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Effect of Alcohol Insoluble Residues from Stem and Root Barks of Elm (*Ulmus davidiana*) on Intestinal Characteristics in Rats

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Abstract Sprague-Dawley rats (n=32) were fed a diet containing basal (control), cellulose (5%), or alcohol insoluble residue (AIR) (5%) extracted from the stem and root barks of elm (*Ulmus davidiana* var. *japonica* Nakai) for 4 weeks. The effects of the diets, on gastrointestinal functions and morphology were evaluated. The weight gains, food intake, and food efficiencies for the cellulose and AIR diet-fed groups were not significantly different from those of the AIR-free (basal) diet. The gastrointestinal transit times of the stem and root bark AIR diets were significantly reduced ($p< 0.01$) compared to the basal diet, and were slower than those of the cellulose diet. The fecal weights of the stem and root bark AIR diets were significantly increased ($p<0.01$) up to 4-fold compared to those of the basal diet. The height of the mucosal villi, and mucosal and muscle layer thicknesses of the colon were greater and more developed in the stem and root bark AIR diets ($p<0.01$) than in the basal diet. The villus heights in the jejunum and the colon mucosal goblet cells were more developed in the order of cellulose > stem bark AIR > root bark AIR diets.

Keywords: alcohol insoluble residue, elm, gastrointestinal transit time, fecal weight, mucosal villi

Introduction

In recent years, the incidence of constipation has increased due to the increased intake of meats and the shift to a westernized, urbanized, and industrialized dietary life. Constipation occurs when there is an abnormal bowel movement because of the stool having a prolonged stay in the bowel, and results in a residue of stool remaining after evacuation of the bowels or hard feces. Constipation can especially occur as a clinical symptom due to overstrain and irregular bowel elimination habits, and can also be caused by a metabolic abnormality, a lack of fiber intake and drug abuse etc. (1). Although regular exercise and intake of dietary fiber are recommended to prevent constipation, the anthraquinone and diphenylmethane derivatives that are stimulative deglutition remedies have been used (2). However, these compounds are known to induce inertia of the colon when taken over a long period of time.

An elm (*Ulmus davidiana* var. *japonica* Nakai) is a widely distributed tree in Korea, and its stem and root barks have been used as oriental medicines for the treatment of edema, mastitis, gastric cancer, and inflammation (3). The stem and root barks of this tree contain large quantities of dietary fiber such as hemicellulose, cellulose, lignin, and pectin (4). Our previous study (5) revealed that alcohol insoluble residues (AIRs) from the stem and root barks of elm contained a high level of dietary fiber (56.2 and 40.8%, respectively), and that the main fraction of this dietary fiber was insoluble dietary fiber (71.3 and 87.5%, respectively), indicating that stem and root barks could be good sources of dietary fiber. Dietary fiber is important not only as a nutrition source for *Bifidobacterium* (6), a useful micro-organism in the bowel, but also to relieve constipation by assisting in the smooth transit of stool via stimulation of the bowel activity. Especially, dietary fiber increases the amount of stool by absorbing much more water than its own weight, which eases evacuation of the bowel.

In this study, we investigated the effect of the AIRs from the stem and root barks of elm on intestinal functions in rats. The gastrointestinal transit time, fecal weight, intestinal morphology, and colon mucosa were also studied for AIR-free (basal), cellulose, and stem and root bark AIR-fed groups.

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Materials and Methods

Preparation of AIR AIR was prepared according to the method of Choi *et al.* (5). Three grams of stem or root bark powders of elm were added to 300 mL of 95% ethanol, heated in a water bath set at 80°C for 1 hr, and filtered through a G3 glass filter. The residues were then washed with boiling ethanol (150 mL), a chloroform/methanol (1:1, v/v) mixture (150 mL), and finally acetone (50 mL). The insoluble materials were air-dried overnight at 40°C.

Animals and diets Male Sprague-Dawley rats (n=32) weighing 180±10 g were fed a non-purified diet for five days followed by either basal or one of three experimental diets (Table 1). Rats were maintained at 22±2°C and 60±5% relative humidity in a room with a 12 hr light: dark cycle and given *ad libitum* access to food and water. Rats were divided into four groups of eight rats each and fed one of four diets for 4 weeks: AIR-free (basal) diet, or a

diet containing cellulose (5%), stem bark AIR (5%), or root bark AIR (5%). Rats were weighed and monitored daily for their general health condition. Daily food intake and food efficiency were monitored weekly.

Gastrointestinal transit time and fecal weight A 1.5% carmine red dye solution (3 mL) was used as a transit marker. The dye was injected into the stomach, and the excretion of the marker was checked. The period of time extending from the administration to the marker's first appearance in the excrement was regarded as the gastrointestinal transit time. White paper sheets were spread under the cages instead of straw to measure the exact weight of feces for each diet group. Fecal weights were determined for daily feces over 4 week (four rats per cage). The feces were collected once per day, were dried for 1 hr and then weighed.

Morphological study To observe any morphological changes of the jejunum and colon, each rat was sacrificed after the fourth week of feeding, and the jejunum and descending colon were dissected, cut into 1-cm segments, washed with physiological saline, fixed in 10% neutral buffered formalin for 24 hr, and then embedded in paraffin through the dehydration process using an average method after washing. A test segment section with the thickness of 7 μm was produced after the embedding and dyed using hematoxylin and eosin. The alcian blue (AB) and periodic acid-Schiff reagent (PAS) staining were applied to observe the changes of the mucous cells. Moreover, a micrometer was installed onto the microscope to measure the changes of villus height and mucous layer thickness. To conduct scanning electron microscopy (SEM) examination of the changes of the jejunum and colon, the rats were sacrificed after the fourth week of their diet, and the jejunum and descending colon were dissected and cut into 1-cm segments, washed in phosphate-buffered saline (PBS) and fixed in 3% phosphate buffered glutaraldehyde. After fixation, any foreign material in the segments was removed by using a soft brush. The post fixing was done by using 15 OsO_4 (pH 7.4) for 2 hr. A test piece was washed using alcohol and acetone, dried by using a critical point dryer (EMS850; Electron Microscopy Sciences, Hatfield, PA, USA) up to the critical point through acetone, coated with gold using an ion sputtering coater (IB-3; Giko Co., Japan) and then observed by SEM.

Statistics and analysis All data are mean±SD. They were analyzed by ANOVA using the statistical analysis system (SAS Institute Inc., Cary, NC, USA). Differences among samples were analyzed using the Duncan's multiple range tests.

Table 1. Composition of experimental diets (unit: g/kg)

Ingredients	Basal	5% cellulose	5% stem bark AIR	5% root bark AIR
Casein	200	200	200	200
DL-Methionine	3	3	3	3
Sucrose	550	550	550	550
Corn starch	150	100	100	100
Corn oil	50	50	50	50
AIN-76 mineral mix[1)]	35	35	35	35
AIN-76 vitamin mix	10	10	10	10
Choline bitartrate	2	2	2	2
Basal	0			
5% cellulose	0	50	0	0
5% stem bark AIR[2)]	0	0	50	0
5% root bark AIR	0	0	0	50

[1)]American Institute of Nutrition (1997).
[2)]AIR, alcohol insoluble residue.

Results and Discussion

Weight gains and food efficiencies Table 2 presents the results of the initial body weights, weight gains, food intake, and food efficiencies in rats fed the four diets (basal, 5% cellulose, and 5% stem and root bark AIR diets). The initial body weights ranged from 182.0±6.3 for the AIR-free (basal) diet to 188.5±7.4 g for the 5% root bark AIR diet, although the differences between the groups were not significant. Both weight gain and food intake were the highest in the group fed a 5% stem bark AIR diet (5.81±1.62 and 18.16±1.77 g/day), but there were no significant differences between the groups. Food efficiencies were highest in the group fed a 5% root bark AIR diet (0.35±0.12 gain/g food), but again there were no significant differences between the groups. These results were in good agreement with previously reported findings (7, 8); in which consumption of dietary fibers such as cellulose and inulin did not significantly affect body weight gain or food consumption.

Table 2. Initial body weight, weight gains, food intakes, and food efficiencies in rats fed 5% AIR or cellulose diets for 4 weeks

Group	Initial body weight (g)	Weight gain (g/day)	Food intake (g/day)	Food efficiency (gain/g food)
Basal	182.0±6.3[1)]	5.54±1.34	16.29±1.38	0.34±0.08
5% cellulose	182.6±7.8	5.32±1.21	16.97±1.62	0.31±0.08
5% stem bark AIR	186.3±5.5	5.81±1.62	18.16±1.77	0.32±0.10
5% root bark AIR	188.5±7.4	5.34±1.68	15.36±2.16	0.35±0.12

[1)]Values are mean ± SD (n=8).

Gastrointestinal transit times and fecal weights Table 3 presents the gastrointestinal transit times and fecal weights in rats fed the four diets. Gastrointestinal transit time for the basal diet group was 16.8±0.68 hr. The transit times for cellulose, and 5% stem and root bark AIR diets, however, were 11.3±1.20, 13.5±0.69, and 14.5±0.82 hr, respectively, which was significantly decreased ($p<0.01$) compared to the basal diet.

Reported studies on the effects of dietary fiber on constipation have mentioned that the addition of dietary fiber to the diet makes evacuation of the bowel much easier by changing the amount and the composition of feces (9). Burrows *et al.* (10) reported that the addition of α-cellulose to the diet at rates of 3, 6, and 9% decreased the gastrointestinal transit times by 41.3, 32.8, and 28.7 hr, respectively. Similarly, Kim (11) also reported that transit times were significantly reduced in rats fed cellulose and chicory, compared to a control diet.

The fecal weight from the basal diet-fed rats was 0.31±0.18 g dry/day, which was significantly lower than that from the cellulose and 5% stem and root bark AIR diets at 1.32±0.25, 1.19±0.15, and 1.06±0.11g dry/day, respectively. Rats fed cellulose, and stem and root bark AIR diets had a fecal weight increased by 3-4-fold relative to the basal diet. This result reflects previous reports (7, 11) of the daily fecal weights being increased by the addition of dietary fibers such as pectin, chicory, inulin, and cellulose. Fecal weight is known to be influenced by the presence of fiber due to its effect on water retention and fiber fermentation (12). Heller *et al.* (13) have reported that the water retention and transit times through the gastrointestinal tract are also affected by the quantity of the dietary fiber.

Table 3. Gastrointestinal transit times and fecal weights in rats fed 5% AIR or cellulose diets for 4 weeks

Group	Time (hr)*	Fecal weight (g /day)*
Basal	16.8±0.68$^{a1,2)}$	0.31±0.18^{c}
5% cellulose	11.3±1.20^{c}	1.32±0.25^{a}
5% stem bark AIR	13.5±0.69^{b}	1.19±0.15^{ab}
5% root bark AIR	14.5±0.82^{b}	1.06±0.11^{b}

[1)]Values are mean ± SD (n=8).
[2)]Different superscript letters within columns are significantly different as compared with the control group; *$p<0.01$.

Table 4. Villus heights of jejunum and mucosal and muscle layer thicknesses of the colon in rats fed 5% AIR or cellulose diets for 4 weeks

Group	Jejunum	Colon
	Villus height (μm)*	Mucosal / Muscle thicknesses (μm)*
Basal	530.0±15.6$^{c1,2)}$	205±14.5/120.0±15.8^{b}
5% cellulose	665.7±19.9^{a}	285±24.1/220.4±15.4^{a}
5% stem bark AIR	646.6±8.10^{a,b}	264.0±23.6/230.2±14.2^{a}
5% root bark AIR	607.5±12.5^{b}	241.2±15.3/150.3±12.5^{b}

[1)]Values are mean ± SD (n=8).
[2)]Different superscript letters within columns are significantly different as compared with the control group; *$p<0.01$.

Morphological changes of the jejunum and colon Table 4 presents the villus height measurements for the jejunum, as well as the mucosal and muscle layer thicknesses of the colon in rats after feeding the four diets. The villus heights in the jejunum of the cellulose, and stem and root bark AIR diets, at 665.7±19.9, 646.6±8.10, and 607.5±12.5 μm, respectively, were all significantly larger (all $p<0.01$) than that of the basal diet, at 530.0±15.6 μm, and also appeared to be more developed. The mucosal and muscle layer thicknesses of the colon for the cellulose, and stem and root bark AIR diets, at 285±24.1/220.4±15.4, 264.0±23.6/230.214.2, and 241.2±15.3/150.3±12.5 μm, respectively, were all significantly increased (all $p<0.01$) compared to that of the basal diet at 205±14.5/120.0±15.8 μm.

Rats fed chicory, inulin, cellulose, and pectin as a source of dietary fiber have been reported to have significantly increased villus height of the jejunum (11, 14). Some investigators have also reported that intestinal morphological characteristics could be influenced by the fiber sources. The villus structure of the small intestine could be changed

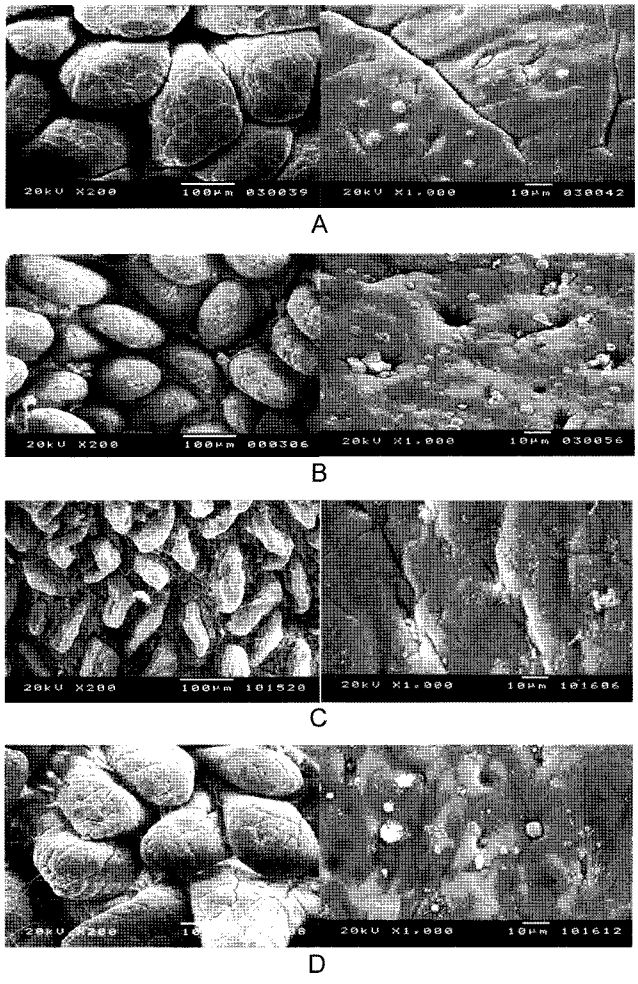

Fig. 1. Scanning electron micrograph showing the morphology of jejunal villi (left) and the number of goblet cells (right) in colonic mucosa of experimental animals. A, basal; B, 5% cellulose; C, 5% stem bark AIR; D, 5% root bark AIR.

by the type of dietary fiber taken in over a long period of time, resulting in increased enterocytes, as well as increased villus thickness and length, suggesting that the function of the intestinal villi might be stimulated (15-17).

The SEM results for the jejunum and colon are presented in Fig. 1. There was no damage to the jejunal villi in rats fed a basal diet (A, left). The mucus secreting goblet cells were oval or round in appearance, and generally surrounded by mucus (A, left). The mucosal surface of the colon was surrounded by microvilli where the goblet cells had not developed well (A, right). The jejunal villi in rats fed a 5% cellulose diet were surrounded by mucus. The jejunal villi were also more developed in these rats compared to rats on the basal diet (B, left). There were many goblet cells at the mucosal surface of the colon (B, right). In addition, the jejunal villi in rats fed a 5% stem bark AIR diet were very developed and surrounded by mucus (C, left). The goblet cells were not as developed as those in rats fed a 5% cellulose diet but they were more developed than those of the basal diet rats, in which the opening shape of the duct of the intestinal glands could be observed (C, right). The jejunal villi (D, left) and the goblet cells inside the mucosa of the colon in rats fed a 5% root bark AIR diet were more developed than those of the basal diet but less developed than those of the other three groups. The opening of the duct of the intestinal glands was also observed at the surface of the mucosa (D, right). Although the mechanism of the structural and functional reactions in response to the long period ingestion of dietary fiber is not well-known, the cell turn over in the crypt of Lieberkuhn is considered an important factor in deciding the shape of villi wherein the mechanism can be controlled partially by the gastrointestinal peptide hormones (18).

Changes of the mucous cells in the mucosa of colon To investigate the changes of the mucous cells in the mucosa of the colon, AB, PAS, and AB/PAS staining methods were conducted, with the results shown in Table 5. The basal and cellulose diets showed a mild staining density (++/++/++; ++/++/++), whereas the stem and root bark AIR diets presented a heavier density (++++/++/++) compared with the other two groups. These results suggest that crypt epithelial cells produced more acid mucin containing sulfomucin. However, there were no changes in staining density by PAS and AB/PAS in any of the experimental groups, indicating no production of alkaline and neutral mucins. According to a previous report (19), the acid mucin content of crypt epithelial cells was increased by injection of carrageenan and chondroitin sulfate. It was also reported that much mucus was secreted by feeding wheat bran due to the increased activity of goblet cells (20). The mucosal surface of the colon plays a role not only as a lubrication that protects the mucosa from mechanical damage but also as a protective barrier against the diffusion of harmful external materials (21).

Table 5. Histochemical density of goblet cells of colon in rats fed 5% AIR or cellulose diets for 4 weeks

Group	Colon
	AB/PAS/AB-PAS
Basal	++/++/++[1)]
5% Cellulose	++/++/++
5% stem AIR	++++/++/++
5% root AIR	++++/++/++

[1)]Staining density: ++, mild; +++, heavy; ++++, more heavy.

In conclusion, the ingestion of stem and root bark AIRs from elm, as a source of dietary fiber, exerted a positive influence on the rat intestinal morphology by increasing the fecal weight, decreasing the gastrointestinal transit time, aiding the development of villi, and raising the stainability of acid mucin in the colon. Therefore, we concluded that stem and root bark AIR diets derived from elm could play an important role in preventing constipation.

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References

1. Rao SS. Constipation evaluation and treatment. Gastroenterol. Clin. N. 32: 659-683 (2003)
2. Carcia-Villar R, Leng-Peschlow E, Ruckebusch Y. Effect of anthraquinone derivatives on canine and rat intestinal motility. J. Pharm. Pharmacol. 32: 323-329 (1980)
3. Lee SJ. Korean folk medicine, Monographs Series No. 3. Publishing Center of Seoul National University, Seoul, Korea. p. 39 (1996)
4. Park JS, Shim CJ, Jung JH, Lee GH, Sung CK, Oh MJ. Antimicrobial activity of Ulmi cortex extracts. J. Korean Food Sci. Nutr. 28: 1022-1028 (1999)
5. Choi YK, Lee CH, Song, GS, Kim YS. Characteristics of alcohol insoluble residue extracted from Ulmus davidiana. Food Sci. Biotechnol. 13: 666-670 (2004)
6. Jun HI, Song GS, Lee YT, Kim YS. Physichochemical properties and intestinal bacterial growth-promoting effect of cell-wall polysaccharides from cucumber peel. Food Sci. Biotechnol. 14: 375-379 (2005)
7. Chau CF, Huang YL, Lin CY. Investigation of the cholesterol-lowering action of insoluble fiber derived from the peel of *Citrus sinensis* L. cv. *Liucheng*. Food Chem. 87: 361-366 (2004)
8. Levrat MA, Remesy C, Remigne C. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J. Nutr. 121: 1730-1737 (1991)
9. Wrick, KL, Robertson JB, Van Soest PJ, Lewis BA, Rivers JM, Roe DA, Hackler LR. The influence of dietary fiber source on human intestinal transit and stool output. J. Nutr. 113: 1464-1479 (1983)
10. Burrows CF, Kronfeld DS, Banta CA, Merritt AM. Effects of fiber on digestibility and transit time in dogs. J. Nutr. 112: 1726-1732 (1982)
11. Kim M. The water-soluble extract of chicory affects rat intestinal morphology similarly to other non-starch polysaccharides. Nutr. Res. 22: 1299-1307 (2002)
12. Slavin JL, Greenber NA. Partially hydrolyzed guar gum: Clinical nutrition uses. Nutrition 19: 549-552 (2003)
13. Heller SN, Hackler LR, Rivers JM, Van Soest PJ, Roe DA, Lewis BA, Robertson J. Dietary fiber: the effect of particle size of wheat bran on colonic function in young adult men. Am. J. Clin Nutr. 31:

1734-1744 (1980)
14. Chun W, Bamba T, Hosda S. Effect of pectin, a soluble dietary fiber, on functional and morphological parameters of small intestine in rats. Digestion 42: 22-29 (1989)
15. Cassidy MM, Lightfoot FG, Grau LE, Story JA, Kritchevsky D, Vahouny GV. Effect of chronic fiber intake on the ultrastructural topography of rat jejunum and colon: a scanning microscopy study. Am. J. Clin. Nutr. 34: 218-228 (1981)
16. Sigleo S, Jackson MJ, Vahouny GV. Effects of dietary fiber constituents on intestinal morphology and nutrient transport. Am. J. Physiol. 246: G34-G39 (1984)
17. Samanya M, Yamauchi K. Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. *natto*. Comp. Biochem. Phys. A 133 A: 95-104 (2002)
18. Loehry CA, Creamer B. Three-dimensional structure of rat small intestine related to mucosal dynamics. Gut 10: 112-120 (1968)
19. Shimotoyodome A, Meguro S, Hase T, Tokimitsu I, Sakata T. Sulfated polysaccharides, but not cellulose, increase colonic mucus in rats with loperamide-induced constipation. Digest. Dis. Sci. 46: 1482-1489 (2001)
20. Schneeman BO, Jacobs LR, Richter BD. Response to dietary wheat bran in the exocrine pancreas and intestine of rats. J. Nutr. 112: 283-286 (1982)
21. Matsuo K, Ota H, Akamatsu T, Sugiyama A, Katsuyama T. Histochemistry of the surface mucosal gel layer of the human colon. Gut 40: 782-789 (1997)