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Efficacy and safety of *Citrus sudachi* peel in obese adults: A randomized, double-blind, pilot study

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ABSTRACT

Objective: This study was undertaken to explore the efficacy and safety of *Citrus sudachi* peel for metabolic risk factors in obese male and female adults.

Background: *Citrus sudachi* Hort. ex Shirai (Rutaceae), called "sudachi", is a small, round, green citrus fruit that is mainly cultivated in Tokushima Prefecture in Japan. Our group reported that *Citrus sudachi* peel powder improved glucose tolerance and dyslipidemia in Zucher-fatty rats and reduced hyperglycemia and hypertriglyceridemia in GK diabetic rats.

Materials and Methods: We conducted a randomized, double-blind, placebo-controlled trial in 40 participants with abdominal obesity and metabolic risk factors including hypertension, impaired glucose tolerance and elevated triglyceride levels. Participants were randomized to receive either tablets that contained 1.3 g dried *Citrus sudachi* peel powder or placebo tablets for 12 weeks. The sudachi peel group included 14 males and 5 females with a mean age of 54.5 years, and the placebo group included 18 males and 2 females with a mean age of 51.9 years.

Results: Physical status including body weight, waist circumference and blood pressure and laboratory markers including metabolic parameters were not different at any observation point between the two groups. However, among participants with serum triglyceride levels of more

than 120 mg/dl, body weight, waist circumference and serum triglyceride levels were significantly decreased at several observation points after the start of treatment in the sudachi peel group but not in the placebo group. No serious adverse events were observed in the sudachi peel group.

Conclusions: *Citrus sudachi* peel has the potential effect to safely improve abdominal obesity and lower serum levels of TG in obese individuals with hypertriglyceridemia. A large-scale randomized, double-blind clinical study targeting subjects with both abdominal obesity and high TG levels is needed to confirm the metabolic effects of *Citrus sudachi* peel.

Trial registration: UMIN Clinical Trials Registry (UMIN-CTR) UMIN000002682. Accession number of the Ethics Committee for Clinical Trials of Food in Tokushima University Hospital is F5.

Key words: health functional food, anti-obesity, triglyceride

BACKGROUND:

Citrus sudachi Hort. ex Shirai (Rutaceae), called "sudachi", is a small, round, green citrus fruit that is mainly cultivated in Tokushima Prefecture in Japan [1]. Slices of this fruit are often used for Japanese dishes as food flavoring in place of vinegar and the peel is also edible. Our group reported that *Citrus sudachi* peel powder improved glucose tolerance and dyslipidemia in Zucher-fatty rats and reduced hyperglycemia and hypertriglyceridemia in GK diabetic rats [2]. However, the effects of *Citrus sudachi* peel on lifestyle-related risk factors in humans have not been clarified. Metabolic syndrome is a combination of lifestyle-related risk factors that increase the risk of developing cardiovascular disease and diabetes [3]. The aim of this study was to explore the efficacy and safety of *Citrus sudachi* peel for improving lifestyle-related risk factors including obesity, hypertension, impaired glucose tolerance and dyslipidemia in humans.

METHODS:

Subjects: Inclusion criteria were males and females aged 20 - 74 years who had abdominal obesity defined as waist circumference >85cm for males or >90cm for females or body mass index (BMI) >25 and any one of the following: 1) fasting plasma glucose (FPG) >100 mg/dL (5.6 mmol/L) or specific treatment for type 2 diabetes, 2) serum triglycerides (TG) >150 mg/dL (1.7 mmol/L) or high-density lipoprotein cholesterol (HDL-C) <40 mg/dL (1.03 mmol/L) or specific treatment for its abnormality, and 3) systolic blood pressure (BP) >130 or diastolic BP >85mmHg or treatment of hypertension. The following subjects were excluded: 1) subjects with complications in the brain, heart, kidney, lungs or liver (cerebrovascular accident, myocardial infraction, angina pectoris, history of cardiovascular intervention, heart failure, arteriosclerosis obliterans, nephropathy, bronchial asthma, pulmonary emphysema, pneumonitis, pulmonary fibrosis, viral hepatitis, cirrhosis, diabetic complications, and diabetes treated with insulin), 2) subjects in whom additional pharmaceutical agents should be considered for hypertension, lipid disorder, diabetes mellitus and/or other diseases, 3) subjects with overt systemic diseases, 4) subjects with a history of malignant diseases, 5) subjects who participated in another clinical trial, and 6) subjects who

were not appropriate in the attending physicians' opinion.

Experimental design: This study was designed as a randomized, double-blind, placebo-controlled trial. Participants were randomized to receive either 5 tablets that in total contained 1.3 g dried *Citrus sud achi* peel powder (sudachi peel group) or 5 placebo tablets (placebo group) every day for 12 weeks. Daily intake in the sudachi peel group was equivalent to 1/3 of a whole *Citrus sudachi*. Since analysis carried out by the Japan Food Research Laboratories showed that the content of synephrine in *Citrus sudachi* peel is 2.5 mg/g, estimated intake of synephrine, which is contained in abundance in citrus peel and has pharmacological effects similar to those of ephedrine, was only 3.25 mg/day in the sudachi peel group. Analysis carried out by the Japan Food Research Laboratories also showed that the content of hesperidin, a flavonoid found abundantly in citrus fruits, was 7.4 mg/g in *Citrus sudachi* peel, indicating that the estimated intake of hesperidin was only 9.62 mg/day in the sudachi peel group. The study period was 17 weeks including a 1-week observation period before treatment, 12-week treatment period with *Citrus sudachi* peel powder or placebo tablets and 4-week observation period after treatment. The subjects made visits to the hospital 1 week before the treatment, 4, 8 and 12 weeks after the start of treatment, and 4 weeks after the treatment period. A life diary including physical activity and exercise was recorded every day during the study period and a diet diary including content of meals was recorded for three days before each visit. The number of remaining tablets was checked at each point of visit to assess the compliance of subjects. Physical examination including measurements of height, body weight, BMI, waist circumference, BP and pulse was performed at each visit. Urine and blood samples were drawn from the antecubital vein after an overnight fast at each visit.

Biochemical analysis: General laboratory tests were also carried out at each visit. The tests included urinalysis to assess urinary protein, occult blood, urobilinogen, sugar and ketone bodies, measurements of peripheral blood including white blood cells, red blood cells, hemoglobin, hematocrit and platelets, assessment of liver function including measurements of GOT, GPT, LDH, total bilirubin, alkaline phosphatase, γGTP, total protein and albumin, assessment of renal function including measurements of BUN and creatinine, measurements of electrolytes including sodium, potassium, chloride, and calcium concentrations, and assessment of metabolic function including measurements of low-density lipoprotein (LDL)-C, HDL-C, TG, FPG, HbA1c (National Glycohemoglobin Standardization Program), immunoreactive insulin (IRI), and uric acid (UA). In addition, blood levels of remnant-like particles (RLP)-C, free fatty acid (FFA), high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor (TNF)-α and adiponectin and urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a marker of oxidative stress were examined 1 week before treatment, 12 weeks after the start of treatment and 4 weeks after the treatment period. Hs-CRP levels were measured at Bio Medical Laboratories (Tokyo, Japan) by nephelometry, a latex particle-enhanced immunoassay (N Latex CRP II). TNF-α was measured by ELISA (Human TNF-α TNFSF1A, R&D Systems, Inc., Minneapolis, USA). Adiponectin was measured by ELISA (Human adiponection ELISA Kit, Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan). Urinary excretion of 8-hydroxydeoxyguanosine (OHdG) was determined by ELISA (new 8-OHdG Check ELISA Kit, Japan Institute for the Control of Aging, Nikken SEIL Corporation, Shizuoka, Japan) and expressed in μg/g

creatinine after correction by urinary creatinine concentration.

Ethics: The present clinical study is in compliance with the Helsinki Declaration. Prior written informed consent was obtained from all subjects before enrollment in this study in accordance with protocols approved by the Ethics Committee for Clinical Trials of Food in Tokushima University Hospital (accession number F5). This study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) with the trial number of UMIN000002682.

Statistical Analysis: All parameters before and after treatment were compared between the sudachi peel group and the placebo group using generalized linear mixed model analysis. Differences in baseline patient characteristics between the two groups were analyzed by the unpaired t-test. The Wilcoxon signed rank test was used to assess significant changes in parameters after the start of treatment in each group. All data are expressed as means ± S.D. The analyses were performed on a Microsoft Windows computer running SPSS software. Differences were considered statistically significant at $p < 0.05$.

RESULTS:

Basal characteristics: Forty subjects were enrolled in this study, but one subject could not visit our hospital due to personal reasons. Finally, the sudachi peel group included 14 males and 5 females with a mean age of 54.5 years (one patient having dropped out), and the placebo group included 18 males and 2 females with a mean age of 51.9 years. The patients' characteristics are summarized in Table 1. The first, second and third quartile of TNFα levels is 1.3, 3.4 and 4.7 pg/mL in the placebo group, respectively, and 2.1, 2.3 and 3.2 pg/mL in the Sudachi peel group, respectively. There were no differences in basal parameters including age, sex, BMI, waist circumference, pulse, BP, TG, HDL-C, HbA1c and FPG between the sudachi peel group and the placebo group.

Intake rate of tablets: There was no difference between the intake rates of tablets in the two groups (95.3±6.7 % in the sudachi peel group and 96.1±6.0 % in the placebo group).

Live and diet diary: Physical activity and dietary intake of total calories and nutrients including carbohydrates, proteins and lipids were not different before, during and after treatment in either the sudachi peel group or placebo group. There were also no differences in these parameters at every observation point between the two groups.

Changes in parameters before, during and after treatment: Physical status including body weight, BMI, waist circumference, pulse and BP and laboratory markers including urinalysis, peripheral blood, liver function, renal function, electrolytes, LDL-C, TG, HDL-C, RLP-C, FFA, HbA1c, FPG, IRI, UA, hs-CRP, urinary 8-OHdG, TNF-α and adiponectin were not different at any observation point between the two groups. There were also no differences in theses parameter before and 4, 8 and 12 weeks after the start of treatment and at 4 weeks after the treatment period in either the sudachi peel group or placebo group.

Adverse effects: One patient in the placebo group developed dizziness and palpitation due to paroxysmal atrial fibrillation. None of the patients in the sudachi peel group showed

expression or worsening of symptoms.

Table 1. Baseline parameters in all subjects

Variables	Placebo group n=20	Sudachi peel group n=19	P-value
Age	51.9±11.0	54.5 ± 8.9	ns
Male : Female	18:2	14:5	ns
Body weight (kg)	76.2 ±10.8	75.6±10.9	ns
BMI	26.2 ± 3.4	26.8 ± 3.0	ns
Waist circumference(cm)	93.7 ± 6.1	97.3 ± 6.3	Ns
Pulse (bpm)	73.5 ± 5.8	71.9 ± 8.2	ns
Systolic BP (mmHg)	128.9 ± 13.0	134.6 ± 10.5	ns
Diastolic BP (mmHg)	82.3 ± 6.2	87.9 ± 8.3	ns
LDL-C (mg/dL)	134.7 ± 25.2	140.8 ± 20.4	ns
TG (mg/dL)	143.8 ± 67.0	146.6 ± 60.3	ns
HDL-C (mg/dL)	62.9 ± 15.1	67.7 ± 15.8	ns
RLP-C (mg/dL)	9.6 ± 5.8	9.8 ± 5.4	ns
FFA (μEq/L)	538.5 ± 133.1	548.6 ± 197.2	ns
HbA1c (%)	6.0 ± 0.3	5.9 ± 0.4	ns
FPG (mg/dL)	112.2 ± 13.3	108.7 ± 14.1	ns
IRI (μg/dL)	8.0 ± 3.1	7.7 ± 3.6	ns
UA (mg/dL)	6.2 ± 1.4	5.8 ± 1.0	ns
hsCRP (μg/dL)	100 ± 116	93 ± 98	ns
U-8OHdG(μg/gCr)	9.9 ± 4.6	11.9 ± 7.5	ns
TNFα (pg/mL)	4.0 ± 4.1	6.2 ± 15.4	ns
Adiponectin(μg/mL)	6.1 ± 2.3	6.5 ± 2.4	ns

Analysis in a subgroup with serum TG levels of more than 120 mg/dL: Since none of the parameters were changed by treatment with *Citrus sudachi* peel, we next analyzed the data in subjects with serum TG levels of more than 120 mg/dl. Although age and blood pressure of the sudachi peel subgroup with serum TG levels of more than 120 mg/dl were slightly higher than those of the placebo subgroup with serum TG levels of more than 120 mg/dl, there were no significant differences in other baseline parameters between the two subgroups (Table 2). The first, second and third quartile of TNFα levels is 3.5, 4.1 and 5.9 pg/mL in the placebo group, respectively, and 2.2, 2.7 and 3.2 pg/mL in the Sudachi peel group, respectively.

Table 2. Baseline parameters in subjects with serum TG levels of more than 120 mg/dl

Variables	Placebo group n=11	Sudachi peel group n=11	P-value
Age	47.3±11.0	56.3 ± 8.2	0.046
Male : Female	9:2	8:3	ns
Body weight (kg)	79.1 ±12.9	76.9±11.9	ns
BMI	26.8 ± 4.0	27.5 ± 3.4	ns
Waist circumference (cm)	95.4 ± 6.8	99.1 ± 6.3	ns
Pulse (bpm)	74.5 ± 6.6	73.8 ± 9.7	ns
Systolic BP (mmHg)	126.7 ± 9.7	135.5 ± 8.1	0.049
Diastolic BP (mmHg)	82.0 ± 6.1	89.5 ± 9.6	0.042
LDL-C (mg/dL)	138.6 ± 27.8	143.4 ± 21.5	ns
TG (mg/dL)	189.4 ± 56.3	184.7 ± 49.4	ns
HDL-C (mg/dL)	58.4 ± 14.5	64.7 ± 11.8	ns
RLP-C (mg/dL)	13.1 ± 5.7	13.0 ± 5.0	ns
FFA (μEq/L)	524.0 ± 141.3	609.5 ± 233.1	ns
HbA1c (%)	6.0 ± 0.4	5.8 ± 0.4	ns
FPG (mg/dL)	110.4 ± 13.0	104.6 ± 4.4	ns
IRI (μg/dL)	8.7 ± 1.8	8.6 ± 4.4	ns
UA (mg/dL)	6.0 ± 1.6	5.8 ± 1.2	ns
hsCRP (μg/dL)	100 ± 89	125 ± 119	ns
U-8OHdG(μg/gCr)	13.6 ± 8.5	10.5 ± 5.7	ns
TNFα (pg/mL)	5.4 ± 3.7	8.8 ± 19.8	ns
Adiponectin(μg/mL)	5.8 ± 2.1	6.7 ± 2.3	ns

In subjects with serum TG levels of more than 120 mg/dl, body weight significantly decreased from 76.9±11.9 kg before treatment to 76.1±11.1 kg ($p<0.05$) and 76.0±11.1 kg ($p<0.05$) at 8 and 12 weeks after the start of treatment, respectively, and the decrease in body weight was maintained 4 weeks after the treatment period (75.6±11.4 kg, $p<0.01$ vs before the treatment) in the sudachi peel subgroup, but no significant changes in body weight were observed in the placebo subgroup (Figure 1A).

Waist circumference also significantly decreased from 99.1±6.3 cm before treatment to 97.5±6.8 cm ($p<0.01$) and 97.3±7.3 cm ($p<0.05$) at 8 and 12 weeks after the start of treatment, respectively, in the sudachi peel subgroup with serum TG levels of more than 120 mg/dL but not in the placebo subgroup (Figure 1B). Serum TG level also significantly decreased from 184.7±49.4 mg/dL before treatment to 158.1±40.7 mg/dL ($p<0.05$) at 8 weeks after the start of treatment and to 141.2±37.1 mg/dL ($p<0.01$) at 4 weeks after the treatment period in the sudachi peel subgroup (Figure 1C). However, no significant changes in serum TG levels were observed in the placebo subgroup with serum TG levels of more than 120 mg/dL (Figure 1C).

However, there was no difference in these parameters at any observation point between the two groups.

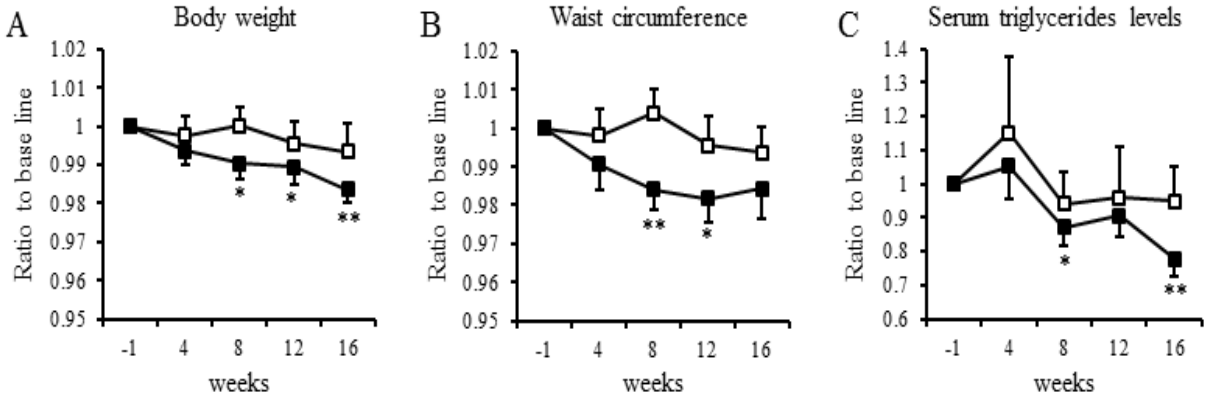

Figure 1. Changes of parameters in the placebo subgroup (□) and the sudachi peel subgroup (■) with serum triglyceride levels of more than 120 mg/dL. Body weight was significantly decreased at 8 and 12 weeks after the start of treatment and the decrease in body weight was maintained 4 weeks after the treatment period in the sudachi peel subgroup (A). Waist circumference was also significantly decreased at 8 and 12 weeks after the start of treatment in the sudachi peel subgroup (B). Serum triglyceride (TG) levels were significantly decreased at 8 weeks after the start of treatment and at 4 weeks after the treatment period in the sudachi peel subgroup (C). Serum TG levels after the start of treatment significantly decreased compared with the baseline level in the sudachi peel group ($p<0.05$) (C). All data are expressed as ratios to baseline level before the start of treatment and means ± SEM. * $p<0.05$ and ** $p<0.01$ vs before treatment.

DISCUSSION:

This study is the first randomized, double-blind, placebo-controlled trial to clarify the effects and safety of *Citrus sudachi* peel in humans. We did not find any significant differences in clinical parameters between the placebo group and sudachi peel group in the present study. However, subgroup analysis using serum TG levels showed that intake of *Citrus sudachi* peel significantly decreased body weight, waist circumference and serum TG level in subjects with serum TG levels of more than 120 mg/dL, but such decreases were not observed in the placebo subgroup. On the other hand, the subgroup analysis did not show significant differences in parameters at any observation point after the start of treatment between the placebo group and the sudachi peel group. These findings suggested that number of enrolled subjects in this study is too small to show a significant difference between the two groups. In addition, Taskinen et al. reported that serum TG level in obese subjects is increased by the combination of increased secretion and severely impaired clearance of TG-rich VLDL1 particles and that increased secretion of TG-rich VLDL1 particles is linked to increased liver and subcutaneous abdominal fat [4], indicating that obesity with high TG levels has a metabolic pathology different from that in other types of obese patients with normal TG levels. Taken together, a large-scale clinical study enrolling obese subjects with high TG levels should be designed to clarify the clinical effects of sudachi peel.

There are several reports about the effects of components in citrus peel on metabolic syndrome. Hesperidin, a flavanone glycoside found abundantly in the peel of citrus fruits, was reported to improve hypercholesterolemia, hypertriglyceridemia or fatty liver in rat models [5,6]. Morand et al. reported that in healthy, middle-aged, moderately overweight men,

orange juice decreases diastolic blood pressure and postprandially increases endothelium-dependent microvascular reactivity [7]. In Morand's study, daily intake of hesperidin was 292 mg. However, in our study, estimated intake of hesperidin was quite low (only 9.62 mg/day) in the sudachi peel group. Synephrine contained in the peel of bitter orange citrus also has beneficial effect on obesity as a thermogenic agent like the action of ephedrine. In our study, estimated intake of synephrine was only 3.25 mg/day. Colker et al. conducted the first study on the effects of a bitter orange extract containing 58.5 mg p-synephrine and 528 mg caffeine daily on body fat loss and lipid levels in 20 overweight adult subjects [8]. A review of human studies on Citrus aurantium (bitter orange) extract and its primary protoalkaloid p-synephrine showed that products containing a low dose of synephrine, such as in our study, have no anti-obesity effect [9]. Taken together, we speculate that the effect of *Citrus sudachi* peel on obesity and TG levels may not be due to synephrine.

Nakagawa et al. identified sudachitin (4',5,7-trihydroxy-3',6,8- trimethoxyflavone) and 3¢-demethoxysudachitin from *Citrus sudachi* peel as the most active compounds with antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori* [10]. Yuasa et al. also reported that sudachitin inhibited nitric oxide production by suppressing the expression of inducible nitric oxide synthase in lipopolysaccharide-stimulated macrophages, indicating that sudachitin has an anti-inflammatory effect [11]. However, no components having an anti-obesity effect or TG-lowering effect have so far been identified from the peel of *Citrus sudachi*. Further studies are needed to clarify the mechanisms by which the peel of *Citrus sudachi* improves obesity and lowers serum TG levels.

In our study, there were no adverse effects of the intake of 1.3 g dried *Citrus sudachi* peel every day for 12 weeks. There are concerns about side effects of citrus peel on the cardiovascular system since it contains a large amount of synephrine with ephedrine-like action. In Guidelines for the Use of Synephrine in Natural Health Products revised by Health Canada in 2010, 30 mg/day is the maximum allowable dose for total synephrine. Peel of *Citrus sudachi* may be safe for consumption because it contains less synephrine than that in other citrus peels. However, since the subjects of our study had no cardiovascular complications, the safety for patients with cardiovascular diseases was not confirmed. In addition, since the period of *Citrus sudachi* peel intake was only 12 weeks in our study, further study is needed to show the safety of long-term *Citrus sudachi* peel intake.

CONCLUSIONS:

Citrus sudachi peel has the potential effect to safely improve abdominal obesity and lower serum levels of TG in obese individuals with hypertriglyceridemia. A large-scale randomized, double-blind clinical study targeting subjects with both abdominal obesity and high TG levels is needed to confirm the metabolic effects of *Citrus sudachi* peel.

Competing interests: The authors have no financial interests or conflicts of interest.

Author's contributions: All authors contributed to this study.

Abbreviations: BMI, body mass index; BP, blood pressure; C, cholesterol; FFA, free fatty acid; FPG , fasting plasma glucose; HDL, high-density lipoprotein; hs-CRP, high-sensitivity

C-reactive protein; IRI, immunoreactive insulin; LDL, low-density lipoprotein; OHdG, hydroxydeoxyguanosine; RLP, remnant-like particles; TG, triglycerides; TNF, tumor necrosis factor; UA, uric acid

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