

Article

Oral Administration of *Ulva pertusa* Kjellman Improves Intestinal Motility Against Loperamide-Induced Constipation in Mice

Eun-Jeong Koh ¹ , In-Yung Sunwoo ¹, Yong-Kyun Ryu ^{1,2} , Won-Kyu Lee ¹ , Taeho Kim ¹
and Woon-Yong Choi ^{1,*} 

¹ Jeju Bio Research Center, Korea Institute of Ocean Science and Technology (KIOST), Jeju 63349, Republic of Korea; kej763@kiost.ac.kr (E.-J.K.); jysunwoo@kiost.ac.kr (I.-Y.S.); ykyou0111@kiost.ac.kr (Y.-K.R.); wonkyulee@kiost.ac.kr (W.-K.L.); kt1024@kiost.ac.kr (T.K.)

² Department of Marine Technology & Convergence Engineering (Marine Biotechnology), KIOST School, University of Science and Technology (UST), Daejeon 34113, Republic of Korea

* Correspondence: cwy@kiost.ac.kr; Tel.: +82-64-798-6109

Abstract: *Ulva pertusa* Kjellman (*U. pertusa*) is a seaweed indigenous to the intertidal zone of the Korean coastline. *U. pertusa* exhibits immune-enhancing and antitumor activities, and its effects on intestinal health have gained attention. However, the mechanisms underlying its beneficial effects on intestinal physiology remain elusive. Here, the effect of *U. pertusa* intake in ameliorating loperamide-induced constipation in male mice was evaluated. Additionally, cellular levels of proinflammatory cytokines, including nuclear factor- κ B and interleukin-1 β , were assessed to decipher the intricate interplay between inflammation and improvements in bowel movement. *U. pertusa* intake increased fecal weight and water content and improved the intestinal transit rate. Moreover, it reduced the levels of proinflammatory cytokines, possibly via short-chain fatty acids implicated in modulating intestinal motility and mucosal inflammation. These findings underscore the efficacy of *U. pertusa* in improving bowel motility and intestinal functionality, and its potential in ameliorating constipation.

Keywords: *Ulva pertusa* Kjellman (*U. pertusa*); constipation; intestinal motility; nuclear factor-kappa B; interleukin-1 β



Citation: Koh, E.-J.; Sunwoo, I.-Y.; Ryu, Y.-K.; Lee, W.-K.; Kim, T.; Choi, W.-Y. Oral Administration of *Ulva pertusa* Kjellman Improves Intestinal Motility Against Loperamide-Induced Constipation in Mice. *Appl. Sci.* **2024**, *14*, 9892. <https://doi.org/10.3390/app14219892>

Academic Editors: Lígia Pimentel and Ana Fontes

Received: 19 September 2024

Revised: 22 October 2024

Accepted: 28 October 2024

Published: 29 October 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Constipation, a prevalent gastrointestinal disorder affecting approximately 16% of the population worldwide, manifests through symptoms such as infrequent bowel movements (<3/week), hardened stool, lumpy consistency, excessive straining, and painful defecation [1,2]. Etiologically intricate, the condition arises from a confluence of genetic predisposition, dietary habits, dynamics of colonic absorption, and multifarious biological, lifestyle, and pharmaceutical factors [3,4]. The contributing factors include the insufficient intake of dietary fiber, inadequate hydration, and irritable bowel syndrome [5]. Diverse interventions encompassing lifestyle modifications, dietary adjustments, laxative use, and surgical interventions have been adopted to address this complexity [6].

The pathophysiology of constipation encompasses multiple underlying mechanisms associated with diverse factors [7]. Notably, intestinal dysmotility, an imbalanced composition of gut microbiota, aberrant mucus secretion, and disruptions in intestinal neural regulation are the key factors [7,8].

Among pharmaceutical agents, loperamide, an opioid receptor agonist, plays a role in treating diarrhea [9]. Used for chronic diarrhea and short bowel syndrome, it reduces longitudinal and circular smooth muscle tone in the intestines, culminating in diminished colonic mass movement and suppressing the gastrocolic reflex, ultimately alleviating the

symptoms of diarrhea [10,11]. Nevertheless, the adverse effects of loperamide administration on bowel motility, including constipation, nausea, abdominal pain, bloating, and distention, have been acknowledged [11,12].

Owing to these adverse effects, loperamide is used to establish constipation models in animal studies, facilitating investigations into the mechanistic underpinnings of constipation and exploration of novel therapeutic compounds [11,13,14]. Increased levels of inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin 1 beta (IL-1 β), and nuclear factor-kappa beta (NF- κ B), have been reported in animal models of loperamide-induced constipation [15,16], delineating an interplay between constipation and inflammation.

Ulva pertusa Kjellman (*U. pertusa*), a common and rapidly proliferating seaweed, is indigenous to the intertidal zone of the Korean coastline [17]. With a rich nutritional profile encompassing proteins, vitamins, minerals, and dietary fibers [18], *U. pertusa* has garnered attention due to its multifaceted attributes, ranging from antioxidant and immune-enhancing effects to antitumor and blood anticoagulant activities [19,20]. However, the mechanisms underlying the effects of the dietary intake of *U. pertusa* on intestinal health are not fully understood.

Recently, an upsurge in *U. pertusa* proliferation has been documented along the western coast of Jeju Island. This proliferation has precipitated concerns ranging from esthetic deterioration and olfactory emissions to harmful repercussions on the local marine ecosystem [21]. Exploring the usage of *U. pertusa* as a functional food source may offer a plausible resolution for the “bloom” phenomenon. Based on these factors, in this study, the ameliorative effects of *U. pertusa* intake on loperamide-induced constipation in mice were evaluated. Additionally, this study focused on unraveling the mechanism underlying intestinal dysmotility induced by alterations in inflammatory responses.

2. Materials and Methods

2.1. Preparation of *Ulva pertusa* Kjellman (*U. pertusa*)

U. pertusa was harvested from Sinyang-ri (33°26'4.70" N 126°55'24.16" E, Jeju, Republic of Korea) [22] between February and April and dried using an air dryer (Jeio Tech, Daejeon, Republic of Korea) at 40 °C for 48 h. *U. pertusa* extract was prepared as per the method presented in Figure 1 [23].

2.2. Compositional Analysis of *U. pertusa*

Compositional analysis was performed at the Institute of Agricultural Science, Chungnam National University, Daejeon, Republic of Korea, using protocols outlined by the American Organization of Analytical Chemists (AOAC). The crude protein content was determined by multiplying the nitrogen (N) value by a conversion factor of 6.25, with nitrogen quantified using the Kjeldahl method (AOAC 2000.11). Crude lipid was measured using the Soxhlet extraction method (AOAC 991.36), and crude fiber was determined in *U. pertusa* extracts using a CSF6 fiber extraction unit (VELP Scientifica, GDE-CSF6, Usmate, Italy) through sequential hydrolysis with 0.26 N sulfuric acid and 0.32 N sodium hydroxide at 100 °C for 30 min each (AOAC 962.09). The total carbohydrate content was calculated by subtracting the combined crude protein, lipid, and ash content from the total composition [24].

2.3. Animals and Experimental Design

Six-week-old male ICR mice (28 g) were purchased from Hana Biotech (Gyeonggi, Republic of Korea) and housed at the Southeast Medi-Chem Institute (Busan, Republic of Korea). The mice were acclimated for 1 week under a 12 h light/dark cycle at a temperature of 22 $\pm$ 2 °C and humidity of 50 $\pm$ 10%. The animal study protocol was approved by the Institutional Animal Care and Use Committee of the Southeast Medi-Chem Institute (IACUC approval number: SEMI-21-009; date: August 2021). All institutional and national guidelines for the care and use of laboratory animals were strictly followed.

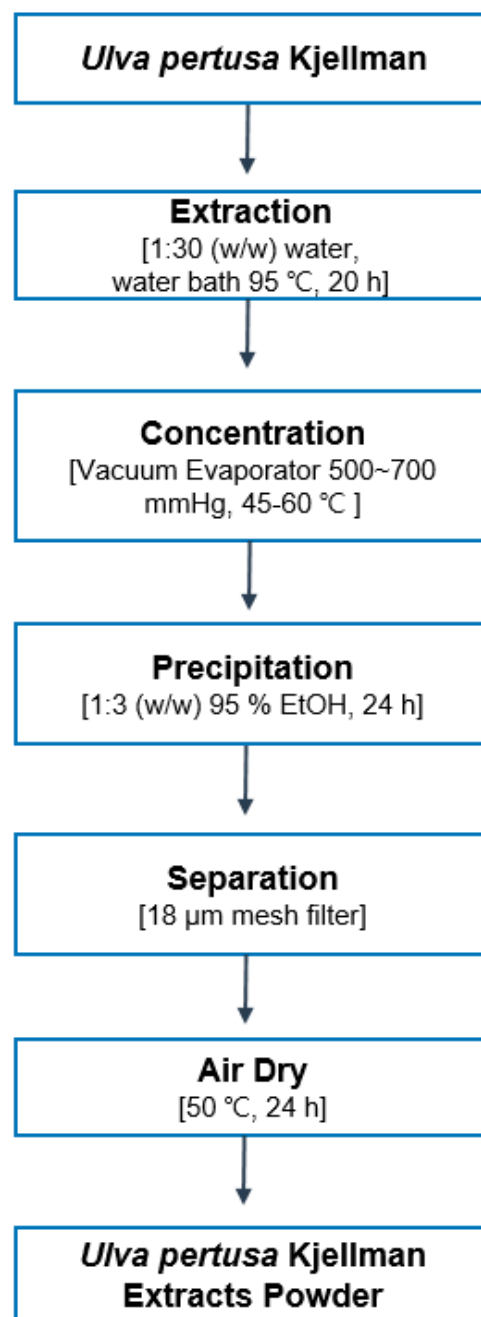


Figure 1. A flow chart summarizing the steps involved in preparing *U. pertusa* extract. *U. pertusa* was extracted in water at 95 °C for 20 h, after which the extracts were concentrated using a vacuum evaporator (EYELA, Gyeonggi, Republic of Korea). The concentrated extracts were precipitated with 95% ethanol at three times the volume of the concentrate for 24 h. Following precipitation, pectin was separated from the precipitate using a mesh filter (TISCH Scientific, Cleves, OH, USA). The samples were then dried at 50 °C for 24 h.

The animal comprised seven mice randomly allocated to each of five groups (Figure 2A). The experimental design is elucidated in Figure 2B. The mice were orally administered $120 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ psyllium husk powder (P), 100 and $500 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ *U. pertusa* (AL and AH, respectively), or an equal volume of vehicle saline solution (N) once daily over an 8-day interval. On day 14, constipation was induced via gavage with loperamide at a dose of $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 2 days, and bowel improvement was comprehensively evaluated.

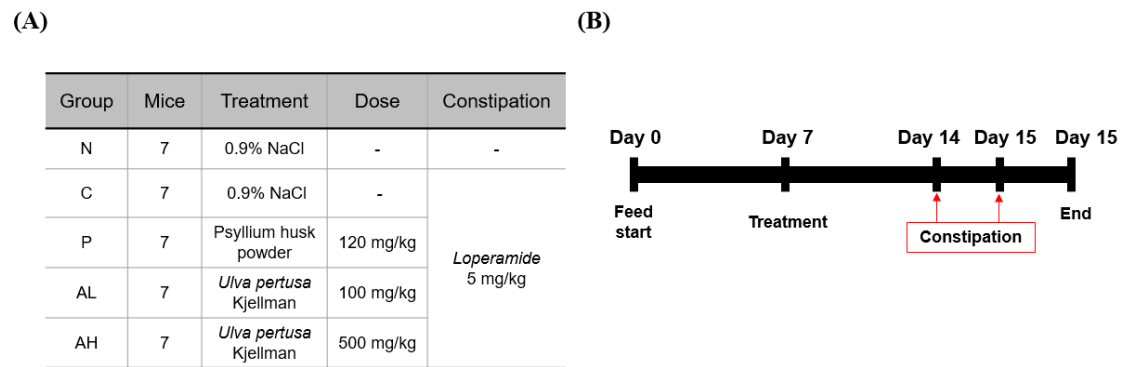


Figure 2. A schematic depicting the experimental design. (A) A cohort of mice was randomly divided into the following five groups: constipation induced via $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ loperamide treatment (C, control), psyllium husk powder treatment at $120 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (P, positive control), *U. pertusa* extract administered at 100 and $500 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ (AL and AH, respectively), and the administration of an equivalent volume of saline solution (N, vehicle). (B) After 7 days of acclimatization, daily oral administration was conducted over 8 days, followed by a 2-day loperamide-induced constipation intervention on day 14 and mice sacrifice on day 15.

2.4. Assessment of Fecal Weight and Water Content

Fecal weight and water content were analyzed after *U. pertusa* treatment on day 15. Following 5 h of constipation induction, fecal samples were collected, weighed, and thoroughly dried in an oven to determine the dry mass. Fecal weight and water content were calculated as follows [25]:

$$\text{Fecal weight (g)} = \text{Wet weight} - \text{dry weight} \quad (1)$$

$$\text{Fecal water content (\%)} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100 \quad (2)$$

2.5. Determination of Intestinal Transit Rate

The intestinal transit rate was measured by the end of *U. pertusa* administration (i.e., day 15). After treatment with P, AL, AH, or saline, mice were administered $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ loperamide for 30 min. Subsequently, 0.5% phenol red in 1.5% methylcellulose was given via gavage. After 20 min, the mice were euthanized using CO_2 and the total length of the small intestine was measured. The intestinal transit rate was then calculated as follows [25]:

$$\text{Intestinal transit rate (\%)} = \frac{\text{Distance of transported phenol red}}{\text{Total length of the small intestine}} \times 100 \quad (3)$$

2.6. Biochemical Analyses

Intestinal tissues were homogenized in phosphate-buffered saline using homogenizer (T-25 basic homogenizer, Ultra-Turrax, IKA-Werke, Wilmington, NC, USA) for 20–40 s and centrifuged at $12,000 \times g$ for 20 min at 4°C , with the supernatant collected for cytokine analysis. The levels of NF- κB and IL-1 β in the intestinal tissues were measured using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions (Cusabio, Houston, TX, USA). These kits utilize the double-antibody sandwich method to quantify inflammatory factors in 100 μL samples. Absorbance was measured at 450 nm using a microplate reader (Molecular Devices, San Jose, CA, USA).

2.7. Statistical Analyses

Statistical analyses were conducted using StatView v.5.0.1 (SAS Institute Inc., Cary, NC, USA). Group comparisons were performed using one-way analysis of variance (one-way ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test to

compare means. The data were normally distributed and exhibit homogeneous variances across the groups according to the Levene's test, respectively. Results are presented as the mean $\pm$ standard deviation. Statistical significance was defined as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results and Discussion

3.1. Nutritional Composition of *U. pertusa*

The nutritional composition of *U. pertusa* was comprehensively investigated, which revealed a moisture, carbohydrate, crude protein, crude fat, crude fiber, and ash content of 9.42, 48.85, 17.15, 1.33, 3.96, and 19.3%, respectively (Table 1). The high carbohydrate content in *U. pertusa* can be ascribed to the presence of intricate polysaccharides including fucoxanthin, galacturonic acid, and dietary fiber. We have previously reported 36.23% galacturonic acid content in *U. pertusa* [23]. Galacturonic acid, a key component of pectin, has various physiological benefits, including the alleviation of constipation, modulation of microbial composition, and regulation of cholesterol level [26].

Table 1. The nutritional composition of *U. pertusa*. The content of moisture, carbohydrate, crude protein, fat, fiber, and ash in *U. pertusa* is expressed as a percentage. Values are expressed as the mean $\pm$ standard deviation (n = 3).

Nutritional Composition (%)	<i>U. pertusa</i>
Carbohydrate	48.85 $\pm$ 0.9
Crude protein	17.15 $\pm$ 3.8
Crude fat	1.33 $\pm$ 0.9
Crude fiber	3.96 $\pm$ 2.1
Crude ash	19.30 $\pm$ 2.7

Recently, it has been reported that *U. pertusa* polysaccharide (UPP) prevents inflammatory bowel disease (IBD) in mice with dextran sulfate sodium (DSS)-induced ulcerative colitis (UC). Notably, UPP improved gut microbiota via regulating *lactobacillus* and short-chain fatty acid [27]. Considering the research findings reported above, the components of *U. pertusa* extract may have potential for improving constipation.

3.2. Effects of *U. pertusa* Administration on Stool Excretion

Loperamide inhibits intestinal smooth muscle contraction, decreases intestinal peristalsis, and reduces fecal water content, making it suitable for establishing constipation models of experimental animals [6]. To analyze bowel movement for confirming model establishment and the improvement effects of *U. pertusa*, we followed the process of generating the constipation model shown in Figure 1. Psyllium husk powder was used as a positive control; it is recognized as a functional ingredient for the enhancement of bowel movement by the Korean Ministry of Food and Drug Safety. Notably, the loperamide gavage group demonstrated a marked reduction in stool weight (C, 0.065 $\pm$ 0.02 g) and water content (C, 41.6 $\pm$ 5.8%) compared with those values of the vehicle group (N, 0.324 $\pm$ 0.07 g; $p < 0.001$) (N, 56.9 $\pm$ 5.5%; $p < 0.01$) (Figure 3). Discernible increments in stool weight and water content were noticed following *U. pertusa* administration (AL, 0.225 $\pm$ 0.06 g; AH, 0.226 $\pm$ 0.04 g; $p < 0.01$) (AL, 53.1 $\pm$ 5.8%; AH, 57.9 $\pm$ 2.7%; $p < 0.001$), mirroring the effects observed after the administration of the positive control (P, 0.288 $\pm$ 0.05 g; $p < 0.001$) (P, 61.6 $\pm$ 5.9%; $p < 0.001$). These findings affirm the successful establishment of the constipation model using loperamide and underscore the facilitative role of *U. pertusa* in augmenting stool excretion.

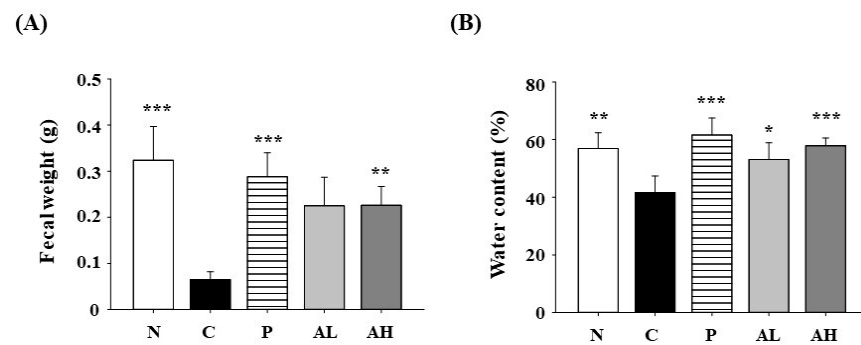


Figure 3. The effects of *U. pertusa* administration on stool excretion in mice. (A) Fecal samples collected after a 5 h constipation-induction treatment were measured. (B) Subsequently, the samples were oven-dried and weighed to determine the dry weight. Water content was determined using the relevant formula. Experiments were conducted in septuplicate. Results are presented as the mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with the control group. Abbreviations: N, vehicle; C, control; P, positive control; AL, 100 mg·kg⁻¹·day⁻¹ *U. pertusa* extract; AH, 500 mg·kg⁻¹·day⁻¹ *U. pertusa* extract.

3.3. Effects of *U. pertusa* Administration on Intestinal Motility

A reduction in stool excretion induced by loperamide leads to the suppression of intestinal peristalsis via several mechanisms such as a decline in calmodulin and paracellular permeability, and the blockade of the calcium channel [28].

To determine the improvement effects of *U. pertusa* on intestinal peristalsis, we examined intestinal length and transit ratio. As shown in Figure 4B, the administration of loperamide resulted in a decreased intestinal length (C, 46.6 $\pm$ 1.7 cm) compared to the vehicle group (N, 52.3 $\pm$ 3.8 cm) ($p < 0.05$). In contrast, treatment with *U. pertusa* (AL, 54.6 $\pm$ 2.2 cm; AH, 53.6 $\pm$ 4.8 cm) ($p < 0.01$) increased the intestinal length reduced by loperamide.

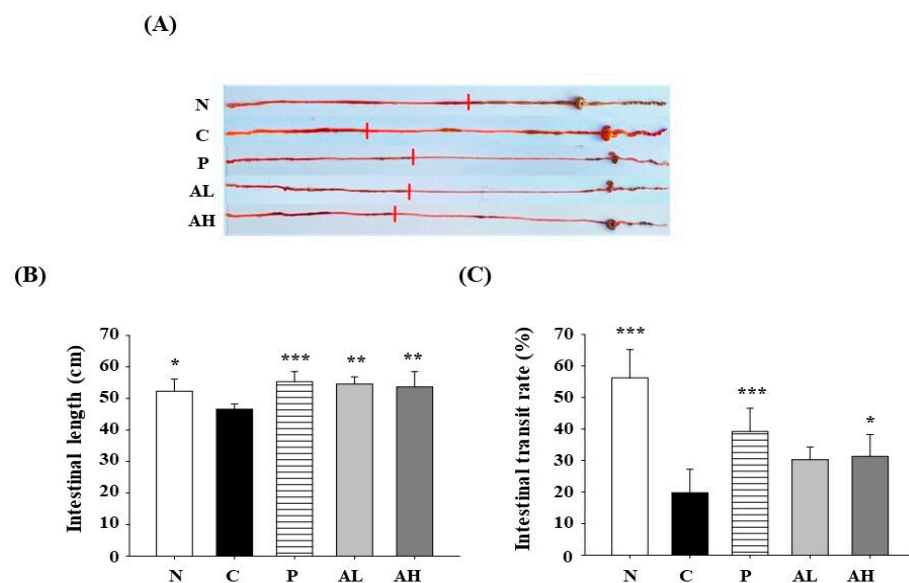


Figure 4. The effects of *U. pertusa* administration on intestinal motility in mice. (A) An illustration portraying phenol red movement within the intestinal tract. The red bar demarcates the location of phenol red. (B) The total distances traversed post-administration of 5 mg·kg⁻¹·day⁻¹ loperamide following treatment with P, AL, AH, or saline solution were measured using 0.5% phenol red. (C) A computation of the intestinal transit rate using the relevant formula. Experiments were conducted in septuplicate. Results are presented as the mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with the control group. Abbreviations: N, vehicle; C, control; P, positive control; AL, 100 mg·kg⁻¹·day⁻¹ *U. pertusa* extract; AH, 500 mg·kg⁻¹·day⁻¹ *U. pertusa* extract.

Additionally, the intestinal transit rate was significantly reduced in the loperamide treatment group (C, $19.8 \pm 7.4\%$) compared with that in the vehicle group (N, $56.2 \pm 9.0\%$; $p < 0.001$) on day 15 (Figure 4C). However, *U. pertusa* treatment increased the intestinal transit rate (AL, $30.3 \pm 4.0\%$; AH, $31.3 \pm 6.9\%$; $p < 0.05$).

It has been reported that carbohydrates, including polysaccharides, alleviate loperamide-induced constipation by modulating the secretion of SCFAs related to gastrointestinal function [29,30]. Our analysis confirmed that *U. pertusa* contains high levels of carbohydrates, particularly polysaccharides, which may stimulate intestinal peristalsis. Overall, our study findings suggest that the improvement in intestinal motility observed upon *U. pertusa* intake is likely attributable to its carbohydrate content, especially its polysaccharide content.

3.4. The Effects of *U. pertusa* on the Levels of Inflammatory Cytokines

SCFAs maintain intestinal homeostasis by modulating the integrity of the intestinal barrier and suppressing nuclear factors, such as NF- κ B, which is pivotal in the inflammatory cascade [31]. Notably, butyrate, an SCFA, may suppress the activation of NF- κ B associated with inflammatory cytokines such as IL-1 β , TNF- α , and IL-6 [32–34].

Recent studies have verified the association between constipation and cytokines, establishing an augmented presence of inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, in participants and mice with constipation [15,29,35,36]. These studies have indicated that constipation may be associated with intestinal motility and inflammatory processes regulated by SCFAs. Therefore, we speculated a possible link between the beneficial effects of dietary intake of *U. pertusa* on intestinal motility and mechanisms underlying the amelioration of inflammatory processes via the enhancement of SCFA generation. To verify the abovementioned hypothesis, we investigated any possible changes in the levels of NF- κ B and IL-1 β in response to the dietary intake of *U. pertusa*. An increased NF- κ B level was observed in the intestinal tissue of the constipation group (C, 1.635 ± 0.292 ng/mL) (Figure 5A). Intriguingly, *U. pertusa* treatment decreased the NF- κ B level (AL, 0.947 ± 0.165 ng/mL; AH, 0.939 ± 0.082 ng/mL; $p < 0.01$) compared with the control group. Furthermore, loperamide significantly increased the IL-1 β level (C, 602.16 ± 220.45 pg/mL) relative to that of the vehicle group (N, 142.20 ± 21.99 pg/mL; $p < 0.001$) (Figure 5B). The administration of *U. pertusa* at low and high concentrations substantially decreased the constipation-associated IL-1 β level (AL, 139.19 ± 23.65 pg/mL; AH, 162.01 ± 14.25 pg/mL; $p < 0.001$).

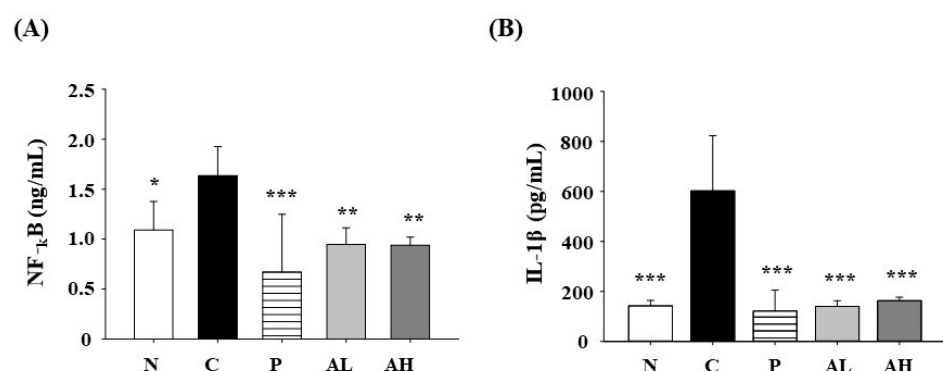


Figure 5. The effects of *U. pertusa* administration on the levels of inflammatory cytokines in intestinal tissues in loperamide-treated mice. (A,B) An assessment of the levels of the inflammatory cytokines NF- κ B (A) and IL-1 β (B) within the colon using enzyme-linked immunosorbent assay kits. Experiments were conducted in septuplicate. Results are presented as the mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group. Abbreviations: N, vehicle; C, control; P, positive control; AL, $100 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ *U. pertusa* extract; AH, $500 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ *U. pertusa* extract; NF- κ B, nuclear factor-kappa B; IL-1 β , interleukin-1 β .

As depicted in Figure 5, the NF- κ B and IL-1 β levels were reduced by *U. pertusa*. These findings indicate that *U. pertusa* improves bowel movement and intestinal motility by regulating NF- κ B and IL-1 β activation in mice with loperamide-induced constipation.

In summary, this study revealed that the dietary intake of *U. pertusa* improves bowel movement and intestinal motility. Therefore, *U. pertusa* can be utilized as a dietary supplement for maintaining normal bowel movement. These effects may be associated with SCFAs, which concurrently reduce the levels of proinflammatory cytokines NF- κ B and IL-1 β , but further study is needed. Future research is needed to elucidate the relationship between short-chain fatty acids and inflammatory factors, as well as to investigate the effects of *U. pertusa* extracts on these mechanisms.

4. Conclusions

The results obtained in this study reveal that the dietary intake of *U. pertusa* improves bowel movement and intestinal motility. These effects may be associated with SCFAs, which concurrently mitigate proinflammatory cytokines, namely NF- κ B and IL-1 β . However, the scope of this exploration remains preliminary, inviting future inquiries to unravel the intricate dynamics at play. Further studies are necessary to investigate the potential of *U. pertusa* dietary intake for elucidating the relationship between SCFAs and proinflammatory cytokines, specifically NF- κ B and IL-1 β .

Author Contributions: Conceptualization, methodology, and the writing of the original draft, E.-J.K. and W.-Y.C.; software and visualization, I.-Y.S.; data curation, Y.-K.R.; methodology and validation, W.-K.L. and T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Korea Institute of Marine Science & Technology Promotion (KIMST), and funded by the Ministry of Oceans and Fisheries (20220128), with research grants from the Korea Institute of Ocean Science and Technology (grant. No. PEA0211).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee of the Southeast Medi-Chem Institute (IACUC approval number SEMI-21-009 and approval date: 11 August 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors have no financial conflicts of interest to declare.

References

- Forootan, M.; Bagheri, N.; Darvishi, M. Chronic constipation: A review of literature. *Medicine* **2018**, *97*, e10631. [[CrossRef](#)] [[PubMed](#)]
- Schiller, L.R. Chronic constipation: New insights, better outcomes? *Lancet Gastroenterol. Hepatol.* **2019**, *4*, 873–882. [[CrossRef](#)] [[PubMed](#)]
- Belsey, J.; Greenfield, S.; Candy, D.; Geraint, M. Systematic review: Impact of constipation on quality of life in adults and children. *Aliment. Pharmacol. Ther.* **2010**, *31*, 938–949. [[CrossRef](#)]
- Wald, A.; Scarpignato, C.; Kamm, M.A.; Mueller-Lissner, S.; Helfrich, C.; Schuijt, C.; Bubeck, C.; Limoni, C.; Petrini, O. The burden of constipation on quality of life: Results of a multinational survey. *Aliment. Pharmacol. Ther.* **2007**, *26*, 227–236. [[CrossRef](#)]
- Tack, J.; Müller-Lissner, S.; Stanghellini, V.; Boeckxstaens, G.; Kamm, M.; Simren, M.; Galmiche, J.P.; Fried, M. Diagnosis and treatment of chronic constipation—A European perspective. *Neurogastroenterol. Motil.* **2011**, *23*, 697–710. [[CrossRef](#)]
- Wang, J.; Bai, X.; Peng, C.; Yu, Z.; Li, B.; Zhang, W.; Sun, Z.; Zhang, H. Fermented milk containing *Lactobacillus casei* Zhang and *Bifidobacterium animalis* ssp. lactis V9 alleviated constipation symptoms through regulation of intestinal microbiota, inflammation, and metabolic pathways. *J. Dairy Sci.* **2020**, *103*, 11025–11038. [[CrossRef](#)]
- Yang, L.; Wang, Y.; Zhang, Y.; Li, W.; Jiang, S.; Qian, D.; Duan, J. Gut microbiota: A new avenue to reveal pathological mechanisms of constipation. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 6899–6913. [[CrossRef](#)]
- Zhao, Q.; Chen, Y.-Y.; Xu, D.-Q.; Yue, S.-J.; Fu, R.-J.; Yang, J.; Xing, L.-M.; Tang, Y.-P. Action mode of gut motility, fluid and electrolyte transport in chronic constipation. *Front. Pharmacol.* **2021**, *12*, 630249. [[CrossRef](#)] [[PubMed](#)]
- Baker, D.E. Loperamide: A pharmacological review. *Rev. Gastroenterol. Disord.* **2007**, *7* (Suppl. 3), S11–S18. [[PubMed](#)]
- Katzung, B.G. *Basic and Clinical Pharmacology*, 9th ed.; McGraw-Hill Publishing Co.: New York, NY, USA, 2004.
- Hanauer, S.B. The role of loperamide in gastrointestinal disorders. *Rev. Gastroenterol. Disord.* **2008**, *8*, 15–20.
- Jaffe, J.H.; Kanzler, M.; Green, J. Abuse potential of loperamide. *Clin. Pharmacol. Ther.* **1980**, *28*, 812–819. [[CrossRef](#)] [[PubMed](#)]

13. Ahmad, M.H.; Zezi, A.U.; Anafi, S.B.; Alhassan, Z.; Mohammed, M.; Danraka, R.N. Mechanisms of anti-diarrhoeal activity of methanol leaf extract of *Combretum hypopilum* diels (combretaceae): Involvement of opioidergic and (α 1 and β)-adrenergic pathways. *J. Ethnopharmacol.* **2021**, *269*, 113750. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Kim, J.-E.; Lee, Y.-J.; Kwak, M.-H.; Jun, G.; Koh, E.-K.; Song, S.-H.; Seong, J.E.; Kim, J.W.; Kim, K.B.; Kim, S.; et al. Metabolomics approach to serum biomarker for loperamide-induced constipation in SD rats. *Lab. Anim. Res.* **2014**, *30*, 35–43. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Yao, Y.; Luo, R.; Xiong, S.; Zhang, C.; Zhang, Y. Protective effects of curcumin against rat intestinal inflammation-related motility disorders. *Mol. Med. Rep.* **2021**, *23*, 391. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Hajji, N.; Wannes, D.; Jabri, M.-A.; Rtibi, K.; Tounsi, H.; Abdellaoui, A.; Sebai, H. Purgative/laxative actions of *Globularia alypum* aqueous extract on gastrointestinal-physiological function and against loperamide-induced constipation coupled to oxidative stress and inflammation in rats. *Neurogastroenterol. Motil.* **2020**, *32*, e13858. [\[CrossRef\]](#)
17. Choi, J.-S.; Ha, Y.-M.; Lee, B.-B.; Moon, H.E.; Cho, K.K.; Cho, I.S. Seasonal variation of antibacterial activities in the green alga *Ulva pertusa* Kjellman. *J. Environ. Biol.* **2014**, *35*, 341–344.
18. Qi, H.; Liu, X.; Ma, J.; Zhang, Q.; Li, Z. In vitro antioxidant activity of acetylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). *J. Med. Plants Res.* **2010**, *4*, 2445–2451. [\[CrossRef\]](#)
19. Hong, J.-K.; Bong, M.-H.; Park, J.-C.; Moon, H.-K.; Kim, D.-W.; Lee, S.-C.; Lee, J.-H. Antioxidant and immunomodulatory effects of *Ulva pertusa* Kjellman on broiler chickens. *J. Anim. Sci. Technol.* **2011**, *53*, 419–428. [\[CrossRef\]](#)
20. Cornish, M.L.; Critchley, A.T.; Mouritsen, O.G. A role for dietary macroalgae in the amelioration of certain risk factors associated with cardiovascular disease. *Phycologia* **2015**, *54*, 649–666. [\[CrossRef\]](#)
21. Kim, J.-D.; Yoon, Y.-H.; Shin, T.-S.; Kim, M.-Y.; Byun, H.-S.; Oh, S.-J.; Seo, H.-J. Bioethanol production from seaweed *Ulva pertusa* for environmental application. *KSBB J.* **2011**, *26*, 317–322. [\[CrossRef\]](#)
22. Kang, J.H.; Jang, J.E.; Kim, J.H.; Byeon, S.Y.; Kim, S.; Choi, S.K.; Kang, Y.H.; Park, S.R.; Lee, H.J. Species Composition, Diversity, and Distribution of the Genus *Ulva* along the Coast of Jeju Island, Korea Based on Molecular Phylogenetic Analysis. *PLoS ONE* **2019**, *14*, e0219958. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Lee, K.-A.; Choi, W.Y.; Park, G.-H.; Jeong, Y.; Park, A.; Lee, Y.; Kang, D.-H. Study on marine pectin extraction and its antioxidant activities from 14 marine algae under different extraction solvents. *Korean Soc. Food Sci. Nutr.* **2020**, *49*, 677–685. [\[CrossRef\]](#)
24. Horwitz, W.; Latimer, G. *Official Methods of Analysis of AOAC International*, 17th ed.; Association of Analytical Chemists International: Gaithersburg, MD, USA, 2000.
25. Koh, E.J.; Shin, K.S.; Sunwoo, I.Y.; Kim, J.; Choi, W.Y. Brown seaweed byproduct extracts improve intestinal motility and auto-inflammation in mice with loperamide-induced constipation. *Foods* **2024**, *13*, 2037. [\[CrossRef\]](#)
26. Srivastava, P.; Malviya, R. Sources of Pectin, Extraction and its Applications in Pharmaceutical Industry—An Overview. *Indian J. Nat. Prod. Resour.* **2011**, *2*, 10–18.
27. Son, S.-U.; Suh, H.J.; Shin, K.-S. Characterization of a novel sulfated-rhamnogalacturonan isolated from Korean seaweed *Ulva pertusa* and its efficacy for treatment of inflammatory bowel disease in mice. *Carbohydr. Polym.* **2024**, *342*, 122373. [\[CrossRef\]](#)
28. Eggleston, W.; Palmer, R.; Dubé, P.A.; Thornton, S.; Stolbach, A.; Calello, D.P.; Marraffa, J.M. Loperamide toxicity: Recommendations for patient monitoring and management. *Clin Toxicol* **2020**, *58*, 355–359. [\[CrossRef\]](#)
29. Liu, B.; Zhang, Z.; Liu, X.; Hu, W.; Wu, W. Gastrointestinal Fermentable Polysaccharide Is Beneficial in Alleviating Loperamide-Induced Constipation in Mice. *Nutrients* **2023**, *15*, 4364. [\[CrossRef\]](#)
30. Zhang, X.; Zheng, J.; Jiang, N.; Sun, G.; Bao, X.; Kong, M.; Cheng, X.; Lin, A.; Liu, H. Modulation of gut microbiota and intestinal metabolites by lactulose improves loperamide-induced constipation in mice. *Eur. J. Pharm. Sci.* **2021**, *158*, 105676. [\[CrossRef\]](#)
31. Markowiak-Kopeć, P.; Śliżewska, K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients* **2020**, *12*, 1107. [\[CrossRef\]](#)
32. Russo, E.; Giudici, F.; Fiorindi, C.; Ficari, F.; Scaringi, S.; Amedei, A. Immunomodulating activity and therapeutic effects of short chain fatty acids and tryptophan post-biotics in inflammatory bowel disease. *Front. Immunol.* **2019**, *10*, 2754. [\[CrossRef\]](#)
33. Kaminsky, L.W.; Al-Sadi, R.; Ma, T.Y. IL-1 β and the intestinal epithelial tight junction barrier. *Front. Immunol.* **2021**, *12*, 767456. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Segain, J.P.; Raingeard de la Blétière, D.; Bourreille, A.; Leray, V.; Gervois, N.; Rosales, C.; Ferrier, L.; Bonnet, C.; Blottière, H.M.; Galmiche, J.P. Butyrate inhibits inflammatory responses through NF κ B inhibition: Implications for Crohn's disease. *Gut* **2000**, *47*, 397–403. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Mokhtare, M.; Alimoradzadeh, R.; Agah, S.; Mirmiranpour, H.; Khodabandehloo, N. The association between modulating inflammatory cytokines and constipation of geriatrics in Iran. *Middle East J. Dig. Dis.* **2017**, *9*, 228–234. [\[CrossRef\]](#)
36. Ferreira, A.C.F.M.; Eveloff, R.J.; Freire, M.; Santos, M.T.B.R. The impact of oral-gut inflammation in cerebral palsy. *Front. Immunol.* **2021**, *12*, 619262. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.