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Effect of Different Molecular Weight Fragments from Corn Bran Hemicellulose on D-galactosamine-induced Hepatitis in Rats in Relation to Intestinal Degradation

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Abstract: The purpose of the present study was to investigate the preventive effect of different Molecular Weight (MW) fragments from dietary fiber Corn Bran Hemicellulose (CBH) on hepatitis induced by D-galactosamine in rats in relation to their degradation and fermentation in gastrointestinal tract. Rats fed the diets supplemented with the fragments with or without neomycin sulfate in experiment 1 and the diets supplemented with Short Chain Fatty Acids (SCFAs) in experiment 2 were administered with D-galactosamine intraperitoneally. The serum transaminase activities, cecal and fecal total and reducing sugar content, cecal SCFAs were analysed. Among the fragments tested, the oligosaccharide fragments with MW ranging from 285.7 to 930.1 significantly suppressed the enhancement of serum transaminase levels caused by D-galactosamine. The ingested oligosaccharide fragments produced striking amount of SCFAs in cecum particularly propionate formation. However, SCFAs tested in experiment 2 could not suppress the elevation of serum transaminase levels by D-galactosamine. The present study demonstrated that the oligosaccharide fragments were effective in preventing D-galactosamine hepatitis and the effect might not be mediated by the action of SCFAs.

Key words: Dietary fiber, corn bran hemicellulose, D-galactosamine hepatitis, oligosaccharides, short chain fatty acids

INTRODUCTION

Recently, water soluble dietary fibers have been paid more and more attention due to the physiological functions. It is considered that they improve glucose tolerance of diabetic patients, decrease serum cholesterol of hypercholesterolemia, and prevent constipation and obesity[1-4]. Corn Bran Hemicellulose (CBH) can be obtained as a kind of water soluble dietary fiber with arabinoxylan as main component. A recent investigation has demonstrated that corn hemicellulose has the protective effect on D-galactosamine-induced hepatitis by suppressing the enhancement of plasma transaminase activities in rats and presumed that the protective effect would occur probably in connection with its intestinal fermentation[5]. Wang *et al.*[6] have reported that some indigestible oligosaccharides inhibited the development of D-galactosamine hepatitis although xylooligosaccharide has not been examined. However, Kajihara *et al.*[7] have demonstrated that xylooligosaccharide decreased blood ammonia levels in patients with liver cirrhosis.

Accordingly, taking these into consideration, it may be interesting and beneficial to investigate whether large molecule polysaccharides or small molecule oligosaccharides or monosaccharides are effective in protecting liver from hepatitis. Hepatitis induced by D-galactosamine in rats is considered to be mediated by inhibiting macromolecular glycoprotein and RNA biosynthesis through depletion in cellular UTP concentration[8] and by increasing absorption of endotoxin from intestine[9]. Keppler *et al.*[10] have revealed that D-galactosamine hepatitis is histopathologically similar to human viral hepatitis in features. Therefore, it has served as one of the experimental models for viral hepatitis studies.

The present study was performed to estimate the preventive effect of different Molecular Weight (MW) fragments, polysaccharides (PS), oligosaccharides (OS) and monosaccharides (MS) from CBH on D-galactosamine-induced hepatitis as assessed by the activities of serum transaminases. The involvement of the intestinal fermentation of CBH fragments in the preventive effect was also conducted with or without neomycin

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sulfate (N), a kind of antibiotics. Furthermore, the present study was carried out to examine whether the liver-protective effect was mediated by the action of Short Chain Fatty Acids (SCFAs) which were the fermentation products of the CBH fragments.

MATERIALS AND METHODS

Materials and chemicals: The CBH (water-soluble type) was kindly supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan). Endo-1, 4-β-xylanase (EC3.2.1.8, from *Thermomyces lanuginosus*), neomycin sulfate, iso-butyric acid, n-valeric acid, iso-valeric acid, naphthoresorcinol and 2,5-dihydroxybenzoic acid were obtained from Sigma Chemical (St. Louis, USA). A kit for transaminase assay (Iatrozyme TA-L_Q) was obtained from Iatron Laboratories Inc. (Tokyo, Japan). Mineral and Vitamin mixtures (AIN-93G) were purchased from Nihon Nosan Kogyo K.K (Yokohama, Japan). Other chemicals (analytical and HPLC grade) were obtained from Wako Pure Chemicals unless otherwise indicated.

Preparation of different MW CBH fragments: The PS was precipitated from a 10% CBH solution according to Kabel *et al.*[11]. The dried matter was ground by a grinder and passed through a 60 mesh sieve. The OS was prepared by incubating 5% PS in 0.02 mol L^{-1} sodium acetate buffer (pH 5.0) with endo-1,4-β-xylanase at 50°C for 24 h and separated by activated charcoal chromatography as described previously[12-14]. The MS was prepared by hydrolyzing PS in its 15% aqueous solution with dilute sulfuric acid (final concentration, 0.5 mol L^{-1}) according to Soderstrom *et al.*[15]. The main component of PS, OS and MS was determined to be arabinoxylan with MW above 20 kDa, a large amount of xylobiose and a small amount of xylo-oligosaccharides or xylo-arabino-oligosaccharides with polymerization degree (DP) of 3-7 with MW ranging from 285.7 to 930.1, and xylose and arabinose with MW of 157.2 and a trace amount of oligosaccharides with MW from 285.8 to 523.1 (DP 2-4), respectively by thin-layer chromatography and MALDI-TOF mass spectrometry according to the methods of Yoshida *et al.*[16] and Kabel *et al.*[17-19].

Animals and diets: Four-week-old male Wistar specific pathogen-free rats, weighing 60-80 g, were obtained from Japan SLC (Shizuoka, Japan). The rats were individually housed and kept in an air-conditioned room (22±1 °C) with a 12 h light and dark cycle (light on 0730 to 1930). All the rats were facilitated to the ambience, given free access to water and a commercial stock diet (CE-2, Clea Japan) for 3 days and then divided into 2 parts for 2 experiments, respectively. In experiment 1, 73 rats were acclimated to the meal feeding of a control diet (AIN-93G) twice daily (0800-0900, am; 2000-2100, pm) for 6 days and then divided into 10 groups. These groups were fed either the control diet or the semipurified experimental diets supplemented with the fragments with or without N by the meal feeding for the next 8 days (Table 1). In experiment 2, 30 rats were given free access to the control diet for 6 days and then divided into 5 groups. These groups were fed either the control diet or the semipurified experimental diets supplemented with sodium acetate, sodium propionate, or sodium n-butyrate at the expense of cellulose at the levels of 27.3, 24.1, 25.0 g kg^{-1}, respectively corresponding to a net amount of each short chain fatty acid of 20.0 g kg^{-1} for the next 8 days. All rats were allowed free access to water during the whole experiment period.

On day 7 after feeding the experimental diets, D-galactosamine adjusted as a sterilized solution of 160 mg mL^{-1} saline with neutral pH was injected intraperitoneally at a dose of 500 mg kg^{-1} body weight. Normal rats were injected with saline. The rats were fasted for 4 h before and 7 h after D-galactosamine administration in experiment 1 and for 4 h before and also 4 h after the administration in experiment 2. Normal and control rats were fed the control diet.

The blood was drawn from rat hearts under pentobarbital anesthesia and cecum and liver were removed carefully, immediately frozen in -80°C liquid nitrogen and weighted 22 h after injection of D-galactosamine on day 8. Serum was obtained from blood by centrifugation at 1,000×g for 20 min at 25°C. Faeces were collected at 10:30 am everyday during the whole experimental diet-period in experiment 1 and body weight and food consumption were measured every two days. The serum, cecum and feces were kept at -80°C till required for analysis. The care and treatment of the rats were carried out according to the National Institutes of Health Guide for the care and use of laboratory animals[20].

Activities of serum transaminases: The serum Asparatate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were measured with the Iatrozyme TA-L_Q kit following the instructions of the manufacturer. One International unit (U/L) is the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

Cecal and fecal total sugar: Feces collected everyday and one part of cecal content were lyophilized and ground. The sample (0.02 g) was suspended in 0.25 mL of 12 mol L^{-1} H_2SO_4 and hydrolyzed as described by

Lebet *et al.*[21]. Total sugar content of the hydrolysate was determined by Anthrone methods for hexose[22] and pentose[23] quantification. Glucose and xylose were used as standards to make standard curves, respectively. The total sugar content of the samples was corrected for basal excretion-the excretion of hexose or pentose when the rats were fed the blank diet which did not contain test substances and cellulose (Table 1).

Cecal and fecal reducing sugar: Distilled water (1.5 mL) was added to 0.01 g lyophilized cecal content or feces sample. It was heated in boiling water to inactivate bacteria. After overnight at 4°C, supernatant was obtained by centrifugation at 9,000×g for 20 min. Reducing sugar content of the supernatant was determined by the dinitrosalicylic acid assay with using glucose and xylose as hexose and pentose standards, respectively[24].

Cecal SCFAs: Distilled water (2.0 mL) was added to 0.5 g of wet cecal content and homogenized at 4°C for 2 min and then 5 μL of 0.005 mol L^{-1} 2-ethyl-butyric acid was added as internal standard solution. The final supernatant was obtained by successive centrifugation at 6,500×g, 9,000×g and 11,500×g for 20 min at 4°C, respectively and passed through the BondElut SCX column (VARIAN, USA). The filtrate was filtered through a 0.45 μm membrane filter (Millipore) and 20 μL of the second filtrate was applied on an HPLC system (Shimadzu, LC-10A) using a column, ULTRON PS-80H (8.0 mm I.D.×30 cm) (SHIN WA CHEMICAL INDUSTRIES. LTD.) (Kyoto, Japan) at 60°C. The sample was eluted with aqueous solution (pH 2.1) adjusted with perchloric acid at a flow rate of 1.0 mL min^{-1}. The SCFAs in eluent were detected at 210 nm.

The sodium salts of citrate, lactate, formate, acetate, propionate and n-butyrate, iso-butyric acid, n-valeric acid and iso-valeric acid were used as standards. Sample identification was confirmed by comparing retention time to those of standards. Quantification was accomplished using standard curves.

Statistical analysis: Means±SE was calculated and Scheffe's multiple range test was used when significant differences were observed.

RESULTS AND DISCUSSION

The growth and food intake were depressed by the MS diet compared with those fed the OS diets (Table 2). The addition of neomycin sulfate had no effect on body weight gain except the MS (N) group and on liver weights except the OS (N) group. Food intakes were reduced by the supplementation of neomycin sulfate except the MS (N) group. Marked increases in cecum weight, wet weight of cecal content and cecasomatic index were found in the OS group compared with D-galactosamine-treated control group. These parameters were significantly higher for neomycin-fed groups than the corresponded diet-fed groups. Moisture of cecal content in the OS and MS groups were almost at the same levels and significantly higher than that of the control group. Neomycin sulfate did not result in the difference in cecal moisture between the neomycin-fed groups and the corresponded diet-fed groups except the control (N) group (Table 2).

The serum AST and ALT activities were dramatically enhanced by D-galactosamine injection compared with the saline-treated normal rats from 47.22±3.51 to 807.33±112.58 U L^{-1} and 12.32±0.50 to 477.72±89.21 U L^{-1}, respectively (Fig. 1). The increases in these enzyme activities were significantly suppressed by OS diet by 544.86 and 335.73 U L^{-1} for AST and ALT, respectively. The MS diet tended to decrease the enhanced enzyme activities caused by D-galactosamine. The PS diet had no effect on these activities compared with the D-galactosamine-treated control rats (Fig. 1A). Among the groups with the addition of neomycin sulfate, the serum AST and ALT activities were significantly lower in the MS group than the control group. Moreover, the serum transaminase activities were found to tend to be lowered by the OS diet (Fig. 1B). The PS (N) and MS (N) diets suppressed the elevation of serum AST and ALT activities compared to the respective PS and MS groups (Fig. 1B).

The cecal total sugar content in PS, OS and MS groups in either case of the presence or the absence of neomycin sulfate were significantly lower than that in the control group as well as the total sugar content in feces of the last day (Fig. 2A and B). However, much more cecal and fecal total sugar was found in the PS (N), OS (N) and MS (N) groups whereas less cecal and fecal total sugar in the control (N) group compared to their corresponding groups, respectively (Fig. 2B).

In contrast to the total sugar, the cecal reducing sugar content in the OS group and the fecal reducing sugar content of last day in the PS, OS and MS groups were significantly higher than those in the control group as shown in Fig. 3A. Among the groups with the addition of neomycin sulfate, low MW fragments, OS and MS, significantly resulted in the increase in reducing sugar content both in cecum and in feces of the last day compared with high MW materials, cellulose and PS. In

Table 1: The composition of diets (g kg^{-1}) (Experiment 1)

Constituents	Blank	Control	Control (N)	PS	PS (N)	OS	OS (N)	MS	MS (N)
Corn starch	447.486	397.486	390.786	397.486	390.786	397.486	390.786	397.486	390.786
Casein	200.000	200.000	200.000	200.000	200.000	200.000	200.000	200.000	200.000
Dextrinized cornstarch	132.000	132.000	132.000	132.000	132.000	132.000	132.000	132.000	132.000
Sucrose	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
Soybean oil	70.000	70.000	70.000	70.000	70.000	70.000	70.000	70.000	70.000
Mineral mixture1	35.000	35.000	35.000	35.000	35.000	35.000	35.000	35.000	35.000
Vitamin mixture1	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Cellulose		50.000	50.000						
Test substances2				50.000	50.000	50.000	50.000	50.000	50.000
L-cystine	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Choline bitartrate	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Tert-butylhydroquinone	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014	0.014
Neomycin sulfate[3]			6.700		6.700		6.700		6.700
Total	1000.000	1000.000	1000.000	1000.000	1000.000	1000.000	1000.000	1000.000	1000.000

[1]AIN-93G, [2]PS, OS, MS. MS was added to the diet as an aqueous solution, [3]A kind of antibiotics. N, neomycin sulfate

Table 2: Body weight gain, food intake, liver and cecum parameters of rats fed the control and experimental diets[1,2] (Experiment 1)

	Non-neomycin sulfate supplemented					Neomycin sulfate supplemented			
Groups	Normal (+saline)	Control (+GalN)	PS (+GalN)	OS (+GalN)	MS (+GalN)	Control (N) (+GalN)	PS (N) (+GalN)	OS (N) (+GalN)	MS (N) (+GalN)
Body weight gain (g/7.5 d)	30.00±0.98a	27.50±2.9ab	28.70±2.68ab	33.00±1.75a	19.30±1.77bc	28.71±1.39	29.57±2.11	31.31±1.90	30.38±2.28**
Food intake (g/7.5 d)	87.20±1.40a	79.90±2.00a	74.40±2.70ab	77.90±1.70a	66.60±1.80b	69.75±1.90**	63.91±2.21*	68.64±2.18**	67.57±2.99
Liver weight (g)	5.87±0.15	4.98±0.20	5.00±0.33	5.71±0.25	4.91±0.20	4.80±0.27	4.78±0.21	5.01±0.14*	4.99±0.31
Hepatosomatic index[3]	4.11±0.07	3.66±0.09	3.63±0.15	3.99±0.13	3.83±0.08	3.48±0.11	3.45±0.09	3.55±0.08*	3.54±0.10
Cecum									
Weight (g)	2.06±0.09a	2.41±0.31ab	3.61±0.24bc	5.14±0.35d	4.26±0.31cd	7.85±0.54a**	11.95±0.58b**	11.22±0.86b**	11.40±0.56b**
Content wet weight (g)	1.54±0.08a	1.90±0.28ab	2.78±0.19b	4.03±0.29c	2.92±0.21b	6.69±0.52a**	10.08±0.62b*	8.16±0.17b**	9.34±0.92b**
Cecasomatic index[4]	1.45±0.07a	1.75±0.19ab	2.63±0.13b	3.60±0.26c	3.34±0.23bc	5.72±0.33a**	8.64±0.35b**	7.94±0.54b**	8.10±0.47b**
Moisture (%)	70.20±0.44a	73.60±0.39a	79.20±0.88ab	85.00±2.11b	87.50±2.10b	78.42±0.82a**	78.25±0.40a	85.02±2.11b	87.35±0.62b

[1]Values are means ± SEM n=7 or 8 (OS, OS (N), MS (N))

[2]Values in a row in non-neomycin sulfate supplemented groups with different letters and in neomycin sulfate supplemented groups with different letters in italics are significantly different at $p<0.05$. Values with asterisk in neomycin sulfate supplemented groups are significantly different from those for the corresponding non-neomycin sulfate supplemented groups at $p<0.05$ (*) or $p<0.01$ (**), [3]Liver weight (g) × 100/final body weight (g), [4]Cecum weight (g) × 100/final body weight (g).
N, neomycin sulfate; GalN, D-galactosamine.

Table 3: Body weight gain, food intake, liver weight, cecum weight and serum transaminase activities of rats fed the control and short chain fatty acid supplemented diets[1,2] (Experiment 2)

					Enzyme activity (U L^{-1})	
Groups	Body weight gain (g/7.5 d)	Food intake (g/7.5 d)	Liver wt. (g)	Cecum wt. (g)	AST	ALT
Normal (+saline)	36.83±0.86	103.30±1.69	5.55±0.11	2.18±0.05	49.96±3.27 a	13.29±0.63a
Control (+GalN)	35.33±1.84	102.67±1.80	4.98±0.18	1.82±0.13	521.84±61.29 b	458.69±78.62b
Acetate (+GalN)	33.67±0.65	101.05±1.32	5.42±0.09	1.76±0.11	659.35±35.70 b	467.68±42.33b
Propionate (+GalN)	32.17±1.01	95.22±1.99	5.65±0.13	1.93±0.09	457.17±68.86 b	321.91±52.11ab
n-Butyrate (+GalN)	35.83±2.31	102.74±3.87	5.70±0.48	1.99±0.03	520.14±132.54 b	434.53±77.79b

[1]Data are means±SEM, n=6, [2]Data in a column with different letter(s) are significantly different at $p<0.05$., AST, asparatate aminotransferase; ALT, alanine aminotransferase; GalN, D-galactosamine.

the presence of neomycin sulfate, both cecal and fecal reducing sugar content for all groups were significantly higher than those for their corresponding groups (Fig. 3B).

Acetate, propionate, n-butyrate and n-valerate were detected in the cecal content of rats fed the control, PS, OS and MS diets. The OS diet influenced the production of SCFAs greatly, namely, the highest pool of each acid was recorded in the rats fed OS diet, whereas no significant difference was observed in rats fed other diets (Fig. 4). For the individual SCFA, CBH fragments all were associated with more propionate production for the molar ratios of acetate, propionate, n-butyrate and n-valerate were 74:18:5:3, 72:19:5:4, 55:36:7:2, 60:33:4:3 and 65:27:6:2 for the normal, control, PS, OS and MS groups, respectively.

Concerning the effect of SCFAs on D-galactosamine hepatitis, none of the SCFAs tested depressed the growth and food intake and affected liver and cecum weight compared with the D-galactosamine-treated control group. The elevation of serum AST and ALT induced by administration of D-galactosamine was not suppressed by

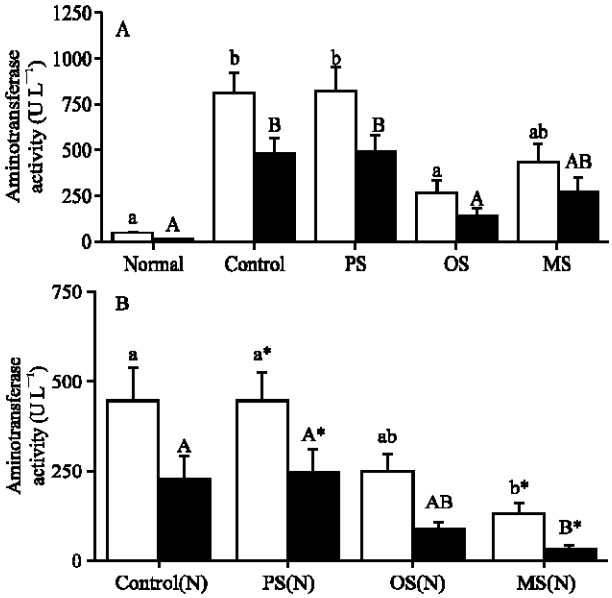

Fig. 1: Effect of different molecular weight fragments from corn bran hemicellulose on serum transaminase activities after D-galactosamine administration without (A) or with (B) neomycin sulfate. □, asparatate aminotransferase (AST); ■, alanine aminotransferase (ALT). Data are means±SEM {n=7 or 8 (OS, OS (N), MS (N))}. Different small and capital letters are significantly different for AST and ALT, respectively at $p<0.05$ in (A) and (B). Columns with asterisk in (B) are significantly different from those of rats fed their corresponding non-neomycin sulfate supplemented diets at $p<0.05$(*)

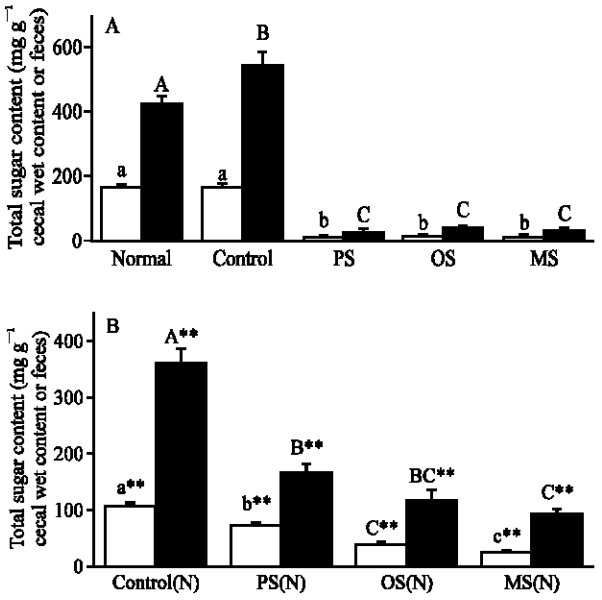

Fig. 2: Total sugar content in cecum and feces of rats fed the control and experimental diets supplemented with different molecular weight fragments from corn bran hemicellulose without (A) or with (B) neomycin sulfate. □ , cecal total sugar content; ■, fecal total sugar content. Data are means±SEM {n=7 or 8 (OS, OS (N), MS (N))}. Different small and capital letters are significantly different for cecum and feces, respectively at $p<0.05$ in (A) and (B). Columns with asterisk in (B) are significantly different from those of rats fed their corresponding non-neomycin sulfate supplemented diets at $p<0.05$ (*) or $p<0.01$ (**)

the supplementation of SCFAs in diets. Propionate was found to tend to normalize them, but no significant difference was observed (Table 3).

The present study was conducted to estimate the preventive effect of different MW fragments from CBH on hepatitis induced by D-galactosamine in rats from the viewpoint of intestinal degradation and fermentation for the first time. This study was taken to indicate that the OS with MW ranging from 285.7 to 930.1 had liver protective effect (Fig. 1A) since the OS depressed the D-galactosamine - induced enhancement of serum transaminase activities the extent of which is generally considered to be directly proportional to the severity of liver injury. Based on the investigation of SCFAs such as acetate, propionate and n-butyrate which are main fermentation products of the OS on hepatitis induced by D-galactosamine, it could not be concluded that the preventive effect of the OS is mediated by the action of SCFAs (Table 3).

All fragments from soluble dietary fiber induced completely different effects on cecum from that insoluble dietary fiber (cellulose) did. The incorporation of PS, OS and MS, particularly OS, in the diet elicited striking increasing effects on cecum weight as well as wet weight of cecum content compared with the control diet (Table 2). Higher cecum weight has been suggested to be caused by either soluble dietary fibers or oligosaccharides compared with that by cellulose or fiber-free diets[6,25-28]. Remesy *et al.*[29] have reported that cecal hypertrophy tended to be highly associated with fermentability rather than with accumulation of dietary fiber. This indicates that low fermentable fibers that accumulate in the cecum have little hypertrophic effect. It was also the case in this study. Moreover, both cecal and fecal total sugar content in rats fed PS, OS and MS diets were much lower, whereas both reducing sugar content were much higher than those in rats fed the control diet (Fig. 2A and 3A) indicating that

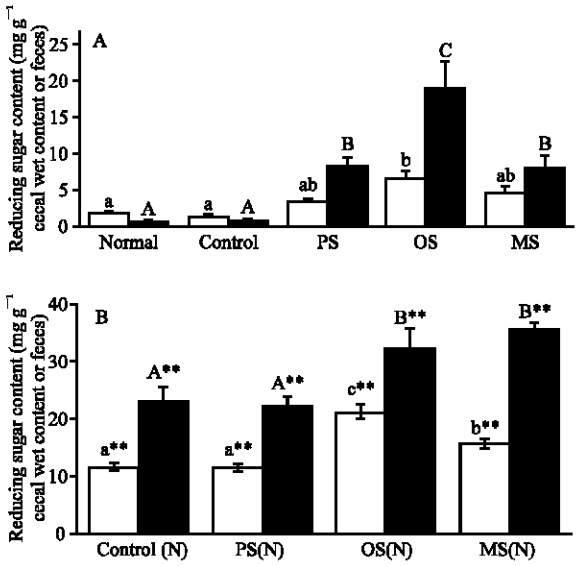

Fig. 3: Reducing sugar content in cecum and feces of rats fed the control and experimental diets supplemented with different molecular weight fragments from corn bran hemicellulose without (A) or with (B) neomycin sulfate. □, cecal reducing sugar content; ■, fecal reducing sugar content. Data are means±SEM {n=7 or 8 (OS, OS (N), MS (N))}. Different small and capital letters are significantly different for cecum and feces, respectively at $p<0.05$ in (A) and (B). Columns with asterisk in (B) are significantly different from those of rats fed their corresponding non-neomycin sulfate supplemented diets at $p<0.05$ (*) or $p<0.01$ (**)

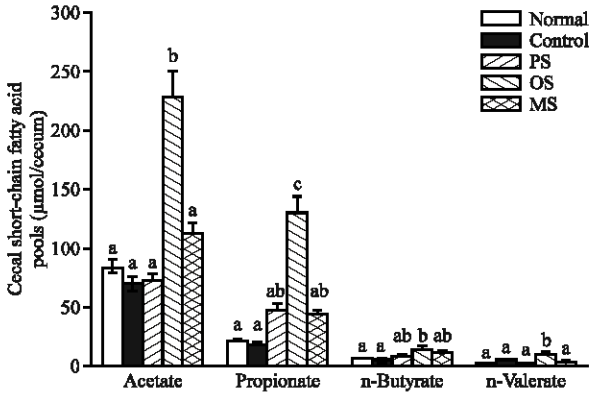

Fig. 4: Effect of different molecular weight fragments from corn bran hemicellulose on production of short chain fatty acids in cecum. Data are means±SEM for 7 or 8 rats (OS). For each short-chain fatty acid, different letters indicate significant differences at $p<0.01$

extensive degradation by gastrointestinal bacteria occurred in the rats fed PS, OS and MS diets.

Furthermore, significant production of SCFAs in cecum was only observed in OS group (Fig. 4) although extensive degradation was found in all rats fed the CBH fragments. In well agreement with Lopez *et al.*[25] and Hopkins *et al.*[30] increased propionate formation was found. Propionic acid is considered to be as glucogenic precursor and secondarily as effector of various metabolic pathways[31]. Acetate largely bypass colonic and liver metabolism, but is metabolized as a source of energy in muscle[32]. Butyrate is preferred as a energy source for colonocytes and thus is extensively metabolized by the colon[33]. Due to the striking production of SCFAs in cecum by the OS diet and their particular physiological functions, the protective ability of SCFAs against D-galactosamine hepatitis was presumed at least in part through the high level production. Therefore, the diets supplemented with acetate, propionate and n-butyrate were fed to rats and preventive effect was tested. None of them protected rats from hepatitis, manifesting that the protective effect of the OS was not mediated by SCFAs. This is not consistent with the findings by Sugiyama *et al.*[5] in which acetate showed liver protective effect against D-galactosamine hepatitis when the rats were fed the diet supplemented with acetate at levels of 15 g kg^{-1}.

The aim of use of neomycin sulfate in the present study was to block the gastrointestinal bacteria activities and fermentation of dietary fiber. Both cecal and fecal total sugar content and reducing sugar content (Fig. 2B and 3B) in CBH fragments-fed groups with neomycin sulfate were significantly higher than those in rats fed their corresponding diets without neomycin sulfate suggesting that little CBH fragments intaked was fermented or utilized in cecum, which could also be explained by the finding that cecal level of SCFAs was completely suppressed by 0.67% neomycin sulfate in the diets (data not shown). In all dietary groups with D-galactosamine, neomycin sulfate tended to lower the serum AST and ALT activities, although no significant depressing effect on the activities was found in rats fed the control and OS diets (Fig. 1B). Katayama *et al.*[34] and Kasravi *et al.*[35] have demonstrated that the enhancement of serum transaminase activities by D-galactosamine was suppressed by neomycin. Grun *et al.*[36] has revealed that endotoxin contributed significantly to the pathogenesis of D-galactosamine hepatitis by comparing its effects in the colectomized, a situation in which Gram negative bacteria and endotoxins were eliminated, the endotoxin resistant and the normal rats. Neomycin sulfate inhibits the proliferation of gastrointestinal bacteria including

endotoxin-producing Gram negative aerobes. Therefore, the results in this work suggested that the lowering effect of neomycin sulfate on the elevation of serum transaminase activities was associated with blocking of transfer of endotoxin from the gut to somatic circulation instead of suppression of fermentation of dietary fiber, although it should be clarified whether neomycin sulfate is absorbed and affects the metabolism in liver.

Wang *et al.*[6,37] have investigated the effect of several oligosaccharides on the development of D-galactosamine hepatitis with or without the presence of neomycin sulfate and pointed out that galactose-containing oligosaccharides such as lactulose, raffinose, and galactooligosaccharide exerted the protective effect via their galactose residue which was released by the action of intestinal bacteria, whereas the protective effect of these oligosaccharides disappeared in the presence of neomycin sulfate. In the present study, the OS obtained from CBH consisted mainly of xylose and arabinose rather than galactose. Accordingly, the preventive role exerted by the OS was not attributed to the galactose-dependent mechanism in which galactose protects liver against D-galactosamine hepatitis by the antagonistic metabolic competition through their uridine diphosphate derivatives.

Neither short chain fatty acid-mediated mechanism found in the present study nor galactose-dependent mechanism was applied to explain the protective effect of the OS from CBH on the D-galactosamine hepatitis. Therefore, there might be other mechanisms behind the preventive effect of the OS.

Sugiyama *et al.*[5] have investigated the effect of different types of dietary fibers including corn hemicellulose, chitin, chitosan, alginate, pectin, guar gum, glucomannan, inulin as well as green tea fiber on D-galactosamine hepatitis and concluded that all the dietary fibers tested had preventive effect suggesting that hepatitis-prevention might be one of the general effects of dietary fibers. However, in their studies, the MW or the active constituents of the dietary fibers supplemented to diets were not designated. It is reasonable to suppose that dietary fibers are a series of mixtures consisting of many different MW fragments such as high MW polysaccharides and low MW oligosaccharides. It might be the oligosaccharides that contribute to the hepatitis-preventive effect of dietary fiber because high MW polysaccharides had no protective effect in the present study.

An acidic polysaccharide, celosian, has been assessed to have hepatitis-preventive effect on hepatitis induced by D-galactosamine and lipopolysaccharides through stimulation of the natural killer cell activities[38]. Further, other studies have suggested that the activation of Kupffer cells in liver[39,40] and release of cytokines such as tumor necrosis factor (TNF-α)[41] might be involved in the D-galactosamine hepatitis.

Hence, the finding that among different MW CBH fragments the oligosaccharide fragments protected rats from D-galactosamine hepatitis and that the preventive effect was not mediated by the action of SCFAs in the present study could propose that the preventive effect of the oligosaccharide fragments might involve the suppression of endotoxin and/or production of cytokines from Kupffer cells avoiding development of liver injury. Further studies on them helping to clear the mechanisms of the preventive effect of the OS from CBH against D-galactosamine hepatitis are under investigation.

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