 ± 25.1	15	–	15	–
	12 w	15	49.9 ± 30.5	15	57.9 ± 27.9	15	-0.3 ± 22.5	15	-0.3 ± 15.5
	24 w	15	51.7 ± 24.4	15	57.6 ± 20.7	15	1.5 ± 13.8	15	-0.5 ± 17.1
Q17. Dandruff	0 w	15	46.1 ± 29.3	15	43.3 ± 27.4	15	–	15	–
	12 w	15	51.9 ± 29.6	15	40.2 ± 28.4	15	5.8 ± 20.7	15	-3.1 ± 31.3
	24 w	15	46.0 ± 25.2	15	38.1 ± 27.6	15	-0.1 ± 14.2	15	-5.2 ± 30.1
Q18. Split ends and hair breakage	0 w	15	53.7 ± 31.6	15	53.9 ± 27.9	15	–	15	–
	12 w	15	44.3 ± 31.0	15	46.4 ± 27.2	15	-9.5 ± 26.3	15	-7.5 ± 25.3
	24 w	15	43.7 ± 23.0	15	49.6 ± 24.1	15	-10.0 ± 29.4	15	-4.3 ± 24.2
Q19. Gray hair	0 w	15	76.2 ± 25.7	15	83.2 ± 17.8	15	–	15	–
	12 w	15	81.4 ± 20.5	15	76.9 ± 21.9	15	5.2 ± 20.9	15	-6.3 ± 13.7
	24 w	15	78.8 ± 16.2	15	77.9 ± 14.0	15	2.6 ± 23.4	15	-5.3 ± 14.9

*p < 0.05, **p < 0.01, ***p < 0.001, significantly different from 0 w by Dunnett-test. †p < 0.05, significantly different between groups by independent t-test. TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol; VAS, visual analogue scale; SD, standard deviation.

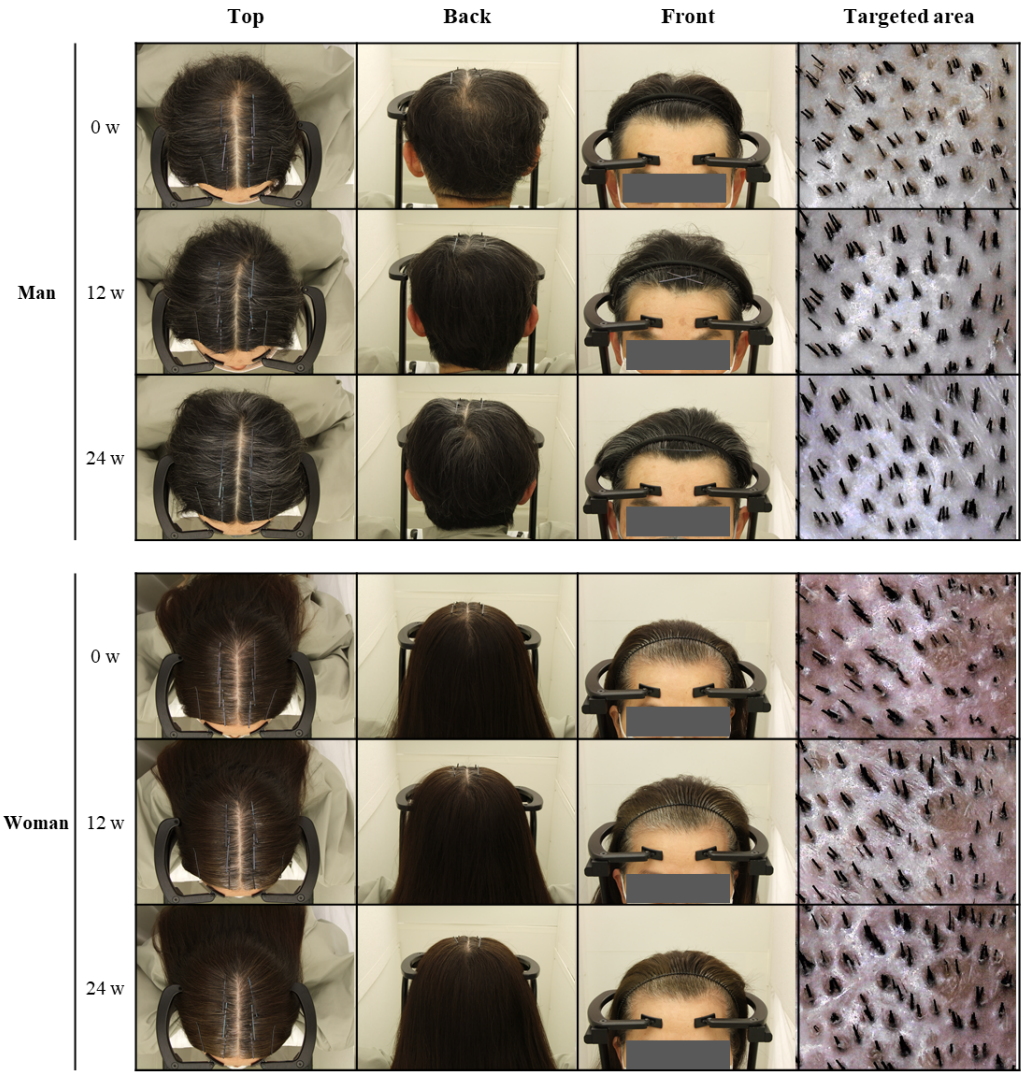

Fig. 2. **Visual changes in the group of subjects who took TBPP (as an example).**
TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol.

Table 4. Evaluation of hair and scalp.

a) Evaluation of hair in target area

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Number of hairs (/cm^2)	0 w	15	177.2 ± 35.9	15	189.8 ± 34.5	15	–	15	–
	12 w	15	182.6 ± 36.8 *	15	195.5 ± 39.4	15	5.4 ± 7.1	15	5.7 ± 8.0
	24 w	15	191.7 ± 39.0 ***	15	207.3 ± 39.4 ***	15	14.5 ± 10.3	15	17.5 ± 11.6
Hair Diameter (μm)	0 w	15	73.4 ± 13.0	15	79.8 ± 8.3	15	–	15	–
	12 w	15	78.8 ± 10.8 **	15	84.1 ± 8.3 ***	15	5.3 ± 6.4	15	4.3 ± 3.8
	24 w	15	83.9 ± 11.1 ***	15	86.0 ± 10.0 ***	15	10.5 ± 6.1	15	6.2 ± 4.8 †

b) Counting the number of fallen hairs

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Number of fallen Hairs	0 w	15	84.9 ± 93.6	15	60.3 ± 47.0	15	–	15	–
	12 w	15	60.3 ± 47.0	15	55.4 ± 35.8	15	-24.6 ± 102.6	15	-1.8 ± 36.3
	24 w	15	66.9 ± 51.1	15	64.4 ± 57.6	15	-18.0 ± 72.6	15	7.2 ± 63.9

c) Measurement of carbonylated protein

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Fluorescent Area (μm^2)	0 w	15	63,350 ± 32,316	15	48,560 ± 25,469	15	–	15	–
	12 w	15	46,836 ± 30,875	15	37,692 ± 21,445	15	-16,514 ± 39,828	15	-10,868 ± 26,321
	24 w	15	36,579 ± 19,176 *	15	34,726 ± 18,269	15	-26,771 ± 35,399	15	-13,835 ± 27,576

*p < 0.05, **p < 0.01, ***p < 0.001, significantly different from 0 w by Dunnett-test. †p < 0.05, significantly different between groups by independent t-test. TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol; VAS, visual analogue scale; SD, standard deviation.

Table 5. Amount of AGEs in blood plasma

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
pentosidine (ng/ml)	0 w	15	49.6 ± 10.6	15	47.3 ± 9.7	15	–	15	–
	12 w	15	49.1 ± 13.1	15	46.1 ± 10.1	15	-0.6 ± 8.9	15	-1.2 ± 8.4
	24 w	15	46.9 ± 11.7	15	49.5 ± 11.7	15	-2.7 ± 6.2	15	2.1 ± 5.4 †
CML (μg/ml)	0 w	15	4.6 ± 1.0	15	5.1 ± 0.5	15	–	15	–
	12 w	15	4.5 ± 0.9	15	4.8 ± 0.6 *	15	-0.1 ± 0.7	15	-0.4 ± 0.4
	24 w	15	4.3 ± 0.7	15	4.6 ± 0.6 **	15	-0.3 ± 0.7	15	-0.5 ± 0.7

*p < 0.05, **p < 0.01, ***p < 0.001, significantly different from 0 w by Dunnett-test. †p < 0.05, significantly different between groups by independent t-test. TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol; AGEs, advanced glycation endproducts; CML, N^{ε}-(carboxymethyl)lysine; SD, standard deviation.

Table 6. Moisture retention of scalp

		Measured value				Amount of change from 0 w			
		TBPP		Placebo		TBPP		Placebo	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Scalp moisture content	0 w	15	50.0 ± 11.1	15	46.3 ± 13.0	15	–	15	–
	12 w	15	37.6 ± 9.9 **	15	40.1 ± 10.2	15	-12.4 ± 11.0	15	-6.2 ± 9.1
	24 w	15	39.8 ± 15.8 *	15	43.1 ± 12.5	15	-10.2 ± 18.7	15	-3.2 ± 18.0
Water transpiration (g/m^2h)	0 w	15	17.5 ± 5.7	15	18.6 ± 7.6	15	–	15	–
	12 w	15	28.7 ± 17.2 **	15	28.1 ± 9.3 ***	15	11.2 ± 15.3	15	9.5 ± 7.6
	24 w	15	18.7 ± 8.4	15	16.5 ± 4.6	15	1.2 ± 8.1	15	-2.0 ± 6.8

*p < 0.05, **p < 0.01, ***p < 0.001, significantly different from 0 w by Dunnett-test. TBPP, *Trapa bispinosa* Roxb. pericarp polyphenol; SD, standard deviation.

In this study, changes in QOL associated with thinning hair served as the primary outcome, assessed through Skindex29 (Japanese version). The English version of Skindex29, developed by MM Chen *et al.* in 1997[19], is a questionnaire exploring the impact of skin condition on individuals' QOL. The Japanese version, introduced by Fukuhara S *et al.* in 2004[20], has been utilized in studies investigating the correlation between Japanese individuals' skin condition and QOL[21]. The Skindex29 questionnaire comprises 29 questions categorized into three subscales: seven questions for "symptoms," 10 questions for "emotions," and 12 questions for "functions." In the Japanese context, it is permissible to adapt the questions from "skin/skin condition" to "scalp/alopecia." This adaptation, known as the Hair Specific Skindex29 in English, is globally accepted, evidenced by its use in countries such as Korea and Spain, with several reports detailing surveys conducted using this questionnaire[7, 16]. In other words, using Skindex29 in the format that surveys "scalp/alopecia" is a method that has gained academic consensus for assessing changes in QOL associated with thinning hair. The results of this study (***Table 2***) demonstrate a significant improvement in the total scores of Skindex29 24 weeks after the intake of TBPP-containing food compared to the placebo group. Skindex29 questions gauge the extent to which subjects were concerned about thinning hair in the past week, and the total scores reflect the condition of hair and scalp within a specified timeframe. The findings indicate that TBPP intake relieves concerns about thinning hair within a certain period. Furthermore, in the temporal comparison to before intake, the subscale "emotions", "symptoms", "functions" and the total scores exhibited significant improvement exclusively in the TBPP group. The "functions" subscale assesses the impact of thinning hair on interpersonal relationships and social activities, suggesting that TBPP intake may mitigate the decline in social activities attributed to thinning hair in this study.

In examining secondary outcomes, the VAS questionnaire results (***Table 3***) reveal a significant improvement in concerns about "low hair volume" 24 weeks after TBPP intake compared to the placebo group. Notably, changes in the appearance of hair volume, particularly at hair whorls, appeared to improve in several cases among both men and women in the TBPP group (***Fig. 2*** top, back).

The phototrichogram results, employed to assess hair condition (***Table 4-a***), indicate no significant difference inthe change of the number of hairs. However, there was a significant increase in hair diameter 24 weeks after TBPP intake compared to the placebo group. In addition, visually, cases of improved hair thickness in the targeted area of haircut have been observed (***Fig. 2*** targeted area). For reference, a study on the efficacy of adenosine in Japanese women, utilizing a similar phototrichogram method, confirmed the suppression of a decrease in hair diameter, denoting an improvement in thinning hair among women[22]. In parallel, this study's outcomes, akin to those with adenosine, affirm that TBPP exhibits the potential to enhance hair thickness, marking an improvement in thinning hair. These results suggest that the increase in hair diameter observed in the TBPP group contributes to a positive perception of hair volume, thereby enhancing hair-related QOL.

The increase in hair diameter observed in individuals consuming TBPP may be attributed to an enhancement in the scalp environment, particularly the hair follicles. As the hair cycle repeats, and the anagen phase shortens, smaller hair follicle sizes may impede the formation of sufficiently thick hair shafts, leading to thinning hair[23]. Hair follicle formation and growth are crucial for maintaining hair thickness. The mechanisms suppressing hair follicle formation and growth include the accumulation of AGEs due to *in vivo* glycation reactions and oxidative stress. AGEs, particularly CML, have been associated with inhibiting hair follicle formation by suppressing the expression of the Sonic Hedgehog gene (SHH gene), a critical gene in this process[9]. AGEs accumulate with age[8], suggesting a potential link between age-related thinning hair and AGEs. In addition, active oxygen is also involved in the suppression of hair follicle formation, and it has been reported that lipid peroxides produced in hair follicles expedite the catagen phase in hair cycles[24, 25]. Thus, oxidative stress caused by UV light and ageing is also considered as a cause of thinning hair. TBPP, with its anti-glycation effect, demonstrated *in vitro* to suppress the generation of pentosidine, CML, and 3-deoxyglucosone, which are AGEs, and the effect of decomposing α-dicarbonyl bonds characteristic of AGEs[10]. Furthermore, TBPP, derived from the pericarp of Trapa, as *Trapa bispinosa* Roxb., possesses antioxidant effect[26], and it has been reported that, among the components of TBPP, polyphenols, such as gallic

acid and ellagic acid, possess strong antioxidant properties [27, 28]. In addition, the authors have previously confirmed that TBPP has antioxidant effects (data not published). Based on these previous reports, focusing on the anti-glycation effect and antioxidant effect of TBPP, indicators for assessing glycative stress and oxidative stress in the subjects were established in this study.

Glycative stress in the subjects was assessed by measuring the plasma AGE content. In addition, oxidative stress in the scalp of the subjects was assessed by measuring the carbonylated protein amount in the stratum corneum of their scalp. As carbonylated protein is produced when proteins and lipid decomposition products on the stratum corneum of the epidermis are combined by active oxygen, it functions as an indicator of skin oxidation level [25]. The study results showed that compared to the placebo group, the blood pentosidine amount significantly decreased 24 weeks after intake in the TBPP group. In a previously conducted placebo-controlled human trial, it has been reported that compared to a placebo, the blood pentosidine amount significantly decreased 12 weeks after intake in the group that consumed 100 mg of water chestnut pericarp extract of the same amount as in this study [12]. Compared to the previous human trial, the period from intake to manifestation of effects was longer in this study, but this is thought to be due to differences in study settings. Thus, the effect of TBPP-containing food in reducing the blood pentosidine amount, as in previous reports, was also demonstrated in this study. In addition, based on the results of carbonylated protein quantification, in the temporal comparison to before intake, the carbonylated protein amount significantly decreased only 24 weeks after intake in the TBPP group. In this study, lipid peroxides in the sebum and *in vivo* active oxygen amount were not measured, so the effects of oxidative stress on the hair follicles of the subjects could not be assessed. However, it has been reported that carbonylated proteins in the stratum corneum of the scalp are produced by the oxidative stress to the scalp [25], thus it was found that TBPP may reduce oxidative stress in the scalp of the subjects in this study. In future studies, by assessing oxidative stress markers and changes in hair/scalp caused by TBPP intake, it may be possible to more accurately confirm the association between the antioxidant effect and thinning hair improvement effect of TBPP. Based on the above results, it can be suggested that TBPP is a food material that contributes to alleviating the suppression of hair follicle formation through its anti-glycation and antioxidant effects.

Conclusion

The results of this study showed that the oral intake of TBPP-containing water chestnut pericarp extract at 100 mg per day for 24 weeks improves temporary concerns about thinning hair. In addition, it is thought that the improvement of hair diameter is involved in the improvement of concerns, and it was suggested that the maintenance of hair follicle formation attributed to the anti-glycation effect of TBPP is involved in the improvement of hair diameter. Furthermore, in this study, in the TBPP group and the placebo group, no adverse event with a cause-and-effect relationship with the test foods occurred. Based on these findings, TBPP is expected to be an effective food material that maintains and improves health by improving concerns about age-associated thinning hair, and contributing to maintaining mental QOL and desires for social activities.

Conflict of interest

This study was outsourced to SOUKEN Co., Ltd. by Hayashikane Sangyo Co., Ltd., and was conducted at SOUKEN Co., Ltd. and Shiba Palace Clinic. Test foods were provided by Hayashikane Sangyo Co., Ltd., and data collection and statistical analysis were performed by SOUKEN Co., Ltd. The authors Takuto Yamasaki, Shouko Takeshita, and Chihiro Tomiya are employees of Hayashikane Sangyo Co., Ltd., and Takashi Koikeda is the director of Shiba Palace Clinic. Shiba Palace Clinic and SOUKEN Co., Ltd. have not received any financial support from Hayashikane Sangyo Co., Ltd. other than for the cost of this study.

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