Lactic Acid Fermentation of Chinese Yam (*Dioscorea batatas* Decne) Flour and Its Pharmacological Effect on Gastrointestinal Function in Rat Model

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Abstract To develop a health-aid preparation of Chinese yam (*Dioscorea batatas* Decne), lactic acid fermentation was attempted using a mixed starter comprising of *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidus*. The anaerobic fermentation of a 5% Chinese yam flour suspension gave a uniform suspension of pH 4.35, containing 7.76×10^6 CFU/mL lactic acid bacteria (LAB), and which was found to be acceptable to the panel from a sensory assessment. During the administration of the lactic acid fermented (LAF) Chinese yam to Sprague Dawley rats for 6 weeks, a smaller body weight gain, but greater excretion of feces were observed, implying the creation of a healthy gastrointestine on the administration of LAF Chinese yam, which was also confirmed by the gastrointestinal motility of the feed in rats fed on LAF Chinese yam. The constipation induced by loperamide was further suppressed in a rat group fed on a LAF Chinese yam diet, which was qualified from healthy gastrointestinal flora established by LAB. A serochemical analysis revealed a slight improvement in the blood glucose, neutral lipid and total cholesterol concentrations on administration of LAF Chinese yam, suggesting LAF Chinese yam could be served as a healthy-aid preparation, even for hyperglycemia or hyperlipidemia patients.

Keywords: Chinese yam, *Dioscorea batatas*, lactic acid fermentation, gastrointestinal function

INTRODUCTION

Yam, the tuber part (rhizome) of the genus *Dioscorea*, is cultivated world-wide as a food source, especially in tropical areas. In Asia, Chinese yam (*D. batatas* Decne) has been used for many years as a traditional medicine (Sanyak in Korean) for the treatment of anorexia, chronic diarrhea, diabetes, seminal emission, and excessive leucorrhea, as described in Pharmacopoeia of the People's Republic of China [1]. Recently, immune response modifying activities of *Dioscorea* rhizomes have been confirmed, including immunomodulation [2,3] and anti-inflammatory [4], anti-tumor [5,6] and anti-osteoporotic effects [7]. In addition, the anti-oxidative activity of dioscorin, a storage protein of *Dioscorea* tubers, has also been extensively investigated [8,9]. Furthermore, the feeding of *Dioscorea* rhizomes has been found to improve certain metabolic abnormalities, includeing hyperglycemia [10], obesity [11], gut function and lipid metabolism [12,13].

Lactic acid bacteria (LAB), as probiotic bacteria, have been known to be beneficial to health, including increased food digestibility and bioavailability of vitamin and minerals, lactose utilization, control of gastrointestinal infection, reduction of liver catabolic products, cancer suppression, reduction of serum cholesterol level, immune stimulation and reduction of blood pressure [14-17]. In Asian countries, lactic acid fermented (LAF) grains, vegetables and fish for their long-term preservation have been taken for many years [18,19]. However, until now, LAF natural herbs have not been reported.

In this study, LAF Chinese yam (*D. batatas* Decne) was prepared, and its pharmacological effect on gastrointestinal function evaluated for the development of a health-aid preparation.

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MATERIALS AND METHODS

Preparation of LAF Chinese Yam

The heat-dried flour of Chinese yam (*D. batatas* Decne) (Sanyak) was purchased from Bukhu Agricultural Cooperative (Andong, Korea). The Chinese yam flour (250 or 500 g) was suspended in 5 L of water and steamed at 100°C for 50 min. After cooling to room temperature, 0.05% of the starter LAB, containing 10^{10} colony forming unit (CFU)/mL, composed of *Lactobacillus acidophilus* ATCC 4356, *Streptococcus thermophilus* ATCC 19258, and *Bifidobacterium bifidus* ATCC 29521 (1:1:1), which was pre-cultured on Lactobacillus MRS broth (Difco, IL, USA) at 37°C for 30 h until the optical density at 620 nm reached approximately 0.61, was then inoculated and fermented statically for an anaerobic culture at 37°C during 20 h.

Analysis of LAF Chinese Yam

After serial dilution of the samples, the microorganisms in the LAF Chinese yam culture were counted as the colony numbers that appeared on Lactobacillus MRS agar (Difco), containing 0.002% bromophenol blue, at 37°C for 48 h [20]. The pH of the LAF Chinese yam culture was directly measured using a pH meter, and the titratable acidity determined as the amount of 0.1 N sodium hydroxide consumed to reach a pH of 8.2.

Experimental Animals, Feeding Schedule, and Examination of Intestinal Flora

Specific pathogen-free male Sprague Dawley rats (4~5 weeks old) were purchased from Samtako, Inc. (Osan, Korea). Three animals were housed per cage and fed a rat chow diet (Netsle Purina PetCare Korea, Ltd., Seoul, Korea) allowed tap water. The animal room was kept at 23 ± 3°C, with a humidity of 50 ± 10% and repeatedly illuminated with 150~300 Lux at 12 h intervals. The Sprague Dawley rats were divided into 3 groups (n = 9 for each group), and the effects on gastrointestinal function and serochemical improvement examined. The first group, as a control, was fed the rat chow diet only, the second group was fed the rat chow diet with the addition of 10% yogurt made from milk using the same procedure and LAB as used for the Chinese yam fermentation, and the third group was fed the rat chow diet mixed with 10% LAF Chinese yam. During the 6-week feeding period, the body weights and amounts of feces produced were measured weekly. In order to compare the number of lactose-utilizing bacteria in the intestine of the rats, feces was collected weekly during the feeding schedule, suspended in saline solution (100 mg/mL), and after serial dilution, were spread on eosin-methylene blue (EMB) agar (Difco, IL, USA) and cultivated anaerobically at 37°C for 24 h.

Examination of Gastrointestinal Function

To test the effect of LAF Chinese yam on gastrointestinal function, after the 6 weeks of feeding, the animals in each group were fasted for 1 day, and then directly injected through the esophagus with as much as 1 mL/100 g of body weight of 10% barium sulfate solution. After 30 min, the rats were anesthetized using urethane, and distance the barium sulfate had moved in the gastrointestinal tract was observed. Also, the animals were administered 0.15 mg loperamide/100 g of body weight twice a day for 5 days, to induce constipation, and the amount as well as the number of feces excreted was observed.

Serochemical Analysis

After 6 weeks of the diets, serum was collected from the abdominal aorta and analyzed for glucose, neutral lipid and total cholesterol. The glucose concentration was determined by the glucose oxidase method, using an YSI Glucose Analyzer (YSI 1500, USA). The serum neutral lipids and total cholesterol concentrations were measured using a kit obtained from the Sigma Chemical Co. (St. Louis, MO, USA).

Sensory Evaluation

The sensory tastes of LAF Chinese yam were evaluated according to the 5-point scores method [21] by a well-trained panel of 25 persons.

RESULTS AND DISCUSSION

Preparation of LAF Chinese Yam and Its Sensory Evaluation

To prepare a homogenous suspension of Chinese yam flour for drinking, lactic acid fermentation using a mixed starter composed of three LAB, *L. acidophilus, S. thermophilus,* and *B. bifidus,* was attempted. When a 5% suspension of Chinese yam flour was subjected to an aerobic fermentation, a uniform pH 4.35 suspension, without precipitates, was obtained, which contained 7.76

Table 1. Lactic acid fermentation of Chinese yam flour

Chinese yam flour (%)	pH	Titratable acidity (% lactic acid)	No. of lactic acid bacteria (log CFU/mL)	Sourness[a]	Sweetness[a]	Acceptability[a]
5	4.35 ± 0.12	0.24 ± 0.01	6.89 ± 0.32	1.42 ± 0.12	2.15 ± 0.21	4.37 ± 0.32
10[b]	4.31 ± 0.10	0.25 ± 0.01	7.02 ± 0.11	1.58 ± 0.15	2.25 ± 0.22	1.25 ± 0.13

[a] Excellent (5), good (4), average (3), poor (2), and very poor (1).
[b] Gruel-shaped.

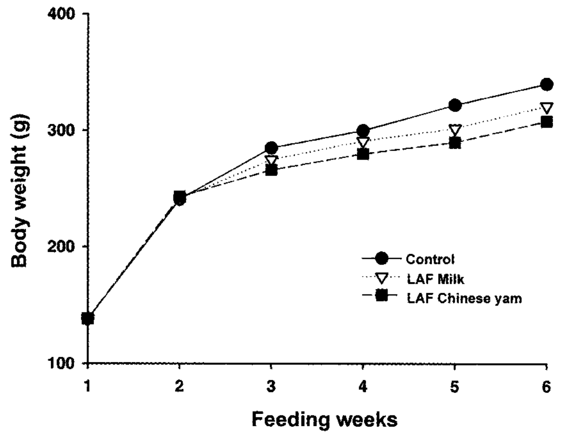

Fig. 1. Body weight change of Sprague Dawley rats during feeding on LAF Chinese yam.

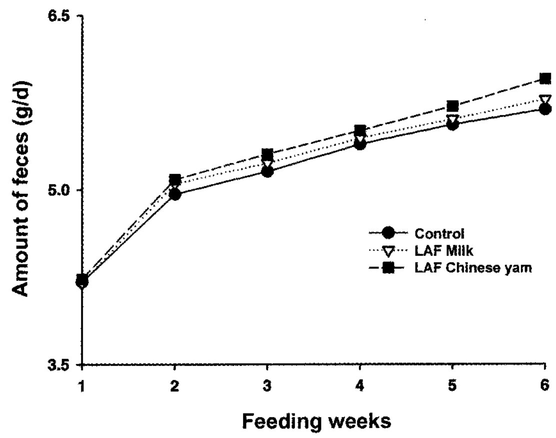

Fig. 2. Amount of fecal excretion of Sprague Dawley rats during feeding on LAF Chinese yam.

$\times$ 10^6 CFU/mL LAB, as shown in Table 1. In contrast, a 10% suspension of Chinese yam flour gave a gruel-like fermented paste of pH 4.31 containing 1.05 $\times$ 10^7 CFU/mL.

The LAF suspension of Chinese yam flour was evaluated by 25 well-trained panelists. The panel reported that the LAF Chinese yam suspension were relatively sour, with low sweetness, but acceptable for taking as a 5% suspension of fermented Chinese yam flour (Table 1). In contrast, the 10% suspension of fermented Chinese yam flour was not acceptable to the panelists because of its gruel-like texture.

The storage of white yam (*D. rotundata* L.), a major food source in tropical countries, in 1.5% brine solution has been reported to establish the microbial flora, including the *Pediococcus*, *Lactobacillus*, *Pseudomonas*, and *Bacillus* species [22]. The final white yam, fermented by naturally established microbial flora, had a pH value and titratable acidity of 5.63 and 0.07, respectively. With respect to the above, the artificial fermentation of Chinese yam (*D. batatas* Decne) using selected beneficial LABs could provide a health-aid preparation, as it has previously been used as a traditional medicine in Oriental countries for many years. However, sweet ingredients, such as oligosaccharide or fructose, should be added to improve its sensory characteristics.

Effect of LAF Chinese Yam Administration in Animal Model

To evaluate the effects of the administration of the LAF suspension from 5% Chinese yam flour, the suspension was fed to Sprague Dawley rats for 6 weeks, and compared with the control and LAF milk groups. The smallest body weight gain was observed in the group fed with the LAF Chinese yam, as shown in Fig. 1. After the 6-week administration, the average body weight of rats was 308 ± 5.3 g, which was 5 and 10% less than the groups fed with LAF milk and the control, respectively. Conversely, the feeding of LAF Chinese yam was proven to increase the amount of fecal excretion by as much as 6.0 ± 0.07 g/d after 6-week, which was 2 and 5% more than in the group fed with LAF milk and the control, respectively (Fig. 2), reflecting that LAF Chinese yam can provide an improved healthy gastrointestinal function, and further prevented rats from becoming so fat.

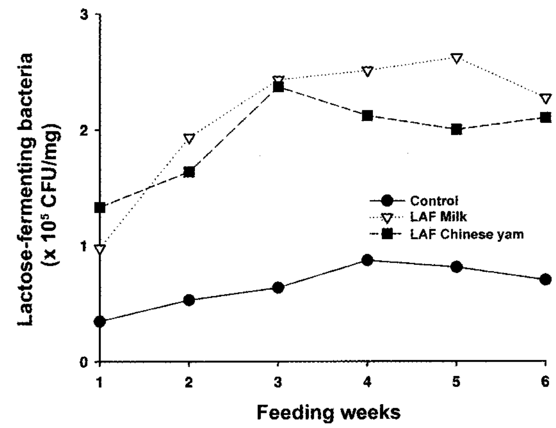

Fig. 3. Change of microbial flora in the intestine of Sprague Dawley rats during feeding on LAF Chinese yam. The number of lactose-utilizing bacteria in rat feces was counted after anaerobic cultivation on EMB agar plates at 37°C for 24 h.

The number of lactose-fermenting bacteria in the rat intestine was significantly increased, to more than 2 $\times$ 10^5 CFU/mg of feces, by the feeding LAF milk and LAF Chinese yam, but this number remained at a similar level (4~8 $\times$ 10^4 CFU/mg of feces) in the control group (Fig. 3). It is clear that both of LAF milk and LAF Chinese yam could be helpful in establishing beneficial flora in the intestine, including lactose-fermenting bacteria, even though the effect of LAF milk was somewhat greater than that of LAF Chinese yam.

To confirm the effect on gastrointestinal function, the gastrointestinal motility was examined after rearing for 6 weeks. The distance moved by the feed in the group administered LAF Chinese yam was 63.5 ± 4.3 cm/h, which was 6 and 40% further than the group fed with LAF milk

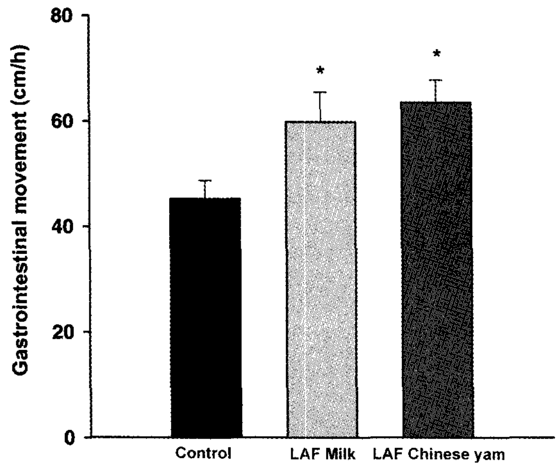

Fig. 4. The gastrointestinal motility of the rat group fed with LAF Chinese yam. The distance the barium sulfate moved in the gastrointestinal tract was measured 30 min after directly injecting a 10% solution through the esophagus.

Table 2. Anti-constipation effect of the lactic acid fermented Chinese yam flour

Group (n = 7)	Fecal excretion (g/d)	No. of fecal pellet
Control	5.72 ± 1.38	3.2 ± 1.8
Rat chow diet + Loperamide	2.91 ± 1.12	5.7 ± 1.7
LAF milk + Loperamide	4.03 ± 1.15	3.2 ± 1.7
LAF Chinese yam + Loperamide	4.33 ± 1.99	3.2 ± 1.2

Table 3. Serochemical effect of the lactic acid fermented Chinese yam flour

Group (n = 7)	Serum glucose (mg/dl)	Serum neutral lipid (mg/dl)	Serum total cholesterol (mg/dl)
Control	124 ± 7.3	36 ± 2.3	122 ± 7.2
LAF milk	119 ± 4.3	32 ± 2.0	118 ± 6.2
LAF Chinese yam	116 ± 3.8	31 ± 1.9	119 ± 6.4

and the control, respectively (Fig. 4), implying that LAF Chinese yam can improve intestinal movement and help the intestinal digestion of food.

Using a rat model of loperamide-induced constipation; the anti-constipation effect was also examined. The treatment of loperamide to the control rat group reduced the amount of fecal excretion by as much as 50.8%, but the number of fecal pellets increased by 78.1% (Table 2). However, the rats fed with LAF Chinese yam or yogurt maintained more than 70% of the amount of fecal excretion, with no significant change in the number of fecal pellets, even though constipation was induced by loperamide. The LAF Chinese yam also provided a better effect than LAF milk, which can be attributed to the healthy gastrointestinal flora established by beneficial LAB.

Serochemical Effect of LAF Chinese Yam

After the 6 week-administration of LAF Chinese yam, the blood of rats was collected and analyzed for serum glucose, serum neutral lipid and serum total cholesterol. As shown in Table 3, the administration of LAF Chinese yam resulted in a slight improvement in the serochemical parameters, even though its effects were not significantly noticeable. This means that LAF Chinese yam could be administered as a health-aid preparation, even to hyperglycemia or hyperlipidemia patients.

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