Study on the Effect of Galacto-oligosaccharide (GOS) in Relieving Constipation and Defecating Feces Excretion

Jin Wang\textsuperscript{1,2}, Ping-Ju Tsai\textsuperscript{3}, Pei-Hsuan Chen\textsuperscript{3}, Manxiang Ye\textsuperscript{4}, Hua Cao\textsuperscript{5}, Jiao Guo\textsuperscript{2}\* and Zhengquan Su\textsuperscript{1}\*

\textsuperscript{1} Guangdong Engineering Research Center of Natural Products and New Drugs, Guangdong Provincial University Engineering Technology Research Center of Natural Products and Drugs, Guangdong Pharmaceutical University, Guangzhou 510006, China
\textsuperscript{2} Guangdong Metabolic Diseases Research Centre of Integrated Chinese and Western Medicine, Guangdong TCM Key Laboratory for Metabolic Diseases, Key Laboratory of Modulating Liver to Treat Hyperlipemia SATCM, Level 3 Laboratory of Lipid Metabolism SATCM, Institute of Chinese Medicinal Sciences, Guangdong Pharmaceutical University, Guangzhou 510006, China
\textsuperscript{3} King-Prebiotics Biotechnology (TW) Co., Ltd 2F.-1, No. 250, Zhongshan Rd., Linkou Dist., New Taipei City 24446, Taiwan, China
\textsuperscript{4} New Francisco (Yunfu City) Biotechnology Co., Ltd Swan-kan-chiau Ind. Dist., Kaofong Village Yunfu City, Guangdong, China
\textsuperscript{5} School of Chemistry and Chemical Engineering, Guangdong Pharmaceutical University, Zhongshan 528458, China

* Correspondence: * Authors to whom correspondence should be addressed.
Professor Jiao Guo, Guangdong Pharmaceutical University, Guangzhou 510006, China. E-mail: gyguoyz@163.com
Professor Zhengquan Su, Guangdong Pharmaceutical University, Guangzhou 510006, China. E-mail: suzhq@scnu.edu.cn. Tel.: +86-20-3935-2067; Fax: +86-20-3935-2067

Abstract. To explore the role of Galacto-oligosaccharide (GOS) in preventing constipation and promoting excretion of feces. The high, medium and low doses of GOS-1000-S (8.8g/kg, 4.4g/kg, 2.2g/kg) were administrated orally to constipation mice for 7 days, and then we measured the propulsive rate of intestines, first defecation time, the number of feces particle and fecal weight in 5 hours. The results showed that GOS-1000-S can significantly promote the small bowel propulsion, shorten the first defecation time and increase the fecal weight number of fecal particle of constipation mice. These indicate that GOS has the effect of relieving constipation and defecating feces excretion. This study provided a basis for the development of GOS with constipation-relieving functions as a health supplement.

1. Introduction

The structure of Galacto-oligosaccharide (GOS) is mainly linked from the galactosyl side of lactose to 1-7 galactosyl groups by $\beta$-1,4 glycosidic bonds [1], and the chemical structure is Gal-(Gal)\textsubscript{n}-Glc/Gal (n=0-6) [2, 3]. GOS is a colorless and translucent substance with a viscosity similar to that of high fructose syrup, and its sweetness is equivalent to 30%-60% of sucrose. It has stable physical and chemical properties [4], is easily soluble in water, has strong stability to acid and heat, and has high moisture retention.
At present, there are five main methods for the synthesis of GOS: natural raw material extraction, natural polysaccharide acid hydrolysis, chemical synthesis, fermentation and microbial enzyme synthesis. The immobilized enzymatic synthesis of GOS is a mainstream method for the synthesis of oligogalactose because it can make full use of β-galactosidase and has good production stability, high yield and few post-treatment processes [5, 6]. The separation and purification techniques of GOS mainly include the following methods: column chromatography, membrane separation, enzymatic separation and microbial fermentation [7].

As one of the most safe, highly recognized and therapeutic therapeutic effects of many functional oligosaccharides, GOS has a variety of physiological functions and has a beneficial effect on the human body[8]. GOS can be utilized by various beneficial bacteria to ferment it to produce short chain fatty acids (SCFAs) and some antibiotic substances, which can inhibit the growth of various harmful bacteria (eg Shigella, Salmonella, Staphylococcus aureus and Escherichia coli) and promote the growth of various beneficial bacteria (such as bifidobacteria and lactic acid bacteria), thereby effectively regulating the balance of intestinal flora and reducing the phenolics produced by the intestinal microbiota and improves skin health [9-11]. In addition, GOS can improve the absorption of mineral elements and improve the incidence of cardiovascular disease, obesity and type 2 diabetes [12-15]. Recent studies have also found that SCFAs produced by the use of beneficial bacteria after GOS enters the human body can also reduce colon cancer by stimulating cancer cell apoptosis [16].

Our research group has long been engaged in the research on the weight loss activity and mechanism of chitosan, chitosan oligosaccharide and GOS, and has systematically studied its weight loss activity [17-21]. In view of the fact that the GOS intestinal metabolite SCFA has a function of affecting intestinal peristalsis [16], our research group began to pay attention to and explore the role of GOS in preventing constipation and promoting excretion.

2. Materials and Methods

2.1. Materials

**King-Prebiotics® GOS1000 syrup (GOS-1000-S)**, which contain <1% monosaccharides and disaccharides, provided by New Francisco (Yunfu City) Biotechnology Co., Ltd, and the corresponding concentration is prepared by using distilled water during the experiment.

**Reagent**: Activated carbon powder (Shanghai Aladdin Biochemical Technology Co., Ltd., C1607128), Acacia gum (Shanghai Aladdin Biotechnology Co., Ltd., A1619001), Loperamide hydrochloride capsule (Xi’an Yangsen Pharmaceutical Co., Ltd., 150827647), Tongbianling Capsules (Anhui Jiren Pharmaceutical Co., Ltd., 1170107).

**Experimental animals**: SPF Male KM mice, 6 weeks old, provided by Guangdong Medical Laboratory Animal Center. Animal production license number: SCXK (Guangdong) 2013-0002, tested after 3 days of normal feeding.

2.2 Methods

**Dosage**: The body recommended dosage for the GOS-1000-S is 13.3 g/d/60 kg. GOS-1000-S corresponds to three dose groups, high, medium and low doses (equivalent of 40, 20, 10 times times to the human body recommended dose), which are 8.8g/kg, 4.4g/kg, 2.2g/kg BW, respectively. The recommended dose for Tongbianling capsules is 1.5g/d/60kg for adults, and the dose for mice is 0.5g/kg (equivalent to 20 times the recommended amount of the human body).

**Raising environment**: During the experimental period, the animal room temperature was 23.0±2.0°C, the relative humidity was 40-70%, the number of air changes was >15 times/hour, 12 hours of illumination/12 hours of darkness, and the brightness and darkness alternated. Laboratory animal use license number: SYXK (Guangdong) 2017-0125.

**Model establishment**: After 7 days of administration of the test samples, the mice were fasted for 16 hours. Except the control group was given distilled water, the other groups were given 3 mg/kg aqueous solution of loperamide hydrochloride capsules by oral gavage, and the mice small bowel peristalsis inhibition model and mice constipation model were established respectively.
The effect of GOS on small bowel movement: 70 male KM mice were randomly divided into 6 groups according to their body weight, namely control group, model control group, Tongbianling capsule positive group, and high, medium and low dose administration groups of GOS-1000-S, with 11 rats in each group. On the 7th day, the mice were fasted for 16 hours before the administration, except for the distilled water in the blank group, and the other groups were given 3 mg/kg aqueous solution of loperamide hydrochloride capsules. After 30 min, each group of mice was given an indicator ink (5% active carbon, 10% gum arabic) containing the corresponding test sample, and the control group and the model group were administered with ink.

After 25 min, the mice were sacrificed by dislocation. The entire gastrointestinal tract of the mice was taken out, and the intestines were plated on a wet glass plate, with the pylorus of the stomach as the starting point and the ileocecal part as the end point, and the total length (cm) of the small intestine and the distance (cm) in which the ink moves in the intestine from the pylorus were measured. The percentage of ink movement distance per mice in the total length of the small intestine was calculated as the gastrointestinal propulsion rate (P) of the mice, and the data was converted (X) and then statistically analyzed X = \sin^{-1}\sqrt{P}.

Effect of GOS on defecation in mice: After the mice in each group were given the ink, they were kept in a single cage, and the feed and water were normally supplied. Record the time of the first black feces, the number of feces particle and weight of feces in the first 5 h after administered with indicator ink in mice.

Statistical processing: The data was expressed as X±SD and statistical analysis was performed using SPSS12.0 software.

3. Results

3.1. The Effect of GOS on Small Bowel Movement

The ink propulsion rate of the model group mice was significantly lower than that of the blank group (P < 0.01), indicating that the constipation model was established. All mice had normal feces and no diarrhea. The ink propelling rates of the GOS-1000-S mice in each dose group were higher than those in the model group (P <0.01). After statistical analysis by data conversion, the difference between the conversion values of the GOS-1000-S groups and the model group was significant (P <0.01), indicating that GOS-1000-S has the effects of promoting small bowel movement in mice, and the effect shows a dose-dependent (the results shown in Table 1).

| Group     | Dose (g/kg) | Propulsion rate (P) (%) | Conversion value (X) |
|-----------|-------------|------------------------|----------------------|
| Control   | -           | 0.73±0.07**            | 1.33±0.05**          |
| Model     | -           | 0.43±0.07              | 1.66±0.13            |
| Positive  | 0.5         | 0.64±0.09**            | 1.40±0.08**          |
| GOS-1000-S-H | 8.8       | 0.86±0.06**            | 1.25±0.03**          |
| GOS-1000-S-M | 4.4       | 0.77±0.07**            | 1.30±0.04**          |
| GOS-1000-S-L | 2.2       | 0.65±0.12**            | 1.40±0.11**          |

Notes: Compare with model group, * P<0.05, ** P<0.01

3.2 Effect of GOS on Defecation in Mice

The first defecation time of the model group was longer than that of the control group (P<0.01), and the number of feces particle and fecal weight were lower than the blank group (P<0.01 and P<0.05), indicating that the constipation model was established. All mice had normal feces and no diarrhea. The first defecation time of each group of the GOS-1000-S was shorter than that of the model group (P<0.01). There was significant difference in the number of feces particle between the GOS-1000-S middle dose group, GOS-1000-S low dose group and the model group (P<0.01), there was no significant difference between the high dose group and the model group (P>0.05). Except for the high...
dose group of GOS-1000-S, the fecal weight of the middle and low dose groups was significantly higher than that of the model group (P<0.01 and P<0.05). The above results indicate that GOS-1000-S can shorten the first defecation time and increase the fecal weight and the number of feces particle of constipation mice (the results shown in Table 2).

### Table 2. Effects of GOS-1000-S on defecation in mice (X±SD, n=10)

| Group       | Dose(g/kg) | First defecation time(min) | Feces particle number(particle) | Fecal weight(g) |
|-------------|------------|-----------------------------|-------------------------------|-----------------|
| Control     | -          | 78.80±13.69**               | 36.00±7.66**                  | 0.44±0.09*      |
| Model       | -          | 153.70±65.93                | 18.90±9.34                   | 0.27±0.15       |
| Positive    | 0.5        | 60.10±25.04**               | 30.40±8.50**                  | 0.52±0.12**     |
| GOS-1000-S-H| 8.8        | 93.90±48.34**               | 16.60±6.38                   | 0.27±0.10       |
| GOS-1000-S-M| 4.4        | 70.00±19.19**               | 34.80±6.65**                  | 0.41±0.11*      |
| GOS-1000-S-L| 2.2        | 91.70±29.99**               | 34.10±6.98**                  | 0.46±0.08**     |

Notes: Compare with model group, * P<0.05, ** P<0.01

### 4. Conclusion

At present, our research shows that GOS-1000-S can promote small bowel propulsion in constipation mice, shorten the first defecation time of constipation mice and increase the fecal weight and the number of feces particle. According to the relevant provisions of the health food functional evaluation procedures and testing methods, these results showed that GOS has the effect of relieving constipation and defecating feces excretion.

### 5. Acknowledgments

This work was financially supported by the Science and Technology Planning Project of Yunfu, Guangdong, China (No. 201702-9); Guangdong Provincial University Engineering Technology Research Center of Natural Products and Drugs, China (No.2017GCZX002); and the Science and Technology Planning Project of Guangdong, China (No.201806040009, 201804010349, 201804010329).

### 6. Reference

[1] Osman A, Tzortzis G, Rastall RA and Charalampopoulos D 2010 A comprehensive investigation of the synthesis of prebiotic galactooligosaccharides by whole cells of Bifidobacterium bifidum NCIMB 41171. J Biotechnol. 150(1):140-148.
[2] Tomomatsu HJFT 1994 Health Effects of Oligosaccharides. 48(10):61-65.
[3] Kimura K, ., Matsumoto K, ., Ishihara C, ., Harada K, . and Miyagi A, . %J Carbohydrate Research 1995 Structure determination of galacto-oligosaccharides by pyridylamination and NMR spectroscopy. 270(1):33-42.
[4] Macfarlane G, Steed H and Macfarlane SJJoAM 2010 Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. 104(2):305-344.
[5] Neri DFM, Balcão VM, Costa RS, Rocha ICAP, Ferreira EMFC, Torres DPM, Rodrigues LRM, Jr LBC and Teixeira JAJFC 2009 Galacto-oligosaccharides production during lactose hydrolysis by free Aspergillus oryzae β-galactosidase and immobilized on magnetic polysiloxane-polyvinyl alcohol. 115(1):92-99.
[6] Krajewska BJE and Technology M 2004 Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. 35(2):126-139.
[7] Martin-Ortiz A, Ruiz-Matute AI, Sanz ML, Moreno FJ and Herrero M 2019 Separation of di- and trisaccharide mixtures by comprehensive two-dimensional liquid chromatography. Application to prebiotic oligosaccharides. Anal Chim Acta. 1060:125-132.
[8] Sabater C, Prodanov M, Olano A, Corzo N and Montilla A 2016 Quantification of prebiotics in commercial infant formulas. Food Chem. 194:6-11.
[9] Bryk G, Coronel MZ, Pellegrini G, Mandalunis P, Rio ME, Portela MLPMd and Zeni SN 2015 Effect of a combination GOS/FOS ® prebiotic mixture and interaction with calcium intake on mineral absorption and bone parameters in growing rats. European Journal of Nutrition. 54(6):913.

[10] Bryk G, Coronel MZ, Lugones C, Mandalunis P, Rio ME, Gualtieri AF and Zeni SN 2016 Effect of a mixture of GOS/FOS ® on calcium absorption and retention during recovery from protein malnutrition: experimental model in growing rats. European Journal of Nutrition. 1-14.

[11] Macfarlane S., Macfarlane GT and Cummings JH 2010 Review article: prebiotics in the gastrointestinal tract. Alimentary Pharmacology & Therapeutics. 24(5):701-714.

[12] Reyes B, Palou M, Rodriguez AM and Palou A 2018 Regulation of Adaptive Thermogenesis and Browning by Prebiotics and Postbiotics. Front Physiol. 9:1908.

[13] Conterno L, Fava F, Viola R and Tuohy KM 2011 Obesity and the gut microbiota: does up-regulating colonic fermentation protect against obesity and metabolic disease? Genes & Nutrition. 6(3):241-260.

[14] Devadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Bäckhed F and Mithieux G 2014 Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. Cell. 156(1-2):84-96.

[15] Cheng W, Lu J, Lin W, Wei X, Li H, Zhao X, Jiang A and Yuan J 2018 Effects of a galacto-oligosaccharide-rich diet on fecal microbiota and metabolite profiles in mice. Food & Function. 9(3):10.1039.C1037FO01720K.

[16] Sun Y and O’Riordan MXD 2013 Chapter Three–Regulation of Bacterial Pathogenesis by Intestinal Short-Chain Fatty Acids. Advances in Applied Microbiology. 85:93-118.

[17] Jian C, Gui-Dong H, Si-Rong T, Jiao G and Zheng-Quan S 2013 The preparation of capsaicin-chitosan microspheres (CCMS) enteric coated tablets. 14(12):24305-24319.

[18] Tao Y, Zhang HL, Hu YM, Wan S and Su Z 2013 Preparation of Chitosan and Water-Soluble Chitosan Microspheres via Spray-Drying Method to Lower Blood Lipids in Rats Fed with High-Fat Diets. 14(2):4174-4184.

[19] S T, B G, Y T, J G and ZQ S 2014 Antiobese effects of capsaicin-chitosan microsphere (CCMS) in obese rats induced by high fat diet. Journal of agricultural and food chemistry. 62(8):1866-1874.

[20] Hong-Liang Z, Yi T, Jiao G, Yin-Ming H and Zheng-Quan S 2011 Hypolipidemic effects of chitosan nanoparticles in hyperlipidemia rats induced by high fat diet. 11(4):457-461.

[21] Yang D, Hu C, Deng X, Bai Y, Cao H, Guo J and Su Z 2019 Therapeutic Effect of Chitoooligosaccharide Tablets on Lipids in High-Fat Diets Induced Hyperlipidemic Rats. Molecules. 24(3).