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Polysaccharide from Seeds of *Plantago asiatica* L. Increases Short-Chain Fatty Acid Production and Fecal Moisture along with Lowering pH in Mouse Colon

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ABSTRACT: Mice (20.0 ± 2.0 g, n = 48 per group) were given 30 days oral administration of polysaccharide from *Plantago asiatica* L. seeds at the dose of 0.4 g/kg body weight by gavage to investigate the effects of the polysaccharide on mouse colon. Results showed that the concentrations of total short-chain fatty acids (SCFA), acetic, propionic, and *n*-butyric acids in mouse colonic content of polysaccharide treated group were all significantly higher than that of control group (water) ($p < 0.05$). In addition, moisture of mouse colonic content of polysaccharide treated group was also notably higher than that of the control group ($p < 0.05$) indicating the intake of polysaccharide from *P. asiatica* L. resulted in a stronger water-holding capacity for colonic content throughout the experimental period. Furthermore, a decreased pH (from 7.5 ± 0.1 to 7.2 ± 0.1) was observed in mouse colon of the polysaccharide treated group compared with the control group (pH from 7.5 ± 0.1 to 7.5 ± 0.1). These results suggested that the intake of the polysaccharide from *P. asiatica* L. might be beneficial for the colon health.

KEYWORDS: *Plantago asiatica L., polysaccharide, short-chain fatty acids, colonic content moisture, colon pH*

■ INTRODUCTION

Increasing interest in dietary fiber has occurred in recent years due to its beneficial effects on the human gastrointestinal tract through its direct and indirect way.[1] The direct effects are due to the dietary fiber itself, for example, many dietary fibers can bind bile acids and increase their excretion in the feces.[2] This can increase the amount of cholesterol the body must devote to bile acid production, and lower serum cholesterol levels, which is one mechanism whereby dietary fiber may exhibit a protective role against cardiovascular disease.[3] The indirect benefits appear to be unrelated to the fiber itself arising from metabolic products of fermentation. The products of fermentation are mainly short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate. Oxidation of these SCFA provides more than 70% of the oxygen consumed by human colonic tissue.[4] Furthermore, acetate is oxidized by brain, heart, and peripheral tissues;[5] propionic acid affects the liver and cholesterol metabolism;[6] butyric acid serves as an energy source for colonic epithelium, regulates epithelial and immune cell growth and apoptosis, provides protection against colonic cancer and colitis, has anti-inflammatory properties, modulates oxidative stress, and affects the composition of the mucus layer.[7] Besides SCFA production, the effects of fermentable fibers on the luminal environment were also paid more and more attention to because they were related to colon health. Fermentable fibers were reported to increase the intestinal transit rate and fecal moisture[8,9] and lower the colonic pH.[10]

Plants of the *Plantago* family are used in folk medicine throughout the world.[11] Some have been studied widely, such as *Plantago afra* L., *Plantago psyllium* L., *Plantago ovata* Forsk. (isabgul), *Plantago indica* L., and *Plantago major* L..[12] Our research group has separated a pure and homogeneous polysaccharide from the seeds of *P. asiatica* L. with a molecular weight of 1894 kDa. The polysaccharide was found to be composed of rhamnose (Rha), arabinose (Ara), xylose (Xyl), manose (Man), glucose (Glc), and galactose (Gal), in a molar ratio of 0.05: 1.00: 1.90: 0.05: 0.06: 0.10. Its uronic acid was glycuronic acid (GlcA). The polysaccharide was highly branched heteroxylan which consisted of a β-1,4-linked Xyl*p* backbone with side chains attached to *O*-2 or *O*-3. The side chains consisted of β-T-linked Xyl*p*, α-T-linked Ara*f*, α-T-linked GlcA*p*, β-Xyl*p*-(1→3)-α-Ara*f* and α-Ara*f*-(1→3)-β-Xyl*p*, and so forth (Figure 1).[13,14] In addition, our recent studies also showed that polysaccharide isolated from the seeds of *P. asiatica* L. could induce maturation of murine dendrite cells have antioxidant activity *in vitro* and promote defecation.[14−17] However, the influence of the fermentation of the polysaccharide from *P. asiatica* L. on SCFA production, fecal moisture, and pH in mouse colon has not been studied.

In this study, the effects of the polysaccharide from *P. asiatica* L. on mouse colons were evaluated by determining its SCFA production in colon of mice. Gas chromatography (GC) method was established and used to determine the SCFA. In addition, colon feces moisture and colon pH were also analyzed to better know the effects of the polysaccharide on colon health.

■ MATERIALS AND METHODS

Chemicals. High purity SCFAs were used to prepare the standard solutions. Acetic acid (100% purity) and *n*-valeric acid (99.9% purity) were obtained from Merck (Darmstadt, Germany). Propionic acid (100% purity) was purchased from Janssen Chimica (Belgium), while i-butyric acid (99.9% purity), *n*-butyric acid (100% purity), *i*-valeric acid (100% purity), and

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Figure 1. Structure of the polysaccharide from the seeds of *P. asiatica* L. proposed by our research group.[13] Rha*p*, rhamno-pyranosyl; Ara*f*, arabino-furanosyl; Xyl*p*, xylo-pyranosyl; Gal*p*, galacto-pyranosyl; Glc*p*, gluco-pyranosyl; GlcA*p*, glycuronic acid pyranosyl.

4-methylvaleric (internal standard) (99.9% purity) were purchased from Sigma (St. Louis, MO, USA). All other reagents used were of analytical grade and purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China).

Animals. Kunming mice, weighing 20.0 ± 2.0 g [Grade II, Certificate Number SCXK (gan) 2006−0001], were purchased from Jiangxi College of Traditional Chinese Medicine, Jiangxi Province, China. All animals used in this study were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institute of Health (NIH, Publication No. 85−23, 1996), and all procedures were approved by the Animal Care Review Committee (Animal application approval number 0064257), Nanchang University, China.

Polysaccharide Preparation. Polysaccharide from *P. asiatica* L. seeds was prepared using our previous published method.[14] The seeds of *P. asiatica* L. (50 g) were defatted with 1 L ethanol at room temperature for 24 h under stirring and then extracted with 500 mL of doubly distilled water at 100 °C for 2 h. The residue was re-extracted. The combined aqueous extract, a gel of high viscosity, was centrifuged (4800*g*, 10 min), prefiltered through a cotton cloth bag, and concentrated in a rotary evaporator under reduced pressure at 55 °C to yield *P. asiatica* L. water extract. The filtrate was mixed with 1.5 g/L papain and heated in water at 60 °C for 2 h. The resulting aqueous solution was extensively dialyzed against doubly distilled water for 72 h and precipitated by adding 4 volumes of anhydrous ethanol at 4 °C for 12 h. After centrifugation, the precipitate was washed with anhydrous ethanol, dissolved in water, and lyophilized to yield the polysaccharide (purity >98.0%).

Animal Experiment. Male 6-week-old Kunming mice (20.0 ± 2.0 g) were individually housed in stainless steel cages in a room with controlled temperature (25 ± 0.5 °C), relative humidity (50 ± 5%), and a 12 h/12 h light/dark cycle. All mice were fed with the same amount of basal diet (Table 1) and randomly divided into two groups: (1) Polysaccharide treated group: mice were given oral administration of the polysaccharide from *P. asiatica* L. seeds (0.4 g/kg body weight). The dose for the polysaccharide administrated was selected according to our previous research.[17] (2) Control group: mice were given distilled water of the same volume as the mean volume of the polysaccharide treated group. Each group had 48 mice, and every mouse was housed in one cage.

Table 1. Composition of the Basal Diet

component	g/kg dry weight (DW) basis
corn starch	454
soybean meal	240
sucrose	100
corn oil	60.0
cellulose	50.0
gelatinized starch	10.0
vitamin mixture[a]	10.0
mineral mixture[b]	76.0

[a]Containing in g/kg (DW basis): 0.250 (2.50×10^5 IU) vitamin A; 0.002 (6.50×10^4 IU) vitamin D_3; 2.00 (2.00×10^3 IU) vitamin E; 0.410 menadinoe; 0.070 folic acid; 1.25 niacin; 0.500 calcium pantothenate; 0.200 riboflavin; 0.250 thiamin; 0.500 pyridoxine; 0.003 cyanocobalamine; 0.025 biotin; 70.0 cholin chloride. [b]Containing in g/kg (DW basis): 69.6 $CaCO_3$; 313 $C_{12}H_{10}Ca_3\cdot 4H_2O$; 115 $CaHPO_4\cdot 2H_2O$; 222 $K_2HPO_4\cdot 3H_2O$; 127 KCl; 78.3 NaCl; 38.9 $MgCO_3$; 0.204 $MnSO_4\cdot H_2O$; 0.042 KI; 0.515 NaF; 0.091 $AlNH_4(SO_4)_2\cdot 12H_2O$.

All of the mice were given oral administration of polysaccharide or water (control) by gavage at about 9:00 a.m. everyday. In addition, we determined the body weight of each mouse everyday before gavage and changed the volume of

the polysaccharide to make sure that the mice were given polysaccharide at the dose of 0.4 g/kg body weight by gavage everyday. Throughout the experiment, the animals' general health status and body mass were observed twice daily. Twelve mice of each group were randomly killed before oral administration, and after 10, 20, and 30 days of oral administration, respectively. Then the colons were aseptically removed immediately and placed on an ice-cold plate, longitudinally opened, and the luminal contents were collected. Each colon feces sample was divided into 3 equal portions for the measurements of colon fecal moisture, pH, and SCFA values.

SCFA Analysis. One third of the stool sample was rapidly put into a round-bottomed stoppered tube in an ice-cold water bath. One milliliter of deionized water was added to the tube and mixed intermittently on a vortex mixer for 2 min. The tube was allowed to stand in the ice-cold water bath for 20 min and then centrifuged at 4800*g* for 20 min at 4 °C. The supernatant was transferred to another round-bottomed stoppered tube. This process was repeated once. The supernatant was analyzed by injection onto the chromatographic system.

Chromatographic analysis was carried out using an Agilent 6890 N GC system equipped with a flame ionization detector (FID) and an N10149 automatic liquid sampler (Agilent, USA). GC column (HP-INNOWAX, 190901N-213, J & W Scientific, Agilent Technologies Inc., USA) of 30 m × 0.32 mm I.D. coated with 0.50 μm film thickness was used. Nitrogen was supplied as the carrier gas at a flow rate of 19.0 mL/min with a split ratio of 1:10. The initial oven temperature was 100 °C and was kept there for 0.5 min and then raised to 180 °C by 4 °C/min. The temperatures of the FID and injection port were 240 °C. The flow rates of hydrogen and air were 30 and 300 mL/min, respectively. The injected sample volume for GC analysis was 0.2 μL, and the running time for each analysis was 20.5 min. The independently replicated determinations were performed three times for each sample. Data handling was carried out with a HP ChemStation Plus software (A.09.xx, Agilent). Mean values and standard deviations were calculated from replicate determinations. Statistical analysis was also performed on triplicate values using Tukey test.

Validation of the GC method was performed according to Food and Drug Administration (FDA) criteria for bioanalytical method validation.[18] For each analyte, the lower limit of detection (LLOD), corresponding to the analyte concentration giving a signal (i.e., peak area) equal to five times the noise signal, was calculated by analyzing five blanks added with a known concentration of analyte.

Calibration curves were made in the range 2−80 mmol/L for acetic acid, 1.5−60 mmol/L for propionic acid, 1−50 mmol/L for n-butyric acid, and 0.1−5 mmol/L for *i*-butyric, *n*-valeric, and *i*-valeric acids (10 concentration levels, 3 replicated for each level), by adding known amounts of the analytes to the blank.

Accuracy was calculated in terms of recovery rate (RR%) as the following:

$$RR\% = c_1/c_2 100 \quad (1)$$

where c_1 is the measured concentration and c_2 is the concentration calculated from the quantity of standard analyte added into the colonic sample. To assess accuracy, the colonic samples used were analyzed with five independent replicate extractions. Intermediate precision, expressed as percent relative standard deviation (% RSD), was evaluated for each analyte using colonic sample, performing five independent replicate extractions of the same sample over different days.

Measurement of Colon pH. Another portion of the stool samples was diluted with distilled water at a ratio of 1:9,[19] and then the pH value was measured using a micro-pH meter (Tianjing analytic corp., Tianjing, China). The determination was performed 3 times for each sample. Mean values and standard deviations were calculated from replicate determinations.

Determination of Colon Fecal Moisture. For determining the fecal moisture content, the final portion of stool samples was put in a 105 ± 2 °C oven until constant weight. The weights before (immediately after collection) and after heating process (dried) were recorded to calculate the moisture content of the sample.[20] The determination was performed 3 times for each sample. Mean values and standard deviations were calculated from replicate determinations.

Statistical Analysis. All the experiments were done in triplicate. Results were expressed as means ± SD. Data were evaluated by 1-way analysis of variance using *SPSS 10.0* software (Version 16.0, Chicago, United States). The difference between polysaccharide treated group and control group was evaluated by Student *t* test, whereas the difference before and after administration in the same group was evaluated by paired *t* test. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Chromatography of the SCFA. Typical chromatograms showing the separation of SCFAs in standard solution and colonic sample are given in parts A and B of Figure 2, respectively. As shown in part A of Figure 2, the HP-INNOWAX column satisfactorily separated the standard

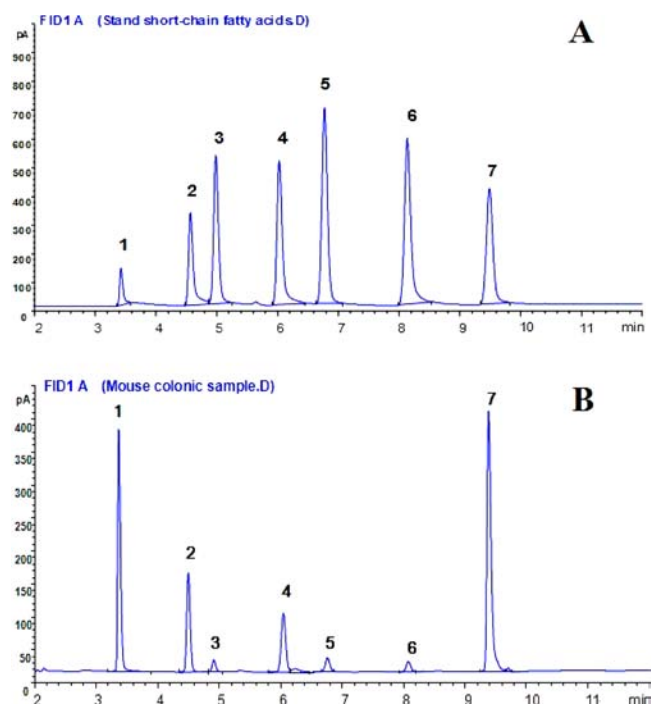

Figure 2. Gas chromatograms of short-chain fatty acids. (A) Standard solution of SCFAs with internal standard. (B) Mouse colonic sample with internal standard. Peaks: 1 = acetic acid, 2 = propionic acid, 3 = *i*-butyric acid; 4 = *n*-butyric acid; 5 = *i*-valeric acid; 6 = *n*-valeric acid; 7 = 4-methylvaleric (internal standard).

Table 2. Retention Time, Calibration Curves, Accuracy, and Precision of Different Short-Chain Fatty Acids in Standard Solution

analyte	retention time[a] (min)	calibration curve	correlation coefficient	RR%	RSD%
acetic acid	3.37 (0.11)	$Y = 64.674x - 84.992$	0.999	98.9 (1.1)	2.81
propionic acid	4.49 (0.11)	$Y = 122.625x - 45.451$	0.999	104 (1)	2.39
i-Butyric acid	4.91 (0.29)	$Y = 151.870x - 20.633$	0.999	99.1 (1.7)	1.20
n-butyric acid	5.93 (0.15)	$Y = 181.650x - 65.895$	0.999	104 (1)	3.19
i-valeric acid	6.67 (0.10)	$Y = 202.327x - 27.347$	0.999	102 (3)	3.58
n-valeric acid	8.01 (0.21)	$Y = 184.732x - 17.363$	0.999	99.1 (1.2)	2.96

[a]Each value represents the mean value, and the value in parentheses is the C.V. of 5 determinations (%).

SCFAs within 10 min. LLOD was calculated for each analyte and tested for accuracy and precision to meet the FDA criteria of bioanalytical validation guidelines.[18] LLOD value of 0.1 mmol/L was obtained for acetic and propionic acids, whereas a LLOD of 0.05 mmol/L was calculated for *n*-butyric, *i*-butyric, *n*-valeric, and *i*-valeric acids. A RSD less than 5% was obtained for all the analytes, in compliance with the requirements of the guidelines for the validation of bioanalytical methods (Table 2). Good linearity was proved in the range 2−80 mmol/L for acetic acid, 1.5−60 mmol/L for propionic acid, 1−50 mmol/L for *n*-butyric acid, and 0.1−5 mmol/L for *i*-butyric, *n*-valeric, and *i*-valeric acids by applying Mandel's fitting test.

SCFAs could be determined by gas chromatography (GC) and high-performance liquid chromatography (HPLC).[21,22] For HPLC methods, troublesome derivatization is often required. While for GC methods, some time-consuming pretreatments, such as acidification,[23] solvent extraction,[24] and headspace extraction [25] were involved in most procedures. The method applied in our study just needed to homogenize the samples in water. Then the supernatants obtained after centrifugation could be directly injected into GC without any other pretreatments, which may reduce the time of determination. In addition, the high recovery values proved the accuracy of the method in this study (Table 2). Therefore, it may be a simple, rapid, and accurate GC analytical method for quantification of SCFA in colon fecal content.

General Health Status of Mice. Throughout the experiment period, no noticeable behavioral or activity changes were observed in the mice, and no treatment-related illness or death occurred. The growth of mice appeared normal throughout the experimental period, and no mouse experienced diarrhea or constipation. There was no observable difference in the animals' body mass and hair luster between the polysaccharide treated group and control group.

SCFA Concentration. Total SCFA concentrations in the mouse colon feces of polysaccharide treated group compared to the control group were presented in Figure 3. The concentrations of acetic, propionic, *n*-butyric, *i*-butyric, *n*-valeric, and *i*-valeric acids were shown in Figure 4. As shown in Figures 3 and 4, the polysaccharide treated group contained significantly higher concentrations of total SCFA as compared with the control group all the time ($p < 0.05$), with acetic acid, propionic, and *n*-butyric acids being the dominant components. It could be seen from Figure 3 that the total SCFA concentration of polysaccharide treated group significantly increased along with the experimental time increasing, and reached the maximum level of 78 mmol/L after 30 days of oral administration ($p < 0.05$), which was nearly 1.8 fold of the control group at the same time (44 mmol/L).

Among the SCFA, acetic acid is found to be oxidized by brain, heart, and peripheral tissues.[5] Results showed that the

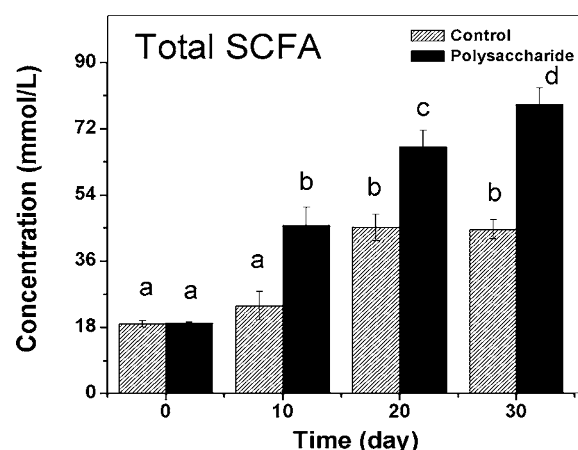

Figure 3. Total SCFA concentrations (mmol/L) in the mouse colon feces of polysaccharide treated groups compared to the control group. Values are expressed as the mean ± standard deviation of twelve mice. Different letters mean significantly different ($p < 0.05$) from each other.

acetic acid concentration of the polysaccharide treated group was significantly higher than the control group after 10, 20, and 30 days of oral administration (part a of Figure 4, $p < 0.05$), and the values for polysaccharide treated group were approximately 2-fold higher than the control group all of the time. Furthermore, the concentration of acetic acid reached the maximum value of nearly 53 mmol/L for polysaccharide treated group compared to 29 mmol/L for the control group after 30 days of oral administration.

Significant difference was also observed between the polysaccharide treated group and control group in the amount of propionic acid produced (part b of Figure 4**b**, $p < 0.05$). In particular, the concentration of propionic acid in mouse colon of the polysaccharide treated group reached the maximum level of 13 mmol/L after 30 days of oral administration, which was 2.2-fold higher than the control group at the same time (6 mmol/L). Additionally, a noteworthy increase was observed in the concentration of propionic acid in mouse colon of the polysaccharide treated group along with the experimental time increasing ($p < 0.05$). On the contrary, in the control group, there was no significant difference between the initial propionic acid concentration and the levels after 30 days of oral administration in mouse colon ($p > 0.05$). According to Mortensen et al., the production of propionic acid could be promoted by the fermentation of glucose, xylose, and arabinose.[26] The polysaccharide from *P. asiatica* L was high in xylose and arabinose content with a relatively lower amount of glucose,[14] so the increase of propionic acid might also result from the fermentation of xylose, arabinose, and glucose in the

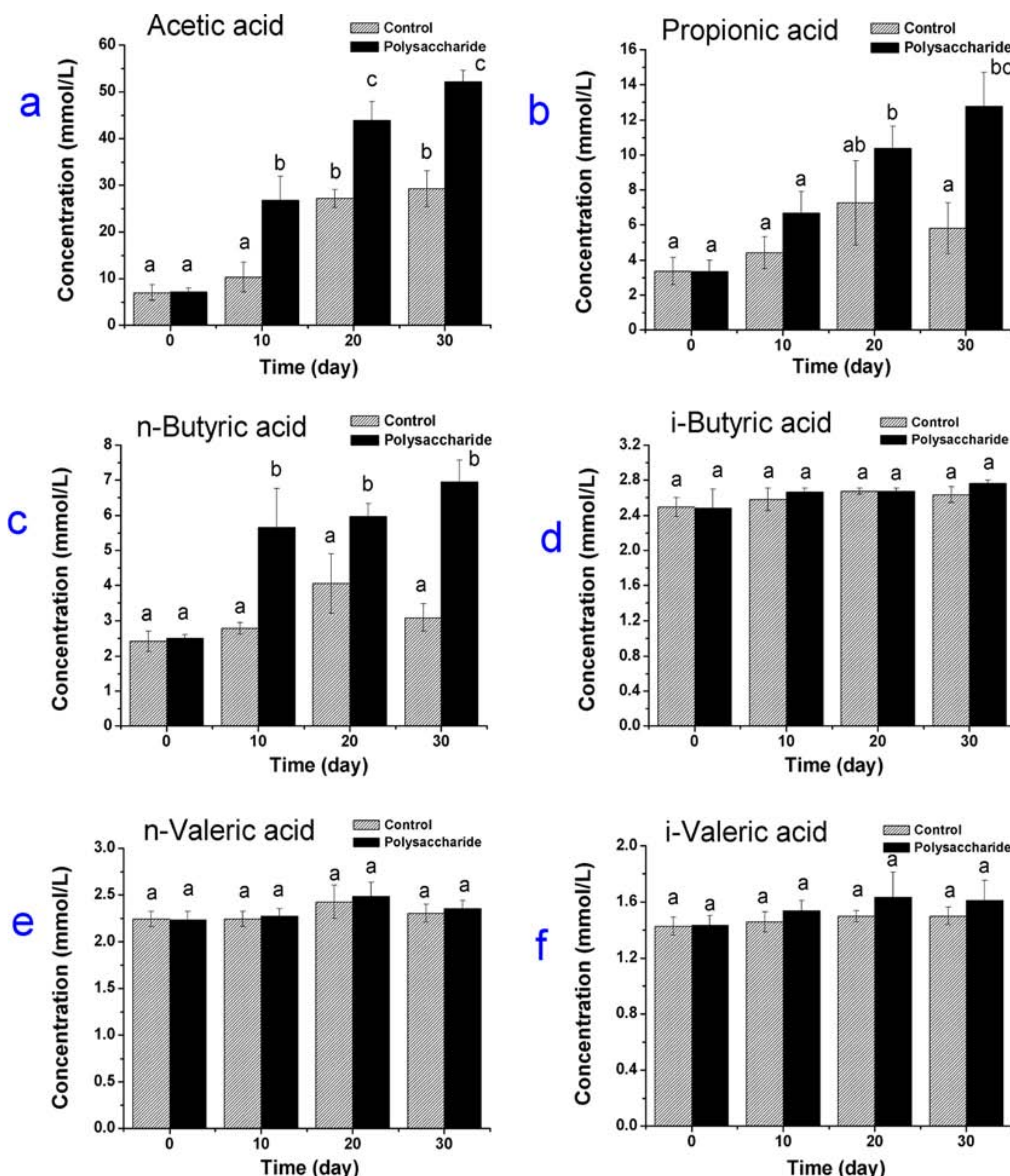

Figure 4. The concentration (mmol/L) of acetic (a), propionic (b), *n*-butyric (c), *i*-butyric (d), *n*-valeric (e), and *i*-valeric acid (f) in the mouse colon feces of polysaccharide treated group compared to the control group. Values are expressed as the mean ± standard deviation of twelve mice. Different letters mean significantly different ($p < 0.05$) from each other.

polysaccharide. Several studies have suggested that propionic acid could have various beneficial effects. For example, propionic acid was found to significantly reduce serum cholesterol levels in rats.[6] Furthermore, in vitro study using rat liver cells has shown that physiological concentrations of propionic acid may attenuate hepatic cholesterol synthesis.[27] Therefore, the increase of propionic acid in mouse colon resulting from the fermentation of the polysaccharide from *P. asiatica* L. may have positive effects on human health.

Butyrate is an important source of energy for the intestinal mucosa and its concentration in the lumen of the large bowel was therefore considered as a preventive factor against the development of colonic diseases.[28] An inhibitory effect of butyrate on the growth of cancer cells has been demonstrated in vitro.[29] Furthermore, subjects with colon cancer and adenomas had a lower amount of butyric acid in feces compared with healthy controls.[28] In our study, similar to acetic and propionic acids, *n*-butyric acid concentration of polysaccharide treated group was always significantly higher than the control group (part c of Figure 4, $p < 0.05$). Obviously, the maximum level of polysaccharide treated group (7 mmol/L, after 30 days of oral administration) was more than 2-fold of the control group (3 mmol/L) at the same time. Salvador et al. studied the relationship between the disappearance of dietary fiber sugars and the production of individual SCFA and found that xylose tended to have a greater impact on the production of butyric acid.[30] The polysaccharide from *P. asiatica* L was rich in xylose amount,[14] so the increase of butyric acids might be due to the fermentation of xylose in the polysaccharide.

Pars d−f of Figure 4 showed that there was no significant difference in the concentration of *i*-butyric acid, *n*-valeric acid, and *i*-valeric acid in mouse colon between the polysaccharide

treated and control groups ($p > 0.05$) throughout the experimental period.

There exists some relevance for SCFA levels in colon between mice and humans. For example, the major colon bacteria of mice and humans both were similar. They are *Bacteroides*, *Eubacterium*, and *Peptostreptococcus*,[31] so the colon bacteria that might utilize the polysaccharide were also similar. In addition, levels of total SCFA concentration in colon were found similar between these two species, and the proportion of the main SCFA acetic acid/propionic acid/butyric acid were both found to vary from 75: 15: 10 to 40: 40: 20 commonly.[32] Therefore, our research which focus on the effects of polysaccharide from *P. asiatica* L. on colon SCFA concentrations of mice could also provide some information for the effects of the polysaccharide on that of humans.

Colon Fecal Moisture. We next examined the effect of polysaccharide from the seeds of *P. asiatica* L. on fecal moistures in the mouse colon. As shown in Table 3, the initial fecal moisture (before oral administration) of the polysaccharide treated group was 60.5 ± 0.3%, which was similar to the control group (60.8 ± 0.4%). This was because the two groups were both fed with the same amount of basal diet (Table 1) before oral administration. Changes in fecal moisture were observed between these two groups after oral administration. Of note was that the fecal moistures of the polysaccharide treated group after 10, 20, and 30 days of oral administration were all significantly higher ($p < 0.05$) than the initial levels. In addition, the highest fecal moisture of polysaccharide treated group mice was 69.5 ± 0.4% after 30 days of oral administration. For the control group, there was no significant difference for the mice colon fecal moisture in the initial time and after 30 days of oral administration ($p > 0.05$).

Table 3. Mouse Colon Fecal Moisture of Polysaccharide Treated Group Compared to the Control Group[a]

time (day)	Weight (mg) wet	dry	moisture (%)
Polysaccharide group			
0	131 ± 0 a	51.5 ± 0.4 a	60.5 ± 0.3 a[b]
10	140 ± 1 b	50.5 ± 0.5 a	64.0 ± 0.1 b
20	150 ± 1 c	46.6 ± 0.3 b	69.0 ± 0.3 c
30	153 ± 1 d	46.5 ± 0.1 b	69.5 ± 0.4 c
Control group			
0	132 ± 0 a	51.5 ± 0.5 a	60.8 ± 0.4 a[b]
10	131 ± 1 a	51.2 ± 0.1 a	60.9 ± 0.3 a
20	131 ± 1 a	52.4 ± 0.4 a	60.1 ± 0.5 a
30	132 ± 0 a	51.7 ± 0.5 a	60.7 ± 0.4 a

[a]Values are means ± SD, $n = 12$. [b]Different letters mean significantly different ($p < 0.05$) from each other in the same column.

The mouse colon fecal moisture of the polysaccharide treated group after 10, 20, and 30 days of oral administration was significantly higher than that of control group (Table 3, $p < 0.05$), indicating that the intake of the polysaccharide resulted in a stronger water-holding capacity for the colon content throughout the experimental period. These results suggested that the polysaccharide intake may be beneficial for the intestinal tract and colon health because the increase of fecal water can increase fecal bulk and the loosely formed stools were caused in part by consumption of an enteral formula.[33]

Some other carbohydrates were also reported to increase the volume of feces excreted and the moisture content of the feces. For example, glucose-based oligosaccharides increased the volume of feces excreted and the moisture content of the feces.[9] Furthermore, fecal moisture content appeared to increase during ingestion of soybean oligosaccharides.[34] The diet supplemented with corn starch resulted in increasing fecal weights and fecal moisture content.[35] These results were similar to ours.

In addition, the wet weight of the mouse colon feces of the polysaccharide treated group was always higher than the control group after 10, 20, and 30 days of oral administration ($p < 0.05$, Table 3) suggesting that the polysaccharide may promote normal laxation. Many carbohydrates, including cereal brans, xylooligosaccharides, and methylcellulose, increase stool weight, thereby promoting normal laxation. Their stool wet weights were also found to increase as these carbohydrates intake increased, and this was considered to be beneficial for the colon health.[19] At the same time, a decrease of colon fecal dry weight was found in the polysaccharide treated group. The dry weight of colon feces decreased from (51.5 ± 0.4) mg at the beginning to (46.5 ± 0.1) mg after one month administration. However, no significant difference was observed for colon fecal dry weight in control group. The decrease of colon fecal dry weight in polysaccharide treated group might result from the promoting defecation effect of the polysaccharide, which has been previously proved in our laboratory.[17] In addition, it has been reported that dietary fiber intake can speed up the rate of material through the intestine and increase the excretion of fecal dry weight,[36] which could also make the fecal dry weight in colon become less than that before the dietary fiber intake.

Colon Fecal pH. A lowering of colonic pH was found to be beneficial, and the subjects with colonic cancer had a higher fecal pH than healthy controls.[8] Therefore, the change of fecal pH might be a helpful indicator for assessing the effects of the polysaccharide from *P. asiatica* L. seeds on mouse colon. The initial colon contents of mice were slightly alkaline (pH 7.5, Figure 5). A moderate decrease in pH (7.5 ± 0.1 to 7.2 ± 0.1, $p < 0.05$) was observed in the mouse colon of polysaccharide

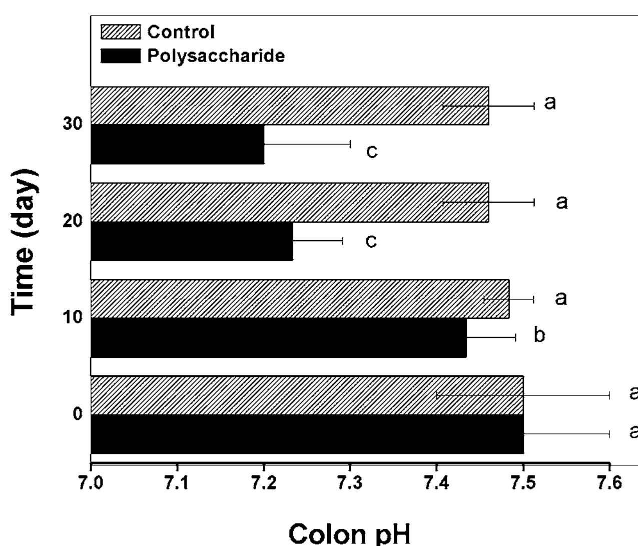

Figure 5. pH change in the mouse colon feces of polysaccharide treated group compared to the control group. Values are expressed as the mean ± standard deviation of twelve mice. Different letters mean significantly different ($p < 0.05$) from each other.

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treated group after 30 days of oral administration compared to the control (pH from 7.5 ± 0.1 to 7.5 ± 0.1). The pH of the colon contents for the control group within 30 days did not differ significantly ($p > 0.05$). However, the colon pH values of the polysaccharide treated group after 30 days of oral administration were relatively lower than the initial levels ($p < 0.05$), which may be due to the fermentation of the polysaccharide in the mouse colon. This could be good for colon health, because a lower colonic pH could inhibit the proliferation of undesirable pathogens and also affect microbial enzymes' activity.[37] In addition, it seems to be helpful for protection against colonic carcinogenesis, which is thought to exert beneficial health effects on human physiology.[7]

A low pH in feces was associated with a reduced incidence of colon cancer in various populations.[31] It was reported that stool pH of the colon cancer patients was 7.0 ± 0.6, whereas the stool pH of controls was 6.7 ± 0.6.[8] Malhotra found that the stool pH of the southern Indians in high risk of colon cancer was 7.8 ± 1.1, whereas the stool pH of northern Indians in low risk was 6.5 ± 1.0.[38] Pietroiosti et al. found that the fecal pH of colon cancer patients was 8.0 ± 0.44, whereas the fecal pH of patients without cancer was 6.6 ± 0.44.[39] Conceivably, fecal pH value could be used as a marker for susceptibility to chronic bowel diseases. Therefore, the maximum reduction of 0.3 for colon pH obtained in this study may be helpful for colon health and protection against colonic cancer in some extents. Similar to our results, some other carbohydrates were also found to enhance lowering colon fecal pH. For example, a decrease in rat fecal pH was observed after intake of oligosaccharides, which may be beneficial in improving gastrointestinal health.[40] Colonic pH was also found to be lowered for the fermentation of the guar gum, oat bran and wheat bran in rat model.[4]

Our results may show the effects of polysaccharide from *P. asiatica* L. on mouse colons. The fermentation of the polysaccharide in mouse colon could significantly increase the concentrations of total SCFA, of which acetic, propionic, and *n*-butyric acids are the dominant components. In addition, fecal moisture in the mouse colon was raised due to the polysaccharide indicating that the intake of the polysaccharide resulted in a stronger water-holding capacity for the colon feces throughout the experimental period. Furthermore, a decreased pH was also observed in the mouse colon resulted from the fermentation of the polysaccharide. These results suggested that the intake of polysaccharide from *P. asiatica* L. may be beneficial for the colon health.

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Notes

The authors declare no competing financial interest.

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Abbreviations Used

SCFA , short-chain fatty acid; GC , gas chromatography; FID , flame ionization detector; LLOD , lower limit of detection; RR , recovery rate; RSD , relative standard deviation; FDA , Food and Drug Administration

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