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SHORT REPORT

Evaluation of the safety and usefulness of *citrus jabara* fruit peel powder cream for patients with atopic dermatitis

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ABSTRACT

Citrus jabara (CJ) is a rare citrus that originally grew wild only in the southern area of the Kii peninsula in Japan. In the relationship between CJ and atopic dermatitis (AD), improvement of AD by oral intake of CJ fruit juice was reported in AD model mice. Our previous study also showed anti-inflammatory potentials of CJ fruit peels *in vitro*. In this study, the applicability of CJ fruit peel powder (CJ powder) for topical application in patients with AD was investigated. After confirming both the safety of CJ powder in preclinical studies and the safety of 5% CJ powder cream in healthy volunteers, the safety and usefulness of 5% CJ powder cream were evaluated in 20 patients with AD. Evaluation of 5% CJ powder cream in patients with AD for 4 weeks showed improvement in the mean severity score of the affected area (from 3.0 to 2.0, p=0.001 by Student's t-test), improvement in skin lesions (11 of 20 participants), usefulness (16 of 20 participants), and safety (16 of 20 participants). Although aggravation of symptoms on application areas were observed on 4 participants, their aggravation were systemic, resulting from causes other than tested cream. These results suggested that 5% CJ powder cream is useful and safe for patients with AD.

Keywords: *Citrus jabara; Atopic Dermatitis; External Cream; Narirutin; Safety; Usefulness*

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Introduction

Citrus jabara (CJ) is a rare citrus that originally grew wild only in the southern area of the Kii peninsula in Japan. Our previous study showed that almost all of flavonoids in CJ fruit peels was narirutin, and CJ fruit peels have anti-inflammatory potentials to inhibit productions of nitric oxide (NO) and interleukin-6 (IL-6), and to inhibit enzymatic activities of soluble epoxide hydrolase (sEH) and hyaluronidase[1]. The inhibitory effect of oral administration of CJ fruit juice was observed on the development of atopic dermatitis (AD) -like skin lesions in NC/Nga mice[2]. Narirutin, which is considered as a main functional component in CJ fruit, is present in peel much more than in juice. These findings expected that CJ fruit peels are effective in improving AD. In this study, we investigated the applicability of CJ fruit peel powder (CJ powder) for topical application in patients with AD.

Materials and Methods

Preparation of CJ powder

The CJ powder was prepared in accordance with Japanese Patent No. 5,323,127 as follows[3]. Briefly, CJ fruits, harvested on early November in 2012, were wholly pressed to remove juice. CJ fruit peels, residue of squeezed CJ fruits, were firstly freeze-dried to remove monoterpenes, next pasteurized, and then powdered to 200 mesh pass particles.

Five percent CJ powder cream

The 5% CJ cream was made by mixing 5% by weight of CJ powder into the base cream consisting of 1,3-butylene glycol, mineral oil, cetyl ethylhexanoate, glycerin, polyacrylamide, dipotassium glycyrrhizate, tocopherol, polysorbate 60, hydrogenated polyisobutene, laureth-7, phenoxyethanol, methylparaben, and purified water. All reagents except for CJ powder were registered in Japanese Standards of Quasi-drug Ingredients (2016).

Component analysis of CJ powder

Amounts of (R)-limonene oxide and (R)-(-)-carvone were quantified by GC-MS. Quantitative analysis was performed with purchased standard samples (Wako Purechemical, Osaka, Japan). The minimum limits of determination of each samples were follows: (R)-limonene oxide, 0.5 μg/ g; (R)-(-)-carvone, 0.3 μg/ g, respectively.

Amount of narirutin was quantified by HPLC with standard sample (Wako Purechemical, Osaka, Japan). Briefly, Chromatographic separation was performed on a COSMOSIL 5C18-AR-II column (φ 4.6 x 250 mm) with a following condition: temperature, 40 ˚C; mobile phase, 20% acetonitrile containing 0.8% acetic acid; flow rate, 1.0 mL/ min. Quantitative analysis was performed with purified standard sample of narirutin. Peaks were detected at 280 nm. The minimum limit of determination was 0.25 μg/ ml.

Preclinical safety evaluation of CJ powder

Following 9 safety tests of CJ powder were carried out in Biosafety Research Center (Sizuoka, Japan) according to OECD Principles of Good Laboratory Practice (GLP) (ENV/MC/CHEM(98)17); acute oral toxicity test, acute skin irritation test, acute eye irritation test, local lymph node assay (LLNA) , Ames test, cumulative skin irritation test, photosensitization test, phototoxicity test, and chromosome aberration test.

Safety evaluation of CJ powder and 5% CJ powder cream in healthy volunteers

Patch tests of CJ powder and 5% CJ powder cream were carried out in Nikoderm Research Inc (Osaka, Japan). Each patch test was carried out with 40 healthy volunteers for 24 hr. Skin sensitization test with 71 healthy volunteers for 6 weeks, and skin phototoxicity test with 32 healthy volunteers for 9 days were carried out in Allergisa Pesquisa Dermato-Cosmética Ltda (Campinas, Brasil).

Clinical evaluation of usefulness and skin safety of CJ powder cream in patients with AD

Participants

The participants were patients with mild to moderate AD who visited Wakayama Medical University Hospital Department of Dermatology between January 2016 and March 2017. The patient met the diagnostic criteria for atopic dermatitis[4,5].The participant, who had been using topical steroid, stopped using steroid during a clinical evaluation period.

CJ powder cream application

The participants applied 1 FTU of 5% CJ powder cream twice a day (morning and evening, or after bathing) on single-site forearm flexor with rash at home for 4 weeks.

Observation and assessments

This was single arm, non-randomized, before-after trial. The usefulness and safety of 5% CJ powder cream were evaluated on interview, visual inspection, and palpation of skin findings by doctor in charge every week. For the severity diagnosis, if erythema, papule, itching, dryness, and lichenization were observed as symptoms of the skin lesions part, 1 point was added to each, and the severity was judged as a minimum of 0 point and a maximum of 5 point. The usefulness of 5% CJ powder cream was comprehensively evaluated by two medical doctors in charge after 4 weeks of use on a five-point scale as follows; extremely useful, useful, slightly useful, not useful, and useless. The safety of 5% CJ powder cream was evaluated by doctor in charge after 4 weeks of use on a four-point scale as follows; safe, almost safe, insufficiently safe, and not safe. Patients and medical doctors didn't know if it's a placebo or an active drug.

Statistical analysis

Student's *t*-test was used to analyze the severity diagnosis between before and after use of 5% CJ powder cream.

Ethics Statement

The ethical committee of Wakayama Medical

University approved this study (Approval number 1716). All study participants were provided informed consent.

Results

Component analysis of CJ powder

The contents of (R)-limonene oxide and (R)-(-)-carvone, inducers of contact dermatitis, in CJ powder were below the lower limit of quantification. The content of narirutin, active ingredient, in CJ powder was 64.4 mg/ g.

Safety evaluation of CJ powder

Safety of CJ powder was assessed by preclinical study and with healthy volunteers. CJ powder was recognized as safe in all evaluation items (**Tables 1** and **2**).

Table 1. Preclinical safety evaluation of CJ powder

Evaluation item	Evaluation result
Acute oral toxicity test	safe
Acute skin irritation test	safe
Acute eye irritation test	safe
LLNA test	safe
Ames test	safe
Cumulative skin irritation test	safe
Photosensitization test	safe
Phototoxicity test	safe
Chromosome aberration test	safe

Safety evaluation of CJ powder cream in healthy volunteers

Safety of 5% CJ powder cream was sessed by patch test, skin sensitization test, and skin phototoxicity test in healthy volunteers. The safety of 5% CJ powder cream was found in all evaluation items (Table 2).

Table 2. Safety evaluation of CJ powder and 5% CJ powder cream in healthy volunteers

Evaluation item	Evaluation result
Patch test of CJ powder	safe
Patch test of 5% CJ powder cream	safe
Skin sensitization test of 5% CJ powder cream	safe
Skin phototoxicity test of 5% CJ powder cream	safe

Background of participants in clinical evaluation

The participants comprised 10 men and 10 women whose mean age was 37.1 ± 10.1 (22-59 years old).

Clinical evaluation of usefulness and skin safety of 5% CJ powder cream in patients with AD

The severity score before and after 5% CJ powder cream treatment, usefulness of 5% CJ powder cream, and the safety of 5% CJ powder cream were assessed in 20 patients with mild to moderate AD. Among them, 2 participants were dropped out before the end of the study.

The mean severity score was 3.0 at baseline and 2.0 at Week 4 (**Figure 1A**). Application of 5% CJ powder cream significantly reduced severity score (p=0.001 by Student's t test). The 5% CJ powder cream was evaluated as useful (extremely useful, useful, and slight useful) in 16 participants (**Figure 1B**). Improvement in skin lesions was observed in 11 participants. The safety of 5% CJ powder cream was observed in 16 of 20 participants (**Figure 1C**). Although aggravation of symptoms on application areas were observed on 4 participants, their aggravation were systemic, resulting from following factors; such as, heat and humidity in summer, overwork because of continuous night shift, and excessive alcohol drinking. Thus, adverse events associated with tested cream were not observed.

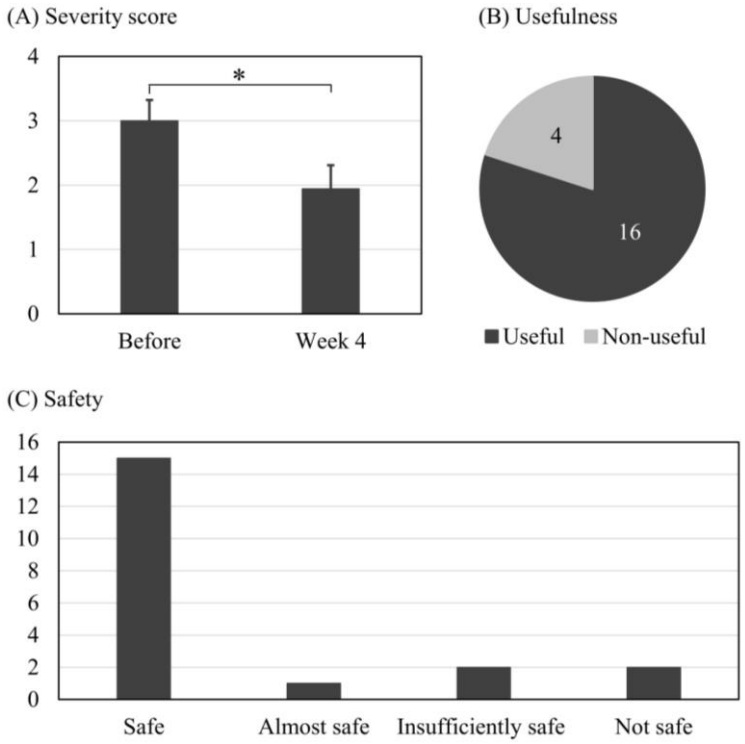

Figure 1. Clinical evaluation of 5% CJ powder cream in patient with AD. (A) Changes in severity scores, (B) usefulness, and (C) safety of 5% CJ powder cream in patients with mild to moderate AD were evaluated for 4 weeks. The participants applied 1 FTU of 5% powder cream twice a day (morning and evening, or after bathing) on single-site forearm flexor with rash at home for 4 weeks. For changes in severity score, Student's *t*-test was used to compare two groups, and significant difference was found (* P=0.001). Bars indicate standard error.

Discussion

Treatments for AD are diverse. We have been studying the usefulness of lactic acid bacteria[5], borage oil[6] and others. Based on these results, we planned this research.

In this study, we investigated the applicability of CJ powder for topical application in patients with AD. Prior to clinical study, the safety of CJ peel powder was examined, because there were no studies on CJ peels as a topical application. When using citrus peels, the following two compounds should be noted: monoterpene oxides and furanocoumarins. Monoterpenes, such as limonene, are aromatic components of citrus, but these oxides can cause contact dermatitis[7]. These oxides are remarkably increased during heating in the presence of oxygen[8]. We solved this problem by removing monoterpenes by the freeze-drying method prior to heat drying[3]. Both (R)-limonene oxide and (R)-(-)-carvone, the oxides of limonene, which are inducers of contact dermatitis, were below the limit of quantification in CJ powder. Furanocoumarins are phototoxic compounds known to be present rich in grapefruit[9]. We did not find any furanocoumarins in CJ peels, although we have comprehensively isolated and identified the compounds in them. Not all citrus fruits contain furanocoumarins, some contain more of them, such as grapefruit and bitter orange, or even none at all, such as *Citrus iyo*[10]. Therefore, it is not surprising that furanocoumarins could not be found in the CJ peel. The results of preclinical studies (Table 1) and patch tests in healthy volunteers (Table 2) also confirmed the safety of CJ peel.

The 5% CJ powder cream was first evaluated for safety in healthy volunteers, and then for usefulness and skin safety in patients with AD. The 5% CJ powder cream was found to be safe on the healthy skin, as it was negative in patch tests, skin sensitization tests, and skin phototoxicity tests in healthy volunteers (Table 2). Evaluation of 5% CJ powder cream in patients with AD for 4 weeks showed improvement in mean severity score of the affected area (from 3.0 to 2.0), improvement of eruption (11 of 20 participants), usefulness (16 of 20 participants), and safety (16 of 20 participants) (Figure 1). During this study, the use of topical steroids was stopped for all participants, thus, it was feared that participants, who had been using topical steroids, were in danger of worsening their symptoms due to the rebound phenomenon, but nonethe-

less, safety and usefulness of 5% CJ powder cream were found in 80% of the participants. Although aggravation of symptoms on application areas were observed on 4 participants, their aggravation were systemic, resulting from causes other than tested cream. These results suggested that 5% CJ powder cream is useful and safe for patients with AD.

Previous studies on CJ and its components have shown that CJ is effective in treating allergic diseases such as AD[2], asthma[11] and hay fever[12], and its functional component is considered to be narirutin. The results of this study are consistent with the results of these previous studies. However, there is a major difference between the previous studies and the present study in the way of ingestion of CJ. That is, all the previous studies were taken orally and the present study was applied to the skin. Comparing the amount of narirutin in the present study with previous studies, it is estimated to be 3.2 mg/day in the present study, 10-20 mg kg^{-1}/day in the AD model mice[2], 10 mg kg^{-1}/day in the asthma model mice[11], and 11 mg/day in human hay fever[12]. Comparing the methods of narirutin administration, application to the skin is considered to be as effective as or more effective than oral administration. In oral intake, narirutin (molecular weight: 580.5) is aglyconized by intestinal bacteria and absorbed as naringenin (molecular weight: 272.3). In *in vitro* experiments, naringenin is significantly stronger than narirutin in terms of anti-inflammatory effects[1]. Therefore, although speculative, this study suggests that the deglycosylation of narirutin may have occurred by the skin flora present in the affected area of the skin and/or enzymes released by immune cells, such as granulocytes and macrophages.

Conclusion

CJ powder cream is considered as useful and safe for patients with AD.

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