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Intestinal Health Benefits of the Water-Soluble Carbohydrate Concentrate of Wild Grape (*Vitis thunbergii*) in Hamsters

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ABSTRACT: The dose−response relationship of the water-soluble carbohydrate concentrate (WSCC) from wild grape (*Vitis thunbergii* Sieb. & Zucc.) on intestinal health was investigated in this study. WSCC contained carbohydrates up to 71.9 g/100 g, including arabinose-rich pectic polysaccharide, hemicelluloses, glucose, and fructose. The consumption of WSCC (0.5 and 1.5 g/100 g of diet) effectively ($P < 0.05$) shortened gastrointestinal transit time (−62.3 to −63.0%), decreased toxic cecal ammonia (−59.3 to −63.0%) and daily fecal ammonia output (−29.7 to −41.4%), decreased the activities of fecal β-glucuronidase (−78.6%), β-glucosidase (−80.5 to −87.5%), mucinase (−64.6 to −72.7%), and urease (−83.2 to −86.0%), increased fecal moisture content (116−129%), and also increased short-chain fatty acid levels in cecal contents (1.8−3.3-fold). These findings suggested that consumption of wild grape WSCC might diminish the exposure of intestinal mucosa to toxic ammonia and other detrimental compounds and, hence exert, favorable effects on improving gastrointestinal milieu.

KEYWORDS: *Vitis thunbergii, wild grape, polysaccharide, bacterial enzyme, intestinal health*

INTRODUCTION

Wild grape (*Vitis thunbergii* Sieb. & Zucc.), also known as a mountain grape, is an edible but underutilized grape species native to Asia. Its fruit is small, sour, and slightly sweet, and it is traditionally used for cooking with other foods for its pleasant flavor. Daily consumption of grape can help relieve chronic constipation.[1,2] Moreover, the wild grapes (unripe or ripened) have also been believed to offer additional therapeutic effects against hepatitis, jaundice, diarrhea, arthritis, and hypertension in dried form.[3] A traditional way of consuming wild grape is to cook ∼25 g of dried wild grapes with water, turning the dried grapes into a hot drink for health maintenance. Our preliminary study has revealed that wild grapes had a total soluble content of up to ∼16.0 g/100 g of dried fruit and were rich in water-soluble carbohydrates (∼71.9% by weight on a moisture-free basis).

Fermentable polysaccharides or carbohydrates in the large intestine might alter cecal pH value and short-chain fatty acids (SCFAs) production (i.e., acetate, propionate, and butyrate), which could assist in maintaining intestinal function and health.[4,5] Some previous studies have also indicated that diet and dietary manipulation can affect the activities of several bacterial enzymes such as β-glucuronidase, β-glucosidase, musinase, and urease in the intestines.[6] They appeared to play a major role in colonic metabolism and were thought to influence colon carcinogenesis.[7] Changes in the activities of these enzymes might therefore alter the enterohepatic recirculation of carcinogenic conjugates, leading to a higher incidence of colon cancer. In addition, different fecal bacterial constituents, enzymes, and metabolites have previously been found to be closely related to various intestinal functions and health conditions.[8] Therefore, further studies on the water-soluble carbohydrates (i.e., polysaccharides or dietary fiber) of wild grapes could provide insight on their health benefits to substantiate their traditional dietary and therapeutic uses.

The aim of the present study was to investigate the potential physiological benefits of the water-soluble carbohydrate concentrate (WSCC) prepared from the wild grapes on intestinal health. More specifically, the dose effects of the wild grape WSCC on fecal microbial enzyme activities, cecal SCFA contents, and certain biochemical parameters in the intestinal tract and feces of hamsters were discussed.

MATERIALS AND METHODS

Preparation of WSCC. Dried *Vitis thunbergii* Sieb. & Zucc., belonging to the Vitaceae family, was kindly provided and had its plant origin confirmed by Dr. Chao-Hsiang Chen (National Chung Hsing University, Taiwan). Dr. Chen has deposited voucher specimens of the plant sample at his own herbarium. The wild grape sample was then processed in a GMP pharmaceutical laboratory at the Standard Chemical and Pharmaceutical Co., Ltd. After the grapes were finely ground to a size small enough to pass through a sieve (0.5 mm in diameter), the wild grape powder sample was suspended in distilled water (1:10, w/v) and extracted with boiling water for 30 min with continuous stirring. After filtration, the filtrate was dried by lyophilization and sealed in a plastic bag. The lyophilized sample was then kept in a desiccator until use.

Proximate Analysis. Moisture was determined by drying the sample to a constant weight at 105 °C. Crude protein content was calculated by multiplying the nitrogen content obtained from a CHN-OS rapid element analyzer (Heraeus F002, Hanau, Germany) by a factor of 6.25. The content of total crude saponin in the extract was determined according to the method of Kwon et al.[9] with slight modifications. Briefly, 5 g of extract was immersed with 50 mL of distilled water and then extracted two times with 50 mL of water-saturated *n*-butanol. After separation, the butanol fraction was evaporated at 55 °C, dried, and weighed quantitatively. The level of

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total polyphenols in the extract was measured spectrophotometrically at 750 nm using the Folin−Cioculteu method.[10] The results were expressed as grams of gallic acid equivalents per 100 g of sample.

For the analysis of water-soluble polysaccharides, the total polysaccharide content was first separated according to the method of Mondal et al.[11] The polysaccharide fraction in the extract solution was precipitated with the addition of 4-fold volumes of 95% ethanol. After centrifugation at 4024*g* for 10 min, the precipitated polysaccharide fraction was separated, collected, and dried in the oven at 40 °C. The amount of free glucose in the supernatant was determined enzymatically using a commercially available glucose assay kit (Megazyme K-GLUC, Wicklow, Ireland). Disaccharides in the supernatant were measured by HPLC using the method of Da Costa Leite et al.[12] with slight modifications. The supernatant was filtered through a 0.45 μm membrane filter and analyzed for sugar components. Fructose and sucrose were used as standards. The HPLC system consisted of a pump (L-6200A, Hitachi, Tokyo, Japan) and a refractive index detector (Bischoff8110, Leonberg, Germany) equipped with an Ultrasep ES100 NH2 column (250 × 4 mm, particle size = 6 μm; Bischoff8110, Leonberg, Germany). Acetonitrile/water (88:12, v/v) was used as mobile phase. An aliquot of 20 μL was injected, and a flow rate of 0.6 mL/min was used in the analysis. The monomeric sugar components of the polysaccharide fraction were determined according to the method described by Chau and Huang.[13] The uronic acid content was determined colorimetrically according to AOAC method 45.4.1[14] using D-galacturonic acid monohydrate as reference.

Diets and Experimental Design. Based on the formulation of the AIN-93 M diet,[15] the control diet (basal formula) was composed of casein (14.0 g), cellulose (5.0 g), sucrose (10.0 g), cornstarch (62.1 g), soybean oil (4.0 g), choline bitartrate (0.25 g), L-cystine (0.18 g), AIN-93 M vitamin mix (1.0 g), and AIN-93 M mineral mix (3.5 g) per 100 g of diet. These ingredients were obtained from ICN Nutritional Biochemicals (Cleveland, OH, USA).

The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. Thirty-two male Golden Syrian hamsters (6 weeks old) weighing 103 ± 2.0 g were obtained from the National Laboratory Animal Center of Taiwan. After an acclimation period of 7 days, animals were divided into four diet groups and housed (in pairs) in stainless steel screen-bottomed cages in a room maintained at 24 ± 1 °C, with a 12 h light/12 h dark cycle. The animals were allowed free access to food and water throughout the experiment.

Because the traditional way of consuming the grapes is to cook ∼25 g of dried wild grapes with water, turning the dried grapes into a hot drink (i.e., around 4 g of WSCC per day) for health maintenance, the dried WSCC intake per day for hamsters was then estimated to be ∼40 mg/day using a conversion factor of 0.01 (for conversion between humans and hamsters). As the food intake of hamsters was determined to be ∼8 g/day, the wild grape-containing diets were prepared by adding different amounts of dried WSCC (i.e., 0.17, 0.5, and 1.5 g WSCC/100 g of diet) into the basal formula. Three test diets, namely, low-, medium-, and high-dose diets, were then prepared by mixing 0.17, 0.5, and 1.5 g of the dried WSCC into 100 g of the basal formula, respectively.

The feeding experiment was carried out for 30 days using the four diets. Body weights and food intakes were recorded daily. Feces were collected and weighed daily and stored at −20 °C until analysis. At the end of the experiment, animals were anesthetized by isoflurane (Halocarbon Laboratories, River Edge, NJ, USA) after fasting for 16 h. After laparotomy, the small intestine, cecum, cecal content, and large intestine of each animal were collected, weighed, and immediately frozen at −80 °C until analysis. A portion of feces was dried in a 105 °C air oven for the determination of the fecal dry weight.

Determination of Gastrointestinal Transit Time. The animals were fed a colored diet containing small amounts of carmine, after fasting for 14 h. The excretion of colored feces was monitored at 30 min intervals. The gastrointestinal transit time was determined as the time it took for colored feces to appear.

Determination of Cecal Ammonia. Ammonia contents in the deproteinized solution of cecal content were determined spectrophotometrically at 630 nm, as described by Okuda and Fujii.[16]

Determination of Fecal Moisture and Ammonia. Fecal moisture contents were determined by drying the fecal samples to constant weights in a 105 °C air oven. As described in the method of Shiau and Chang,[17] fecal samples were mixed in cold 0.01 M phosphate buffer (pH 7.2, 1:50, w/v) for 30 min. After homogenization (Glas-Col, Terre Haute, IN, USA) and centrifugation at 1006*g* for 10 min, the fecal ammonia content in the supernatant was then determined according to the method of Okuda and Fujii.[16]

Determination of Bacterial Enzyme Activities in Feces. Following the method of Shiau and Chang,[17] fresh fecal samples collected during the last three days of the experimental period were homogenized (Glas-Col) in 0.1 M phosphate buffer (pH 7.2, 1:150, w/v). After centrifugation at 1006*g* for 10 min, the supernatants were assayed for different bacterial enzyme activities. Proteins in the supernatants were determined using protein assay kit (catalog no. 500-0006, Bio-Rad, Hercules, CA, USA).

According to the method of Goldin and Gorbach,[18] β-D-glucuronidase activity, defined as micromoles of phenolphthalein produced per minute per milligram of fecal protein, was determined by the amount of phenolphthalein released from 0.01 M phenolphthalein β-glucuronide (no. P0501, Sigma Chemical Co., St. Louis, MO, USA). Using the method of Goldin and Gorbach,[18] β-glucosidase activity, defined as nanomoles of nitrophenol produced per minute per milligram of fecal protein, was measured as the release rate of nitrophenol from 1 mM 4-nitrophenyl β-D-glucopyranoside (no. N7006, Sigma). Then, mucinase activity, defined as micromoles of reducing sugar released per minute per milligram of fecal protein, was determined by the amount of reducing sugar released from porcine gastric mucin (no. M1778, Sigma) using the method of Shiau and Chang.[17] Urease activity, defined as nanomoles of ammonia released per minute per milligram of fecal protein, was determined by the contents of ammonia released from 0.01 M urea (no. U0631, Sigma) using the methods of Okuda and Fujii[16] and Ling et al.[19]

Determination of SCFA Contents. The SCFA concentrations in the cecal contents were determined according to the method of Whitehead et al.[20] with slight modifications. Briefly, a portion (0.3 g) of cecal content was homogenized with cold saline (0.9%, w/w) and centrifuged at 1006*g* for 10 min. The supernatant was then mixed with a known amount of isocaproic acid as an internal standard. The SCFA was extracted with diethyl ether, and 1 μL of the ether layer was assayed by a packed column (GP10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb) using a gas chromatograph (Hitachi G-5000, Tokyo, Japan) fitted with a flame ionization detector. The conditions were as follows: oven temperature, initially held at 80 °C for 3 min and then raised to 130 °C at a rate of 2 °C/min; injector temperature, 200 °C; detector temperature, 250 °C; gas flow rate, 20 mL/min (carrier gas, nitrogen).

Statistical Analysis. All results expressed as the mean ± standard derivation were analyzed by one-way analysis of variance using the Statistical Analysis System (SAS). Values of $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In our study, the amount of freeze-dried WSCC prepared from the wild grape sample was about 16.0 g/100 g of dried fruit. Table 1 reveals that the WSCC contained a substantial amount of carbohydrates up to 71.9 g/100 g, including water-soluble polysaccharides, glucose, and fructose. The small amounts of monosaccharide (e.g., glucose and fructose at 5.40 and 4.29 g/100 g of WSCC, respectively) explained the low sweetness of the traditional folk drink prepared from the under-ripe wild grapes. The glucose to fructose ratio >1 agreed with the observations that glucose predominated in unripe grapes, whereas fructose constituted the major sugars in fully ripe grapes.[21] In addition, the wild grape WSCC also possessed low

Table 1. Proximate Composition[a] of the Wild Grape WSCC[b]

proximate composition	g/100 g of WSCC
protein	4.52 ± 0.17
ash	2.94 ± 0.58
polysaccharide fraction	62.2 ± 1.0
di- and monosaccharides	
sucrose	tr[c]
glucose	5.40 ± 0.23
fructose	4.29 ± 0.24
total crude saponin	3.71 ± 0.82
total polyphenols	10.1 ± 2.3

[a]Means ± SD of triplicate determinations. [b]The moisture content of freeze-dried WSCC sample was 8.35 g/100 g of WSCC. [c]tr, trace amount (< 0.1).

levels of crude saponin (3.71 g/100 g) and total polyphenols (10.1 g/100 g) and small amounts of residual proteins.

In Table 2, the contents of various monomeric sugars released from the polysaccharide fraction after acid hydrolysis

Table 2. Monomeric Sugar Components[a] of the Polysaccharide Separated from the Wild Grape WSCC[b]

monomeric sugar component	g/100 g of polysaccharide fraction
rhamnose	1.77 ± 0.11
arabinose	4.45 ± 0.32
xylose	1.41 ± 0.21
mannose	1.48 ± 0.06
galactose	6.37 ± 0.19
noncellulosic glucose	5.25 ± 0.06
uronic acid	30.4 ± 0.8

[a]Means ± SD of duplicate determinations. [b]The amount of polysaccharide separated from the wild grape WSCC was 62.2 g/100 g of WSCC.

are presented. The total sugar content (51.1 g/100 g) accounted for ~82% of the wild grape polysaccharide fraction (62.2 g/100 g). The monomeric sugars in the polysaccharide fraction were predominantly uronic acid, followed by galactose, noncellulosic glucose, and arabinose (59.5, 12.5, 10.3, and 8.70% of total sugar contents, respectively). On the basis of the above monomeric sugar profile, it was determined that wild grape polysaccharides were mainly composed of pectic substances. In general, the sugar components of pectic polysaccharides are galacturonic acid, rhamnose, arabinose, and galactose, whereas those of hemicelluloses are glucose, xylose, and mannose.[22] In terms of composition, it was therefore inferred that the polysaccharides in the wild grape WSCC were mainly constituted of arabinose-rich pectic polysaccharides followed by hemicelluloses (i.e., xyloglucan). However, it should be noted that the above monomeric sugar profile was not sufficient to speculate about a polysaccharide structure. A structural and functional characterization, involving purification steps and structural analysis, should be performed to determine the polysaccharide structure in future studies.

Throughout the entire experimental period, all of the animals remained active and healthy. After 30 days of feeding, there were no significant changes in the food intakes (7.53−7.65 g/day) and body weight gains (0.80−1.11 g/day) in all four diet groups. Hence, similar final body weights were observed among the four diet groups. Moreover, no significant discrepancies were observed in the relative weights of small intestines (1.27−1.44 g/100 g bw), cecums (0.71−0.81 g/100 g bw), and colons and rectums (1.68−1.75 g/100 g bw) across the four diet groups.

Next, the daily fecal outputs (0.85−1.02 g/day, dry weight) of the four diet groups are summarized in Table 3. The fecal

Table 3. Effects of the Wild Grape WSCC on Gastrointestinal Transit Time,[a] Fecal Moisture Content,[a] and Fecal Dry Weight[a]

diet group[b]	fecal dry weight (g/day)	fecal moisture content (g/100 g of feces)	gastrointestinal transit time (h)
control	0.85 ± 0.16w	30.1 ± 2.51w	11.2 ± 0.12w
low dose	0.86 ± 0.06w	31.0 ± 1.50x	10.0 ± 1.14w
medium dose	1.02 ± 0.03w	34.9 ± 3.42x	4.22 ± 0.06x
high dose	0.98 ± 0.09w	38.8 ± 1.71y	4.15 ± 0.02x

[a]Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$). [b]The low-, medium-, and high-dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of WSCC into 100 g of the control diet (basal formula), respectively.

moisture contents of the WSCC-supplemented groups were found to be 103−129% higher ($P < 0.05$) than that of the control group (30.1 g/100 g feces). The higher the WSCC consumption, the higher was the fecal moisture. As compared to the control group, the feeding of WSCC (at 0.5 and 1.5 g/100 g of diet) brought about a significant ($P < 0.05$) reduction in the gastrointestinal transit time (by 62.3 and 63.0%, respectively). The consumption of polysaccharide fractions obtained from other edible plants such as *Zizyphus jujuba*, *Salvia plebeia*, and *Amomum villosum* has also been reported to increase intestinal motility and to reduce gastrointestinal transit time.[3,23,24]

In Table 4, it was demonstrated that the consumption of wild grape WSCC at medium and high doses could lead to a

Table 4. Effects of the Wild Grape WSCC on the Cecal Ammonia,[a] Fecal Ammonia,[a] and Daily Fecal Ammonia Output[a,b]

diet group[c]	cecal ammonia (μmol/g cecal content)	fecal ammonia (μmol/g fresh feces)	daily fecal ammonia output (μmol/day)
control	2.7 ± 0.5w	40.1 ± 2.5w	50.5 ± 3.4w
low dose	2.6 ± 0.1w	30.8 ± 1.8x	38.5 ± 2.9x
medium dose	1.1 ± 0.1x	22.6 ± 1.7y	35.5 ± 2.4x
high dose	1.0 ± 0.1x	18.5 ± 0.4z	29.6 ± 0.6y

[a]Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$). [b]The total fecal outputs (fresh weight) per day with the low-, medium-, and high-dose diets were 1.25, 1.57, and 1.60 g/day, respectively. [c]The low-, medium-, and high-dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of WSCC into 100 g of the control diet (basal formula), respectively.

significant ($P < 0.05$) reduction of cecal ammonia content by 59.3−63.0%. Fecal sample analyses also demonstrated that consumption of wild grape WSCC at three different doses significantly ($P < 0.05$) reduced the ammonia concentration in fresh feces by 23.2−53.9%, when compared to the control group (40.1 μmol/g of fresh feces). On a daily basis, the total amount of fecal ammonia excreted per day among the test

groups was significantly ($P < 0.05$) reduced by 23.8−41.4%. The production of ammonia in the intestinal tract was in fact related to the growth of undesired intestinal microflora, implying that any decrease in both the cecal and fecal ammonia levels would be beneficial to intestinal health.[23,25]

Furthermore, Table 5 demonstrates that the feeding of wild grape WSCC at three different doses could significantly ($P < 0.05$) reduce the activities of β-D-glucuronidase (by 57.1−78.6%) and β-D-glucosidase (by 77.8−87.5%) in fecal samples. These fecal bacterial enzymes are responsible for the hydrolysis of conjugated products such as glucuronide and glucoside in the intestinal lumen, leading to the generation of toxic and carcinogenic substances as well as a higher risk of colorectal tumors.[18] Compared with the control group, the WSCC-supplemented groups had a significantly ($P < 0.05$) lower level of fecal mucinase activity (−36.4 to −72.7%). As mucinase could hydrolyze the protective mucin, which functions as a major local defense barrier to prevent most bacterial invasion,[26] the ability of wild grape WSCC to lower mucinase activity was desirable for the maintenance of the protective gel coating against bacteria and toxins.

Table 5. Effects of the Wild Grape WSCC on the Activities[a] of Different Fecal Bacterial Enzymes

diet group[b]	β-D-glucuronidase[c]	β-D-glucosidase[c]	mucinase[c]	urease[c]
control	1.4 ± 0.1w	129 ± 2w	1.1 ± 0.0w	214 ± 5w
low dose	0.6 ± 0.0x	28.6 ± 5.8x	0.7 ± 0.1x	105 ± 4x
medium dose	0.3 ± 0.2y	25.2 ± 3.5x	0.4 ± 0.1y	36 ± 2y
high dose	0.3 ± 0.0y	16.1 ± 2.5y	0.3 ± 0.1y	30 ± 3y

[a]Values (means ± SD, $n = 8$) in the same column with different letters are significantly different ($P < 0.05$). [b]The low-, medium-, and high-dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of WSCC into 100 g of the control diet (basal formula), respectively. [c]Enzyme activities were as described under Materials and Methods.

As shown in Table 5, the basal urease activity (214 nmol of ammonia released per minute per milligram of fecal protein) was significantly ($P < 0.05$) reduced by 50.9−86.0% with the consumption of wild grape WSCC at different doses. A correlation ($r = 0.84$; $P < 0.05$) was observed between the urease activity and fecal ammonia contents (Tables 4 and 5). Because bacterial urease can hydrolyze the urea produced from amino acid degradation into ammonia, which could then enter the bloodstream and cause harmful effects to animal health, a lower level of basal urease activity in cecum and feces might be beneficial for intestinal health. Dietary fermentable polysaccharides have also been reported to reduce urease activity and net ammonia production in the hindguts of hamsters.[3,26]

Finally, the SCFA profiles from the cecal contents among the four diet groups are displayed in Figure 1. The inclusion of WSCC at 0.17−1.5 g/100 g of diet markedly ($P < 0.05$) elevated the total SCFA concentrations by 1.8−3.3-fold (79.5−145 μmol/g of cecal content) as compared to the control (44.6 μmol/g of cecal content).

Analyses of volatile fatty acids (i.e., acetate) have indicated that the concentration of acetate, which might stimulate mucin secretion,[23] was significantly ($P < 0.05$) elevated up to 3−5-fold with increased consumption of WSCC. However, apparent ($P < 0.05$) increases in the concentrations of propionate and butyrate were observed only in the high-dose group (2.5- and 1.4-fold, respectively) when compared to the control (5.43 and 17.0 μmol/g of cecal content, respectively). Butyrate, which is preferentially used in colonic energy metabolism, can nourish the colonic epithelium. Moreover, SCFAs can be rapidly absorbed and chemically stimulate the mucosal cells and, in turn, trigger a peristaltic relaxation and increase gut motility, leading to a reduction in gastrointestinal transit time.[23] The apparent decrease in gastrointestinal transit time (−10.7 to −62.9%, Table 3) by feeding the hamsters with wild grape WSCC might therefore be partly attributed to the significant increase in SCFA levels.

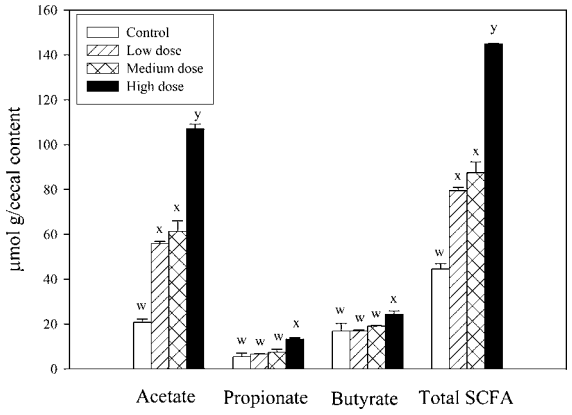

Figure 1. Effects of the wild grape WSCC on different SCFA concentrations in the cecal content of hamsters. Values (mean ± SD) with different letters are significantly different ($P < 0.05$).

In the present study, the consumption of the wild grape WSCC (preferably at 0.5 g/100 g of diet) effectively increased fecal moisture content and various SCFAs in hamster hindguts, shortened gastrointestinal transit time, lowered the activities of colonic bacterial enzymes in feces, and reduced the exposure of intestinal mucosa to toxic ammonia along the intestinal tract. This effective dosage for hamsters was determined to be equivalent to about 4 g of WSCC (~30 g of dried wild grape) for a human adult, per day. These findings suggested that the consumption of the wild grape WSCC might exert a favorable effect on maintaining normal, or improving, gastrointestinal milieu. A structural and functional characterization, involving purification steps and structural analysis on the water-soluble polysaccharide, should be performed in future studies.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

WSCC, water-soluble carbohydrate concentrate; SCFA, short-chain fatty acid.

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