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Laxative effects of silk-shaped sea tangle (*Laminaria japonica*) on loperamide-induced constipation in rats.

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ABSTRACT

In the present study, we investigated the laxative effects of silk-shaped sea tangle (*Laminaria japonica*) in a rat model of loperamide-induced constipation. The loperamide-induced constipated rats were supplemented with 10 and 20 g/kg diet of the silk-shaped sea tangle or commercial sea tangle powder for 5 weeks during which the body weight, fecal properties, and gastrointestinal transit (GIT) ratio were evaluated. The silk-shaped sea tangle and commercial sea tangle significantly increased the fecal pellet number, wet weight of fecal pellets, and water content of feces in rats with loperamide-induced constipation. GIT ratio significantly increased after supplementation with sea tangle in loperamide-induced constipated rats. Especially, the GIT ratio of the constipated rats supplemented with 2% silk-shaped sea tangle was similar to the result of the normal group. These results indicated that silk-shaped sea tangle is a functional material that is derived from a natural food product and maybe effective in ameliorating constipation.
Keywords: Constipation, Laxative effects, Loperamide, Silk-shaped sea tangle (*Laminaria japonica*)

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INTRODUCTION

Constipation is a highly prevalent functional gastrointestinal disorder affecting 3-15% of the general population [1]. Constipation increases during aging and it can be a chronic condition requiring the use of laxatives over the long term [2]. In general, constipation is often induced by insufficient dietary fiber and fluid intake, decreased physical activity, hypothyroidism, several side effects of medication, and obstruction by colorectal cancer [3]. The use of chemical drugs such as correctol, senna, exlax, senokot, and gaviscon is commonly administered for the treatment of constipation, but these drugs are very limited due to their high cost and undesirable side-effects [4]. Thus, many researchers have focused on the development of new therapeutic agents for constipation without side effects and improved laxative effects.

Sea tangle (*Laminaria japonica*) is an edible brown seaweed and has long been utilized as an important food resource to promote maternal health in Asian countries. Sea tangle has recently attracted much attention due to its high contents of dietary fiber, minerals, carbohydrates, and protein, bioactive and nutraceutical materials [5]. It has been known that the major active compounds in sea tangle include sulfated polysaccharides such as fucoidans and polyphenols that possesses anti-oxidant, anti-inflammatory, antibacterial, anti-mutagenic, anti-coagulation, anti-proliferation, hypotensive and antiproliferative activities [6-8]. However, it has not been reported whether silk-shaped sea tangle is effective in preventing loperamide-induced constipation in animal model.

Loperamide is well known as an agonist of μ-opioid receptors. In the intestine, agonized μ-opioid receptors inhibit release of endogenous acetylcholine granules [9]. Loperamide inhibits colonic peristalsis and intestinal water secretion, which extends the fecal evacuation time and delays intestinal transit [10, 11]. In the present study, therefore, we investigated the laxative effects of silk-shaped sea tangle, using an animal model of loperamide-induced constipation.

MATERIALS AND METHODS

Materials

The silk-shaped sea tangle (*Laminaria japonica*) was supplied by Woorim F&B Co. (Goheung, Korea). Commercial sea tangle was purchased from local market (Wando, Korea). Loperamide was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All reagents used were of analytical grade.

Animals

Thirty six male Sprague Dawley rats (5 weeks old) were purchased from SamTacho (Osan, Korea). The animals were housed at an animal room with controlled temperature (23 ± 2 °C) and humidity level (55 ± 10 %) under a 12-h light/dark cycle. All rats were provided with standard irradiated chow diet (Purina Mills, Seoungnam, Korea) and tap water were provided *ad libitum*. All animals were treated in accordance with the Guidelines for Care and Use of Laboratory Animals of Chonnam National University, Yeosu, Republic of Korea.

Induction of constipation and experimental design

After one week of acclimation to pelleted commercial diet, the rats were randomly divided into six treatment groups, and fed *ad libitum* access to water and AIN-76A basal diets. One group (n = 6, Normal control) was fed AIN-76A basal diet and constipation was induced in the animals through experimental diet containing loperamide (0.3g/kg diet), respectively. Control group (n =6) were fed experimental diets containing loperamide to induce constipation and simultaneously, four groups (n =6, respectively) were fed a silk-shaped sea tangle powder (SST1; 10 g/kg diet and SST2; 20 g/kg diet) or commercial sea tangle powder (CST1; 10 g/kg diet and CST2; 20 g/kg diet) for 5 weeks. The composition of experimental diet was shown in Table 1. Weekly measurement of body weight and daily food intake monitoring were obtained throughout the experiment. Feed efficiency ratio (FER) was calculated by dividing the dietary intake amount during the same period of body weight gain during the treatment period.

Table 1: Composition of the experimental diet (g/kg diet)

	Groups[1)]					
	NOR	CON	SST1	SST2	CST1	CST2
Casein, lactic	200	200	200	200	200	200
DL-Methionine	3	3	3	3	3	3
Corn Starch	150	150	150	150	150	150
Sucrose	500	500	500	500	500	500
Cellulose	50	50	50	50	50	50
Corn Oil	50	50	50	50	50	50
Mineral Mix[2)]	35	35	35	35	35	35
Vitamin Mix[3)]	10	10	10	10	10	10
Choline Bitartrate	2	2	2	2	2	2
Loperamide		0.3	0.3	0.3	0.3	0.3
Silk-shaped sea tangle (SST)			10	20		
Commercial sea tangle (CST)					10	20

[1)] The experimental diet groups are as follow: NOR: normal diet group, CON: constipation group induced by loperamide, SST1: 1% of silk-shaped sea tangle diet and loperamide-treated group, SST2: 2% of silk-shaped sea tangle diet and loperamide-treated group, CST1: 1% of commercial sea tangle diet and loperamide-treated group, CST2: 2% of commercial sea tangle diet and loperamide-treated group.
[2)] Based on AIN-76A vitamin mixture.
[3)] Based on AIN-76A mineral mixture.

Number of fecal pellets, weight of fecal pellets, and fecal water content

Number of fecal pellets and wet weight of fecal pellets were measured every day during the experiment. The fecal water content was determined by drying the fecal pellets at 70°C for 24 hours in an oven and calculating the difference between the weight before drying and the weight after drying. The fecal water content was calculated as follows: Fecal water content (%) = [(fecal wet weight – fecal dry weight)/fecal wet weight]×100.

Gastrointestinal transit (GIT) ratio

GIT ratio was measured according to the method of Nakagura et al. [12], with minor modifications. Animals fasted for 18 h prior to the experiment, but consumed water *ad libitum*. On the 35th day, the rats were orally administered 1 ml of carmine (3 g suspended in 50 ml of 0.5% carboxymethylcellulose). One hour after administering the marker, the rats were sacrificed and then the small intestines were quickly removed. Heart, kidneys, liver and fat pad were rapidly removed and washed in saline buffer, collected into cryovials, weighed and immediately stored in liquid nitrogen. The distance over which the carmine had moved and the total length of the small intestine were measured. The GIT ratio was expressed as the percentage of the distance moved by the carmine relative to the total length of the small intestine.

Statistical analysis

All data are expressed as means ± SD. The data were evaluated by one-way analysis of variance. Differences between mean values were assessed using the Tukey-Kramer multiple comparison test. Differences were considered statistically significant when the P value was less than 0.05.

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RESULTS AND DISCUSSION

Body weight, food intake, and food efficiency

In general, food intake and water consumption are considered to be major factors for evaluation of constipation symptoms. However, loperamide-induced constipation models can have many different effects on food intake and water consumption [13]. In the present study, final body weight and body weight gain of the normal control group were significantly higher compared to those of the other groups after inducing constipation (Table 2). However, there were no significant differences in body weight and food intake among the experimental groups during the experiment period, although the control group showed a slightly lower food intake than the other groups. In a previous study, it was also reported that SD rats with constipation ate markedly less food than the non-constipation group, however, loperamide treatment did not induce any alteration of body weight and food intake [14]. In the present study, the food efficiency ratio among the experimental groups was not significantly different. However, the food efficiency ratio of the control, SST2 and CST2 supplemented groups were significantly increased after inducing constipation compared to that of the control group. Taken together, these results show that sea tangle consumption had weight lowering effects without any alteration of food intake.

Table 2: Effects of silk-shaped sea tangle on body weight gain, food intake and food efficiency ratio in loperamide-induced constipated rats

	Groups					
	NOR	CON	SST1	SST2	CST1	CST2
Initial body weight (g)	291.2±7.5^{NS}	287.0±9.4	289.4±11.2	286.2±11.5	290.4±13.2	292.4±9.7
Final body weight (g)	488.8±21.9^{a}	387.4±9.4^{b}	380.6±28.4^{b}	365.8±25.1^{b}	381.8±33.2^{b}	384.6±14.5^{b}
Body weight gain (g)	197.6±18.5^{a}	100.4±11.3^{b}	91.2±19.9^{b}	79.6±24.5^{b}	91.4±28.0^{b}	92.2±15.8^{b}
Food intake (g/day)	24.9±0.8^{a}	18.8±1.6^{b}	21.3±2.6^{ab}	22.7±1.8^{ab}	20.0±2.1^{ab}	22.6±1.8^{ab}
FER[1)]	0.23±0.06^{a}	0.15±0.02^{b}	0.19±0.02^{ab}	0.21±0.01^{a}	0.18±0.03^{ab}	0.22±0.04^{a}

NOR: normal diet group, CON: constipation group induced by loperamide, SST1: 1% of silk-shaped sea tangle diet and loperamide-treated group, SST2: 2% of silk-shaped sea tangle diet and loperamide-treated group, CST1: 1% of commercial sea tangle diet and loperamide-treated group, CST2: 2% of commercial sea tangle diet and loperamide-treated group. Values are means ± SD (*n* = 6). NS: not significantly different among groups. Values not sharing a common letter are significantly different at $P < 0.05$ by the Tukey-Kramer multiple comparison test.

[1)] FER: Food efficiency ratio = body weight gain (g)/food intake (g).

Effect of silk-shaped sea tangle on organ weight of constipated rats

After five weeks of silk-shaped sea tangle or commercial sea tangle supplemented to constipated rats, the organ weight including liver, kidney, heart and fat was not significant differences among the groups (Table 3). Similar to our results, blue-green algae supplementation did not affect to the body and liver weight in C57BL/6J mice [15].

Table 3: Effect of silk-shaped sea tangle on organ weight of constipated rats

	Groups					
	NOR	CON	SST1	SST2	CST1	CST2
Liver	10.4±1.4[NS]	9.9±1.1	9.6±1.3	9.8±0.8	9.5±1.7	9.6±0.5
Kidney	2.8±0.2[NS]	2.5±0.3	2.6±0.3	2.5±0.3	2.5±0.3	2.6±0.1
Heart	1.5±0.1[NS]	1.3±0.1	1.3±0.2	1.3±0.1	1.2±0.2	1.4±0.1
Total fat	25.9±5.8[NS]	34.2±5.4	21.1±6.0	21.6±5.2	22.3±9.6	23.4±8.2

NOR: normal diet group, CON: constipation group induced by loperamide, SST1: 1% of silk-shaped sea tangle diet and loperamide-treated group, SST2: 2% of silk-shaped sea tangle diet and loperamide-treated group, CST1: 1% of commercial sea tangle diet and loperamide-treated group, CST2: 2% of commercial sea tangle diet and loperamide-treated group. Values are means ± SD (*n* = 6). NS: not significantly different among groups.

Table 4: Effects of silk-shaped sea tangle on number of fecal pellet, wet weight of fecal pellets and fecal water content in loperamide-induced constipated rats

	Groups					
	NOR	CON	SST1	SST2	CST1	CST2
Number of fecal pellet (count/day)	33.7±5.2[a]	10.4±1.8[d]	19.2±3.5[c]	25.4±4.3[b]	21.8±3.0[c]	25.5±4.0[b]
Wet weight of fecal pellet (g/day)	3.6±0.7[a]	1.7±0.4[c]	2.7±0.6[b]	3.5±0.7[a]	2.8±0.6[b]	2.9±0.6[b]
Fecal water content (%)	40.7±6.8[a]	22.5±4.1[c]	31.2±6.2[b]	37.9±6.0[a]	34.2±7.1[b]	36.2±6.0[a]

NOR: normal diet group, CON: constipation group induced by loperamide, SST1: 1% of silk-shaped sea tangle diet and loperamide-treated group, SST2: 2% of silk-shaped sea tangle diet and loperamide-treated group, CST1: 1% of commercial sea tangle diet and loperamide-treated group, CST2: 2% of commercial sea tangle diet and loperamide-treated group. Values are means ± SD (*n* = 6). NS: not significantly different among groups. Values not sharing a common letter are significantly different at $P < 0.05$ by the Tukey-Kramer multiple comparison test.

Effects of silk-shaped sea tangle on fecal parameters in the rat model of loperamide-induced constipation

To examine the effects of slk-shaped sea tangle on number of fecal pellets, wet weight of fecal pellets, and fecal water content, constipation was induced in the rats by maintaining them on the loperamide containing diet with silk-shaped sea tangle or commercial sea tangle for 5 weeks. The number of fecal pellets, wet weight of fecal pellets, and fecal water content are shown in Table 4. Fecal pellet number in the normal group (33.7 count/day) was significantly different from that of the control group (10.4 count/day), suggesting the induction of loperamide-induced constipation. After inducing constipation, fecal pellet number in the SST and CST groups was significantly increased compared to that of the control group. Similarly, wet weight of fecal pellets in the control group was markedly decreased than the normal control group after inducing constipation. On the other hand, wet weight of fecal pellets were significantly increased compared to that of the control group (1.7 g/day) after inducing constipation by supplementation with silk-shaped sea tangle and commercial sea tangle in a dose dependent manner. Especially, supplementation of 2% silk-shaped sea tangle (SST2) was observed to increase wet weight of fecal pellets by normal range (3.5 g/day in SST2 vs. 3.6 g/day in NOR). In a previous study, it has been reported that both the total number and dry weight of fecal pellets were significantly decreased in the morphine-induced constipated rats, however, these were markedly increased by apple fiber supplementation [16]. In the present study, fecal water content of normal control group was significantly different from that of the control group. In addition, the fecal water content markedly increased in the SST and CST groups in a dose dependent manner after inducing constipation. It has been also reported that the fecal water content was increased by dietary fiber which has a water-holding effects [17]. Moreover, it has been reported that dietary fiber improved bowel movement and swelled feces by its water-holding effect [18]. Wintola et al. [19] reported that the supplementation of aqueous extract of *Aloe ferox* to the constipated rats was effective in influencing increased fecal volume and motility of the colon. These findings indicated that the preventive effect on constipation induced by loperamide was due to the water-holding effect of silk-shaped sea tangle enriched in dietary fiber.

Effect of silk-shaped sea tangle on gastrointestinal transit ratio in loperamide-induced constipated rats

In this study, we used carmine as the marker to measure the gastrointestinal transit ratio. It has been reported that transit through the gastrointestinal tract reflects the overall gastrointestinal motor activity, and measurement of the gastrointestinal transit ratio is useful for the diagnosis of constipation [13]. A decrease in the gastrointestinal charcoal transit ratio is indicative of constipation [20]. In the present study, loperamide treatment significantly reduced gastrointestinal motility in the untreated constipated rats as shown in Table 5. On the other hand, the supplementation with the sea tangle powder significantly increased the gastrointestinal motility in a dose dependent manner which compared to that of constipated rats. Especially, the GIT ratio of the constipated rats were normalized following the supplementation with 2% silk-shaped sea tangle. Lee et al. [21] reported that loperamide administration decreased food intake and gut transit time, whereas dietary mulberry leaf supplementation increased gut transit time and transit speed in rats. In addition, Oh & Lim [22] reported that sea tangle showed useful roles as treatment for chronic idiopathetic constipation. These results showed that silk-shaped sea tangle is useful and functional material that is derived from a marine food sources and is effective in ameliorating constipation. However, the present study was limited in that it used only one type of animal model for constipation. Therefore, more studies are necessary to clarify the laxative effects of silk-shaped sea tangle to improve human health.

Table 5: Gastrointestinal transit ratio following supplementation with silk-shaped sea tangle in loperamide-induced constipated rats

	Gastrointestinal motility (during 2 h)		
	Total small intestine length (cm)	Transit distance (cm)	Gastrointestinal transit ratio (%)
NOR	131.1±4.7^{NS}	126.0±4.9^{a}	96.1±0.8^{a}
CON	132.5±5.8	87.6±3.5^{c}	64.6±3.6^{c}
WST1	134.0±3.7	115.0±4.3^{ab}	85.9±4.5^{b}
WST2	133.1±5.7	122.0±6.8^{ab}	93.2±3.5^{a}
ST1	133.9±8.6	111.8±8.8^{b}	83.5±4.0^{b}
ST2	133.2±3.1	113.0±6.3^{b}	87.9±4.5^{b}

NOR: normal diet group, CON: constipation group induced by loperamide, SST1: 1% of silk-shaped sea tangle diet and loperamide-treated group, SST2: 2% of silk-shaped sea tangle diet and loperamide-treated group, CST1: 1% of commercial sea tangle diet and loperamide-treated group, CST2: 2% of commercial sea tangle diet and loperamide-treated group. Values are means ± SD (*n* = 6). NS: not significantly different among groups.

Values not sharing a common letter are significantly different at $P < 0.05$ by the Tukey-Kramer multiple comparison test.

CONCLUSIONS

In conclusion, silk-shaped sea tangle (*Laminaria japonica*) supplementation showed a laxative effect on rats with loperamide-induced constipation. This study demonstrated that silk-shaped sea tangle enhanced gastrointestinal transit and alleviated constipation in loperamide-treated rats, with greater fecal excretion and fecal water content. In addition, silk-shaped sea tangle could be recommended as a cost-effective alternative for alleviation of constipation due to its easy availability and no side effects.

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