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Effects of Radish Seed Ethanol Extracts on Gastrointestinal Function in Rats

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Abstract The effects of ethanol extract from radish seeds on gastrointestinal function in rats were investigated. Rats were divided into 2 groups of 8 rats each, the basal group and the radish seed ethanol extract group. The radish seed ethanol extract group had a significantly decreased gastrointestinal transit time, but a significantly increased fecal weight compared with the basal group. The radish seed ethanol extract group also had significantly higher duodenal villus height and greater colonic mucosal thickness than the basal group. The radish seed ethanol extract group had a greater proliferation of 5-bromo-2-deoxy-uridine (BrdU) immunoreactive cells in the gastric mucosa as well as in the mucosa and submucosa of the small and large intestine than did the basal group. Thus, radish seed ethanol extract may be useful in preventing constipation based on the observation of an increase in fecal weight, a decrease in gastrointestinal transit time, and positive changes in the intestinal mucosa.

Keywords: radish seed, gastrointestinal function, villus height, mucosal thickness, immunoreactive cells

Introduction

It is generally known that constipation gives rise to low bowel frequency, irregular stool expulsion, difficulty defecating (often requiring straining), a hard stool consistency, a feeling of incomplete rectal evacuation, and the passage of abnormally small stools (1). Regular exercise and intake of dietary fiber are recommended to prevent constipation. The derivatives of anthraquinone, diphenylmethane, and other stimulant laxatives have been commonly used for the treatment of constipation, but result in colonic inertia if taken for long periods of time. Accordingly, research has been focused on a search for medicinal plants that can accelerate intestinal motility and improve gastrointestinal function to treat and prevent gastrointestinal disorders (2, 3).

Radish (*Raphanus sativus* L.), which belongs to the Cruciferae, is widely available throughout the world. This plant has been used as a folk remedy for the treatment of various urinary disorders (4). Its leaves and roots have been used as a laxative, stimulant, and digestive aid (4-6). Radish seeds have also been reported to reduce the feeling of abdominal distension and to inhibit *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pneumoniae* activities as well as the activity of many pathogenic fungi (7). Previous studies have shown that radishes contain important medicinal compounds such as peroxidase and isothiocyanates that have several biological effects including antihyperlipidemic, antimutagenic, anticarcinogenic, and antioxidant activities (8-12).

However, little evidence is available in the literature regarding the role of radishes in gastrointestinal activity. It is therefore important to provide a scientific basis for its traditional use in constipation. In this study, we examined the effect of an ethanol extract from radish seeds on gastrointestinal function in rats to determine the possibility of radish seeds as a functional food source effective in the improvement of intestinal function and the prevention of constipation.

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Materials and Methods

Animals and diets Male Sprague-Dawley rats (Samtako, Korea) weighing approximately 190 g were housed in a room with controlled temperature (22±2°C) and relative humidity (60±5%). The experimental groups were the basal and radish seed ethanol extract groups, which were administered with saline and radish seed ethanol extract, respectively. Sixteen animals were assigned to each group (4 animals per cage) and observed for 4 weeks. They were allowed to consume food and drinking water freely. The diets used in this study consisted of the following ingredients in g/100 g diet: 20 g casein, 0.3 g DL-methionine, 55.2 g sucrose, 15 g corn starch, 5 g corn oil, 3.5 g American Institute of Nutrition (AIN)-76 mineral, and 1 g AIN-76 vitamin mix. The freeze-dried radish seed ethanol extract (100 mg) was dissolved in 0.5 mL distilled water and administered orally 2 times daily (200 mg/day) for 4 weeks.

Preparation of the radish seed extract Radish seeds were purchased from a local herbal medicine store, then dried and ground. The extract was prepared by soaking radish seeds (3 kg) in 80% ethanol for 2 weeks, stirring this mixture 4 times a day, concentrating it in a rotary vacuum evaporator, and freeze-drying.

Measurement of body weight, food intake, and food efficiency Rats were monitored daily for their general health, and their body weights were examined weekly throughout the study. Food intake and food efficiency were measured at 1 week intervals, and the average amount

of food consumed daily and food efficiency were calculated.

Gastrointestinal transit time and fecal weight Gastrointestinal transit time was determined by measuring the time until the initial appearance of dye in feces after 1.5% carmine red dye (2 mL) was injected into the stomach. The fecal weight was measured by collecting feces once daily and then air drying them for 1 hr. A sheet of white paper was spread out under the cage instead of straw so that the exact fecal weight for each group could be measured.

Morphological characteristics and immunohistochemical staining Rats were sacrificed after the 4th week of feeding, and their gastrointestinal tracts were dissected and cut into segments (1 cm). The segments were washed with physiological saline, fixed in 10% neutral buffered formalin for 24 hr, and then embedded in paraffin by means of a dehydration process. A test piece with the thickness of 7 μm was produced after the embedding process, and a hematoxylin and eosin stain were applied. Alcian blue (AB) and periodic acid-Schiff reagent (PAS) staining were applied to observe changes in mucous cells. A 1×1 mm graticule was attached to a microscope, which was used to monitor changes in villus height and mucous layer thickness.

Immunohistochemical staining was performed using the procedure described by Ito *et al.* (13). The 5-bromo-2-deoxy-uridine (BrdU, 50 μg/g body weight) was injected intraperitoneally twice daily for 2 days prior to being sacrificed. Tissues were treated with 0.05% proteinase K for 20 min and rinsed with phosphate buffered saline (PBS). The primary antibody against BrdU (mouse anti-BrdU antibody, Santa Cruz, CA, USA) was diluted to 1:50, and then added to tissue sections at room temperature for 2 hr. Subsequently, the secondary antibody, biotinylated anti-mouse IgG (Vector Laboratory, Burlingame, CA, USA), was diluted to 1:200 and added at room temperature for 1 hr. The samples were then soaked in avidin-biotin-horseradish peroxidase complex (ABC) solution at room temperature for 1 hr. Tissues were stained with 3-3' diaminobenzidine and counterstained with hematoxylin, then observed by using a light microscope.

Statistical analysis All data are presented as mean±SD. They were analyzed by analysis of variance (ANOVA) using the SAS statistical analysis system (SAS Institute Inc., Cary, NC, USA). Differences among samples were analyzed using the Duncan's multiple range test ($p<0.05$).

Results and Discussion

Body weight, food intake, and food efficiency Table 1 presents the initial body weight, weight gain, food intake, and food efficiency in rats administered physiological saline (basal) and radish seed ethanol extract solutions. The initial body weight was approximately 190 g and was not significantly different between these 2 groups, while the final body weight was lower for the radish seed ethanol extract group compared with the basal group. The amount of weight gain was slightly lower in the radish seed ethanol extract group (3.43±1.04 g/day) compared with the basal group (4.08±1.52 g/day). Food intake and efficiency were also slightly lower in the radish seed ethanol extract group compared with the basal group. The low weight gain rate in the radish seed ethanol extract group might be due to both low food intake and low food efficiency.

Gastrointestinal transit time and the fecal weight Gastrointestinal transit times and fecal weights for the basal and radish seed ethanol extract groups are shown in Table 2. The gastrointestinal transit time in the basal group was 14.75 hr compared with 8.87 hr in the radish seed ethanol extract group, which was a significant decrease in comparison with the basal group ($p<0.01$). Fecal weight, based on excretions collected over an 18 hr period following injection of the dye, was 0.87 g for the radish seed ethanol extract group, which was significantly higher than the basal group (0.55 g, $p<0.01$). Daily fecal weight was also significantly higher for the radish seed ethanol extract group (1.070 g) compared with the basal group (0.895 g, $p<0.01$). Accordingly, the gastrointestinal transit time decreased and the amount of defecation increased with the administration of the radish seed ethanol extract.

Baik *et al.* (14) reported that the administration of radish water extract to mice resulted in the acceleration of the movement along the intestinal transit and that the 3-10 kDa fraction of the extract was the most effective in inducing movement. Jung *et al.* (15) also reported that radish extract increased the contraction of duodenum, jejunum, and ileum in rats in a dose dependent manner in vitro and that oral administration of the radish extract improved intestinal transit time in animal studies. In addition, the radish extract appeared to accelerate activity in the muscarinic pathway and, thus, stimulate movement along the gastrointestinal tract. It was thus inferred that

Table 1. Food intake and growth in rats fed a basal diet compared with radish seed ethanol extract for 4 weeks

Experimental group	Final body weight (g)	Weight gain (g/day)	Food intake (g/day)	Food efficiency (gain/g food)
Basal	313.7±13.6[1)]	4.08±1.52	15.05±1.02	0.271±0.04
Radish seed ethanol extract	280.3±11.5	3.43±1.04	13.79±1.34	0.249±0.05

[1)]Values are mean ± SD, n=8.

Table 2. Gastrointestinal transit time and fecal weight in rats fed a basal diet compared with radish seed ethanol extract for 4 weeks

Experimental group	Transit time (hr)	Red fecal weight (g dry/18hr)	Whole fecal weight (g dry/day)
Basal	14.75±1.43[1)]	0.55±0.04	0.895±0.04
Radish seed ethanol extract	8.87±1.05**	0.87±0.06**	1.070±0.06**

[1)]Values are mean ± SD, n=8. **$p<0.01$.

Table 3. Villus heights in the duodenum and mucosal and muscle layer thickness in the colon in rats administered a basal diet compared with radish seed ethanol extract for 4 weeks

Experimental group	Duodenum	Colon	
	Villus height (μm)	Mucosal thickness (μm)	Muscle thickness (μm)
Basal	883±13.7[1)]	208±13.3	115±10.4
Radish seed ethanol extract	942±10.4**	236±11.5*	146±13.2*

[1)]Values are mean ± SD, n=8. *$p<0.05$, **$p<0.01$.

the reduction in gastrointestinal transit time induced by radish seed ethanol extract observed in this study might be related to stimulation of gastrointestinal movement.

Morphological changes in the jejunum and ileum Table 3 presents villus height measurements in the duodenum and the thickness of the mucosal and muscle layer in the rat colon after being fed basal and radish seed ethanol extract for 4 weeks. The villus height in the duodenum of rats in the radish seed ethanol extract group was 942 μm, which was significantly higher than that in the control group (883 mm, $p<0.01$). The mucosal and muscle layer thickness of the colon in the radish seed ethanol extract group (236±11.5 and 146±13.2 μm, respectively) appeared to be significantly larger than in the control group (208±13.3 and 115±10.4 μm, respectively, $p<0.05$).

It has been reported in morphological studies that rats fed dietary fibers for a long time have an altered villus structure in the small intestine (16). Sigleo *et al.* (17) have stressed that the long term intake of dietary fibers such as cellulose and pectin induced an increase in the surface area, marked by an increase in the number of cells in each villus and a significant increase in the thickness and length of villus, thereby improving nutrient absorption. It has also been reported that cell turnover in the crypts of Lieberkuhn help determine villus morphology and that the mechanism involved might be controlled partially by the gastrointestinal peptide hormones (18). Therefore, the radish seed ethanol extract may cause changes in intestinal morphology, such as increase in the thickness of intestinal villi, and thus cause intestinal movement and absorption to be enhanced.

Immunohistology of the gastrointestinal tract The BrdU staining method is used to locate 5-bromo-2-deoxy-uridine and thus determine the cell proliferation rate (19). The results of mucosal staining of the gastrointestinal tract using BrdU are shown in Fig. 1 to 3. The basal group exhibited weak BrdU staining (Fig. 1a) in the gastric mucosa, whereas the radish seed ethanol extract group showed strong BrdU staining (Fig. 1b). This suggests that strong BrdU staining might be induced by the intake

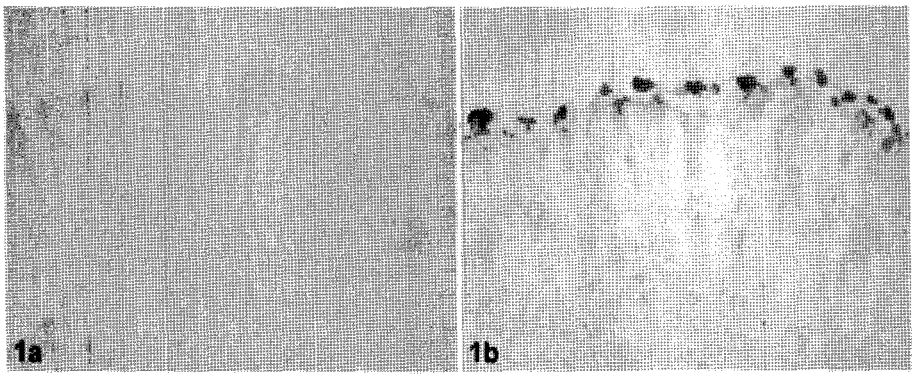

Fig. 1. BrdU immunoreactivity of gastric mucosa in rats. 1a, basal group; 1b, radish seed ethanol extract group.

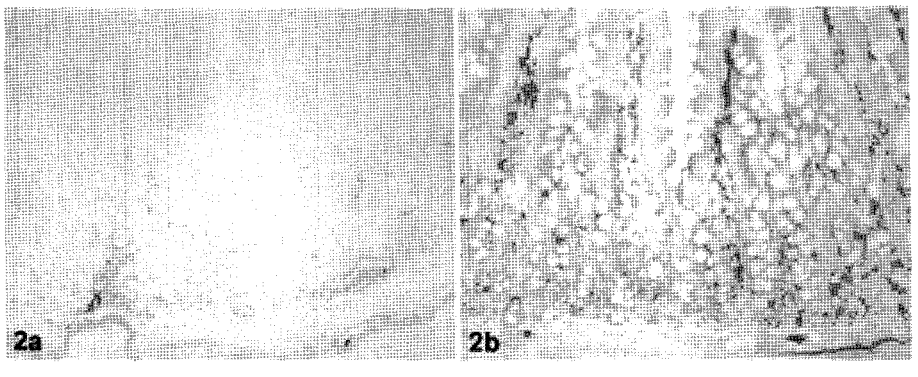

Fig. 2. BrdU immunoreactivity of lamina propria of duodenal mucosa in rats. 2a, basal group; 2b, radish seed ethanol extract group.

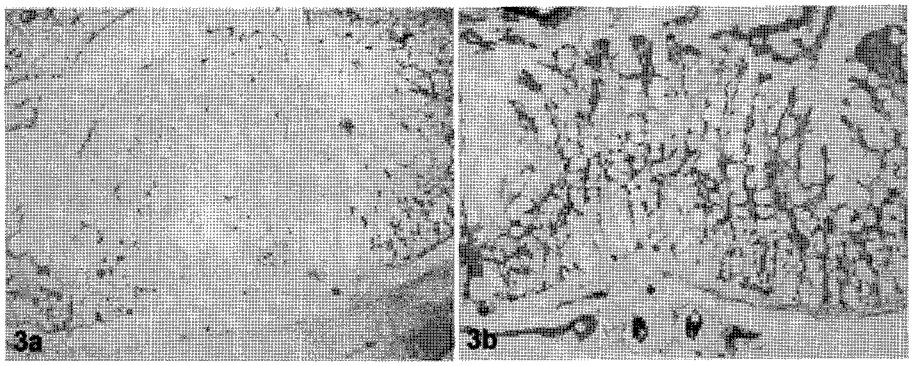

Fig. 3. BrdU immunoreactivity of lamina propria of colonic mucosa in rat. 3a, basal group; 3b, radish seed ethanol extract group.

of radish seed ethanol extract. The basal group presented weak BrdU staining in the lamina propria of the duodenal and jejunal mucosa and mild BrdU staining in the blood vessels of submucosal and connective tissues (Fig. 2a), whereas radish seed ethanol extract group showed strong BrdU staining in the lamina propria cells of the mucosa and submucosa (Fig. 2b). In the colon, the basal group exhibited weak BrdU staining in the lamina propria cells (Fig. 3a), but showed strong BrdU staining in lamina propria cells of the mucosa and blood vessels of submucosa (Fig. 3b).

Potten (20) has reported that an increase in the length of intestinal villi causes the depth of mucosal crypts to increase and enlarge the area responsible for cell proliferation in the intestinal mucosa. Thus, both cell proliferation and cell death also increased, which results in an increase in the turnover of intestinal mucosal cells. Accordingly, strong BrdU staining along the surface of gastric mucosal epithelium and the mucosal and submucosal layers of the small and large intestines indicates that mitotic proliferation in the intestinal mucosa may progress rapidly as a result of accelerated DNA synthesis in the mucosal epithelial cells of the intestine and an increase in the turnover of mucosal epithelial cells.

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