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Original article

The effects and safety of food containing a labdane type diterpenoid (labdane) on knee joint problems of healthy adults: An uncontrolled open-label trial.

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Abstract

The knee joint problem is one of the major problems for elderly people. The labdane 8 (17),12-diene-15,16-dial (hereinafter referred to as "the Labdane"), which is contained in the Japanese white turmeric leaf and rhizome has demonstrated to inhibit degradation of hyaluronic acid. Since hyaluronic acid is important for lubricating joints, the Labdane is expected to have a role of maintaining joint health. Here, we firstly report the clinical effect of the Labdane on knees. In an uncontrolled single-arm open-label trial, 60 healthy subjects (ages 48 to 88 years) with a discomfort in the knee joint received 0.065 mg of the Labdane-rich LTDT (labdane-type diterpene compounds) per day for 8 weeks. The discomfort of the knee joint was measured every 4 weeks from the start of intake until 8 weeks after beginning the test food ingestion using the visual analog scale (VAS). The results of each VAS score were 4.7 ± 2.2 at 0 weeks, 3.4 ± 2.3 at 4 weeks and 2.6 ± 2.3 at 8 weeks. Statistically significant differences were observed at 4 weeks ($p < 0.001$) and 8 weeks ($p < 0.001$) against the 0 weeks of intake. No adverse effects were observed. This preliminary study provided a basic finding that taking the Labdane-rich LTDT 0.065 mg/day for 4 and/or 8 weeks in healthy adult men and women with a discomfort in the knee joint is confirmed to be significantly effective and safe.

***KEY WORDS*:** foods with functional claims, labdane, joint, knee, labdane-type diterpene compounds

Introduction

Japan faces the challenges of a super-aging society. One of these issues is known as locomotive syndrome, which is a salient feature when considering a healthy life expectancy. Locomotive syndrome, also referred to as motor organ syndrome, and commonly known as "Locomo" was first proposed by the Japanese Orthopedic Association in 2007. It is defined as "the need for long-term care and a high risk of long-term care due to musculoskeletal disorders" [1]. The major cause of Locomo is cartilage exhaustion due to aging.

It has been observed that cartilage exhaustion occurs most often in the knees. It is estimated that a total of 25.3 million people (42.6% male, 62.4% female) of Japanese over the age of 40 have problems with their knee joints [2]. Since the knee joint bears a higher load than the other joints, the frequency of occurrence of knee problems is higher than that of other joints. As symptoms worsen, discomfort increases. These adverse conditions affect walking and cause daily activities to be restricted. Locomo is one of the main factors that result in a significant deterioration in the overall quality of life (QOL) in elderly people [3].

The "Overview of the 2013 National Living Basic Survey", listed the main cause for the need for nursing care, as well as the amount of required care, to be "joint problems" with 20.7% of the population. This was followed by "weakness due to aging" at 15.4%, and "fractures/falls" at 14.6% [4]. According to the "Physical Activity Standards for Health Promotion 2013", the ability to maintain and increase appropriate physical activity, as appropriate to the life stage, can reduce the risk of Locomo [5].

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These reports indicate that maintaining and improving the health of the knee joint is a key factor when considering the improvement of healthy life expectancy. In turn, this is consistent with the concept of the functional labeling food system. Since its launch in Japan in 2015, the new functional labeling food system has had a reduction of knee discomfort as one of its most important goals.

Problems with the knee joint can occur not only for the elderly but for the general public as well. Knee joint problems can result in psychosocial factors, such as stress, as well as physical limitations. Finally, it has been empirically observed that problems with knee joints reduce not only personal QOL, but also work productivity.

Curcumin is a major component of spring turmeric and autumn turmeric. However the Japanese white turmeric contains little or no curcumin. The Japanese white turmeric does contain a labdane-type diterpene compound (LTDT), which is an essential oil component. It contains 85 to 90% labdane 8 (17), 12-diene-15, 16-dial as its main component (hereinafter referred to as ("the Labdane"). Labdane is found in trace amounts in ginger, turmeric, and plants of the genus *Hana-myoga*. The Japanese white turmeric contains 80-100 times the concentration of labdane in comparison with them. Additionally, white turmeric leaf and/or rhizome extract, which contains these large quantities of labdane, has demonstrated the ability (*in vitro*) to inhibit the enzyme that causes the degradation of hyaluronic acid (HA). Since HA is considered an important fluid that is known to lubricate joints, its preservation is considered important in maintaining good joint health[6]. However, there are still no reports of clinical studies focusing on the consumption of labdane. Although this labdane containing food has already been sold for 4 years from 2015 to 2019, with no reported adverse effects, no organized studies have been done to determine its efficacy. The purpose of this study is to examine the effects of the labdane on discomfort in the knee joint. In this study, food containing the Labdane-rich LTDT (0.065 mg/day) was continuously taken for 8 weeks by healthy adult males and females. The population ranged in age from 48 to under 90 years. All had knee discomfort as accessed using the visual analog scale (VAS).

Methods

Participants

Subjects were recruited from those who wished to purchase healthy food and had discomfort in their joints. Among the 91 applicants, 60 healthy subjects, who met the selection criteria shown in ***Fig. 1*** and did not violate the exclusion criteria, were enrolled.

Written and verbal explanations were given as to the purpose, method, and expectations of the study. The 60 enrollees, ages 48 to 88 years, all signed informed consent forms.

During the observation period, subjects were instructed not to make substantial changes in lifestyle habits such as eating, exercising, smoking, and taking medicine. They were also told not to engage in excessive exercise that greatly deviated from their normal daily routine. Subjects were also asked to ingest the test food per daily allotment and to avoid

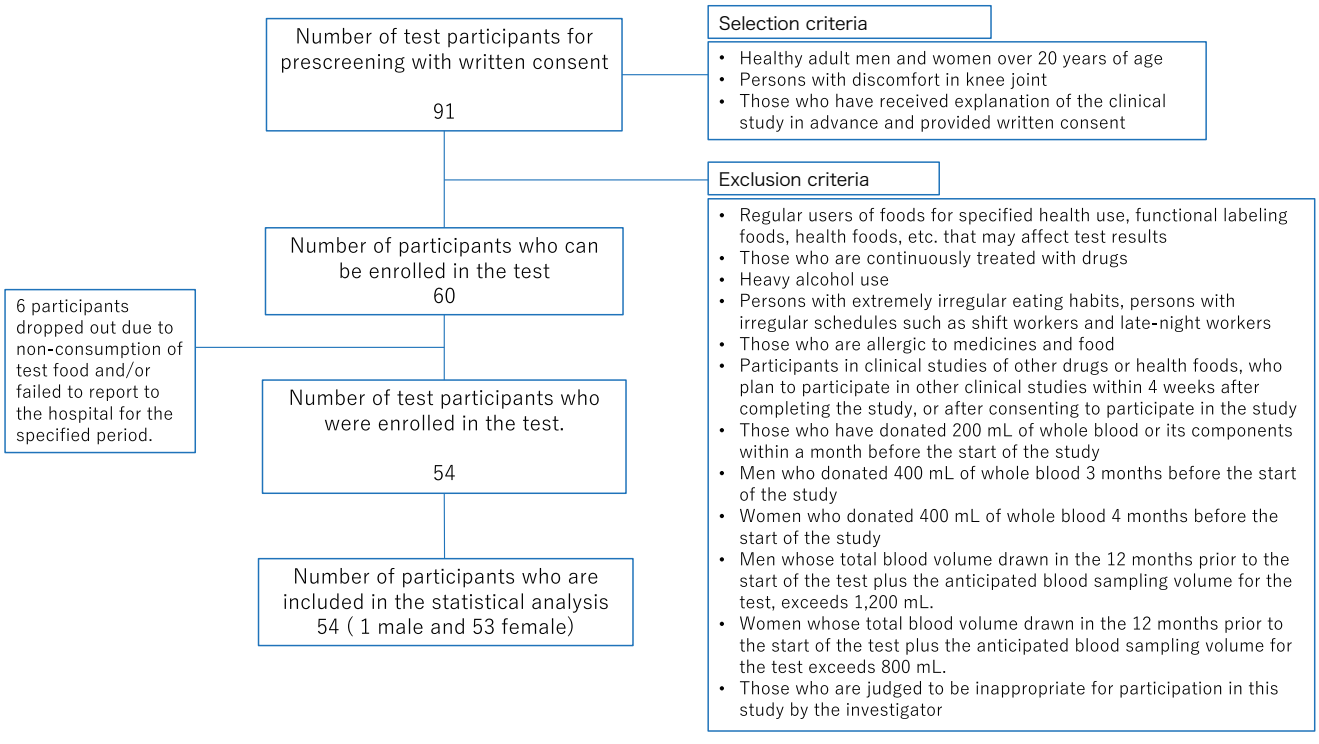

Fig. 1. **Flow chart of the clinical trial.**

consuming new health foods or continuous intake of food for specified health use. During the observation period, they were instructed to generally conduct their life as before the test as much as possible.

Materials and test food

The test food used was manufactured by Aliment Industries Co., Ltd as a soft capsule and includes the Labdane-rich LTDT 0.0325 mg/capsul as test food. Since the daily intake was 2 capsules, the daily total intake of the Labdane-rich LTDT was 0.065 mg.

Study design

The test food intake period for this study was 8 weeks, with observations was conducted every week from the start of intake until 8 weeks after beginning the test food ingestion. The test food was administered as 2 capsules per day with no time of day specified for consumption.

Although originally planned as a randomized, double-blind, parallel-group comparison study, the trial design was changed to an uncontrolled one-arm open-label test. This was because during the informed consent, subjects voiced a strong desire to receive the Labdane containing food in order to see if their knee discomfort could be ameliorated. In consideration of the fact that knee cartilage does not improve, but rather continues to deteriorate, the study methodology was modified.

Besides the ethical considerations, it was concluded that the purpose of the test could be adequately achieved by comparing it with the VAS values from the beginning of the study to the final evaluations.

Visual analog scale (VAS)

Questionnaire survey

The discomfort of the knee joint was measured by using VAS. VAS corresponds to a graphic rating method, which is a psychological rating system [7]. VAS generally uses a 10 cm line segment. VAS assesses the subjective degree of a question item by marking it on a straight line. VAS is a way of taking difficult to rate symptoms, such as pain, and quantitatively simplifying them into easy to understand, and easy to measure judgments [8].

Analysis of data

Analysis of target group

The full analysis set (FAS) excludes all study subjects who did not consume the test food as well as those who failed to report to the hospital for the specified period. Six subjects dropped out due to non-consumption of the test food and/or failed to report to the hospital for the specified period, and 54 (1 male and 53 female) subjects were analyzed. Consequently, 54 subjects completed the study and had the data analyzed.

Analysis method

The results of VAS on knee joint discomfort were used as evaluation indices, and the values at 4 and 8 weeks were compared with 0 week. SPSS Statics 26 (IMB Japan, Tokyo, Japan) was used for the statistical analysis. The data analysis was conducted by Friedman test with Bonferroni correction. The numerical values determined were: mean, standard deviation or frequency, with a significance level of +/– 5%.

Results

Background of test subjects

Of the 54 test subjects, there was 1 male and 53 females. Their ages ranged 48-88 years old with an average age of 64.6 ± 8.2 years.

Effectiveness of analysis

Analysis in all case

Comparison of VAS scores relating to the enrollees' knee discomfort was made between test food intake 0 week (4.7 ± 2.2), 4 weeks (3.4 ± 2.3), 8 weeks (2.6 ± 2.3). In the analysis, statistical differences were observed between 4 weeks ($p < 0.001$), 8 weeks ($p < 0.001$) and 0 weeks of intake (***Fig. 2***). Since the difference was statistically significant, subgroup analysis on age and gender was not performed.

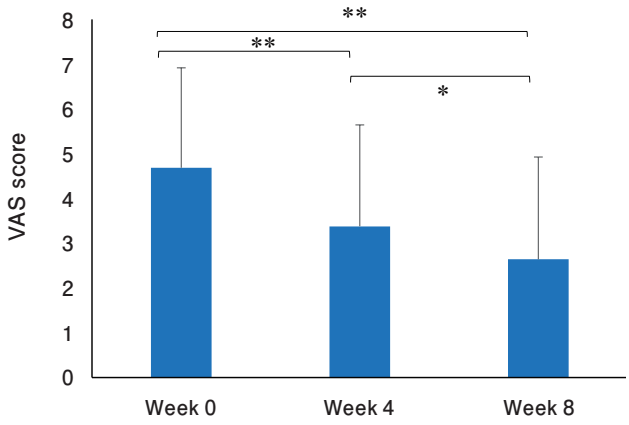

Fig. 2. **VAS evaluation average.**

Results are expressed as mean ± SD, n = 54, *p < 0.05, **p < 0.001 by Friedman test. VAS, visual analog scale; SD, standard deviation.

Safety evaluation

No adverse events were reported by any study subjects during the intake period. Under the test conditions, long-term (8 weeks) intake of the test food was considered safe.

Discussion

This study examined the effects that consumption of white turmeric with labdane had on healthy adults with discomfort of the knee joint. In all cases, a significant improvement was observed in the measured values of VAS scores compared to the start of the test food intake to 4 and 8 weeks. The study results indicate the ingestion of the Labdane-rich LTDT 0.065 mg/day alleviates knee joint discomfort.

The reduction of discomfort in the knee joint was notable in subjects who took food containing the Labdane. Efficacy was seen 4 weeks after ingestion, and it is considered that the subjects felt a reduction in knee joint discomfort even earlier.

In this study, female subjects greatly outnumbered male subjects [9-11]. This is thought to reflect the fact that there are gender differences in epidemiological cases involving the knee joint and that women outnumber men in this category. The subjects were considered elderly with an average age of over 70 years. This resulted from the fact that individuals often have knee problems caused by aging. Conversely, younger persons with discomfort with knee joints have often suffered from some kind of trauma or disease. As such, they usually were already receiving treatment for their ailment. No invasive evaluation methods were selected because the study was designed for healthy subjects. In addition, evaluation using MRI, etc. was not performed because of patient measurement burden and test cost. Future studies will be necessary to further verify the effectiveness of the Labdane. If it is possible to obtain an understanding of the subject and the ethics committee, it is necessary to consider a study based on objective evaluation criteria using a randomized parallel-group comparison design. Furthermore, based on the findings obtained in this study, it is necessary to verify the mechanism of action of the Labdane on the discomfort of the knee joint.

Labdane is contained in various plants as well as the Japanese white turmeric [12-21]. The effects reported for labdane are as follows: antitumor effect [15], cytotoxicity [16], antibacterial effect [17, 20], anti-inflammatory effect [18], α-glucosidase activity inhibition [19, 21], lipase activity inhibition [21], and blood compatibility enhancement [20]. In the evaluation of blood compatibility, the risk of thrombus formation is evaluated by the index of hypercoagulation and platelet activation, against which labdane acts suppressively. Furthermore, labdane has recently been reported to have an inhibitory effect on HA degradation [6]. HA, in the joint cavity, has actions of cartilage lubrication, anti-inflammation, and anti-glycation, and, therefore, plays an important role in maintaining joint homeostasis [22]. When, based on these reports, estimating the mechanism of inhibitory action for joint pain, the important factors are actions of anti-inflammation and inhibition of HA degradation.

A person who has discomfort of joints often complains of symptoms characteristic of neuropathic pain, where GABAA receptors are involved [23, 24]. GABAA receptor is one of ion channel type receptors and ion channel built-in type receptors, and the ligand is γ-aminobutyric acid (GABA), an inhibitory neurotransmitter. Pain is often chronic, and it has been reported that central sensitization mechanisms work followed by overlapping psychological factors [25]. That is, it becomes sensitive to pain, which is generated by a stimulus that normally does not cause pain (allodynia). As a result, the pain becomes wider and longer lasting [26].

Chronic pain excites sympathetic tone and vasomotor nerves, causing vasoconstriction and muscle tone elevation. Consequently, blood circulation deteriorates, leading to the generation of "algesic substance." Some of the LTDTs have actions of platelet aggregation inhibition [27] and vascular smooth muscle relaxing [27, 28], thus contributing to maintenance of blood flow. It is necessary to verify the effects of labdane on vascular smooth muscle.

It has been reported that sclareol and andrographolide, which are LTDTs, are effective for osteoarthritis (OA) [29] or rheumatoid arthritis (RA) [30, 31]. Additionally, another report showed the GABAA receptor modulation by *Curcuma kwangsiensis* rhizome extract [32].

Under physiological conditions, GABA acts, via the GABAA receptor, to suppress the nerve excitation. However, once peripheral nerves are damaged, microglial cells become activated in the spinal cord, via activation of P2X4 (purinergic receptor 4) receptor, and release brain derived neurotrophic factor (BDNF). As a result, the effect of GABA released by tactile stimulation on secondary neurons changes from inhibitory to excitatory, and accordingly tactile stimulation causes stronger pain [33]. These findings suggest that GABAA receptor modulators may alleviate neuropathic pain [34], that is interesting. The action of labdane on GABAA receptors needs to be verified in the future.

In Japan, there are about 30 products bearing functional food labeling that claim or imply relief of the discomfort of the knee joint. Most of them are chondroitin, HA, and/or cartilage components such as collagen or fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). However, low molecular weight compounds like labdane are entirely new.

Conclusion

The statistically significant results of this study at 4 and/or 8 weeks indicated that healthy individuals, with knee joint discomfort, experienced safe and effective relief after taking 0.065 mg/day of Labdane from the extract of the Japanese white turmeric.

Conflict of Interest Statement

The present study was partly supported by Veritas Co., Ltd., and Veritas Hanbai Co., Ltd.

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