Journal of Functional Foods 71 (2020) 104021

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Oligosaccharides from *Gracilaria fisheri* ameliorate gastrointestinal dysmotility and gut dysbiosis in colitis mice

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ARTICLE INFO

Keywords:
Prebiotic
Red algae
Ulcerative colitis
Gastrointestinal transit
Gut microbiota
Short-chain fatty acids

ABSTRACT

Oligosaccharides from red algae have demonstrated prebiotic activity. In this study, the prebiotic effects of *Gracilaria fisheri* oligosaccharides (GFO) on gastrointestinal (GI) motility in acetic acid (AA)-induced colitis mice were investigated through GI transit time, *ex vivo* propulsive motility, *in vitro* colonic smooth muscle contractility, composition of colonic microbiota, and production of short-chain fatty acids (SCFAs). Treatment with GFO attenuated histological change and shortening of the colon, reduced body weight loss, and lowered the disease activity index. Notably, GFO prevented reductions in gut transit, propulsive motility, and smooth muscle contractility in colitis mice. GFO treatment also modulated Enterobacteria populations and SCFAs production in the GI tract. The results suggested that the prebiotic effect of GFO could prevent and attenuate colitis symptoms and GI dysmotility in AA-induced colitis, perhaps by reducing populations of harmful bacteria and increasing SCFAs production.

1. Introduction

Ulcerative colitis (UC) is a subcategory of inflammatory bowel disease (IBD). IBD involves inflammation of the large intestine (Head & Jurenka, 2003). It is widely accepted that inflammatory mediators can disturb gastrointestinal (GI) motility (Kinoshita et al., 2006; Cheon, Cui, Yeon, Kwon, & Park, 2012; Mawe, Strong, & Sharkey, 2009; Ozaki, Hori, Kinoshita, & Ohama, 2005) and that GI motility disorder may lead to gut dysbiosis (Ozaki et al., 2005). Previous studies have demonstrated that gut dysbiosis may contribute to the severity of inflammation by increasing harmful bacteria in the GI tracts of patients with IBD (Sasaki & Klapproth, 2012). The most common medicine used to treat UC is mesalamine or 5-aminosalicylic acid (5-ASA). However, the common side effects of mesalamine treatment include headache and GI symptoms such as diarrhea, bloating, nausea, and hypersensitivity (Karagozian & Burakoff, 2007). This study focuses on gut microbiota modulation with prebiotic supplements as a potential therapeutic aid in the treatment of IBD.

Prebiotics were defined as substrates that are selectively fermented by gut microbiota to confer health benefits (Gibson et al., 2017). Neither digested nor absorbed in the small intestine, prebiotics were fermented well by beneficial intestinal microbiota and poorly by harmful microbiota (Markowiak & Śliżewska, 2017). The best known prebiotics are fructo-oligosaccharide (FOS), galacto-oligosaccharide (GOS), and inulin (Markowiak & Śliżewska, 2017). The major fermentation products of prebiotic metabolism in the colon are short-chain fatty acids (SCFA), which are reported to play an important function in gut physiology. In addition, they could regulate immune cell development and suppress inflammation (Sant'Anna and Ferreira, 2014; Louis and Flint, 2017). A recent study reported that GOS directly inhibited adherence to epithelial cells by *Escherichia coli in vitro* and *Citrobacter rodentium in vivo*. By acting as a molecular receptor decoy, GOS inhibited

Abbreviations: GFO, *Gracilaria fisheri* oligosaccharide; GI, gastrointestinal; AA, acetic acid; UC, ulcerative colitis; IBD, inflammatory bowel disease; 5-ASA, 5-aminosalicylic acid; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; SCFA, short-chain fatty acid; *E. coli*, *Escherichia coli*; qPCR, quantitative polymerase chain reaction; DW, distilled water; DP, degree of polymerization; DAI, disease activity index; GIMM, Gastrointestinal Motility Monitor; COX-2, cyclooxygenase-2; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6

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https://doi.org/10.1016/j.jff.2020.104021
Received 6 November 2019; Received in revised form 3 May 2020; Accepted 11 May 2020
Available online 30 May 2020

competitively the adherence of intestinal pathogens to host cells (Monteagudo-Mera, Rastall, Gibson, Charalampopoulos, & Chatzifragkou, 2019).

Gracilaria fisheri (known as Pom Nang seaweed in Thailand) is a red algae widely distributed along the coast of the Gulf of Thailand (Sriprach, Kitikoon, Poungsuk, & Suppadit, 2012). Many studies have revealed the multiple biological activities of oligosaccharides from red algae. As well as demonstrated anti-inflammatory, anti-oxidant and anti-tumor properties, oligosaccharides also showed prebiotic effects by stimulating the growth of beneficial bacteria and the production of SCFAs (Li et al., 2014). However, no study has been carried out to investigate the prebiotic effects of *Gracilaria fisheri* oligosaccharides (GFO) on GI motility. Therefore, using an acetic acid (AA)-induced colitis mouse model, this study investigates the prebiotic effects of GFO on GI motility, GI transit time, *ex vivo* propulsive motility, *in vitro* colonic smooth muscle contractility, composition of colonic microbiota, and production of SCFAs.

2. Materials and methods

2.1. Chemicals

AA was purchased from J.T.Baker (Avantor, UK). Reference prebiotic and the supplements: inulin, Evans blue, methylcellulose, activated charcoal powder, and arabic gum were purchased from Sigma (St. Louis, MO, USA). Krebs solution (119 mM NaCl, 2.5 mM $CaCl_2$, 4.5 mM KCl, 2.5 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11.1 mM glucose) was purchased from Merck, Co., Ltd., Darmstadt, Germany. qPCR Master Mix: SensiFAST™ SYBR® No-ROX Kit was purchased from Bioline (Taunton, MA, USA).

2.2. GFO preparation

The preparation of GFO followed an acid hydrolysis method described in previous reports (Yun et al., 2013; Al-Alawi, Chitra, Al-Mamun, Al-Marhubi, & Rahman, 2018). *Gracilaria fisheri* was harvested from Songkhla lake, Kohyor island, Songkhla, Thailand. Briefly, *Gracilaria fisheri* agar was extracted with distilled water (DW) at a temperature of 130 °C. The agar extract was hydrolyzed with 0.5% AA under high pressure at 130 °C in a hydrothermal reactor. The obtained extract was filtered using a membrane filter. The filtrate of hydrolyzed oligosaccharide was collected and labeled (GFO). HPLC analysis showed GFO was composed of 8.09% DP1, 6.37% DP3, 12.67% DP4-5, 18.14% DP6, 21.07% DP10, and 33.65% ≥ DP10 (Figure S1). The molecular weight distribution of GFO extended from 540 to 1800 Daltons. The monosaccharide composition in the GFO was D-galactose. The sulfate content of GFO was 2.01% (dry weight).

2.3. Animals and experimental design

Adult male ICR/Mlac mice (*Mus musculus*; 6–8 weeks old, weighing 30–35 g) were purchased from the National Laboratory Animal Center, Mahidol University, and housed at the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. All mice were housed in a stainless steel cage in a humidity- and temperature-controlled room, under a circadian cycle of 12 h in light and 12 h in darkness. They had free access to food and water.

After acclimatization for a week, the mice were divided into six groups: normal control, colitis control, 1000 mg/kg inulin, 100 mg/kg GFO, 500 mg/kg GFO, and 1000 mg/kg GFO. The diets of the normal and colitis control groups were supplemented with DW (both groups). The diets of the other groups were supplemented with either 1000 mg/kg inulin or GFO at 100, 500, or 1000 mg/kg. All treatments continued for two weeks. At day 10, colitis was induced with 5% AA in all groups except the normal control group. The induction method was based on a previous study (Colombo et al., 2018). The severity of colitis was scored according to the disease activity index (DAI) (Zhang et al., 2016). In brief, the DAI was the combined scores of weight loss, stool consistency, and occult/gross bleeding divided by three.

Before the mice were euthanized, gastrointestinal transit was measured. The mice were then anesthetized with 70 mg/kg thiopental sodium and the abdominal cavity was dissected to study upper gut transit, intestinal length, *ex vivo* colonic motility patterns, followed by *in vitro* colonic smooth muscle contractility. To study the composition of colonic microbiota and production of SCFAs, gut contents were collected from the proximal and distal colon, kept in a microtube, and stored at −80 °C. This study was approved and guided by the Animals Ethics Committee of the Prince of Songkla University, Thailand (Project license number MOE 0521.11/1069). The timeline and a brief summary of the experimental procedure are presented in Figure S2.

2.4. Histopathological analysis

Colonic fragments were collected and immediately fixed in 10% formalin for 24 h at room temperature, then transferred to 50% ethanol for another 24 h. The tissues were processed and embedded in a paraffin block and sectioned at a thickness of 5 μm. Colonic sections were placed on slides and stained with hematoxylin and eosin (H&E) using standard procedures. All colonic sections were observed for histopathological changes using an Olympus DP73 microscope equipped with cellSens software. For each treatment group, 27 fields (n = 3/group) were randomly selected and histological changes were scored to determine the colonic inflammation severity.

2.5. Gastrointestinal transit measurement

To measure total gut transit time, mice received by gavage 0.3 mL semiliquid solution containing 2.5% Evans blue in 0.5% methylcellulose and the time until the expulsion of the first blue pellet was recorded. To measure small intestinal transit, mice received by gavage a 0.3 mL charcoal meal containing 10% w/v charcoal in 5% w/v arabic gum 30 min before euthanization. Mice were dissected and charcoal transit (%) was measured and calculated by (the distance of charcoal meal/total length of the small intestine) × 100. For distal colonic transit time measurement, a 3-mm glass bead was inserted 2 cm into the colon proximal to the anus using a plastic tip lubricated with pure petroleum jelly. The time to bead expulsion was recorded.

2.6. Ex vivo colonic motility patterns

After dissecting an abdominal cavity, the whole colon was removed and placed in ice-cold Krebs solution (pH 7.4 with an osmolality of 289–292 mmol/kg H_2O). The colon was placed in a Gastrointestinal Motility Monitor (GIMM) organ bath (Catamount Research and Development, St. Albans, VT. USA) and continuously perfused with fresh oxygenated Krebs solution at 10 mL/min. The colon was allowed to equilibrate in 37 °C Krebs solution for 30 min without flushing out natural fecal pellets. The movement of the pellets was recorded from above using a video camera connected to a computer and analyzed by GIMM software. Spatiotemporal maps of motility were constructed from recordings acquired from individual runs. The pattern of contractions was composed of propagating contractions (peristalsis) and non-propagating contraction (segmentation).

2.7. In vitro colonic smooth muscle contractility

After the study of *ex vivo* colonic motility, the colon was cleared of luminal content, and the colonic segments (1 cm) from proximal and distal colon were suspended in the direction of longitudinal smooth muscle fibers in a 10 mL organ bath containing oxygenated Krebs solution at 37 °C. Spontaneous contractions were analyzed for the amplitude of contraction (g), frequency of contraction (times/min), and

duration of contraction (s) which were recorded by the PowerLab® System (AD Instruments, Australia) and analyzed by LabChart7 program software.

2.8. Composition of colonic microbiota analyses

Bacterial DNA samples were extracted from colonic content (Peerakietkhajorn et al., 2020). qPCR of bacterial 16S rDNA of Bifidobacteria, Lactobacilli, and Enterobacteria was performed using a qPCR machine (LineGene 9600 Plus, BIOER) and SensiFAST™ SYBR® No-ROX Kit (Bioline). PCR amplifications were performed using primer sets to determine the total number of Bifidobacteria (LM26 5′-GATTCTGGCT CAGGATGAACGC-3′and Bif228 5′-CTGATAGGACGCGACCCCAT-3′), Lactobacilli (FW 5′-CGATGAGTGCTAGGTGTTGGA-3′and RV 5′-CAAG ATGTCAAGACCTGGTAAG-3′) and Enterobacteria (F-ent 5′-ATGGCTG TCGTCAGCTCGT-3′ and R-ent 5′-CCTACTTCTTTTGCAACCCACTC3′) under the following conditions: 3 min at 95 °C, and 40 cycles of a two-temperature condition (5 s at 95 °C and 30 s at 60 °C) following the manufacturer's procedure.

2.9. Production of short-chain fatty acids analysis

Short-chain fatty acids, especially butyrate and propionate, in the colonic contents were measured by high-performance liquid chromatography (HPLC). Colonic contents (100 mg) were mixed with 400 mL sterile PBS buffer (pH 7.4) in a microtube and vortexed for 1 min or until the suspension was homogeneous. The suspension was centrifuged at 12,000*g* for 10 min at 4 °C and the supernatant was filtered through a syringe filter with a 0.22 μm pore size mixed cellulose esters membrane. The concentration of butyrate was determined using HPLC (Agilent 1200 Series) with a DAD detector, equipped with an Aminex HPX-87H column. H_2SO_4 at 0.005 M was used as the mobile phase. The flow rate was 0.6 mL/min at 50 °C and the UV detector was set to 215 nm. The quantitative analyses of butyrate and propionate using standards of butyrate and propionate at 10, 20, 40, 80, 100, and 200 mM, sample (5 μL) was injected into the column.

2.10. Statistical analysis

The results were expressed as means ± SEM (standard error of the mean). Statistical comparisons between groups were performed by using a one-way or two-way analysis of variance (ANOVA) followed by Bonferroni's test. The level of significance for all statistical tests is $p < 0.05$. Data were analyzed by GraphPad Prism 5.

3. Results

3.1. GFO attenuated AA-induced colitis symptoms

Food intake and body weight were reduced amongst the colitis control group (Fig. 1A and 1B). At day 14, food intake and body weight in the colitis control group were 66.93 ± 12.12% and 96.07 ± 1.90%. These figures were significantly lower than the results from the normal control group (107.82 ± 5.55%and 104.68 ± 0.67%), ($p < 0.001$). In addition, stool consistency was more liquid and occult blood was observed in the stool in the colitis control group. Therefore, the DAI score in the colitis control group was higher than the score in the normal control group ($p < 0.001$), (Fig. 1C). The colon length in the colitis control group was 8.18 ± 0.23 cm, which was significantly shorter than the length in the normal control group (9.84 ± 0.25 cm.), ($p < 0.001$), (Fig. 2). However, at day 14, compared with the colitis control group, food intake and body weight were restored in the colitis groups treated with 1000 mg/kg inulin, and 100 and 500 mg/kg GFO ($p < 0.001$, $p < 0.01$, and $p < 0.05$). The DAI score was lower in colitis groups treated with 100, 500 and 1000 mg/kg GFO than in the colitis control group ($p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively). Compared with the colitis control group, colon length recovered in colitis groups treated with 1000 mg/kg inulin, and 100, 500 and 1000 mg/kg GFO ($p < 0.05$, $p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively). Therefore, we concluded that GFO treatment of AA-induced colitis mice stimulated food intake, attenuated body weight loss and colon shortening, lowered DAI scores, restored stool pellets, and reduced occult blood in stool.

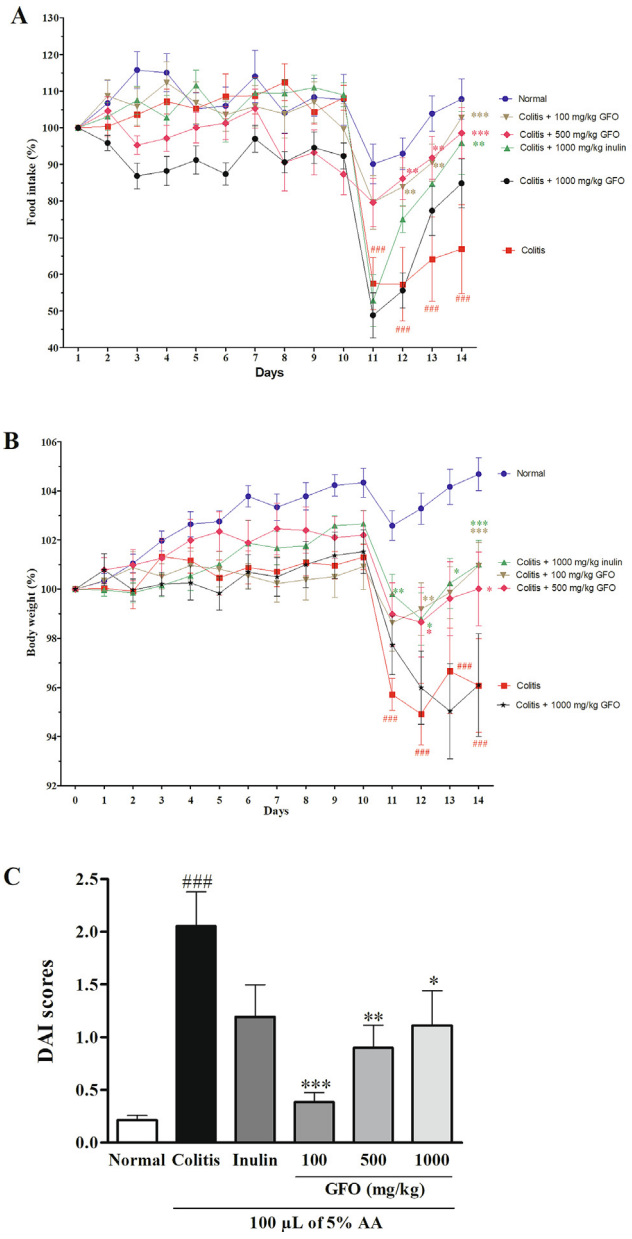


Fig. 1. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation for 14 days in acetic acid (AA)-induced colitis mice. Plots show the effects of supplementation on (A) food intake (%), (B) body weight (%). The bar chart (C) shows effects on disease activity index (DAI) scores at day 12. Data were shown as means ± SEM (n = 10–14). Symbols indicate significant differences from normal control or colitis control groups (### means $p < 0.001$ when compared with the normal control group, and *, ** and *** means $p < 0.05$, 0.01 and 0.001 when compared with the colitis control group.

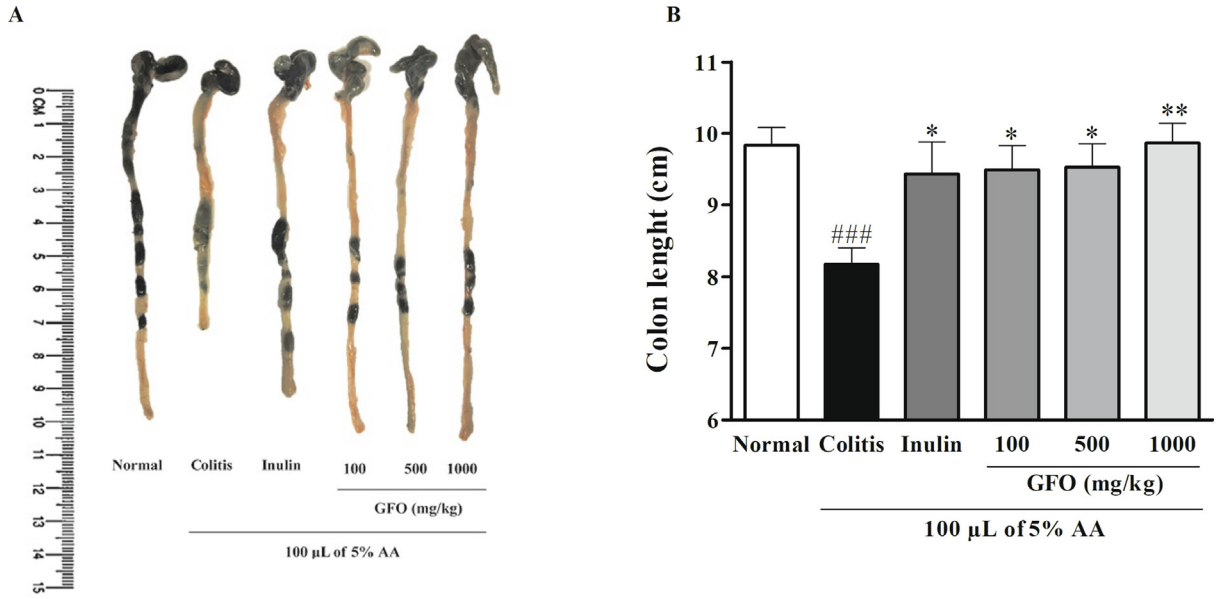


Fig. 2. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation in acetic acid (AA)-induced colitis mice on colon length. The photograph (A) shows the effects of supplementation on colon length in representatives of different treatment groups and the bar chart (B) shows colon length differences between all experimental animal groups. Data were shown as means ± SEM (n = 10–14). Symbols indicate significant differences from normal control or colitis groups (### means $p < 0.001$ when compared with the normal control group, and * *and* **means $p < 0.05$, and 0.01 when compared with the colitis control group.

3.2. Effects of GFO on colonic histological damage in AA-induced colitis mice

The colonic tissue of the colitis control group showed greater histopathological damage when compared to the tissue of the normal control group ($p < 0.001$), (Fig. 3B). The colonic tissue of colitis mice showed severe inflammation of the mucosal layer and even the submucosal layer. Infiltration of inflammatory cells to the mucosal layer, crypt loss, and destroyed epithelium could be observed (Fig. 3A). The severity of inflammation, epithelium destruction and crypt loss was attenuated in colitis mice treated with 100, 500, and 1000 mg/kg GFO ($p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively).

3.3. Effects of GFO on gastrointestinal transit in AA-induced colitis mice

The effects of GFO on gastrointestinal transit were investigated by determining total gut transit time, upper gut transit, and distal colonic transit time (Fig. 4A, 4B and 4C, respectively). The total gut transit time in the colitis control group was 242.2 ± 26.42 min which was significantly longer than total gut transit time in the normal control group (131 ± 16.27 min), ($p < 0.01$). Upper gut transit was determined by the distance a charcoal meal traveled along the small intestine. This parameter did not significantly differ among all groups. The distal colonic transit time in the colitis control group was 226.5 ± 48.96 s, which was slightly shorter than the time in the normal control group (334.43 ± 35.85 s), ($p > 0.05$) but significantly shorter than distal colonic transit time in the inulin group (661.1 ± 87.71 s). However, total gut transit time in the colitis group treated with 100 mg/kg GFO was the same as the time in the normal control group ($p < 0.01$). Interestingly, GFO treatment could attenuate and even restore total gut transit time in AA-induced colitis mice.

3.4. Effects of GFO on colonic motility pattern in AA-induced colitis mice

Colonic motility patterns were characterized by spatiotemporal maps (Figure S3). The maps represented three parameters: the total number of contractions, the number of non-propagation contractions (segmentation), and the number of propagation contractions (peristalsis) (Fig. 5A, B, and C, respectively). The total number of contractions, non-propagation contraction, and propagation contraction were significantly lower in the colitis control group (37 ± 4.94, 35.28 ± 4.82, and 2.4 ± 0.6 times/30 min, respectively) than in the normal control group (64.4 ± 3.88, 56.3 ± 4.07, 8.1 ± 0.73 times/30 min), ($p < 0.001$). However, by comparison with the colitis control group, the total number of contractions, and non-propagation and propagation contractions in the colitis group treated with 100 mg/kg GFO were almost fully restored ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively). Moreover, the number of propagation contractions was restored in colitis groups treated with 1000 mg/kg inulin, and 500 and 1000 mg/kg GFO when compared with the colitis control group ($p < 0.001$). The results suggested that GFO treatment can recover colonic motility patterns both of propulsion and non-propulsion in AA-induced colitis mice.

3.5. Effects of GFO on colonic smooth muscle contractility in AA-induced colitis mice

Colonic smooth muscle contractility was investigated on amplitude, frequency, and duration of contraction of longitudinal smooth muscle fibers of the proximal and distal colon, and the representative traces of the contractions are shown in Figure S4. Frequency of contraction of proximal colon was slightly decreased in colitis control group (3.07 ± 0.92 times/min) when compared with normal control group (5.5 ± 0.89 times/min) ($p > 0.05$) (Fig. 6A). In distal colon showed that the colitis control group (1.8 ± 0.56 times/min) was significantly decreased when compared with normal control group (10.5 ± 2.4 times/min) ($p < 0.01$) (Fig. 6B). Amplitude of contraction of proximal colon (0.05 ± 0.01 g) was significantly decreased in colitis control group when compared with normal control group (0.22 ± 0.05 g), ($p < 0.001$) (Fig. 6C). Similar to the distal colon which showed that the colitis control group (0.04 ± 0.01 g) was significantly decreased when compared with the normal control group (0.18 ± 0.06 g), ($p < 0.01$) (Fig. 6D). The duration of the contraction of proximal and distal colon did not show a significant difference in any groups (Fig. 6E and F). However, the frequency of contraction of the proximal colon was not significantly increased in colitis treated with 1000 mg/kg

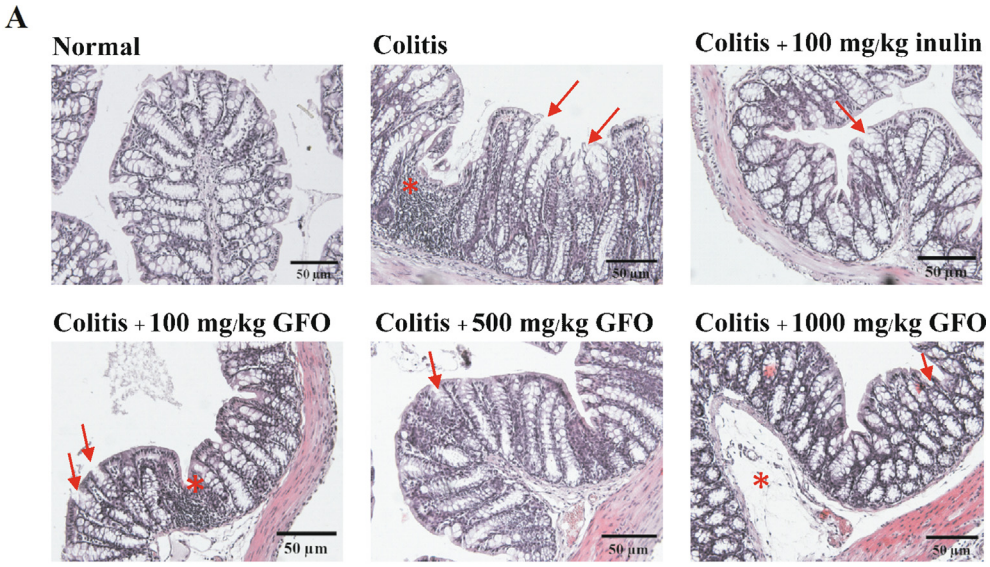
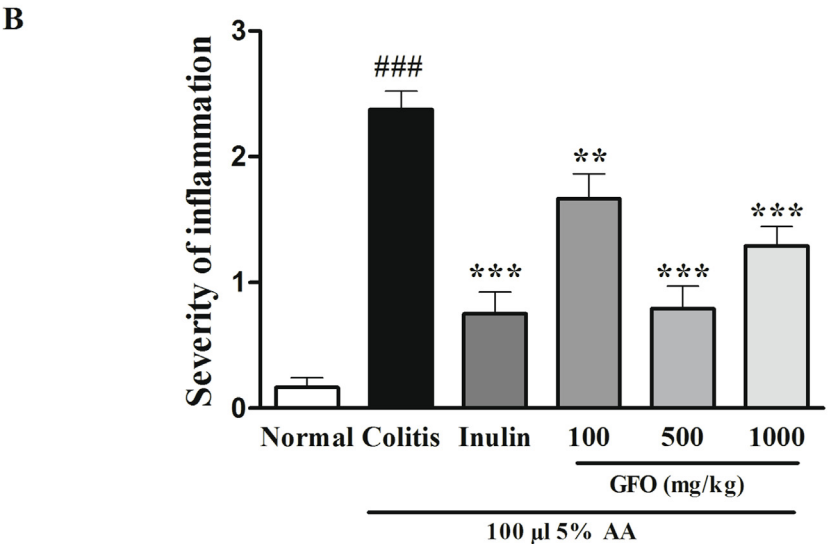


Fig. 3. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation in acetic acid (AA)-induced colitis mice on histopathological changes of the colon. (A) Representative pictures of hematoxylin and eosin (H&E) staining of histopathology under a microscope and (B) Severity scores of inflammation in normal control, colitis, and colitis-treated mice. Data were shown as means ± SEM (n = 3). Symbols indicate significant differences from normal control or colitis groups (### means $p < 0.001$ when compared with the normal control group and ** and *** means $p < 0.01$ and $p < 0.001$ when compared with the colitis control group. Red arrows indicate crypt loss and distortion; red asterisks indicate the infiltration of inflammatory cells. Scale bar: 50 μm.



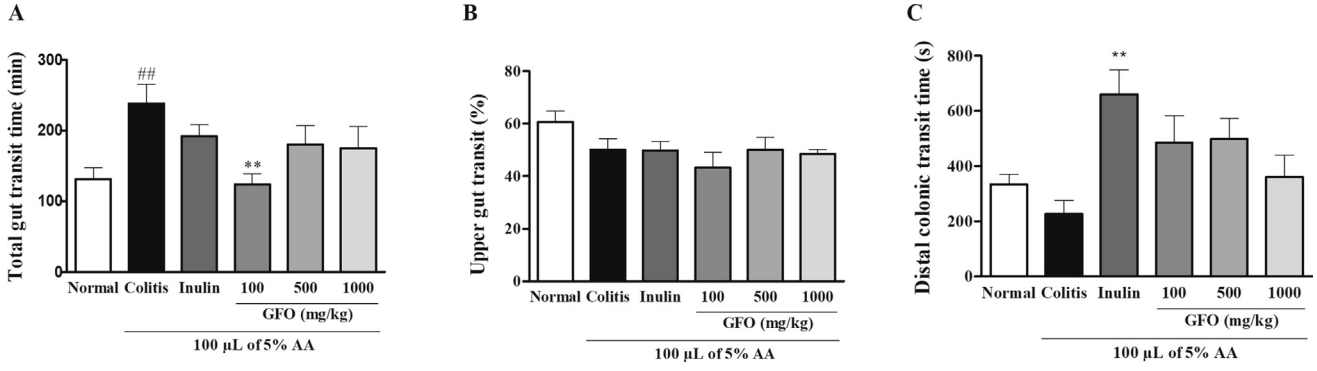


Fig. 4. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation in acetic acid (AA)-induced colitis mice on gastrointestinal transit. (A) Total gut transit time, (B) upper gut transit, and (C) distal colonic transit time of normal control, colitis and colitis-treated mice. Data were shown as means ± SEM (n = 5–9). Symbols indicate significant differences from normal control or colitis groups (### means $p < 0.001$ when compared with the normal control group, and ** means $p < 0.01$ when compared with the colitis control group.

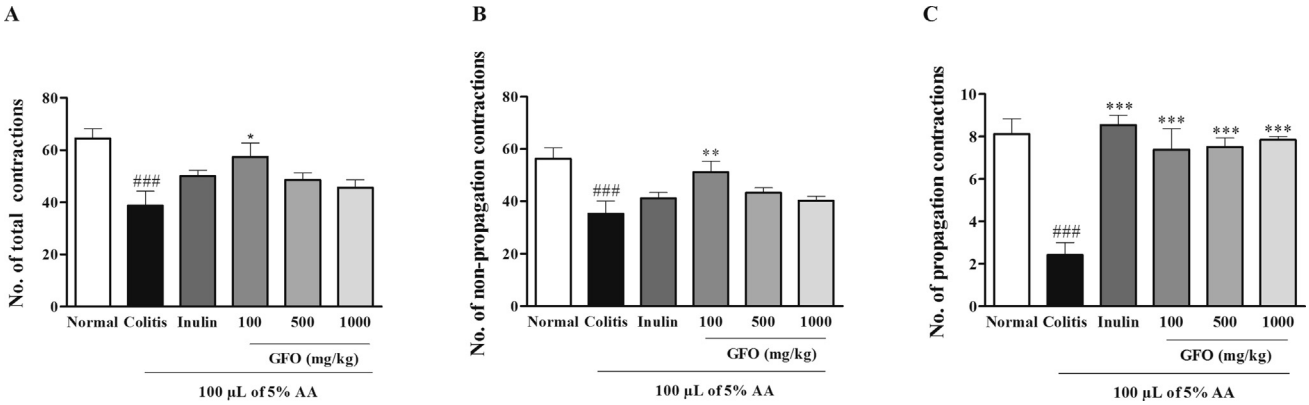


Fig. 5. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation in acetic acid (AA)-induced colitis mice on colonic motility patterns. (A) Total number of contractions, (B) number of non-propagation contractions (segmentation), and (C) number of propagation contractions (peristalsis) of normal control, colitis, and colitis-treated mice. Data were shown as means ± SEM (n = 5–9). Symbols indicate significant differences from normal control or colitis groups (### means $p < 0.001$ when compared with the normal control group, and *, ** and *** means $p < 0.05$, 0.01 and 0.001 when compared with the colitis control group.

inulin, 100, 500, and 1000 mg/kg GFO groups when compared with the colitis control group ($p > 0.05$). In distal colon, the frequency of contractions was significantly increased in colitis treated with 1000 mg/kg inulin, 100 and 500 mg/kg GFO groups when compared with colitis control group ($p < 0.01$, $p < 0.001$ and $p < 0.01$, respectively). The amplitude of contraction of the proximal colon was significantly increased in colitis treated with 1000 mg/kg inulin and 100 mg/kg GFO groups when compared with the colitis control group ($p < 0.05$). In distal colon, the amplitude of contractions was not significantly increased in colitis treated with 1000 mg/kg inulin, 100, 500, and 1000 mg/kg GFO groups when compared with the colitis control group ($p > 0.05$). The result suggested that GFO treatments can recover colonic smooth muscle contractility in AA-induced colitis mice.

3.6. Effects of GFO on composition of colonic microbiota in AA-induced colitis mice

Gut contents were collected to investigate of Bifidobacteria, Lactobacilli, and Enterobacteria in the composition of microbiota in the proximal and distal colon of GFO-treated mice. The results showed that the numbers of Bifidobacteria in the proximal and distal colon of mice were not significantly different among the groups ($p > 0.05$) (Fig. 7A and B). The number of Lactobacilli in the colon was not significant between the colitis control group and the normal control group (Fig. 7C and D). The numbers of Enterobacteria in proximal and distal colon were significantly higher in the colitis control group (11.28 ± 0.22 and 10.35 ± 0.38 log cells/mg DNA) when compared with the normal control group (7.81 ± 0.06 and 7.84 ± 0.65 log cells/mg DNA) ($p > 0.001$ and $p > 0.01$), (Fig. 7E and F). However, when compared with the colitis control group, the number of Lactobacilli was significantly lower in the proximal colon in the colitis group treated with 1000 mg/kg inulin ($p < 0.05$) and significantly lower in the distal colon in the colitis group treated with 500 mg/kg GFO ($p < 0.01$). The numbers of Enterobacteria in the proximal colon were significantly lower in colitis treated with 1000 mg/kg inulin, 100, 500 and 1000 mg/kg GFO groups when compared with colitis control group ($p < 0.05$, $p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively). However, numbers in the distal colon were significantly lower only in colitis groups treated with 100 and 1000 mg/kg GFO ($p < 0.05$ and $p < 0.05$). Interestingly, GFO treatments modulated the composition of colonic microbiota, especially Enterobacteria, in AA-induced colitis mice.

3.7. Effects of GFO on production of SCFAs in AA-induced colitis mice

Gut contents were collected to evaluate the production of butyrate and propionate in the colon of GFO-treated mice. The concentration of butyrate in the colon was not significant between the colitis control group and the normal control group (35.95 ± 1.26 and 36.10 ± 0.77 mmol/kg feces), (Fig. 8A). The concentration of butyrate in the colon was significantly higher in the colitis groups treated with 100, 500, and 1000 mg/kg GFO when compared with the colitis control group (62.77 ± 1.62, 55.66 ± 6.81, and 53.59 ± 1.85 mmol/kg feces, respectively), ($p < 0.01$, $p < 0.05$, and $p < 0.05$, respectively). The concentration of propionate in the colon was not significant between the colitis control group and the normal control group (45.28 ± 4.30 and 40.59 ± 1.36 mmol/kg feces), (Fig. 8B). The concentration of propionate in the colon was significantly higher in the colitis groups treated with inulin, and 100 and 500 mg/kg GFO when compared with the colitis control group (58.27 ± 2.78, 67.35 ± 2.86, and 58.10 ± 3.23 mmol/kg feces, respectively), ($p < 0.01$, $p < 0.001$, and $p < 0.01$, respectively).

4. Discussion

Since a previous study reported that colitis mice suffered disturbance of GI motility (Cheon et al., 2012), the aim of this study was to evaluate changes in colonic motility in colitis mice as a result of the prebiotic effects of *Gracilaria fisheri* oligosaccharides, or GFO. The present study used an AA-induced colitis mouse model in which the colitis symptoms were characterized by decreased body weights, shortened colonic length, and increased DAI scores (Zhang et al., 2019). Based on observation of the above symptoms, colitis induction was successful. It was suggested that the inflammatory phase in this model is similar to acute intestinal inflammation in humans who participated in the initial screening of new drugs (Elson, Sartor, Tennyson, & Riddell, 1995; Dodda, Chhajed, & Mishra, 2014). Treatment with the prebiotic positive control (inulin) and GFO in this study improved the condition of colitis mice by improving food intake, restoring body weight and colonic length, decreasing DAI scores, and preventing histological changes. Therefore, this study indicated that GFO has the potential to attenuate colitis in mice.

Dodda et al. (2014) demonstrated shortening of the colon length resulting from mucosal necrosis and inflammation which extended into the lamina propria, submucosa, or muscle layers through activation of the arachidonic acid pathway. The involvement of the arachidonic acid pathway in AA-induced colitis mice was similar to the involvement in human IBD of the cyclooxygenase pathway with prostaglandins and thromboxanes (Sharon & Stenson, 1985; Dodda et al., 2014). The cyclooxygenase pathway, especially cyclooxygenase-2 (COX-2), reduced the rate of propulsive motility by stimulating the hyperexcitability of myenteric afterhyperpolarization neurons present in the myenteric

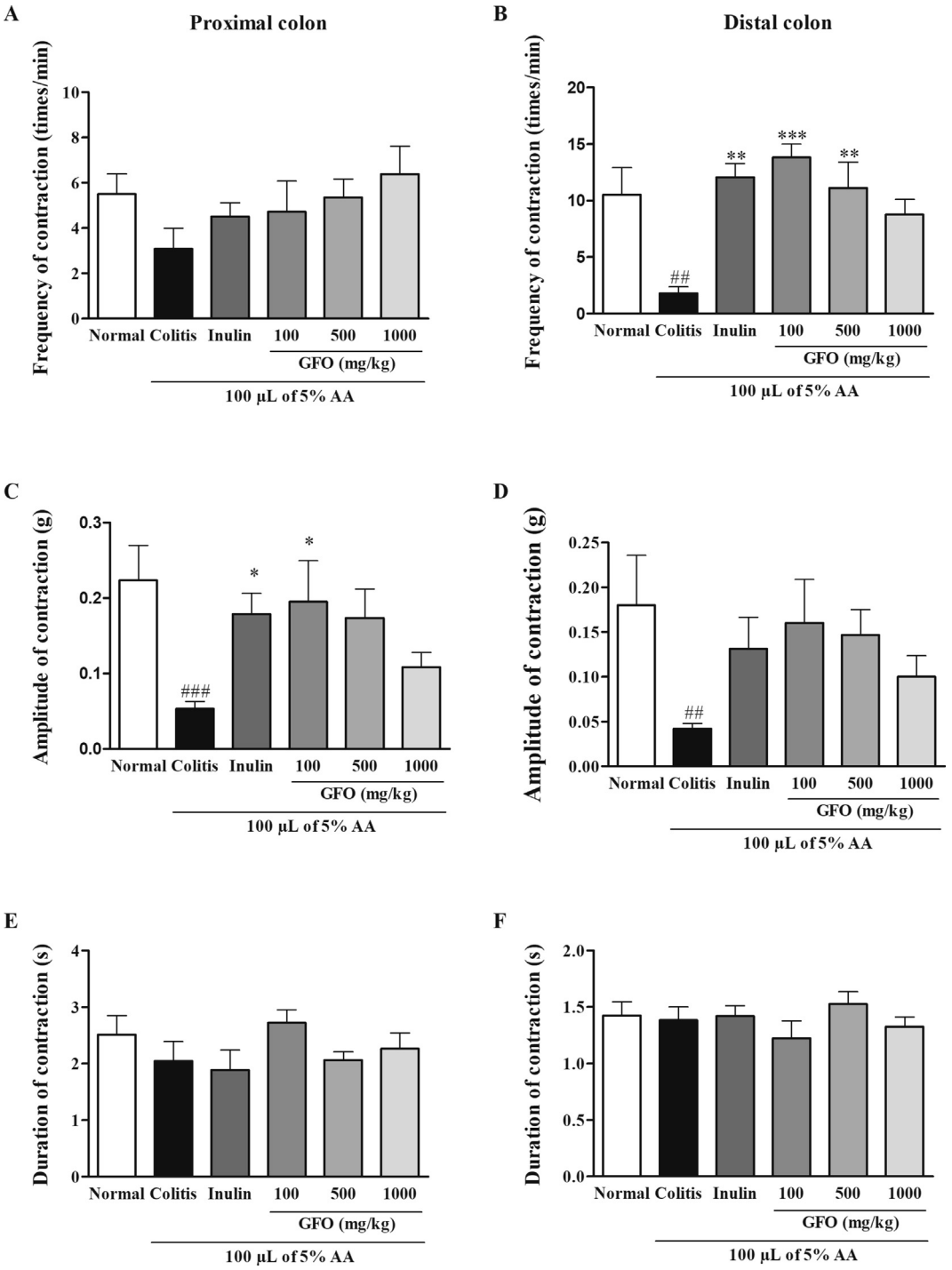


Fig. 6. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation on colonic smooth muscle contractility in acetic acid (AA)-induced colitis mice. (A and B) The amplitude of contraction in proximal and distal colon, (C and D) the frequency of contraction in proximal and distal colon, and (E and F) the duration of contraction in proximal and distal colon in normal control, colitis, and colitis-treated mice. Data were shown as means ± SEM (n = 5–8). Symbols indicate significant differences from normal control or colitis groups (## and ### means $p < 0.01$ and 0.001 when compared with the normal control group, and *, ** and *** means $p < 0.05$, 0.01 and 0.001 when compared with the colitis control group.

plexus and submucosal plexus on the smooth muscle layer, which decreased smooth muscle contractility (Mawe et al., 2009; Ozaki et al., 2005). The investigation of colonic dysmotility and abnormal smooth muscle contraction in the colon of colitis mice in this study found significantly decreased total contractions, propagation contractions, and non-propagation contractions. These findings were consistent with the findings of a previous study (Kashyap et al., 2013; Ruyssers et al., 2010; Mawe et al., 2009). Frequency and amplitude contractions of the colonic smooth muscle in the colitis mice were also significantly decreased.

Other mediators, in addition to the arachidonic acid pathway, cause the reduction of GI motility in colitis mice. Expression of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), was increased in the inflamed mucosa and muscle layers, and induced intestinal dysmotility by reducing smooth muscle contractility. TNF-α, in particular, directly

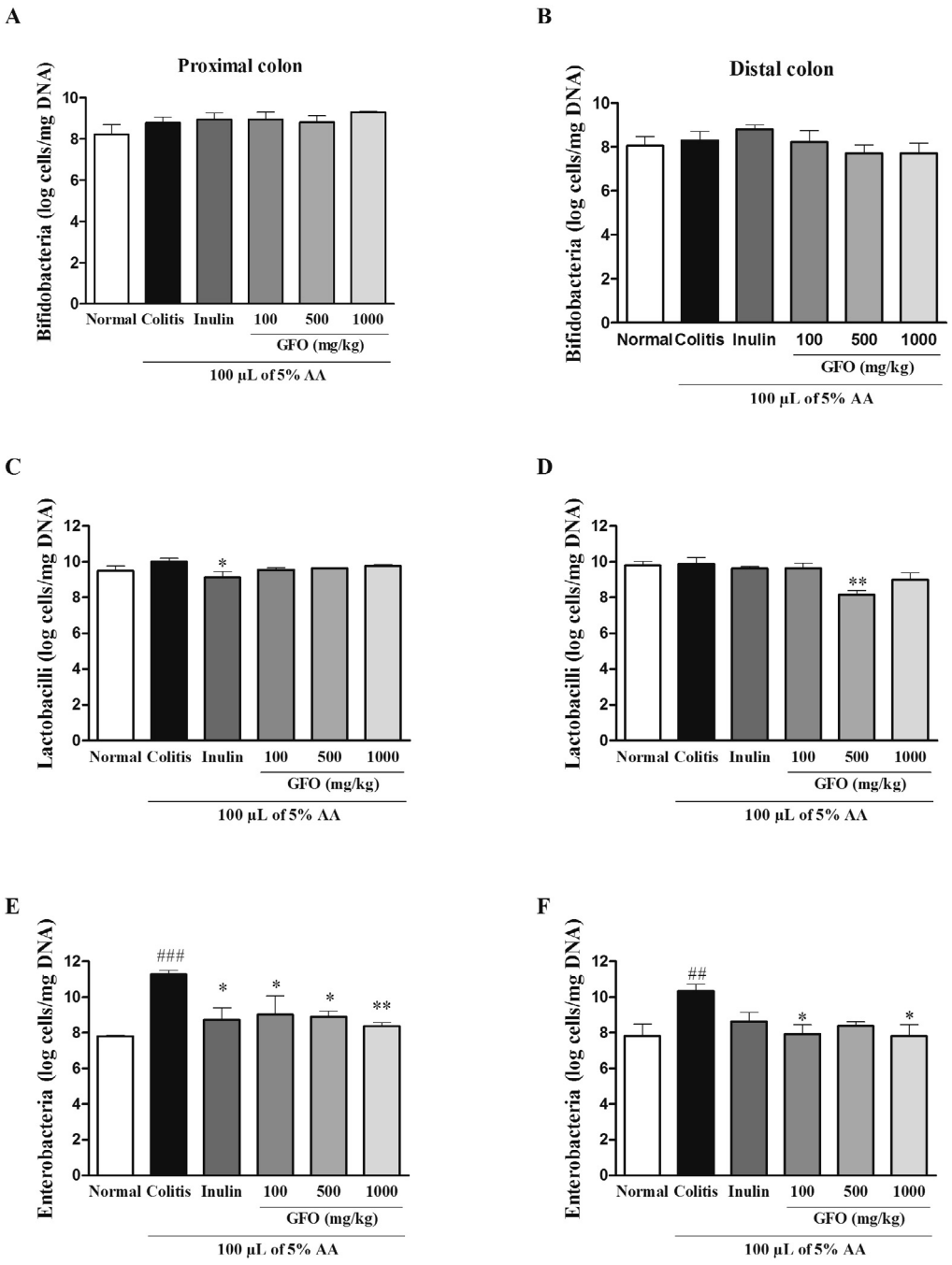


Fig. 7. Bar charts show effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation in acetic acid (AA)-induced colitis mice on the amount of gut microbiota. Bifidobacteria, Lactobacilli, and Enterobacteria in the proximal colonic contents (A, C, and E, respectively) and the distal colonic contents (B, D, and F, respectively) in normal control, colitis control, and colitis-treated mice. Data were shown as means ± SEM in a log scale (n = 4). Symbols indicate significant differences from normal control or colitis groups (## and ### means $p < 0.01$ and 0.001 when compared with the normal control group, and * and ** means $p < 0.05$ and 0.01 when compared with the colitis control group.

disturbed smooth muscle function by inhibiting the activity of L-type Ca^{2+} channels on the smooth muscle membrane (Kinoshita et al., 2006; Ozaki et al., 2005). Interestingly, GFO can prevent the reduction of propulsive motility and smooth muscle contractility. Previous studies demonstrated that besides stimulating the growth of beneficial bacteria through prebiotic effects, oligosaccharides from red algae have anti-inflammatory properties (Li et al., 2014). Thus, GFO may attenuate GI dysmotility through anti-inflammatory activity.

Furthermore, Ozaki et al. (2005) demonstrated that inflammation of the colon suppressed GI motility which induced secondarily an imbalance of gut microbiota, increasing harmful bacteria that produced a toxic substance that impaired the mucosal barrier and contributed to the severity of inflammation. Therefore, the severity of colitis may possibly be a consequence of gut dysbiosis. Analysis of gut microbiota in this study found that Enterobacteria populations were significantly higher in colitis mice but, notably, not in colitis mice treated with GFO. A recent study found that numbers of *Escherichia coli* (*E. coli*; a member of *Enterobacteriaceae* family, Enterobacteria) increased in colitis mice and IBD patients (Khan et al., 2019). Sasaki and Klapproth (2012) revealed that dysbiotic gut microbiota in human colitis patients showed increased populations of *E. coli. E. coli* acts as a pro-inflammatory agent. Moreover, *E. coli* can degrade mucins to produce toxic substances, such as necrotoxins and haemolysin, and has mannose-resistant adhesins. Additionally, the presence of the adhesin increased the release of inflammatory mediators (Burke & Axon, 1987).

Taken together, these results suggested that GFO has prebiotic effects that induce specific changes in the composition of the gut microbiota. GFO also showed effects on smooth muscle contractility, altering colonic motility. A study of prebiotic properties of red algae showed that oligosaccharides from red algae can stimulate the growth of bifidobacteria and lactobacilli (Han et al., 2019). However, we did not find any change in the growth of these bacteria in this study. Many

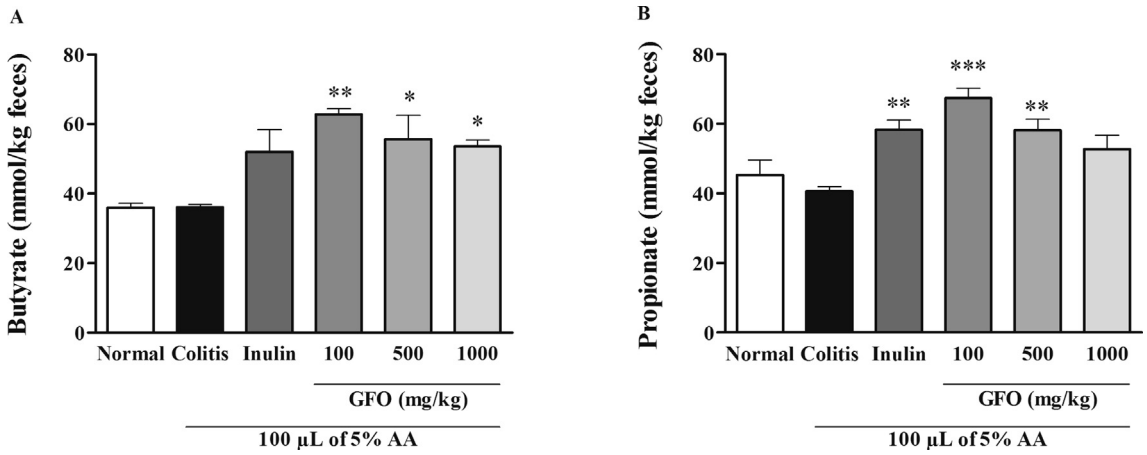


Fig. 8. Effects of *Gracilaria fisheri* oligosaccharides (GFO) supplementation in acetic acid (AA)-induced colitis mice on production of short-chain fatty acids. The charts show concentrations of (A) butyrate and (B) propionate in the colonic content in normal control, colitis, and colitis-treated mice. Data were shown as means ± SEM (n = 3). Symbols indicate significant differences from colitis group (*, **, and *** mean $p < 0.05$, 0.01, and 0.001 when compared with the colitis control group).

studies have demonstrated that populations of Bifidobacteria and Lactobacilli were increased by prebiotic treatment. Conversely, in this study, the numbers of Lactobacilli were reduced by inulin treatment. Goto et al. (2010), studying DSS-induced colitis mice, found that the numbers of Lactobacilli were reduced by FOS treatment. However, they have yet to find an explanation for the reduction.

Other gut microbiota, including Bacteroidetes, could ferment carbohydrates and had an anti-inflammatory effect (Kedia, Rampal, Paul, & Ahuja, 2016). Bacteroidetes might possibly have played a role in GFO fermentation that attenuated AA-induced colitis mainly by producing acetate and propionate (Den Besten et al., 2013). Also, Rios-Covian et al. (2015) demonstrated that inflammation in the gut was prevented by enhanced butyrate formation (Ganesan, Chung, Vanamala, & Xu, 2018) from cross-feeding between Firmicutes (*Faecalibacterium prausnitzii* (*F. prausnitzii*)) and Bifidobacteria (*Bifidobacterium adolescentis* (*B. adolescentis*)). They investigated the interaction between *Bifidobacterium* and *Faecalibacterium* strains using *in vitro* co-cultures with FOS. They found that *B. adolescentis* was able to ferment FOS, producing acetate and that *F. prausnitzii* could further influence cross-feeding effects by using the acetate to form butyrate.

Ganesan et al. (2018) revealed that in an acidic environment provided by SCFAs production, Bacteroidetes grew rapidly. Possibly, our treatment was fermented by Bacteroidetes and promoted the growth of Bacteroidetes, which mainly produce acetate and propionate, which restrict the growth of *Lactobacillus*. Monteagudo-Mera et al. (2019) reported that GOS directly inhibited adherence to epithelial cells by *Escherichia coli in vitro* study and *Citrobacter rodentium in vivo* study. GOS acted as a molecular receptor decoy that competitively inhibited the adherence of intestinal pathogens to host cells. Additionally, SCFAs is known to be a product of bacterial prebiotic fermentation that provides an important function in gut physiology. Tao et al. (2016) demonstrated that the concentration of SCFAs (butyrate and propionate) in colitis mice were decreased remarkably compared to the normal group. On the other hand, we did not find any change in the concentration of these SCFAs especially butyrate of colitis mice in this study. As described above about butyrate formation by cross-feeding between Firmicutes and Bifidobacteria. Probably, the butyrate formation might not halt in colitis mice since the number of Bifidobacteria in colitis mice did not decrease in this study. However, this study found that treatment with GFO significantly increased the concentration of butyrate and propionate, which was consistent with the findings of Long, Yu, Wang, Liu, and He (2018). They reported that chito-oligosaccharides enhanced the concentration of SCFAs in colitis mice.

Many studies have demonstrated that butyrate has immunomodulatory functions. In both *in vivo* and *in vitro* studies, butyrate could treat IBD (Sant'Anna and Ferreira, 2014; Sun et al., 2016). In addition, Ji et al. (2016) reported that butyrate treatment reduced levels of the pro-inflammatory cytokines IL-6, TNF-α, and IL-1β. These processes may act through downstream activation of G-protein-coupled receptors (GPCRs) and inhibition of histone deacetylases (HDACs). Moreover, HDACs inhibition could suppress NF-κB, modulate oxidative stress, and also associate with mucus synthesis, which may prevent histological change. Furthermore, Hurst et al. (2014) revealed that butyrate can enhance peristalsis by stimulating choline acetyltransferase (ChAT) positive cholinergic neurons in the enteric nervous system. Propionate can enhance segmentation by stimulating Peptide YY release from the enteroendocrine L cells which is consistent with the results of this study.

5. Conclusion

This study showed that dietary supplementation with GFO could ameliorate colitis and reverse motility defects in AA-induced colitis mice. GFO was provided for 10 days before and for 4 days after the induction of colitis. Food intake reduction was alleviated, body weight loss and colon shortening were attenuated, and high DAI scores were lowered. Production of stool pellets recovered, occult blood in stool, and histological change were diminished. There was increased transit speed, total, propagating and non-propagating contractions increased and the frequency and amplitude of contractions increased. Populations of harmful Enterobacteria decreased and the production of butyrate and propionate were increased. These might be direct effects or produced indirectly by the reduction of harmful bacteria. The results suggest that GFO could be used as a food supplement in the treatment of UC and IBD and also GI dysmotility. Its therapeutic value could reside in its anti-inflammatory and prebiotic effects. Further studies are indicated.

Acknowledgements

This work was supported by the grants from Faculty of Science Research Fund, Prince of Songkla University (Contract number 1-2560-02-001 to S. K-da); the National Research Council of Thailand for the year 2019 (to S. K-da); Thailand Education Hub-ASEAN Countries (TEH-AC) Scholarship, Prince of Songkla University (grant number TEH AC 012/2017 to B. Siringoringo); and Prince of Songkla University (grant number SCI6202058S to P. Khuituan). The authors are grateful to Mr. Thomas Coyne, Faculty of Science, Prince of Songkla University for providing assistance in proofreading and providing feedback on the

manuscript.

Disclosure

None of the authors had conflicts of interest throughout the study process.

Authorship contributions

The authors' responsibilities:
S.P., P.M., S.W, and P.K. - conceptualized and designed research;
S.K., S.P., B.S., S.W, and P.K. - conducted research;
S.K., S.P., B.S., S.W, and P.K. - analyzed data;
S.K., S.P., S.W, and P.K. - wrote the paper;
P.M., and P.K. - reviewed final content;
All authors: reviewed and approved the final version of the manuscript.

Ethics Statements

This study was approved and guided by the Animals Ethics Committee of the Prince of Songkla University, Thailand (Project license number MOE 0521.11/1069).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104021.

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