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Original Article

Effect of *Lythrum salicaria* Extract on Body Fat Reduction: A Protocol for a Randomized, Double-Blinded, Placebo-Controlled Clinical Trial

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체지방 감소에 대한 털부처꽃 추출물의 효과: 무작위배정, 이중눈가림, 대조군 비교 인체적용시험 프로토콜

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Objectives: Obesity is a globally prevalent public health issue. Hence, there is a need for the development of safer and more effective anti-obesity drugs. *Lythrum salicaria*, a traditional medicinal herb used for centuries, has been reported to improve lipid metabolism and fat accumulation. It also has a low toxicity profile. Therefore, its potential as a functional ingredient in health functional foods needs to be evaluated.

Methods: In this randomized, double-blind, placebo-controlled clinical trial, 90 participants will be randomly assigned to either the experimental or control group. Each subject will orally receive *L. salicaria* extract (1,350 mg/day) (500 mg *L. salicaria*+850 mg lactose as vehicle) or lactose (1,350 mg/day) as a hard capsule formula for 84 days (12 weeks). The primary outcome will be body fat mass (kg), which will be assessed using dual-energy x-ray absorptiometry (DXA) (performed only at visits 2 and 4). Secondary outcomes include body mass index, body weight, waist-to-hip ratio, body fat percentage (%) measured using DXA, lean body mass (kg) measured using DXA (assessed only at visits 2 and 4), lipids (total cholesterol, triglyceride, high-density lipoprotein cholesterol, and calculated low-density lipoprotein cholesterol), free fatty acid, high sensitivity C-reactive protein, adiponectin, and leptin.

Conclusions: This protocol will be implemented after approval of Institutional Review Board of Pusan National University Korean Medicine Hospital (approval number: PNUKHIRB-2022-08-002) and registration with the Korean National Clinical Research Information Service (CRIS) (CRIS-KCT0008060). The results of this trial will provide potential of *L. salicaria* as a new anti-obesity functional food with fat-reducing effects and low toxicity.

Key Words: *Lythrum salicaria*, Body fat reduction, Randomized controlled trial, Obesity

Introduction

Obesity is defined as abnormal or excessive fat accumulation in individuals with a body mass index (BMI) of 30 kg/m^2 or greater[1]. More than one in two adults in the Organization for Economic Cooperation and Development (OECD) countries are classified as overweight or obese. The obesity rate in Korea was 5.3% in 2015, which is the lowest among all OECD countries except Japan. Although the prevalence of obesity in Korea has stabilized over the past decade, the obesity rates have been projected to increase at a faster pace[2]. The prevalence of obesity depends on education levels and occupational conditions, which could affect household income level, food intake pattern, and the quality of food people intake[3]. Due to the widespread prevalence of obesity worldwide, there is a need to discover and develop safe and effective anti-obesity drugs. However, anti-obesity drugs have various side effects[4]. Hence, studies searching for medicines derived from natural products have been conducted. These are hypothesized to be beneficial for human health and have fat-reducing effects.

Purple loosestrife-*Lythrum salicaria* L. is a perennial grass that has a worldwide distribution, including in South Korea, Japan, China, Siberia, Iran, Middle Asia, Europe, and North America. It has been used to treat diarrhea, external bleeding, and hemorrhage in Eastern countries[5]. It has also been used for gastrointestinal disorders and wound healing in Europe. Components of *L. salicaria* are salicarinin, lythrine, lythranine, lythranidine, lythramine, lythrancine, flavonoids, phenolic acids, anthocyanins, etc.[6] In previous studies, ethanol and water extracts of *L. salicaria* were reported to have potential antioxidant, anti-inflammatory, antidiabetic, anti-obesity, and antibacterial activities[7]. The leaf extract of *L. salicaria* has been reported to improve lipid metabolism and fat accumulation in a high-fat diet mouse model[8]. In a previous single oral dose toxicity test carried out by our research team, no abnormal clinical signs or mortalities were observed among the animals. Hence, *L. salicaria* demonstrated low toxicity in the animal body[9].

Therefore, the aim of this study is to determine the potential of *L. salicaria* as a fat-reducing ingredient in functional foods and to analyze and evaluate its oral intake safety by designing a protocol for a randomized, double- blinded, placebo-controlled clinical trial. This study not only observes BMI, body measurements, and various blood test indicators, but also measures body fat mass, body fat percentage using dual-energy x-ray absorptiometry (DXA) to provide actual quantitative change in fat in the human body.

Materials and Methods

1. Research overview and ethics

This is a randomized, double-blind, placebo-controlled clinical trial. This study is being conducted in accordance with the Declaration of Helsinki, after approval of Institutional Review Board (IRB) of Pusan National University Korean Medicine Hospital (approval number: PNUKHIRB-2022-08-002) on September 14, 2022. The study protocol was registered with the Korean National Clinical Research Information Service (CRIS) (CRIS-KCT0008060) on January 2, 2023. All participants will sign an informed consent form for participation in the study. Any participant in this study can withdraw consent or voluntarily stop participation at any time for any reason. The trial will begin by recruiting patients on February 16, 2023. Once the participants voluntarily signs a written consent form, they will undergo a baseline assessment. If they fulfill the inclusion criteria, they will be randomly assigned to either the experimental or the control group in a 1:1 ratio using a sealed envelope method by a researcher not involved in the assessments and interventions. The flow chart of clinical trial process is described in Fig. 1. The overall schedule is described in Table 1.

2. Selection of subjects

1) Inclusion criteria

(1) Individuals who consented to participate in this study and voluntarily signed a written consent form

(2) Adult men and women between the ages of 19 and 75

(3) Individuals with a BMI of 23 to 30 kg/m^2

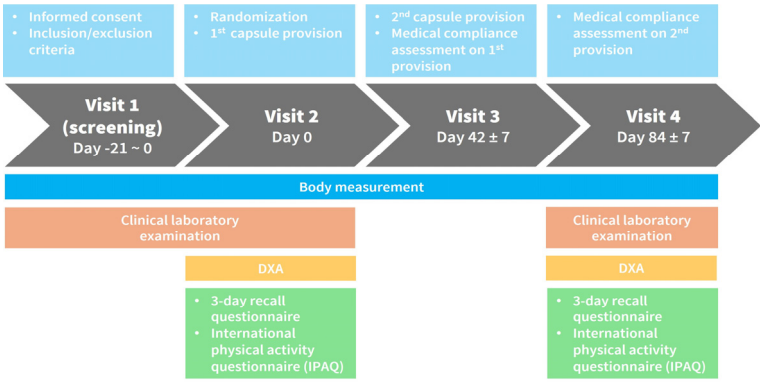

Fig. 1. Flow chart of the clinical trial process. DXA: dual-energy x-ray absorptiometry.

Table 1. Clinical Trial Schedule

Visit	Visit 1 (screening) (−21~0 day)	Visit 2 (0 day)	Visit 3 (42±7 day)	Visit 4 (84±7 day)
Informed consent	○			
Incousion/exclusion criteria	○	○		
Demographic information	○			
Past history investigation	○			
Physical examination	○	○	○	○
Vital signs	○	○	○	○
Clinical laboratory examination	○	○		○
Experimental/control capsule provision		○	○	
Outcome measures		○	○	○
Questionnaires		○		○
Adverse events			○	○
Co-administraion drug investigation		○	○	○
Medication compliance assessment			○	○

2) Exclusion criteria

(1) Individuals with severe cerebrovascular disease (cerebral infarction, cerebral hemorrhage, etc.), heart disease (angina pectoris, myocardial infarction, heart failure, arrhythmia requiring treatment), or malignant tumors present within the last six months

(2) Patients with uncontrolled hypertension (blood pressure of 160/100 mmHg or higher, measurement obtained after the subject had rested for 10 minutes)

(3) Diabetic patients with poor blood sugar control (fasting blood sugar of 160 mg/dl or higher)

(4) Individuals who are being treated for hypothyroidism or hyperthyroidism

(5) Individuals with creatinine measurement values more than twice the upper limit of normal values determined by the research institution

(6) Participants with aspartate aminotransferase (glutamic-oxaloacetic transaminase) or alanine transaminase (glutamic-pyruvic transaminase) measurement values more than three times the upper limit of normal established at the research institution

(7) Participants with the complaint of severe gastrointestinal symptoms such as heartburn and indigestion (lactose intolerance)

(8) Participants who have been taking drugs that affect weight within the past month

(9) Individuals who have participated in a commercial obesity program within the last 3 months

(10) Individuals who have participated in or plan to participate in other clinical trials within the last month
(11) Participants with a history of alcohol abuse
(12) Individuals with a history of smoking within the last 3 months
(13) Participants who are pregnant/lactating or plan to become pregnant during the trial period
(14) Participants with an allergic reaction to component foods
(15) Individuals who are judged to be unsuitable by the tester for other reasons

3) Dropout criteria

(1) Request by the subject to withdraw from the trial or stop participation/testing
(2) Violation of inclusion/exclusion criteria
(3) Occurrence of adverse effects which were directly related to the experimental drug and were judged to highly affect the test (including serious adverse effects)
(4) Non-compliance (less than 80%) or unobservable trial food intake

4) Sample size calculation

This study aimed to collect data from 90 subjects, with 45 subjects included in the experimental group and 45 in the control group. According to Min et al.[10], the difference in body fat mass measured at week 8 of the trial as compared with the baseline values was 1.21 kg; the pooled standard deviation was approximately 2.38. Sargassum confusum extracts used in the study of Min et al.[10] showed suppressive effects on lipase and α-amylase activity, and *L. salicaria* extract also suppresses pancreatic lipase activity. Thus, in this study, we estimated that the body fat mass in week 12 of the trial as compared with the baseline would be approximately 1.5 kg with a 10% dropout rate.

3. Study design

1) Randomization and blinding procedures

To conduct this clinical trial scientifically and objectively, the final selected participants will be assigned to either the experimental or the control group in a 1:1 ratio using the block randomization method. Since the block size can allow for predictions about randomization, it will be determined arbitrarily or randomly by a statistical expert. Therefore, the size and number of blocks for randomization are unknown. The total number of random assignments will be generated to approximately 120% of the target recruitment. Subjects will be assigned a three-digit registration number (randomized number) based on the order they are recruited according to the randomization details, and the test food or control food according to the corresponding code to each subjects. Capsules prescribed to both the experimental and control groups have been produced with the same appearance and packaging to maintain double blindness. Once a participant is registered and assigned a randomization number, the number cannot be used again even if the participant drops out. The assignment of the subject's registration number has been kept sealed by the principal investigator. It will not be disclosed until the end of the trial. In case of a serious adverse event, when disclosure of an issued registration number is inevitably required, it will be managed in a separate blinded envelope format for each subject, so that only the random assignment information of the subject can be browsed.

2) Intervention

The experimental group will be prescribed oral *L. salicaria* extract (1,350 mg/day) (500 mg *L. salicaria*+850 mg lactose as vehicle) as a hard capsule formula. The control group will be orally administered lactose (1,350 mg/day) as a hard capsule formula. Each participant will be instructed to consume capsules with water at breakfast, lunch, and dinner for 84 days (12 weeks).

Treatments or oral drugs already administered at the time of enrollment or those added during the trial period will be considered as concomitantly administered. If co-administration is required, the treatment information will be written in a case report form or any other form of document, and the existing therapy should remain unchanged. Co-administration during the trial will be minimized; it will only be done if

it is considered necessary for the participants' welfare and does not affect the intake of the trial capsules.

4. Outcome measures

1) Primary outcome

Body fat mass (kg), measured using DXA (performed only at visits 2 and 4, before starting and after completing the trial capsules, an approximately 84 day interval. DXA will be carried out in Pusan National University Yangsan Hospital.)

2) Secondary outcomes

(1) BMI
(2) Body weight
(3) Waist-to-hip ratio
(4) Body fat percentage (%), and lean body mass (kg), measured using DXA (performed only at visits 2 and 4)
(5) Lipids (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and calculated low-density lipoprotein cholesterol)
(6) Free fatty acid
(7) High sensitivity C-reactive protein
(8) Adiponectin
(9) Leptin

The body weight and BMI is measured and calculted by anthropometer. Wast-to-hip ratio of each participants is calculated after the waist and hip circumference are measued by a researcher. Lipids, free fatty acid, high sensitivity C-reactive protein, adiponectin, and leptin are measured via clinical laboratory examination. Every secondary outcomes are measured at visit 1, 2, and 4, except for body fat percentage and lean body mass measured by DXA at visit 2 and 4.

5. Data collection

The demographic variables and medical histories of the participants will be collected during the screening assessment. A physical examination will be performed, and vital signs will be recorded. At visits 2 (the day participants receive the trial capsules) and 4 (the day participants visit after completing the trial capsules), the participants will complete a 3-day recall questionnaire and the International Physical Activity Questionnaire. Every document related to the trial is considered confidential and will be stored in locked cabinets. Documents will be classified only by the subjects' registration numbers and not by their names, to preserve anonymity.

This trial must be in compliance with the test plan, except for cases where it is necessary to immediately eliminate risks to participants. If any violation occurs, details and reasons shall be recorded and reported to the IRB.

6. Statistical analysis

A per-protocol analysis has been adopted as the main analysis method. An intention-to-treat analysis will also be performed, but only for reference. The subjects for efficacy evaluation will consist of participants who have been included in the intention-to-treat (ITT) analysis and have completed the clinical trial without any severe adverse events until the 4th visit. For ITT analysis of participants with at least one trial food intake, data regarding the primary outcome should be obtainable. All statistical significance tests will be conducted at the 5% confidence level.

Demographic variables will be summarized by group and compared between the groups. Continuous data will be tested using the t-test (or Wilcoxon's rank-sum test), while categorical data will be tested using the chi-square test (or Fisher's exact test). If the data follows a normal distribution, an independent t-test will be performed to test differences in continuous variables between the two groups, and a paired t-test will be performed to test differences across time within the groups. Otherwise, the Mann-Whitney U test and Wilcoxon signed-rank test will be performed. The normality of continuous variables will be assessed using the Shapiro–Wilk test with a 5% confidence level. Variables that could affect each main outcome measurement will be considered as control variables if there is a difference between the groups, and analysis of covariance will be additionally performed.

7. Safety

The occurrence of side effects will be assessed both dur-

ing the food intake period and at the last visit. Adverse events will be classified as mild, moderate, or severe. Every severe adverse event, regardless of its association with the trial capsule, will be reported to the IRB and the subject will be requested to withdraw from the trial. The researcher must continue to report the adverse event until the end, such as when the adverse drug reaction resolves or follow-up is no longer possible.

Discussion

L. salicaria has long been used as a traditional medicinal herb in Eastern and Western countries[9]. It is a common plant with a worldwide distribution. It is mainly used to treat external bleeding, hemorrhage, and gastrointestinal illness[4-6]. *L. salicaria* has previously demonstrated fat-reducing potential by inhibitory action against pancreatic lipase, which disassembles triacylglycerol into 2-monoacylglycerol and fatty acid[7]. The ethanol extract of *L. salicaria* significantly decreased the levels of serum triglycerides, total cholesterol, and liver triglycerides in rats fed a high-fat diet, thereby improving lipid metabolism and reducing fat accumulation and body weight[8]. In the single oral dose toxicity limit test, each rat was orally administered 5,000 mg/kg water extract of *L. salicaria* and survived. No abnormal signs or mortalities were observed among the animals, indicating a lethal dose only higher than 5,000 mg/kg and no toxicity after single-dose intake[9].

Existing anti-obesity drugs have been associated with adverse effects such as cardiovascular disease, fetal toxicity, increased systolic blood pressure, and tachycardia[11]. Hence, natural herbs are receiving more attention because of their safety profile. *L. salicaria* has potential as an anti-obesity functional food with fat-reducing effects and low toxicity. Since this study uses a single herb, not the form of mixture of various herbs or decoction, it suggests the possibility of conducting larger-scale clinical trials in the future. Additionally, this study sets lean body mass measured by DXA as an outcome measure, as well as changes in BMI, body measurements, and body fat percentage. This helps to actually verify the quantitiative changes in fat in the human body.

Although the fat-reducing effect of *L. salicaria* is relatively recently discovered and further research is needed compared to its other effects, its excellent toxicity and side effect in animal study suggest significant research value. This study aims to introduce new herbal medicine and provide clinical evidence for Korean Medicine treatment on obesity.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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