IMPROVEMENT IN IMMUNE FUNCTION BY SUPPLEMENT CONTAINED TAHEEBO NAPHTHOQUINONE AND TAHEEBO POLYPHENOL

— A RANDOMIZED PLACEBO-CONTROLLED STUDY —

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Abstract

Objectives: The objective of this research was to investigate the effectiveness of daily ingestion of a Taheebo tablet, which contains Taheebo naphthoquinone and Taheebo polyphenol on immunological vigor.
Methods: In this randomized, placebo-controlled, double-blind trial, 28 were subjected. The SIV score (combination of the number of T cells, naive T cells, $CD8^+CD28^+$ T cells, B cells, and NK cells, and ratios of CD4/CD8 T cell and naive/memory T cell) was measured as primary outcomes. The SEIV score (subjective reporting) was also tested as primary outcomes.
Results: 5 subjects were withdrawn due to personal reasons and the remaining 23 subjects completed the study. The SIV score of the subjects showed a significant difference between test and placebo samples after 12-week ingestion. Moreover, in the intergroup comparison of SEIV, 8 items out of 40 illustrated a significant difference after 12-week ingestion.
Conclusion: The present results suggest that daily ingestion of Taheebo tablets including Taheebo naphthoquinone and Taheebo polyphenol can improve some respects of immunological vigor.

Key words: Taheebo, immunological vigor, immune

1. INTRODUCTION

Taheebo (the botanical name: *Tabebuia avellanedae*) is a tree that belongs to the *Tabebuia* species of the bignonia family, and its place of origin is the Amazon river basin of Brazil. Taheebo has been used for more than 1.5 thousand years, and in the era of the ancient Inca Empire, the Indians made the Taheebo tea by brewing the inner bark of Taheebo and drank it for the cure of diseases and the health maintenance. In the ancient aboriginal language, Taheebo means "gift from God" or "light of God", and the Taheebo trees were said to be called as "the tree of gift from God".

The recent studies applying the animal testing reported that Taheebo owns various functionalities such as analgesic and anti-inflammatory effects[1)2)], antidepressant-like action[3)] and anti-obesity effect[4)]. A part of these effects are thought to have a close relationship with human immune function. In addition, there is a report that the ingestion of Taheebo inhibits the progress of cancer and the improvement of $CD4^+/CD8^+$ T-cell ratio[5)], and this illustrates the possibility that Taheebo contributes to the activation of the immune function. Immunity is a self-protection system of human body. Although it is an essential factor for maintaining our health, its level decreases due to inevitable matters of daily life such as aging, stress and irregular lifestyle habit. Therefore, the ingestion of foods with the capacity to activate the immune function is thought to be an effective measure against the weakening of immunological vigor.

Although Taheebo is thought to have a function of activating human immune function as illustrated above, there are few reports that discuss the functionality of Taheebo for the immune function of healthy Japanese men and women with daily tiredness and fatigue; such reports include the study utilizing animal testing, or the study focuses on the medical functions of certain ingredients contained in Taheebo. The objective of this research was to investigate the effectiveness of ingestion of a Taheebo tablet, which includes Taheebo naphthoquinone and Taheebo polyphenol, on immunological function of healthy human. As the measurement, we adopted the immune score measurement method by Hirokawa and Utsuyama[6)]. The test targets were healthy Japanese men and women who

feel tiredness and fatigue on a day-to-day basis, and the test method was a randomized, placebo-controlled, double-blind study. We also examined the safety of the test product.

2. METHOD

2.1. Trial Design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from TAHEEBO JAPAN CO., LTD. (Osaka) at two centers (OZ clinic, Tokyo, and JACTA, Tokyo). The study period was 12 weeks, from August 3rd to October 26th, 2015.

This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc.

2.2.1. Inclusion criteria

(1) Healthy Japanese men and women aged between 35 and 59 years with daily tiredness and fatigue;
(2) With relatively low self-examination of immunological vigor (SEIV) ≥ 90.

2.2.2. Exclusion criteria

(1) Previously suffered with malignant tumors, heat failure, or cardiac infarction;
(2) Under the care of a doctor for the treatment of chronic diseases such as atrial fibrillation, uneven heartbeat, rheumatism, diabetes, high blood pressure and diseases of the liver, kidney, cerebral system, circulatory system, and lipid metabolism;
(3) Taking medicines, including herbal medicines;
(4) Pregnant, nursing, or were likely to become pregnant during the trial;
(5) Judged to be unsuitable to participate in the test by the doctor responsible for the present study.

2.3. Randomization

Recruited subjects were 42 persons. Subjects who fulfilled eligibility criteria were 28 persons. The inclusion was judged by the principle investigator. All subjects were sequentially assigned based on a random number table to one of the masked products and randomized to group T (Test sample: 14) and group P (Placebo: 14). The allocation was pre-assigned on the basis of randomized numbers.

2.4. Description of test foods and blinding

The test food, "Taheebo NFD Premium" ("TNP") is a tablet containing Taheebo naphthoquinone and Taheebo polyphenol. The amount of a daily intake is 6 tablets (1 tablet weighs 350 mg, therefore 6 tablets weigh 2.1 g).

Placebo does not contain Taheebo naphthoquinone and Taheebo polyphenol. **Table 1** shows the nutritional content of the sample. Both tablets were indistinguishable in shape, color or taste. Tablets were managed by the identification symbol. All involved were blinded.

Table 1 Nutritional content of the sample per 100 g

Item	TNP	Placebo
Energy	404 kcal	321 kcal
Protein	1.3 g	0.8 g
Lipid	5.0 g	3.8 g
Ash	1.2 g	21.0 g
Carbohydrates	88.5 g	70.9 g
Salt equivalent	0.0605 mg	0.404 mg

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 6 tablets of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to maintain a daily record of lifestyle factors such as what they ate and pedometer measurement during the test period; and to send the diary to the study coordinator every Friday by mobile email.

2.5.2. Outcome

The objective of this study is to verify immunological vigor of ingesting food containing Taheebo naphthoquinone and Taheebo polyphenol. Immunological vigor was set as the primary outcome. SIV and SEIV were used to evaluate that. SIV is the scoring system that can combine seven immunological parameters, the number of T cells, naive T cells, $CD8^+CD28^+$ T cells, B cells, and NK cells, and ratios of CD4/CD8 T cell and naive/memory T cell, and express the immune status of individuals as a simple numeral. This system also shows T lymphocyte age. Due to this, we can compare the real age with the age evaluated from immunological vigor's point of view (**Fig. 1**).

The SIV scoring system is an index system, which utilizes the database of test results for every particular item, which are accumulated by Hirokawa and Utsuyama[6], and by using the database, its index is calculated from several subsets. Therefore it enabled us to evaluate the condition of a variety of immune cells in a comprehensive and multilateral manner, and it has already been introduced in past research as the new testing method of immune strength[7]. SEIV is a self-examination of immunological vigor with Likert scales. Blood biochemical and urine parameters were recorded to evaluate the safety of the test foods as the secondary outcome. These assessments were conducted upon entry into the study (pre-intervention) and after 12 weeks

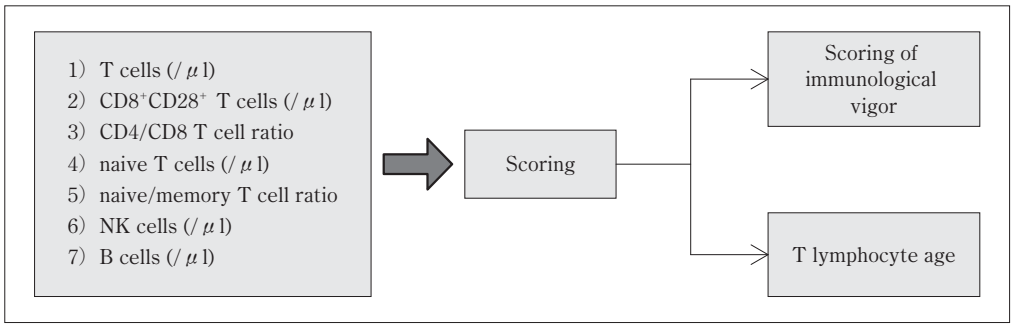

Fig. 1　SIV

Table 2　Schedule for the study.

Item \ Term	Screening	Pre Trial Test	Test period (12 w)
SEIV	●		●
Informed consent	●		
Selection and/or allocation	●		
immunological parameters		●	●
Biochemical analysis of blood		●	●
Urine analysis		●	●
Ingestion of test foods			↔
Log			↔

● : Implementation
↔ : Daily practice during the test period

(post-intervention).

To evaluate the safety of the test foods, adverse events were collected by means of a written questionnaire during the study.

According to the schedule shown in **Table 2**, we measured parameters on efficacy and safety.

2.6. Data Analysis

The full analysis set principal was adopted in the present study and no sample size was used.

Data is expressed as mean ± SD. For SIV, immunological parameters of blood, T lymphocyte age and biochemical analyses of blood and urine, changes from the baseline in the same group were assessed using the paired t-test. Student's t-test was used for intergroup comparisons of changes from the baseline. For SEIV, changes from the baseline in the same group were assessed using the Wilcoxon signed-rank test. The Mann-Whitney U test was used for intergroup comparisons of changes from the baseline. Student's t-test was used to compare subject's backgrounds between groups. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at the $<5\%$ level in the two-sided test.

3. RESULTS

3.1. Participant Demographics

From all of 42 applicants, 14 were eliminated according to the SEIV criteria (<90). 28 subjects were randomly assigned to an intervention group and made a start with ingestion. 5 were withdrawn due to personal reasons and remaining 23 subjects completed the study. These 5 subjects (all in the Placebo) were withdrawn for the following reasons: urgent work commitment (3 subjects); due to a cold (2 subjects).

Thus, data obtained from the 23 subjects was used for the analysis of efficacy (**Fig. 2**). There were no significant differences in the mean age, gender ratio or SEIV between groups (**Table 3**).

3.2. SIV

The results of the statistical analysis of SIV, other immunological parameters and T lymphocyte age are shown in **Table 4.** In TNP, the SIV score increased significantly after 12-week ingestion. Furthermore significant difference of ⊿ SIV was observed between two groups after 12-week ingestion. Also, the within-group analysis showed that only in the TNP the numbers of T cells and naive T cells increased significantly after 12-week ingestion. As for $CD8^+CD28^+$ T cells, B cells and T lymphocyte age, there were significant tendency in TNP after 12-week ingestion. In other items, any significant changes or differences were shown neither in TNP nor in Placebo.

3.3. SEIV

Table 5 shows the results of SEIV. After 12-week ingestion, 8 items out of 40 illustrated a significant changes in values of intergroup comparison: 1,

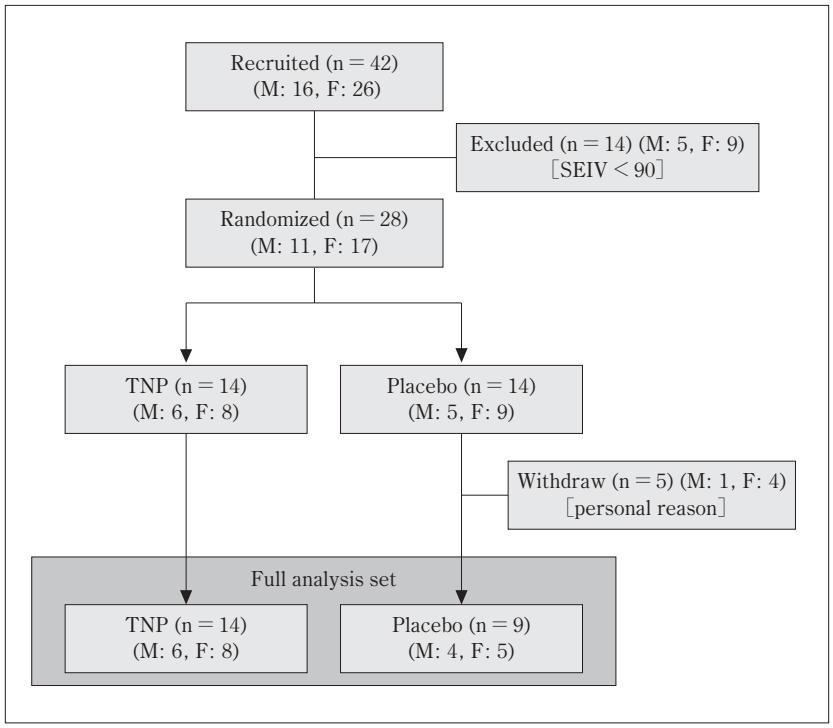

Fig. 2 Flow diagram of subject disposition

Table 3 Subject demographics

Item	Unit	TNP	Placebo
Subjects*	—	14	9
Male: female*	—	6:8	4:5
Age	years	45.0 ± 4.6	47.8 ± 6.7
SEIV	scores	115.1 ± 9.6	110.9 ± 10.0

* Number of subjects
mean ± SD

"enjoyment of meal"; 12, "wake up energetically without fatigue from the previous night"; 13, "feeling of fatigue is reduced by resting on weekends"; 17, "less frequency of stiff shoulder and lower back pain"; 18, "catching a cold"; 20, "less symptoms of stomatitis"; 28, "indifference to standing in the train"; 38, "want to be helpful to people and society".

3.4. Blood Test and Urine Test

Table 6 and 7 shows blood biochemical and urine parameters.

With respect to blood test, a significant difference was observed in Total Cholesterol, Potassium, Inorganic Phosphorus, Urea Nitrogen, Creatinine (Female) and Blood Sugar serum of TNP after 12-week ingestion. The same difference was found in urinary specific gravity after 12-week ingestion. In either case, since the difference was minor one, the investigator judged it as the range of physiological variation (or clinically safe).

3.5. Adverse Event

No adverse event was reported during this trial.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study to verify the effects of the Taheebo tablet which contains Taheebo naphthoquinone and Taheebo polyphenol, on immunological vigor of human. As the primary outcome, the study showed significant changes in values of several items of SIV (Scoring of Immunological Vigor), a comprehensive index of immune function, and SEIV (Self-examination of immunological vigor), a subjective assessment, after 12-week ingestion period. At the same time, as the secondary outcome the observation of clinical findings such as medical interview, blood and urine test revealed no abnormal change had been triggered by the ingestion of test product.

In this study, we evaluated the effect of the test product containing Taheebo naphthoquinone and Taheebo polyphenol for the immunological vigor, by comparing scores of SIV and SEIV between the test group (TNP) and the placebo group (Placebo).

Table 4　SIV, other immunological parameters and T lymphocyte age

Item	Unit	Time points	Scores/Values		P-value
			TNP (n = 14)	Placebo (n = 14)	
SIV	scores	Baseline	15.5 ± 1.8	16.3 ± 2.4	
		Week 12	16.8 ± 1.6 **	16.6 ± 2.1	
		Change	1.3 ± 1.1	0.2 ± 1.0	0.025 #
T cells	numbers/μl	Baseline	1226.1 ± 370.8	1291.6 ± 370.4	
		Week 12	1406.9 ± 353.4 *	1451.3 ± 499.5	
		Change	180.8 ± 232.1	159.8 ± 355.6	0.865
Naive T cells	numbers/μl	Baseline	276.8 ± 173.3	363.6 ± 121.7	
		Week 12	350.0 ± 179.4 *	411.1 ± 176.6	
		Change	73.2 ± 112.3	47.6 ± 123.8	0.612
$CD8^+CD28^+$ T cells	numbers/μl	Baseline	195.4 ± 68.4	214.9 ± 147.4	
		Week 12	226.5 ± 94.2 †	202.3 ± 91.7	
		Change	31.1 ± 54.3	− 12.6 ± 108.4	0.286
B cells	numbers/μl	Baseline	236.4 ± 105.1	264.0 ± 87.8	
		Week 12	279.9 ± 96.2 †	348.9 ± 129.7	
		Change	43.6 ± 85.8	84.9 ± 139.4	0.387
NK cells	numbers/μl	Baseline	249.9 ± 67.8	285.2 ± 183.8	
		Week 12	241.6 ± 110.8	282.2 ± 152.4	
		Change	− 8.4 ± 87.3	− 3.0 ± 111.2	0.898
CD4/CD8 T cell ratio	ratio	Baseline	2.77 ± 1.50	5.05 ± 5.03	
		Week 12	3.17 ± 2.31	3.16 ± 1.11	
		Change	0.41 ± 1.84	− 1.89 ± 5.31	0.208
Naive/memory T cell ratio	ratio	Baseline	0.52 ± 0.26	0.80 ± 0.43	
		Week 12	0.63 ± 0.33	0.76 ± 0.29	
		Change	0.11 ± 0.24	− 0.04 ± 0.33	0.220
T lymphocyte age	years	Baseline	52.3 ± 6.7	52.6 ± 12.9	
		Week 12	49.9 ± 8.4 †	54.1 ± 10.0	
		Change	− 2.4 ± 4.3	1.4 ± 6.8	0.176

Scores and values are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.

# $p < 0.05$ between-group differences in change from baseline.

SIV score is a comprehensive index of immune function and it means that the higher the score, the more the immune function is comprehensively good. The result showed that in TNP the score of SIV increased after 12-week ingestion, and this suggests there was a significant improvement in the immune function of TNP. As for Placebo, on the other hand, there was no significant change in the score, and therefore it indicates there was no improvement in the immune function. T lymphocyte age is an index which matches the immune function to the actual age, and it means the lower the year is, the younger the immune function, is equipped. In this study, although we could not statistically find any significant difference, we observed that T lymphocyte age is younger in TNP (i.e. improvement tendency) whereas that of Placebo did not change. In addition, the number of T cells and their subsets (such as Naive T cells, $CD8^+CD28^+$ T cells and B cells) increased significantly or tended to increase in TNP. This study used healthy Japanese men and women with daily tiredness and fatigue as its test subject, therefore it is likely that the immune function of the subject among Placebo deteriorated during the test period. However, this result supports the possibility that the ingestion of TNP activated the immune function of subjects, especially among T-type cells. At the same time, in SEIV (an index for the awareness of immune system), significant improvement was observed in the items that may be related to fatigue, stress or QOL. Since it is generally said that the fatigue or stress have a major effect on the human immune function[8], this result of SEIV should have a relationship with the improvement of SIV score as discussed above.

Table 5 SEIV ①

	Item	Time points	Scores	
			TNP (n = 14)	Placebo (n = 9)
1	Enjoyment of meal	Baseline	1.6 ± 0.5	1.3 ± 0.5
		Week 12	1.1 ± 0.4 *	2.1 ± 0.8 *
		Change	− 0.4 ± 0.5	0.8 ± 0.7 ##
2	Meal is often 3 times per day.	Baseline	2.0 ± 1.4	2.0 ± 1.5
		Week 12	1.9 ± 1.3	1.2 ± 0.4
		Change	− 0.1 ± 0.7	− 0.8 ± 1.6
3	Nutritional balance of the meal is a considerable point.	Baseline	2.6 ± 1.2	2.3 ± 1.3
		Week 12	2.3 ± 1.1	1.9 ± 0.6
		Change	− 0.3 ± 1.0	− 0.4 ± 1.1
4	Eat more meat than fish.	Baseline	3.1 ± 1.2	3.1 ± 0.9
		Week 12	3.1 ± 1.0	2.9 ± 0.9
		Change	0.0 ± 1.3	− 0.2 ± 0.8
5	Meal is low in salt.	Baseline	2.9 ± 1.4	2.8 ± 1.4
		Week 12	2.5 ± 0.9	2.7 ± 0.9
		Change	− 0.4 ± 1.4	− 0.1 ± 1.2
6	Eat moderately in quantity.	Baseline	2.9 ± 1.1	3.1 ± 1.5
		Week 12	2.6 ± 0.9	2.7 ± 1.4
		Change	− 0.3 ± 1.2	− 0.4 ± 1.0
7	Eat more vegetables.	Baseline	2.6 ± 1.5	1.9 ± 0.9
		Week 12	1.9 ± 0.7	1.7 ± 0.9
		Change	− 0.7 ± 1.5	− 0.2 ± 1.0
8	Limit intake of animal fat.	Baseline	2.7 ± 1.2	2.9 ± 1.3
		Week 12	2.5 ± 1.0	1.9 ± 0.8 *
		Change	− 0.2 ± 1.1	− 1.0 ± 1.0
9	No food before sleep	Baseline	3.1 ± 1.0	2.8 ± 1.3
		Week 12	3.0 ± 1.0	2.0 ± 0.7
		Change	− 0.1 ± 1.0	− 0.8 ± 1.4
10	No alcohol	Baseline	2.6 ± 1.7	3.0 ± 1.8
		Week 12	2.6 ± 1.5	3.0 ± 1.7
		Change	0.0 ± 1.2	0.0 ± 1.8
11	Amount of drinking less than the standard quantity i.e. beer < 500 ml, wine < 180 ml, sake < 180 ml, or whiskey < 50 ml	Baseline	1.3 ± 1.1	1.9 ± 1.8
		Week 12	1.6 ± 0.7	2.4 ± 1.9
		Change	0.4 ± 0.9	0.6 ± 1.3
12	Wake up energetically without fatigue from the previous night.	Baseline	3.5 ± 1.5	3.2 ± 1.3
		Week 12	2.3 ± 0.8 **	3.8 ± 1.0
		Change	− 1.2 ± 1.1	0.6 ± 0.9 ##
13	Feeling of fatigue is reduced resting on weekends.	Baseline	4.3 ± 0.8	2.7 ± 1.1
		Week 12	2.4 ± 1.0 **	3.6 ± 1.0
		Change	− 1.9 ± 1.2	0.9 ± 0.6 ##
14	Going to bed before 12 pm	Baseline	3.6 ± 1.5	3.1 ± 1.4
		Week 12	3.3 ± 1.4	2.2 ± 1.0 †
		Change	− 0.4 ± 0.9	− 0.9 ± 1.2
15	Getting sufficient quantity of sleep.	Baseline	3.6 ± 0.9	2.8 ± 1.5
		Week 12	2.9 ± 0.9 *	2.1 ± 1.1 †
		Change	− 0.7 ± 1.1	− 0.7 ± 0.9
16	No smoking	Baseline	1.3 ± 1.1	2.3 ± 2.0
		Week 12	1.3 ± 1.1	2.2 ± 1.9
		Change	0.0 ± 0.0	− 0.1 ± 2.0
17	Less frequency of stiff shoulder and lower back pain	Baseline	3.9 ± 1.3	3.6 ± 1.3
		Week 12	2.9 ± 1.5 *	4.0 ± 0.9
		Change	− 1.0 ± 1.2	0.4 ± 0.7 #
18	Catching a cold.	Baseline	2.6 ± 1.1	3.3 ± 1.5
		Week 12	1.7 ± 1.1 *	3.4 ± 1.4
		Change	− 0.9 ± 1.0	0.1 ± 0.3 #
19	Less symptoms of gastro-intestinal problem	Baseline	2.2 ± 1.1	3.0 ± 1.5
		Week 12	1.9 ± 1.0	3.1 ± 1.6
		Change	− 0.4 ± 0.9	0.1 ± 0.3
20	Less symptoms of stomatitis	Baseline	2.2 ± 1.1	2.6 ± 1.1
		Week 12	1.6 ± 0.6 *	3.0 ± 1.2
		Change	− 0.6 ± 0.7	0.4 ± 1.0 #
21	No record of the following diseases: diabetes mellitus, liver disease, kidney disease, hypertension, hyperlipidemia, cancer, heart disease, autoimmune disease, depression.	Baseline	1.3 ± 1.1	1.0 ± 0.0
		Week 12	1.5 ± 1.2	1.7 ± 1.4
		Change	0.2 ± 1.7	0.7 ± 1.4

Scores are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.

# $p < 0.05$, ## $p < 0.01$ between-group differences in change from baseline.

Table 5　SEIV ②

Item		Time points	Scores	
			TNP (n = 14)	Placebo (n = 9)
22	BMI ＜ 25　BMI = BW{Kg}/(BL{m}x BL{m})	Baseline	2.4 ± 2.0	2.3 ± 2.0
		Week 12	2.4 ± 1.6	2.0 ± 1.0
		Change	0.0 ± 1.7	− 0.3 ± 1.4
23	Normal bowel movement	Baseline	2.2 ± 1.1	2.9 ± 1.4
		Week 12	2.1 ± 1.1	3.2 ± 1.2
		Change	− 0.1 ± 0.9	0.3 ± 0.5
24	Try to take the stairs.	Baseline	3.5 ± 1.1	3.7 ± 1.2
		Week 12	2.7 ± 1.1 †	3.9 ± 0.9
		Change	− 0.8 ± 1.4	0.2 ± 0.7 ‡
25	Try to walk instead of vehicles.	Baseline	2.5 ± 1.3	3.0 ± 1.4
		Week 12	1.7 ± 0.8 †	3.1 ± 1.3
		Change	− 0.8 ± 1.5	0.1 ± 0.9
26	Fast pace walk	Baseline	2.9 ± 1.4	3.0 ± 1.3
		Week 12	2.3 ± 1.0 †	2.1 ± 1.3 †
		Change	− 0.6 ± 1.2	− 0.9 ± 1.4
27	Indifference to walking	Baseline	2.8 ± 1.3	2.9 ± 1.3
		Week 12	1.8 ± 0.6 *	2.4 ± 1.1
		Change	− 1.0 ± 1.2	− 0.4 ± 1.0
28	Indifference to standing in the train	Baseline	3.4 ± 1.6	3.7 ± 1.0
		Week 12	2.3 ± 1.1 *	3.6 ± 0.9
		Change	− 1.1 ± 1.4	− 0.1 ± 0.3 #
29	Using pedometer	Baseline	3.6 ± 2.0	4.1 ± 1.8
		Week 12	1.4 ± 1.1 *	1.2 ± 0.4 *
		Change	− 2.2 ± 2.0	− 2.9 ± 1.7
30	Indifference to running when needed	Baseline	3.5 ± 1.1	3.3 ± 1.2
		Week 12	2.4 ± 1.2 *	1.7 ± 0.9 *
		Change	− 1.1 ± 1.5	− 1.7 ± 1.1
31	Have a hobby containing physical exercise.	Baseline	3.9 ± 1.6	4.0 ± 1.6
		Week 12	3.4 ± 1.5	3.0 ± 1.0 †
		Change	− 0.6 ± 1.6	− 1.0 ± 1.6
32	Less distress / worry than usual	Baseline	3.6 ± 1.2	3.2 ± 0.7
		Week 12	3.0 ± 1.1 †	3.0 ± 0.9
		Change	− 0.6 ± 1.1	− 0.2 ± 0.7
33	Ability to forget problems / worries and move forward	Baseline	3.7 ± 1.3	2.9 ± 1.5
		Week 12	2.9 ± 1.0 *	1.8 ± 0.8 *
		Change	− 0.9 ± 1.3	− 1.1 ± 1.2
34	Enjoy talking with family and friends.	Baseline	2.4 ± 1.1	1.7 ± 1.1
		Week 12	2.1 ± 1.1	1.7 ± 0.7
		Change	− 0.3 ± 0.6	0.0 ± 1.0
35	Have a friend listen to your negative feelings / thoughts.	Baseline	3.4 ± 1.4	2.7 ± 1.6
		Week 12	2.5 ± 1.0 †	1.8 ± 0.8 †
		Change	− 0.9 ± 1.5	− 0.9 ± 1.3
36	Satisfied with your daily job.	Baseline	3.4 ± 1.3	3.4 ± 1.0
		Week 12	2.8 ± 1.1 †	2.0 ± 0.9 *
		Change	− 0.6 ± 1.2	− 1.4 ± 0.9
37	Have optimism for the future.	Baseline	3.6 ± 1.3	2.7 ± 1.2
		Week 12	2.6 ± 1.3 *	2.0 ± 0.7
		Change	− 1.0 ± 1.3	− 0.7 ± 1.2
38	Want to be helpful to people and society.	Baseline	3.4 ± 1.2	2.6 ± 1.1
		Week 12	2.6 ± 1.1 †	2.9 ± 1.1
		Change	− 0.7 ± 1.2	0.3 ± 0.5 #
39	Have a hobby, not related with your job.	Baseline	2.4 ± 1.3	2.3 ± 1.8
		Week 12	1.8 ± 1.1 †	1.2 ± 0.4
		Change	− 0.6 ± 1.2	− 1.1 ± 1.8
40	Daily activities except job decided by yourself.	Baseline	2.7 ± 1.4	1.9 ± 1.3
		Week 12	2.7 ± 1.3	1.9 ± 0.6
		Change	0.0 ± 1.4	0.0 ± 1.4
−	Total score of SEIV	Baseline	115.1 ± 9.6	110.9 ± 10.0
		Week 12	92.0 ± 18.5 **	98.0 ± 9.3 **
		Change	− 23.1 ± 14.7	− 12.9 ± 7.7 ‡

Scores are expressed as the mean ± SD.

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.

‡ $p < 0.1$, # $p < 0.05$ between-group differences in change from baseline.

Table 6 Changes in biochemical blood test

Item	Unit	Std. Value	Gender	Time points	Values	
					TNP (n = 14)	Placebo (n = 14)
Total Bilirubin	mg/dL	0.2–1.2	M/F	Baseline	0.66 ± 0.24	0.72 ± 0.31
				Week 12	0.64 ± 0.27	0.66 ± 0.38
				Change	− 0.02 ± 0.33	− 0.06 ± 0.25
Total Protein	g/dL	6.3–8.3	M/F	Baseline	7.6 ± 0.4	7.6 ± 0.4
				Week 12	7.6 ± 0.4	7.7 ± 0.4
				Change	− 0.0 ± 0.2	0.1 ± 0.3
Albumen	g/dL	3.8–5.3	M/F	Baseline	4.7 ± 0.2	4.6 ± 0.3
				Week 12	4.6 ± 0.3	4.6 ± 0.2
				Change	− 0.1 ± 0.2	0.0 ± 0.2
AST (GOT)	U/L	8–38	M/F	Baseline	21.4 ± 7.0	20.3 ± 5.5
				Week 12	22.5 ± 6.1	20.0 ± 5.0
				Change	1.1 ± 4.3	− 0.3 ± 5.0
ALT (GPT)	U/L	4–43	M/F	Baseline	20.1 ± 9.3	21.2 ± 15.3
				Week 12	21.7 ± 11.8	20.4 ± 10.8
				Change	1.6 ± 6.1	− 0.8 ± 8.2
ALP	U/L	110–354	M/F	Baseline	182.6 ± 46.6	198.2 ± 69.4
				Week 12	193.6 ± 54.3 †	211.6 ± 89.1
				Change	10.9 ± 23.0	13.4 ± 37.8
LD (LDH)	U/L	121–245	M/F	Baseline	184.2 ± 33.9	183.8 ± 32.7
				Week 12	179.8 ± 31.0	175.1 ± 32.7
				Change	− 4.4 ± 17.5	− 8.6 ± 20.4
γ-GT (γ GTP)	U/L	86 and under	M	Baseline	39.0 ± 13.9	41.4 ± 26.1
				Week 12	37.5 ± 15.1	30.0 ± 13.0
				Change	− 1.5 ± 6.4	− 11.4 ± 18.1
		48 and under	F	Baseline	17.1 ± 6.2	16.9 ± 6.5
				Week 12	16.9 ± 7.4	17.7 ± 8.7
				Change	− 0.3 ± 2.3	0.8 ± 3.6
CK (CPK)	mg/dL	38–196	M	Baseline	167.5 ± 112.7	154.6 ± 62.0
				Week 12	154.3 ± 78.8	116.6 ± 69.5
				Change	− 13.2 ± 119.1	− 38.0 ± 65.5
		30–172	F	Baseline	98.3 ± 72.9	84.6 ± 26.9
				Week 12	90.4 ± 39.4	87.3 ± 38.6
				Change	− 7.9 ± 41.7	2.8 ± 14.8
Total Cholesterol	mg/dL	130–219	M/F	Baseline	195.6 ± 25.9	222.7 ± 38.8
				Week 12	211.6 ± 22.1 **	235.7 ± 42.4
				Change	15.9 ± 19.4	13.0 ± 27.8
Neutral Fat (TG)	mg/dL	30–149	M/F	Baseline	127.9 ± 42.2	170.9 ± 91.4
				Week 12	160.5 ± 114.2	171.4 ± 125.9
				Change	32.6 ± 87.4	0.5 ± 107.5
Sodium	mEq/L	135–150	M/F	Baseline	144.5 ± 2.4	145.2 ± 1.6
				Week 12	144.1 ± 2.0	144.2 ± 1.6 †
				Change	− 0.4 ± 1.9	− 1.0 ± 2.0
Chloride	mEq/L	98–110	M/F	Baseline	105.2 ± 1.7	106.1 ± 2.6
				Week 12	104.5 ± 1.9	104.7 ± 2.3 *
				Change	− 0.7 ± 2.3	− 1.4 ± 2.2
Potassium	mEq/L	3.5–5.3	M/F	Baseline	4.1 ± 0.3	3.8 ± 0.2
				Week 12	4.3 ± 0.4 *	4.1 ± 0.2 **
				Change	0.2 ± 0.3	0.3 ± 0.2
Calcium	mg/dL	8.4–10.2	M/F	Baseline	9.9 ± 0.2	9.8 ± 0.4
				Week 12	9.8 ± 0.3	9.9 ± 0.4
				Change	− 0.1 ± 0.3	0.1 ± 0.3
Inorganic Phosphorus	mg/dL	2.5–4.5	M/F	Baseline	3.7 ± 0.5	3.5 ± 0.4
				Week 12	3.4 ± 0.7 *	3.3 ± 0.6
				Change	− 0.3 ± 0.4	− 0.2 ± 0.6
Urea Nitrogen	mg/dL	8.0–20.0	M/F	Baseline	16.8 ± 5.8	15.4 ± 4.7
				Week 12	14.1 ± 4.2 *	13.8 ± 3.4
				Change	− 2.8 ± 4.8	− 1.5 ± 3.4
Creatinine	mg/dL	0.61–1.04	M	Baseline	0.91 ± 0.13	0.91 ± 0.12
				Week 12	0.87 ± 0.13	0.90 ± 0.07
				Change	− 0.04 ± 0.08	− 0.01 ± 0.09
		0.47–0.79	F	Baseline	0.66 ± 0.06	0.67 ± 0.08
				Week 12	0.63 ± 0.06 *	0.66 ± 0.10
				Change	− 0.03 ± 0.02	− 0.01 ± 0.05
Blood Sugar (Serum)	mg/dL	60–109	M/F	Baseline	68.6 ± 8.8	69.7 ± 21.4
				Week 12	74.7 ± 8.5 *	74.1 ± 13.4
				Change	6.1 ± 6.9	4.4 ± 22.2

Values are expressed as the mean ± SD.
† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ against baseline.

Table 7　Transition of Urinalysis

Item	Unit	Std. Value	Gender	Time points	Values	
					TNP (n = 14)	Placebo (n = 14)
Specific Gravity	mg/dL	1.010–1.025	M/F	Baseline	1.026 ± 0.007	1.022 ± 0.006
				Week 12	1.016 ± 0.007 **	1.019 ± 0.007
				Change	− 0.010 ± 0.007	− 0.004 ± 0.009 #
pH	g/dL	4.5–8.0	M/F	Baseline	5.9 ± 0.9	5.9 ± 0.8
				Week 12	6.0 ± 0.7	6.5 ± 1.0 *
				Change	0.0 ± 1.1	0.6 ± 1.0

Values are expressed as the mean ± SD.

* $p < 0.05$, ** $p < 0.01$ against baseline.

# $p < 0.05$ between-group differences in change from baseline.

The Taheebo tablet used for this study consists of Taheebo bark extract and Taheebo-derived ingredients such as Taheebo naphthoquinone and Taheebo polyphenol. Taheebo polyphenol contains acteoside, isoacteoside, echinacoside and salidroside[9], all of which are known to have strong anti-oxidative property[10]. In addition, there are several study reports that emphasize the function of Taheebo polyphenol such as its anti-allergic effect which is achieved by its affection to immunoglobulin E[11], or its enhancement of the cellular/humoral immune response by affection of salidroside to T-cells, which is evaluated by the experiment using old rats[12]. Taheebo naphthoquinone, on the other hand, is a kind of quinone (plant pigment), and especially in Taheebo "2-(1-hydroxyethyl)-5-hydroxy-naphtho [2,3-b] furan-4,9-dione (NQ801)" is included[13]. Naphthoquinone reportedly owns antitumor effect[14], anti-inflammatory effect and anti-oxidative effect[15], and as an indirect action, immunostimulating effect[14)16)]. In addition, it was confirmed that Taheebo contains fat, tannin and minerals[17], and these ingredients are thought to maintain bodily functions. Furthermore, there is a report that the ingestion of Taheebo lowers the anti-oxidative effect of macrophage and enhances the cell-mediated immunity of lymphocyte[18]. All of the above findings support the hypothesis that the immunity was activated by the ingestion of the test product (TNP) containing Taheebo naphthoquinone and Taheebo polyphenol.

In this study we examined the safety of the test product by blood and urine test. In the blood test, significant difference was observed in total cholesterol, potassium, inorganic phosphorus, urea nitrogen, creatinine (female) and blood sugar serum. On the other hand, in the urine test we observed significant difference in the change of urine specific gravity. In either case, since the difference was minor one, the investigator judged it as the range of physiological variation (or clinically safe). During the study, 5 subjects were excluded during the test due to personal reasons such as work or a cold, and they were not related to the adverse event caused by the test product. Therefore, based upon the medical interview, blood and urine test, we observed no harmful influence against biochemical and/or physiological matters of the subjects. These results indicated the safety of the ingestion of the test product (TNP) for the 12-week test period.

It is generally said that the immunity weakens with advancing age. As Japanese society faces aging of population, it is increasingly important to grow old while maintaining good health. Non-prescription medicines such as cold medicine are all symptomatic treatment and therefore have a function of alleviating symptoms, but do not boost disease resistance in a fundamental way. Increasing natural immunity of human and boosting disease resistance by ingesting "snackable foods" like tablet-type supplements may contribute to increase the population of physical wellness, and eventually contribute to increase QOL and reduce the overall medical costs.

This study examined an immunostimulating effect of human by the ingestion of the test product (TNP) for 12 weeks. However, the immune function of human tends to be affected by their environment and/or stress. It may also be affected depending upon the ingestion period. Therefore, 12-week test period should be not enough for obtaining solid evidence and we need to continue further investigation with different test arrangement, such as the longer test period or the changing of season and environment. In addition, since there is a report concluding that the extracts of Taheebo is more effective than the individual ingredients from Taheebo[16], the functional mechanism of Taheebo ingredients inside the body is still unknown, and this point should also be further scrutinized in the future.

In conclusion, we found out that the ingestion of the Taheebo tablet (TNP) containing Taheebo naphthoquinone and Taheebo polyphenol for 12 weeks contributed to the immunostimulating effect. In addition, no safety-related matter occurred during 12-week test period.

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