別紙様式（Ｖ）－２【添付ファイル用】

特定保健用食品とは異なる臨床試験（ヒト試験）方法とした合理的理由に関する説明資料

１．製品概要

商品名	冷えケア
機能性関与成分名	ヒハツ由来ピペリン類
表示しようとする機能性	本品にはヒハツ由来ピペリン類が含まれるので、寒さにより低下した血流（末梢血流）を改善し、体温（末梢体温）を保つ機能があります。本品は冬期や冷房による末梢の冷えが気になる方に適しています

２．特定保健用食品とは異なる臨床試験（ヒト試験）方法（科学的合理性が担保されたものに限る。）とした合理的理由

特定保健用食品の保健の用途には、当該製品で表示しようとする機能性が含まれていない。そのため、「特定保健用食品の届出等に関するガイドライン」に記載されている「特定保健用食品の表示許可等について」の別添 2「特定保健用食品申請に係る申請書作成上の留意事項」に示された特定保健用食品の試験方法に準拠することはできなかった。

しかし、下記の理由により、当該食品の臨床試験方法には科学的合理性が担保されていると判断している。

① 試験方法(評価系)

冷えを客観的に測定する方法として、冷水負荷後、サーモグラフィを用いた皮膚表面温度の測定、回復率の算出、非接触型レーザー血流計を用いた末梢血流動態の測定を行った。皮膚表面温度は冷えを自覚していても実際の皮膚表面温度は高い場合があり、環境や体調に影響を受けやすい。しかし、冷水などの負荷を加えることで皮膚表面温度の不安定さを少なくし、評価することができると報告されている[1)]。また、冷えを自覚する者では血流量が低く、冷水負荷後の皮膚表面温度の回復率が低いことが確認されている[2)]。本試験方法を用いた報告は多く、当該分野において学術的にコンセンサスの得られている評価項目であると判断し、本臨床試験にて採用した。

②対象者

当該製品での試験では、冷えを自覚する 20 歳以上 65 歳未満の健康な日本人の女性を対象に試験を行ったが、病者等を含まない。また、当該製品の試験では、排卵による体温変動の影響を除外するため検査日が排卵予定日前後 2 日に

該当する者は対象としなかった[3)]。

一般的に冷えを訴えるのは男性より女性の方が多いとされていることから、本試験では女性を対象に試験を行った[4)]。また、機能性関与成分の摂取で、寒さにより低下した末梢血流の改善作用についても性差の影響は報告されておらず、当該製品の試験結果は末梢の冷えが気になる方に用いることができる。

③試験期間

当該製品の機能性関与成分であるヒハツ由来ピペリン類を用いた臨床試験では、単回摂取により末梢血流量及び皮膚表面温度の増加を確認した。また、機能性関与成分がNO産生に関与していると考えられることから、摂取直後の機能を評価することは適切であるといえる。したがって、単回摂取での機能性をクロスオーバー試験で評価した。

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別紙様式（Ｖ）－３【添付ファイル用】

表示しようとする機能性の科学的根拠に関する補足説明資料

１．製品概要

商品名	冷えケア
機能性関与成分名	ヒハツ由来ピペリン類
表示しようとする機能性	本品にはヒハツ由来ピペリン類が含まれるので、寒さにより低下した血流（末梢血流）を改善し、体温（末梢体温）を保つ機能があります。本品は冬期や冷房による末梢の冷えが気になる方に適しています。

２．補足説明

【科学的根拠に用いた試験食品と当該製品の同一性について】

科学的根拠資料に記載される試験食品と当該製品は、同一原料及び同一製法を用いて製造した製剤である。

試験食品には、機能性関与成分であるヒハツ由来ピペリン類 120μｇが含まれており、当該製品の同一性は担保されている。

Effects of Long Pepper Extract on Peripheral Coldness in Japanese Healthy Women

—A Randomized Double-blind Placebo-controlled Crossover Trial—

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Hiroshige Kuwahara[1)] Shuji Nakata[2)]

ABSTRACT

Objective Peripheral coldness can sometimes be unbearable, and may be associated with decreased peripheral blood flow. Because long pepper extract is known to improve blood flow, it might be a useful approach in the treatment of peripheral coldness. However, clinical data regarding the effects of long pepper extract on peripheral coldness are lacking. The objective of the present study was to assess the effects of long pepper extract on peripheral coldness in healthy women.

Methods Thirty healthy women aged 20-64 years completed this randomized, double-blind, placebo-controlled, crossover trial. The primary endpoint was assessment of peripheral blood flow and skin temperature after cold water stress. This trial was registered at UMIN Clinical Trials Registry as UMIN000025893.

Results All subjects completed this study without abnormal events regarding subjective and objective symptoms during the observation period. Peripheral blood flow and skin temperature were statistically significantly higher after cold water stress with consumption of long pepper extract than with consumption of placebo.

Conclusion Our data suggest that a single oral intake of long pepper extract increases the peripheral blood flow and skin temperature after cold stress, and is a potential tool for the prevention and improvement of peripheral coldness in healthy women.

(Jpn Pharmacol Ther 2018 ; 46 : 219-25)

KEY WORDS Blood flow, Healthy human, Long pepper extract, Peripheral coldness, Randomized controlled trial

INTRODUCTION

Peripheral coldness is a symptom commonly referred to as "Hie" in Japan. Peripheral coldness is also a common symptom worldwide, because it can be defined as a feeling of coldness or chill in a particular part of the

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body. It can sometimes be unbearable, and is often complicated by other symptoms such as headache, depression and fatigue.[1-3)]

The mechanisms of peripheral coldness have not been clearly established, but it may be associated with decreased peripheral blood flow. Women with peripheral coldness had about 50% lower blood flow in the upper extremities and about 60% lower blood flow in the lower extremities, as compared to those without peripheral coldness.[4,5)] It has been reported that body weight and body mass index (BMI) were significantly lower in peripheral coldness, thus suggesting a relationship between leanness and peripheral coldness.[6)] In Japan, more than 20% of women aged 20-29 years are underweight (BMI<18.5), and that percentage was much higher than that in most developed countries, highlighting the importance of effective treatment for peripheral coldness.[7)] However, appropriate therapy for healthy humans suffering from such peripheral coldness has been insufficient. This is a prevalent problem affecting improvements in quality of life (QOL).

As peripheral coldness markedly impacts QOL and it is under the influence of many factors, various strategies have been implemented to enhance and maintain peripheral blood flow and peripheral coldness. In Japan, administration of traditional herbal medicines such as Tokishigyakukagoshuyushokyoto is one of the approaches in the treatment of peripheral coldness and related symptoms.[4,8)] Previous reports suggest that α-Glucosylhesperidin, royal jelly, ginger extract and piperine promoted the recovery of peripheral blood flow and body temperature after cooling stress.[9-16)] Like these materials, among various strategies, phytomedicine or herbal therapy, which has long been used in traditional folklore to enhance peripheral blood flow, has gained much attention.

Long pepper (*Piper retrofractum*), belonging to the Piperaceae family, is widely distributed in tropical and subtropical regions of the world.[17)] The fruits of long pepper have been used for their anti-flatulent, expectorant, antitussive, antifungal, uterus-contractile, sedative-hypnotic, appetizing and counter-irritant properties in traditional medicine.[18)] The aqueous extract of long pepper showed high levels of activity against mosquito larvae.[19,20)] Furthermore, it has been reported that constituents of long pepper extract protect against high-fat diet induced obesity and enhanced neuroprotective activity.[21,22)] Long pepper contains piperine,[22)] which was previously reported to improve blood flow.[15,16)]

Table 1 Characteristics of the study subjects

Characteristics	Baseline values
Number	30
Age (Years; Indicated as mean age and range)	44 (20-60)
Hight (cm)	159.4±3.8
Weight (kg)	51.6±7.6
Body mass index (kg/m^2)	20.3±2.6
Body fat ratio (%)	28.8±4.8
Systolic blood pressure (mmHg)	100.5±14.5
Diastolic blood pressure (mmHg)	63.8±8.6
Pulse (beats/min)	70.4±9.5

Values are presented as mean±standard deviation (SD).

We therefore hypothesized that long pepper extract might enhance peripheral blood flow and suppress peripheral coldness, and we aimed to investigate the effects of a single administration of long pepper extract on peripheral blood flow and skin temperature in healthy women.

MATERIALS AND METHODS

1 Ethics statement

This study was carried out in accordance with the Declaration of Helsinki of 1995 (as revised in 2013) and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. This study was approved by the Human Ethics Committee of the Oriental Ueno Detection Center (Tokyo, Japan; Date of approval: January 17, 2017; Approval No: 2017-02). Volunteers were given a full explanation of the study's procedures and tests in advance, and all volunteers gave their signed informed consent before participating in the study.

2 Subjects

Sixty-two healthy Japanese female subjects aged 20-64 years were recruited for the study. Prior to the enrolment into the study, all subjects were assessed by a medical physician and classified as clinically healthy. This medical entrance examination included a standard medicinal history questionnaire, height, weight, body mass index (BMI), blood pressure measurements and pulse. The subjects also completed a cold water stress test. This entrance examination was performed two weeks before the study. Exclusion criteria were smoker, use of medicinal products, history of ovarian resection or hysterectomy, and serious diseases such as diabetes, hepatopathic disease, kidney damage, heart failure, endocrine disease, thyroid dis-

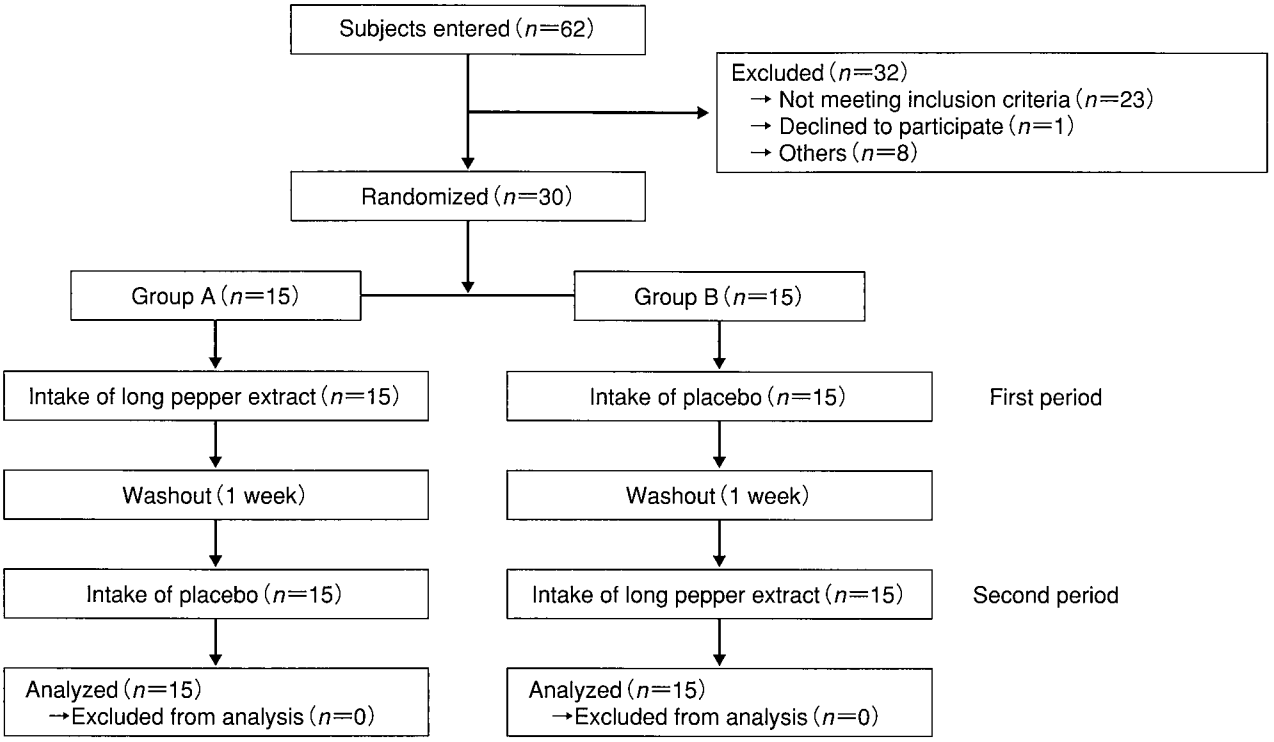

Fig. 1 Flow diagram of the study

ease, adrenal disease and/or metabolic disease.

The volunteers were not undergoing ovulation for the 2 days before and after measurements. They were not pregnant or lactating during the study period. Subjects did not take any health-promoting foods, foods for specified health uses, health foods or supplements for the 3 months before and during the experimental period.

Based on these exclusion criteria, twenty three subjects were excluded from the study. One other subject declined from this study. Finally, we selected 30 women subjects from the remaining 38 subjects. The subjects' characteristics are summarized in **Table 1**. The disposition of the subjects enrolled in this study is shown in **Fig. 1**.

3 Study design

This study was a randomized, double-blind, placebo-controlled, crossover trial. Subjects were randomly divided into group A and group B by the stratified permutated block randomized method. In the first period of the study, group A ingested the long pepper extract and group B ingested the placebo. Then, after a washout period of one week, group A ingested the placebo and group B ingested the long pepper extract in the second period of the study. The randomization list was kept confidential and was unsealed only after closure of the database.

This study was performed in February 2017 at TES holdings Co., Ltd.(Tokyo, Japan). This study was registered in the UMIN Clinical Trials Registry as UMIN ID: UMIN000025893.

4 Test foods

We prepared two test foods; either containing 150 mg of long pepper extract (Long pepper extract powder®; Maruzen Pharmaceuticals Co., Ltd., Hiroshima, Japan) in the case of the active food, or without long pepper extract for use as placebo. The placebo contained 150 mg dextrin powder. The long pepper extract was prepared from fruits of long pepper (*Piper retrofractum*) with water. Standardization and conformity of the extract was assured by strict in process controls during manufacture, and complete analytical control of the resulting dry extract. The active food and the placebo were indistinguishable in terms of flavor and appearance including color, size and packaging. Subjects ingested each test food with 50 mL water.

5 Experimental procedure

This study was conducted under controlled conditions (room temperature of 26°C) under doctor's supervision. Subjects were instructed not to eat or drink anything from 21:00 on the preceding day to the begin-

Table 2 Peripheral blood flow and body temperature before intake in first and second period

Index	First period (n=30)	Second period (n=30)	P-value
Peripheral blood flow (mL/min/100 g)	10.63±1.26	10.35±1.77	0.46
Hand skin temperature (°C)	32.81±1.62	32.77±0.95	0.86
Underarm temperature (°C)	36.53±0.33	36.44±0.28	0.11

Values are presented as mean±standard deviation (SD).
No significant differences were observed in any parameters between the two period.

ning of the experimental day. On the examination morning, each subject's height, weight, BMI, blood pressure, pulse and underarm temperature were measured before intervention. We continued to measure left hand temperature and blood flow for 10 minutes after water cold stress.

Cold water stress test was performed in a controlled temperature room (26±1°C). After 30 minutes rest, thermography and blood flow were measured on left hand before intake. Subjects placed both hands into water (18°C for 1 minute) at 10 minutes after intake of the test food. Peripheral blood flow and skin temperature of the left hand were measured for 10 minutes after cold water stress.

Peripheral blood flow in the left hand was measured using the 2 dimensional laser blood flow imager (OMEGAZONE OZ-1; OMEGAWAVE INC., Tokyo, Japan). Skin temperature in the left hand was measured using a thermal imaging camera (InfReC-R300SR; Nippon Avionics Co., Ltd., Tokyo, Japan).

The percentage recovery of skin temperature after cold water stress was calculated using the following formula: Percentage recovery of skin temperature=(value at 10 minutes after cold water stress−value at 0 minute after cold water stress)/(value before intake−value at 0 minute after cold water stress)×100.

6 Statistical analysis

All data were expressed as mean values and standard deviation (Mean±SD). In **Fig. 2**, these values are expressed as means and standard errors (Mean±SE).

The sample size was calculated based on preliminary data on recovery rates of peripheral blood flow following cold water stress with long pepper extract intake (Mean=46%, SD=250) in healthy adults compared with placebo (Mean=−49%, SD=174). For detecting a difference with a power of 80% at significant level of alpha=0.05, and the conservative assumption of a correlation of 0.9, a sample size of 30 was required.

To assess the adequacy of the crossover trial, both period effects and carry over effects with peripheral blood flow, skin temperature and underarm temperature were carried out by Welch's t-test. Paired t-test was used to determine the effect of recovery of peripheral blood flow and skin temperature. We have not considered the adjusting for multiplicity. Statistical analyses were performed using BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan) and IBM SPSS Version 21.0 (IBM Japan, Tokyo, Japan). The level of statistical significance was set at $P<0.05$.

RESULTS

All subjects completed this study without any adverse events regarding subjective and objective symptoms during the observation period. **Table 1** shows the mean values of baseline characteristics in all subjects.

Table 2 shows the baseline values of peripheral blood flow of left hand, skin temperature of left hand and underarm temperature of subjects at the first period and the second period. There were no significant differences in any parameters at baseline between the first period and second period. Moreover, no period effects and carry over effects were observed in peripheral blood flow, skin temperature, and underarm temperature. Therefore, it was indicated that this crossover trial was properly performed.

Table 3 shows the changes in peripheral blood flow and skin temperature of left hand after cold water stress at intake of long pepper extract and intake of placebo. There were no significant differences in any parameters at baseline between long pepper extract intake and placebo intake (data not shown). The mean

Table 3　Recovery of peripheral blood flow and skin temperature after cold water stress

Group	After cold water stress					
	0 min	2 min	4 min	6 min	8 min	10 min
Peripheral blood flow（mL/min/100 g）						
Long pepper extract（n=30）	9.55±0.92	9.38±0.91*	9.52±0.86*	9.41±0.82	9.14±0.98	8.97±0.79
Placebo（n=30）	9.57±0.94	9.02±1.04	9.07±0.85	9.45±0.95	9.21±0.95	9.11±0.93
Skin temperature（℃）						
Long pepper extract（n=30）	25.61±0.84	28.29±0.99	29.00±1.07	29.12±1.32	29.22±1.44*	29.23±1.52*
Placebo（n=30）	25.56±0.85	28.08±0.88	28.76±0.89	28.84±0.86	28.77±0.98	28.75±1.06

Values are presented as mean±standard deviation（SD）.
*P<0.05 vs. Placebo group

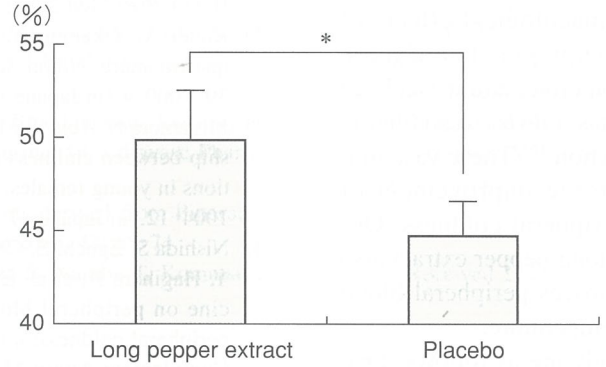

Fig. 2　Recovery rate of skin temperature after cold water stress
Values are presented as mean±standard error（SE）.
*P<0.05 vs. Placebo group

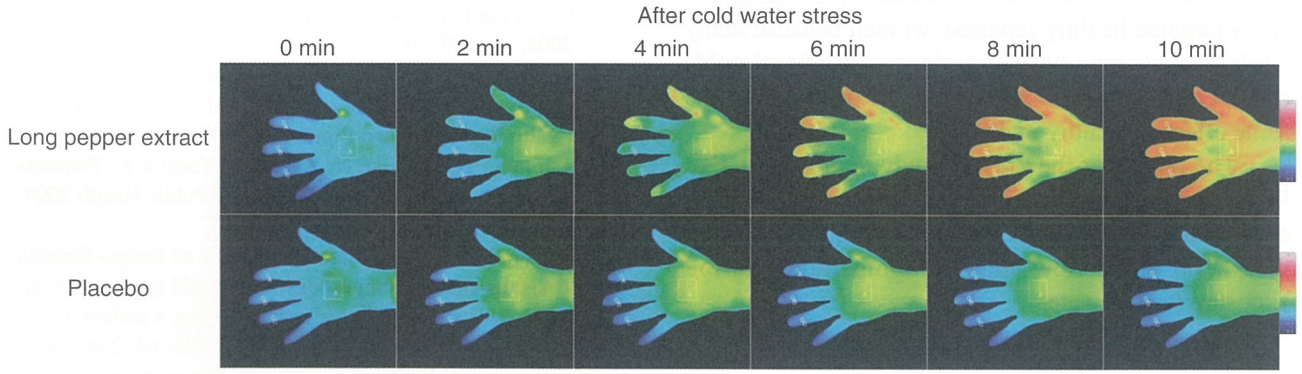

Fig. 3　The typical example on a thrmograph after cold water stress

value of peripheral blood flow at 4 minutes after cold water stress with intake of long pepper extract was 9.52 mL/min/100 g, but it decreased to 9.07 mL/min/100 g with placebo. Moreover, mean value of skin temperature at 10 minutes after cold water stress with intake of long pepper extract was 29.23℃, but it remained at 28.75℃ with placebo intake.

Fig. 2 shows the recovery rate of skin temperature of left hand at 10 minutes after cold water stress. Mean value of recovery rate of skin temperature at 10 minutes after cold water stress with intake of long pepper extract was 49.9%, but it remained at 44.7% with placebo intake. **Fig. 3** shows the typical example on a thermograph after cold water stress with intake of long pepper extract and intake of placebo.

DISCUSSION

In the present randomized crossover trial, subjects at intake of long pepper extract showed significant recovery of peripheral blood flow and skin temperature in the left hand after cold water stress. Our study is the first to analyze the effects of long pepper extract on peripheral coldness in a randomized controlled trial. Therefore, our study provides robust evidence of the effectiveness of long pepper extract in improving peripheral coldness.

The mechanism responsible for the effects of long pepper extract on peripheral coldness is not well established. One of the pharmacological effects of long pepper extract may be the progression of nitric oxide. Long pepper contains piperine, and it has been reported in rats that piperine has a direct vasodilating effect by enhancing NO production.[15] These vasodilator effects may contribute to the improvement of peripheral blood flow and peripheral coldness. Our results support the notion that long pepper extract also enhances NO production, improves peripheral blood flow, and elevates peripheral temperature.

The limitations of this study are as follows: Fist, the measurement errors for peripheral blood flow and skin temperature were inevitable. Secondly, we did not evaluate the vasodilator effects of long pepper extract directly because of the technical difficulty. That evaluation would have clarified the mechanisms. Third, this study targeted healthy Japanese women because many adolescent women have experience peripheral coldness. It is necessary to verify whether or not it has the same effect in healthy males.

The results of the present study demonstrate that long pepper extract is effective for improving peripheral coldness, as well as for preventing decreases in peripheral blood flow and skin temperature during exposure to cold water.

CONCLUSION

We conducted the randomized controlled trial to assess the long pepper extract on peripheral coldness in healthy women. The thirty female subjects were received 150 mg of the long pepper extract or placebo. This study showed that the long pepper extract improved significantly peripheral blood flow and skin temperature in the left hand after cold water stress.

The present study's findings indicate that long pepper extract is an effective for improving and/or preventing peripheral coldness.

[Conflict of interest] This trial conducted with the financial support of Maruzen Pharmaceuticals Co., Ltd.

[Acknowledgements] We would like to thank the volunteers for their participation and tolerance.

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