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Effect of Ethanol Extract of Dried Chinese Yam (*Dioscorea batatas*) Flour Containing Dioscin on Gastrointestinal Function in Rat Model

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In this study, a 40% ethanol extract of Chinese yam flour (*Dioscoreae* rhizoma), containing 177±58 μg/mL of dioscin, was tested in order to evaluate its pharmacological effects on the gastrointestinal tracts of Sprague-Dawley rats. Via the ingestion of the Chinese yam extract, the secretion of gastric acid was suppressed in the rats, and gastrointestinal motility increased by as much as 10%. The fecal quantity of rats fed on the Chinese yam extract also increased, by more than 40% as compared with that of the controls. The Chinese yam extract was found not to affect the growth of normal intestinal bacteria. However, a great deal of lactose-fermenting bacteria was observed in the fecal samples of rats fed for 6 weeks on 2% Chinese yam extract. This finding would appear to suggest that Chinese yam extract not only induces an improvement in digestive capability, but also affects the conversion of some intestinal flora to helpful bacteria. Our serochemical analyses indicated that serum glucose, neutral lipid, and total cholesterol levels were reduced to some degree by long-term feeding on Chinese yam extract. This finding bolsters the notion that Chinese yam extract may prove helpful as a digestion-aiding agent for patients suffering from hyperglycemia or hyperlipidemia.

Key words: *Dioscoreae* rhizoma, Chinese yam, Gastric function, Gastrointestinal motility, Intestinal bacteria

INTRODUCTION

The tuber portion (rhizome) of the genus *Dioscorea*, which has been extensively cultivated as a food in Asia, has been also employed in traditional medicine for the treatment of anorexia, chronic diarrhea, diabetes, seminal emissions, and excessive leucorrhea, as has been described in the Pharmacopoeia of the People's Republic of China (1997). In Oriental regions, the most commonly cultivated species of *Dioscorea* is the Chinese yam (*D. batatas* Decne or *D. opposita* Thunb, Sautour *et al.*, 2004a). Thus far, four important groups of active constituents of the Chinese yam have been isolated and characterized *via* phytochemical analyses. These constituents are: mucilage polysaccharide (Zhao *et al.*, 2005), dioscorin as a storage protein (Gaidamashvili *et al.*, 2004), streoidal saponins, including dioscin (Kawasaki and Yamauchi, 1968; Sautour *et al.*, 2004b), gracillin (Yang *et al.*, 2003) and furostanol (Dong *et al.*, 2001; Liu *et al.*, 2003), and phenanthrene derivatives, such as the batatasins (Sautour *et al.*, 2004).

Recently, *Dioscorea* rhizomes were determined to exert immune response-modifying effects. These effects include immunomodulation (Choi *et al.*, 2004; Zhao *et al.*, 2005), anti-inflammatory effects (Kim *et al.*, 2004), anti-tumor effects (Hu and Yao, 2003a, 2003b), and anti-osteoporotic effects (Yin *et al.*, 2004). In addition, the anti-oxidative effects of dioscorin, a storage protein harbored by *Dioscorea* tubers, have been also extensively investigated (Hou *et al.*, 1999, 2001). It has been demonstrated that the consumption of *Dioscorea* rhizomes may ameliorate some metabolic abnormalities, including hyperglycemia (McAnuff *et al.*, 2005), obesity (Kwon *et al.*, 2003), gut function, and lipid metabolism (Chen *et al.*, 2003).

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The primary objective of this study was to confirm the effects of ethanol extract from heat-dried *D. batatas* Decne flour on gastrointestinal tract functions, as this substance has been widely used, in Korea, as health food.

MATERIALS AND METHODS

Materials

Heat-dried flour of the Chinese yam (*D. batatas* Decne) was purchased from the Bukhu Agricultural Cooperative (Andong, Korea). The yam flour was extracted *via* refluxing with 40% ethanol, and the extract was recovered by filtration through Whatman filter paper. After maintaining the filtrate for 1 week in a refrigerator, the precipitate was removed *via* centrifugation at 3,000 rpm. The resulting solution was subjected to a high performance liquid chromatography (HPLC) system for the analysis of dioscin content (Nam *et al.,* 2006), and then used in this study.

Experimental animals

Specific pathogen-free male Sprague-Dawley rats (4~5 weeks old) were obtained from Daehan Laboratory Animal Research Center Co. Ltd. (Eumsung, Korea). Four animals were housed in each cage, and fed with a tap water and rat chow diet (Samyang Co., Ulsan, Korea). The animal room was maintained at a temperature of 23±3°C with 50±10% humidity, and illuminated repeatedly at 150~300 Lux with 12 h intervals. All animals were adapted for at least 1 week prior to the experiment.

Gastrointestinal motility test

In order to evaluate the effects of Chinese yam extract on gastric pH, the Chinese yam ethanol extract (corresponding to 50 mg of Chinese yam flour/mL), diluted 10-fold with milk fat, was injected directly into the stomachs of experimental Sprague-Dawley rats (n=8) through the esophagus, at a dosage of 1.5 mL/100 g, whereas the control group received injections consisting only of milk fat containing 4% ethanol. The animals were sacrificed after 30 min, and the gastric juices were collected for pH measurements. Another group of animals (n=8) was treated with the same method as above, except that 0.5% charcoal was also added to their diet. After 30 min, these rats were anesthetized with urethane, and the moving distance of the charcoal in the gastrointestinal tract was determined. As an alternative procedure for the evaluation of gastrointestinal motility, the fecal quantity during the 24 h after feeding was measured. In this case, rats were pre-fasted for 24 h, and were fed on the same diet as above, which was mixed with 0.5% charcoal in order to allow for the discrimination of feces by color.

Serochemical and toxicological test

The effects of long-term administration of Chinese yam extract on serochemical changes in the rats was evaluated *via* the monitoring of 3 divided groups (n=8) of Sprague-Dawley rats. The first group, the controls, was fed only with a rat chow diet, and the second group was fed on a rat chow diet to which 2% Chinese yam flour had been added. The third group ate a rat chow diet mixed with 2% of Chinese yam extract, which corresponds to 10% Chinese yam flour, as the extract was concentrated to 1/5 the weight of the original yam flour. After maintaining the rats' diets for 6 weeks, sera were collected from the abdominal aortas of the rats, and the sera were analyzed for the glucose, neutral lipid, and total cholesterol contents. Glucose concentration was determined *via* the glucose oxidase method, using an YSI Glucose Analyzer (YSI 1500, U.S.A.). Serum neutral lipids and total cholesterol concentrations were determined using a kit, which was purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

The rats were then sacrificed, and biopsies were conducted on the gastric cells, small intestine epithelial cells, liver cells, lung cells, kidney cells, spleen cells, and brain cells. The tissue cells were stained using hematoxylin-eosin, and the presence of abnormal cells was detected with a Nikon optical microscope (TE-2000, ×400).

Intestinal microorganisms and cultivation

The typical intestinal bacteria, *Lactobacillus plantarum* KCCM12116, *Enterobacter cloacae* KCCM12178, and *Bacteroides fragilis* KCCM12102 were obtained from the Korea Culture Collection of Microorganisms (Seoul, Korea). The media used for the culturing of *L. plantarum* was Lactobacillus MRS broth (1% peptone, 1% beef extract, 0.5% yeast extract, 2% glucose, 0.1% Tween 80, 0.2% ammonium citrate, 0.5% sodium acetate, 0.2% K_2HPO_4, 0.01% $MgSO_4 \cdot 7H_20$, 0.005% $MnSO_4 \cdot 4H_20$, pH 6.5), whereas for *E. cloacae* we employed Nutrient broth (0.3% beef extract, 0.5% peptone, pH 7.0), and for *B. fragilis* Oxoid CM149 broth (0.3% yeast extract, 1% beef extract, 1% peptone, 0.1% starch, 0.5% glucose, 0.05% cystein HCl, 0.5% NaCl, 0.3% sodium acetate, pH 6.8) was used. These bacteria were all cultivated in anaerobic flasks at 37°C, with shaking at 100 rpm.

Gastrointestinal flora test

In the intestinal bacteria culture broths, 1% or 3% (v/v) of Chinese yam extract was added, in order to determine its effects on bacterial growth. The degree of cell growth was measured as turbidity at 600 nm (A_{600}), using a spectrophotometer. The feces of the rats (n=8) were collected after 6 weeks of treatment with Chinese yam extract, as described in the section titled "Serochemical and toxicological tests", then dispersed in saline solution at a concentration of 100 mg/mL. On McConkey agar (Difco, IL, U.S.A.) plates, 100 μL of fecal suspension was spread and aerobically cultivated at 37°C.

RESULTS

Effect of Chinese yam extract on gastrointestinal motilty

The gastric pH of Sprague Dawley rats, 30 min after the injection of the Chinese yam extract, containing 177±58 μg/mL of dioscin, through the esophagus, was observed to be as high as 4.50±0.1, whereas the control group evidenced a pH value of 3.95±0.1 (Fig. 1). This weak acidity in the gastric juice may exert a protective effect on the stomach epithelial cells.

The administration of Chinese yam extract also increased gastrointestinal motility. The moving distance of feed in rats fed on the Chinese yam extract was 44.8±1.4 cm/h, which was 10% faster than in the control group, in which the movement of the feed was measured at 40.5±1.7 cm/h (Fig. 2). This would appear to suggest that Chinese yam extract both improves intestinal motility and facilitates intestinal digestion.

Simultaneously, the consumption of Chinese yam extract was shown to increase fecal quantity. In the rats fed on Chinese yam extract, the fecal quantity was measured at 12.63±0.78 g/day/rat, a quantity 40% larger than was detected in the control group, in which the measured fecal quantity was 8.85±0.52 g/day/rat (Fig. 3). This is also reflective of the ability of Chinese yam extract to increase the gastrointestinal motility, and foster healthier gastrointestinal function.

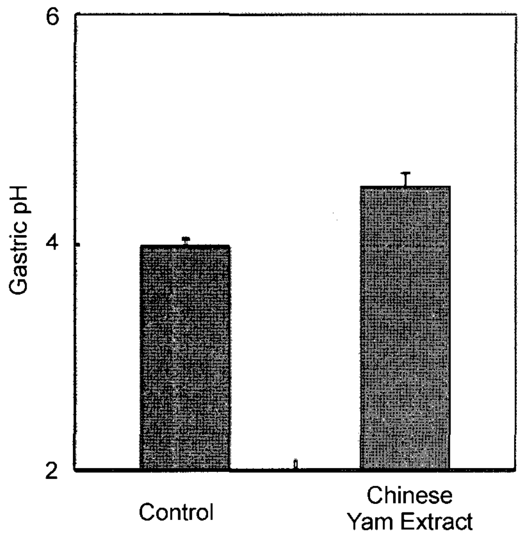

Fig. 1. The pH value of gastric juice after the administration of Chinese yam extract. The gastric juice from sacrificed rats (n=8) was collected 30 min after the injection of 1.5 mL of Chinese yam extract (corresponding to 75 mg Chinese yam flour/100 g rat weight) through the esophagus.

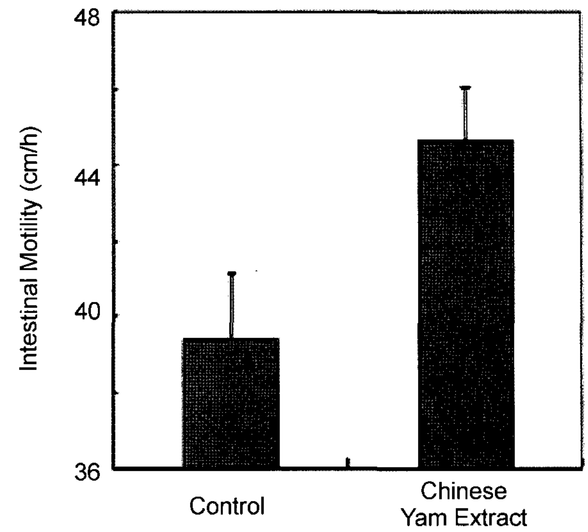

Fig. 2. Gastrointestinal motility after the administration of Chinese yam extract. The moving distance of charcoal in the gastrointestinal tracts of rats (n=8) was measured 30 min after the feeding of 1.5 mL of Chinese yam extract (corresponding to 75 mg Chinese yam flour/100 g rat weight) through the esophagus.

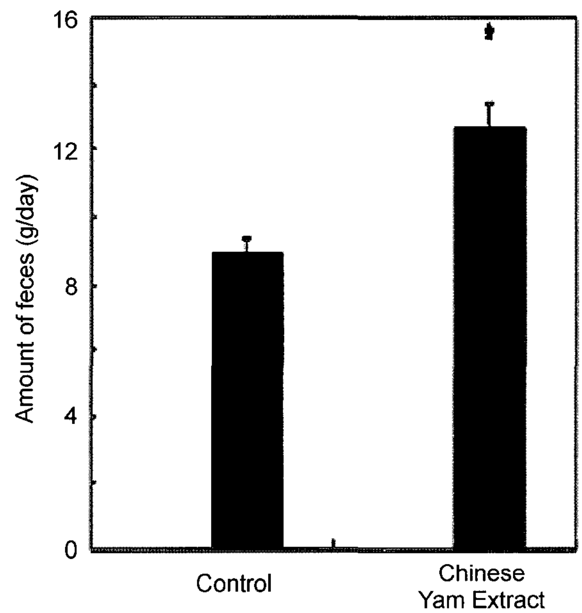

Fig. 3. Fecal quantity after the administration of Chinese yam extract. Fecal quantity over 24 h was measured after the ingestion of 1.5 mL of Chinese yam extract (corresponding to 75 mg Chinese yam flour/100 g rat weight) by rats (n=8) that had been subjected to 24 h of starvation.

Effects of Chinese yam extract on intestinal flora

The abundant intestinal microbial community not only aids in digestion, but also protects the intestine against

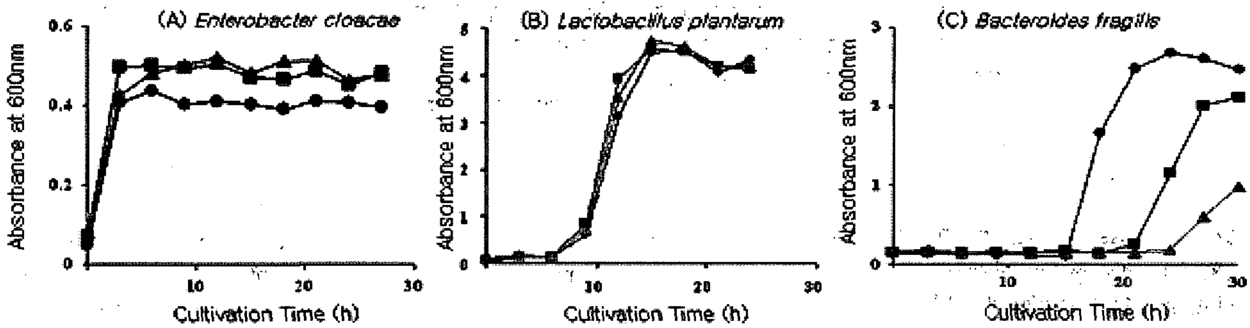

Fig. 4. Growth curve of intestinal bacteria when cultivated anaerobically in media supplemented with Chinese yam extract. (A) *Enterobacter cloacae*; (B) *Lactobacillus plantarum*; (C) *Bacteroides fragilis*. Round symbol (●); without Chinese yam extract, rectangular symbol (■); with 1% Chinese yam extract supplementation, triangular sympol (▲); with 3% Chinese yam extract supplementation.

harmful microorganisms. In order to determine the effects of Chinese yam extract on the growth of intestinal bacteria, we selected three bacterial strains: *L. plantarum* as a helpful bacterium, and *E. cloacae* and *B. fragilis* as normal bacterial representatives. When 1% and 3% (v/v) of Chinese yam extract was added to the culture broths of each of the bacteria, no significant variations in growth pattern were observed in the cases of *L. plantarum* and *E. cloacae,* as is shown in Fig. 4. However, the growth of *B. fragilis* was noticeably retarded by the addition of Chinese yam extract, which suggests that the Chinese yam extract may possess some compounds that inhibit the growth of strictly anaerobic bacteria, such as *B. fragilis*.

Microbial floral change was also investigated in conjunction with a long-term administration of Chinese yam extract. After 6 weeks of feeding with Chinese yam extract, fecal microorganisms were examined on McConkey agar plates, in which lactose-fermenters form pink colonies, and lactose-non-fermenters produce white ones. As can be seen in Fig. 5, in the plates to which Chinese yam extract was administered, the number of pink colonies in the fecal samples increased, but the white colonies disappeared almost completely. This indicates that the microbial flora had been converted largely to lactose-fermenting strains, which are known to be beneficial with regard to intestinal function.

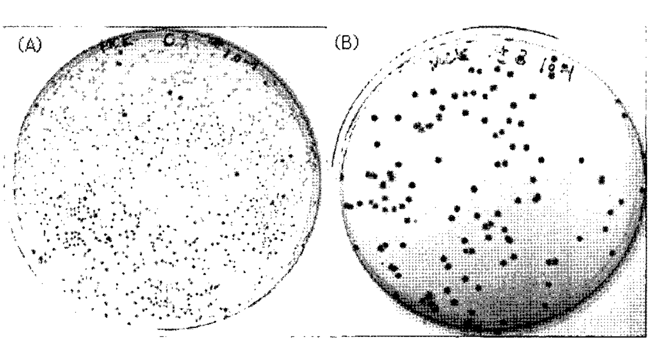

Fig. 5. Microbial floral changes in the feces of rats. After 6 weeks of administration of Chinese yam extract, the feces of rats (n=8) were collected, suspended at 100 mg/mL, and spread onto McConkey's agar plates after dilution.

Serochemical and toxicological effects of Chinese yam extract

After 6 weeks of administration of Chinese yam extract, blood was collected from the rats and analyzed for serum glucose, neutral lipid, and total cholesterol content. As can be seen in Table I, the administration of Chinese yam flour and Chinese yam extract resulted in improvements in blood levels of glucose, neutral lipids, and total cholesterol in the rats. Also, the group fed on Chinese yam extract exhibited serochemical results superior to those observed in the group fed on the Chinese yam flour. This result can probably be attributed to the fact that the Chinese yam extract contained 5 times more active components than did the Chinese yam flour.

Another interesting finding was that body weight gain was reduced in the group to which Chinese yam extract was administered. After 6 weeks on a diet featuring Chinese yam extract, the average body weight of the experimental rats was 302.3±6.4 g, less than that of the control group, which was measured to be 325.9±7.9 g.

Upon microscopic observations of body tissues collected from the rats fed on the 6-week Chinese yam extract diet, no significant clinical deteriorations or alterations were observed in the gastric cells, small intestine epithelial cells, liver cells, lung cells, kidney cells, spleen cells, and

Table I. Serochemical effect of Chinese yam flour and its ethanol extract

Group	Serum glucose (mg/dL)	Serum neutral lipid (mg/dL)	Serum total cholesterol (mg/dL)
Control	115±7.3	25±1.2	120±4.9
2% Chinese yam flour	117±5.4	23±1.0	115±6.4
2% Chinese yam extract	107±5.7	21±1.1*	113±5.5

Values are mean±SE (n=8).
*p<0.05 vs control.

brain cells (data not shown). This suggests that the long-term administration of Chinese yam extract induced no significant toxicological effects in the body tissues of the experimental rats.

DISCUSSION

As the Chinese yam (*Dioscoreae* rhizoma) has been traditionally employed in the treatment of a host of gastrointestinal malfunctions, including anorexia and chronic diarrhea, this study attempted to determine its effects on gastrointestinal tract function and intestinal floral composition. When a 40% ethanol extract of Chinese yam flour containing 177±58 μg/mL of dioscin was ingested by Sprague Dawley rats after starvation, gastric acid secretion was significantly suppressed, and gastrointestinal motility and fecal quantities were substantially increased, as compared to control values. This implies that the Chinese yam extract might prove to be a useful digestive-aid agent, which may serve to increase gastrointestinal motility.

The Chinese yam extract had no effects on the growth of normal intestinal bacteria. However, the intestinal floral mix shifted toward lactose-fermenting bacteria after 6 weeks of a diet featuring 2% Chinese yam extract. This indicates that Chinese yam extract not only contributes to improvements in digestive capability, but also alters the intestinal floral mix toward helpful bacteria.

After 6 weeks of the feeding of Sprague-Dawley rats on Chinese yam extract, our serochemical analyses produced results indicating that the serum levels of glucose, neutral lipids, and total cholesterol had all decreased to some degree. This result was similar to that of Chen *et al.* who assessed the effects of the Taiwanese yam on upper gut function and lipid metabolism in Balb/c mice (2003), as well as the results of the study of Kwon *et al.* (2003), who described the effects of *Dioscorea nipponica* Makino in Sprague-Dawley rats. However, the precision of serochemical studies is best served by employing hyperglycemic and/or hypercholesteremic rat models.

During the 6 weeks for which Chinese yam extract was added to the diets of the experimental mice, a general reduction in body weight gain was observed. This appears to corroborate to some degree the noted anti-obesity effects of the *Dioscorea* yam, previously reported in the study of Kwon *et al.* (2003).

Our results provide basic data necessary for the development of Chinese yam extract as a digestion-aiding agent for the treatment of gastrointestinal disorders, particularly in patients suffering from hyperglycemia or hyperlipidemia.

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