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Anti-Oxidant And Laxative Effects Of Taurine-Galactose On Loperamide-Induced Constipation In Rats.

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ABSTRACT

Constipation is one of the most common functional gastrointestinal disorders caused by improper water intake, fiber deficiency, metabolic imbalances, and drugs. The objective of the present study was to investigate *in vitro* anti-oxidant activities and laxative effects of Taurine-Galactose (T-G) on loperamide-induced constipation in Sprague Dawley rats. Animals were divided into three groups: normal (NOR), loperamide treated control (CON), and loperamide with T-G (15 mg/kg). The laxative activity was determined based on feeding characteristics, body weight, fecal properties, gastrointestinal transit (GIT) ratio, and serum metabolic parameters. T-G showed potent reducing power and free radical scavenging activities against DPPH (1,1-diphenyl-2-picrylhydrazyl) and $ABTS^+$ (2,2'-azino-bis (3-ethylbenzothiazoline 6-sulfonic acid ammonium salt)) radicals. T-G supplementation markedly increased the number, weight, and water content of fecal pellets in constipation rats treated by loperamide. GIT ratio and serum metabolic parameters including gastrin (GAS), motilin (MTL), and somatostatin (SS) were significantly improved by T-G supplementation. These findings indicate that T-G has potent *in vitro* antioxidant activities against various free radicals with *in vivo* laxative effects against loperamide-induced constipation by increasing gastrointestinal motility.

Keywords: Taurine-galactose, Anti-oxidant effects, Constipation, Loperamide, Laxative effects

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INTRODUCTION

Constipation results from infrequent stools, difficult stool passage, or both [1]. It is often caused by inappropriate water intake, fiber deficiency, metabolic imbalance, and drugs [2]. Constipation symptoms include rare bowel movements, reduction in the amount of stool, dyspnea, and dry stools. Constipation is a symptom that affects about 5 ~ 20% of the whole population, although it varies somewhat according to the standard. It has an increasing trend with westernized diet habit of the modern society [3, 4]. In general, loperamide is widely used as a drug to induce constipation [5]. However, inhibition of intestinal secretion and peristalsis induced by loperamide are accompanied by increased MDA and H_2O_2 levels, decreased sulfhydryl groups, and reduced glutathione and intracolonic injuries with deleterious effects on activities of colic antioxidant enzymes [6]. Currently, drugs with constipation-improving effects are available in the market. They have been widely used. However, therapeutic drugs can cause side effects such as abdominal distension and diarrhea [7]. Therefore, it is necessary to actively search for food or natural materials that can effectively prevent and treat constipation. Taurine is affluent in blood cells, the brain, skeleton, and cardiac muscles [8]. In addition, it is known to play important roles in neuronal control, cell membrane stabilization, detoxification, and osmotic pressure control. It also possesses antioxidant activity and protection against diabetic complications, atherosclerosis, and gastrointestinal damage [8-10]. The objective of the present study was to investigate antioxidant and laxative effects of Taurine-Galactose (T-G) in constipated rats induced by loperamide.

MATERIALS AND METHODS

Materials

Loperamide hydrochloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline 6-sulfonic acid ammonium salt (ABTS), potassium persulfate, sodium phosphate buffer, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, and carmine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All reagents used were of analytical grade.

DPPH radical scavenging assay

DPPH assay was performed according to the method of Brand-Williams et al. [11] with some modifications. Briefly, 100 µL of Taurine-Galactose (3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 mg/mL) was added to 200 µL of 0.2 mM DPPH solution dissolved in methanol. Samples were incubated at room temperature for 30 min in the dark. The absorbance was then measured at wavelength of 517 nm using a microplate reader (Multiskan FC 357, Thermo Scientific, China).

ABTS radical scavenging assay

ABTS assay was determined according to the method of Re et al. [12] with some modifications. Briefly, ABTS radical cation was prepared by mixing 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution in equal quantities. The mixture was allowed to react at room temperature for 24 h in the dark before use. $ABTS^+$ solution was diluted with deionized water to obtain an absorbance of 0.7 ± 0.02 at 734 nm. After 50 µL of Taurine-Galactose (3.125, 6.25, 12.5, 25 and 50 mg/mL) was added to 950 µL of the diluted $ABTS^+$ solution, samples were incubated at room temperature for 30 min in the dark and the absorbance was then measured at 734 nm using a microplate reader.

Measurement of reducing power

Reducing power assay was determined according to the method of Oyaizu [13] with some modifications. Briefly, 100 µL of Taurine-Galactose (3.125, 6.25, 12.5, 25, and 50 mg/mL) was added to 0.2 M sodium phosphate buffer (pH6.6) and 10% potassium ferricyanide in equal quantities with sample. The mixture was allowed to react at 50°C for 20 min. The resulting solution was mixed with 100 µL of 10% trichloroacetic acid and centrifuged at 1,000 g for 10 min. Then 200 µL of the supernatant was mixed with 40 µL of 0.1% ferric chloride and the absorbance was measured at 700 nm using a microplate reader.

Animals

Twenty-four male Sprague-Dawley rats (5 weeks old) were purchased from Samtacho (Osan, Korea) and individually housed in a temperature-controlled room (23 ± 2°C) with humidity of 55 ± 10% under 12 h light/dark cycle. Animals were provided a standard irradiated chow diet (Purina Mills, Seongnam, Korea) and water *ad libitum* to stabilize their metabolic condition for one week. All animals were handled following Guidelines for Care and Use of Laboratory Animals of Chonnam National University.

Induction of constipation in rats

After one week of adaptation, rats were divided into four groups (n = 6 per group) at random. They were provided AIN-76A basal diets and water *ad libitum*. Constipation in rats was induced by oral administration with 1 mL loperamide (4 mg/kg) suspended in 0.9% NaCl (sodium chloride) twice daily at 9 am and 6 pm for 2 weeks. The NOR group was administered normal saline only. Taurine-Galactose (15 mg/kg) was administered to rats by daily oral administration at 10:00 am during the experimental period. A comparison group was supplied with Dulcolax S (5.5 mg/kg) as a standard medicine. Daily feed intake, water intake, and body weight gain of all rats were measured. Treatment continued for 2 weeks. Feed efficiency ratio (FER) was calculated during the experimental period by dividing the dietary intake amount by body weight gain.

Number, weight, and water content of fecal pellets

Number and weight of fecal pellets of individual rats were measured daily at 9:00 am during the experiment period. Fecal pellets were dried at 70°C for 24 h to determine water content. Water content of fecal pellets was calculated with the following equation: fecal water content (%) = [(fecal wet weight – fecal dry weight)/fecal wet weight] × 100.

Gastrointestinal transit (GIT) ratio

Gastrointestinal transit ratio was determined according to the method of Nakagura et al. [14] with some modifications. Briefly, on the 15th day, 1 mL of carmine (3g suspended in 50 mL of 0.5% carboxymethylcellulose) was orally administered to rats as a marker. After half an hour, animals were sacrificed and their small intestines were quickly removed. We measured the distance traveled by carmine and the total length of the small intestine to calculate the GIT ratio. GIT ratio was calculated as the percentage of the distance traveled by carmine compared to the total length of the small intestine.

Assessment of serum levels of GAS, MTL, SS, and CGRP

Serum levels of GAS (gastrin), MTL (motilin), SS (somatostatin), and CGRP (calcitonin gene related peptide) were valued by ELISA using commercially available kits.

Statistical analysis

All data are expressed as mean ± SD. All statistical analyses were performed using IBM SPSS Statistics (Chicago, IL, USA). Multiple group comparisons were conducted by one-way analysis of variance followed by Tukey-Kramer multiple range test to determine significant differences in all parameters. Statistically significant differences were considered at $P < 0.05$.

RESULTS

Anti-oxidant activities of Taurine-Galactose

We investigated antioxidant activities of Taurine-Galactose, including DPPH, ABTS, and reducing power. Results of these antioxidant activities are shown in Figure 1. With increasing concentration of Taurine-Galactose, free radical scavenging activities against DPPH and ABTS tended to be increased. Its IC_{50} values against DPPH and ABTS were 305.84 ± 0.69 mg/mL and 19.15 ± 0.48 mg/mL, respectively. Absorbance value of reducing power tended to increase with increasing concentration of Taurine-Galactose. These results demonstrate that Taurine-Galactose has concentration-dependent antioxidant effect.

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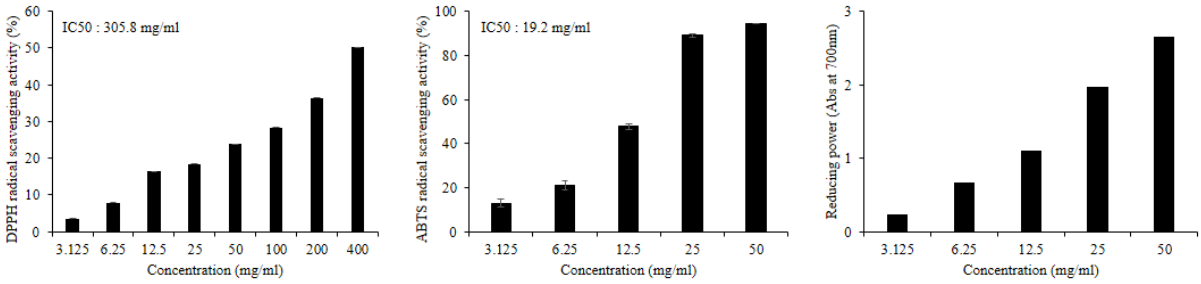

Figure 1: Antioxidant activities of Taurine-Galactose at different concentrations. Values not sharing a common letter are significantly different at P < 0.05 by Tukey-Kramer multiple comparison test.

Body weight, food intake, and food efficiency

After constipation was induced by loperamide, final body weight and weight gain were significantly higher in the control group than those in other groups (Table 1). Although the Control group showed lower food intake than the T-G group, final body weight or food intake did not differ significantly between the Control group and the T-G group during the experiment period. FER was not significantly different either between experiment groups. These results show that Taurine-Galactose supply does not alter food intake or FER.

Table 1: Effects of Taurine-Galactose on body weight gain, food intake, and food efficiency ratio in loperamide-induced constipated rats

	Groups			
	NOR	CON	T-G	DS
Initial body weight (g)	78.0±1.7^{NS}	80.7±2.1	76.0±1.4	76.7±1.2
Final body weight (g)	216.3±4.2^{a}	191.7±14.5^{b}	175.3±7.6^{b}	183.7±9.3^{b}
Body weight gain (g)	137.0±3.6^{a}	111.0±13.9^{b}	99.3±8.9^{b}	107.0±8.9^{b}
Food intake (g/day)	18.6±0.5^{a}	14.9±0.8^{b}	15.6±0.5^{b}	15.0±1.2^{b}
FER[1)]	7.44±0.11^{NS}	7.51±1.24	6.39±0.69	7.12±0.18

NOR: normal diet group; CON: loperamide-induced constipation group (4 mg/kg, p.o.); T-G: taurine-galactose (15 mg/kg, p.o.) and loperamide-treated group; DS: Dulcolax S (5.5 mg/kg, p.o.) 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 Tukey-Kramer multiple comparison test. [1)]FER: food efficiency ratio = body weight gain (g)/food intake (g).

Table 2: Effect of Taurine-Galactose on organ weight of constipated rats

	Groups			
	NOR	CON	T-G	DS
Liver	8.72±0.14^{NS}	7.77±1.33	7.29±1.98	7.46±1.09
Kidney	2.03±0.19^{NS}	2.00±0.18	1.74±0.23	1.87±0.18
Heart	0.98±0.08^{NS}	0.98±0.08	0.85±0.09	0.89±0.03

NOR: normal diet group; CON: constipation group induced by loperamide (4 mg/kg, p.o.); T-G: taurine-galactose (15 mg/kg, p.o.) and loperamide-treated group; DS: Dulcolax S (5.5 mg/kg, p.o.) and loperamide-treated group. Values are means ± SD (n = 6). NS: not significantly different among groups.

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Table 3: Effects of Taurine-Galactose on number, weight, and water content of fecal pellets in loperamide-induced constipated rats

	Groups			
	NOR	CON	T-G	DS
Number of fecal pellets (count/day)	22.9±0.3^{a}	18.1±0.6^{c}	21.0±0.5^{b}	20.1±0.9^{b}
Weight of fecal pellet (g/day)	1.31±0.08^{a}	0.99±0.06^{b}	1.25±0.08^{a}	1.11±0.07^{ab}
Water content of fecal pellet (%)	18.8±0.8^{a}	14.1±0.5^{b}	21.4±2.2^{a}	18.1±1.6^{a}

NOR: normal diet group; CON: constipation group induced by loperamide (4 mg/kg, p.o.); T-G: taurine-galactose (15 mg/kg, p.o.) and loperamide-treated group; DS: Dulcolax S (5.5 mg/kg, p.o.) and loperamide-treated group. Values are means ± SD (n = 6). Values not sharing a common letter are significantly different at $P < 0.05$ by Tukey-Kramer multiple comparison test.

Table 4: Gastrointestinal transit ratio following supplementation with taurine in loperamide-induced constipated rats

	Gastrointestinal motility (during 2 h)		
	Total small intestine length (cm)	Transit distance (cm)	Gastrointestinal transit ratio (%)
NOR	121.3±5.1^{NS}	90.7±5.0^{a}	74.9±6.2^{a}
CON	118.3±5.1	73.0±1.0^{b}	61.8±2.2^{b}
T-G	124.2±7.0	96.6±9.0^{a}	77.7±4.6^{a}
DS	120.7±4.0	85.8±2.4^{ab}	71.2±4.4^{ab}

NOR: normal diet group; CON: constipation group induced by loperamide (4 mg/kg, p.o.); T-G: taurine-galactose (15 mg/kg, p.o.) and loperamide-treated group; DS: Dulcolax S (5.5 mg/kg, p.o.) 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 Tukey-Kramer multiple comparison test.

Effect of Taurine-Galactose on organ weights of constipated rats

Constipated rats were treated with Dulcolax as a standard drug. Organ weight of liver, kidney, or heart was not significantly different between groups (Table 2).

Effects of Taurine-Galactose on fecal parameters in loperamide-induced constipated rats

Effects of Taurine-Galactose on the number, weight, and water content of fecal pellets in loperamide-induced constipated rats are shown in Table 3. The number of fecal pellets in the control (CON) group was significantly lower than that in the normal (NOR) group, suggesting the occurrence of loperamide-induced constipation. In addition, weight and water content of fecal pellets in the CON group were significantly lower than those in the NOR group. After induction of constipation, the fecal pellet count, weight, and water content of Taurine-Galactose (T-G) group and Dulcolax S (DS) group tended to be higher than those in the CON group. Especially, T-G groups showed higher water content of fecal pellets than the NOR group.

Effect of Taurine-Galactose on GIT ratio in loperamide-induced constipated rats

As shown in Table 4, the CON group had markedly decreased gastrointestinal motility compared to other groups. On the contrary, T-G group and DS group tended to show increased transit distance and GIT ratio. Especially, T-G group had higher transit distance and GIT ratio than the NOR group. These results indicate that Taurine-Galactose is a competent substance in improving constipation.

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Effect of Taurine-Galactose on colonic motility index in loperamide-induced constipated rats

In the present study, we investigated the effect of Taurine-Galactose on serum levels of gastrointestinal hormones such as MTL, GAS, CGRP, and SS. It has been known that digestive juice secretion, movement of gastrointestinal contents, and gastrointestinal motility are stimulated by MTL and GAS, but inhibited by CGRP and SS [15]. As shown in Fig. 2, serum levels of MTL and GAS in the CON group were significantly lower than those in the NOR group. Serum levels of MTL and GAS in the T-G group were also significantly higher than those in the CON group. Serum levels of SS in the CON group were significantly higher than those in the NOR group and T-G group. Although serum levels of CGRP in the CON group were higher than those in the NOR group, T-G did not affect serum concentration of CGRP in loperamide-induced constipated rats.

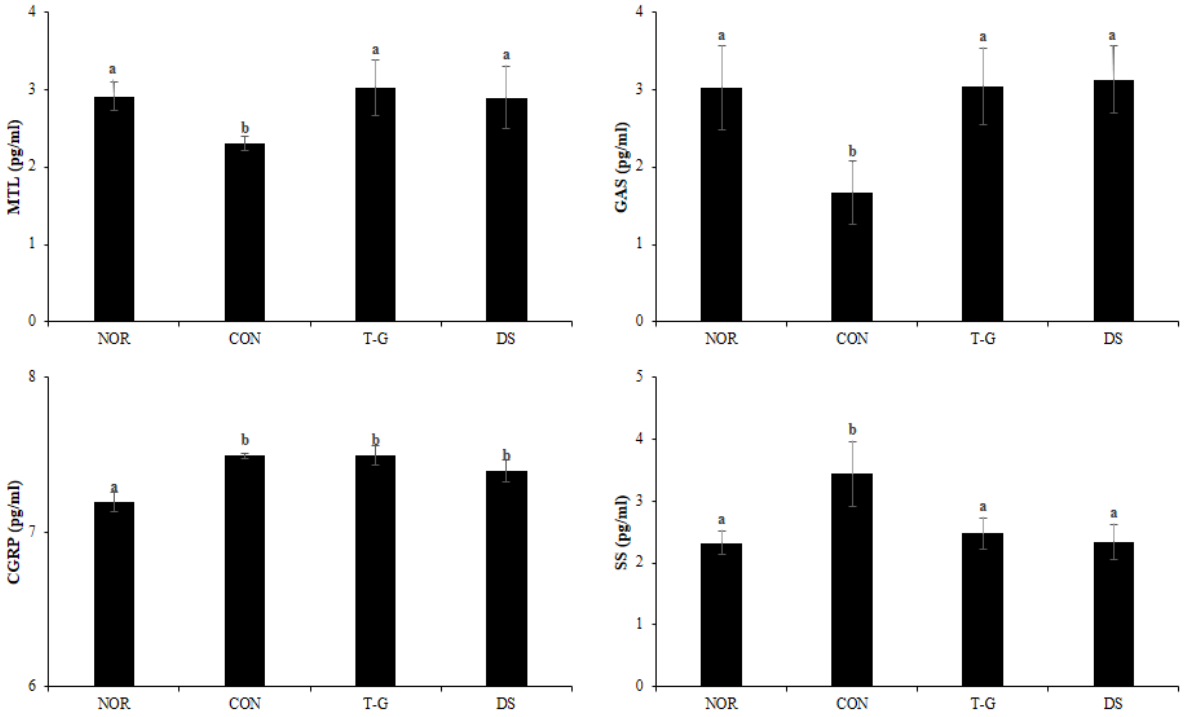

Figure 2: Colonic motility index function following Taurine-Galactose in loperamide-induced constipated rats. NOR: normal diet group; CON: constipation group induced by loperamide (4 mg/kg, p.o.); T-G: taurine-galactose (15 mg/kg, p.o.) and loperamide-treated group; DS: Dulcolax S (5.5 mg/kg, p.o.) and loperamide-treated group. Values not sharing a common letter are significantly different at P < 0.05 by Tukey-Kramer multiple comparison test. Concentrations of gastrin (GAS), motilin (MTL), somatostatin (SS), and calcitonin gene related peptide (CGRP) in the serum were estimated by ELISA using commercially available kits.

DISCUSSION

Loperamide is known to cause intestinal obstruction, intestinal absorption, or secretion leading to oxidative stress in the intestine [16, 17]. In addition, free radicals produced by cellular metabolism are known to be toxic to biological tissues. They can damage DNA, lipid, cell membrane, and protein [18]. In general, reactive oxygen species cause membrane lipid peroxidation, resulting in cellular damage. They can also cause gastrointestinal disorders [19]. Therefore, we studied antioxidant activities of Taurine-Galactose such as DPPH, ABTS, and reducing power. Antioxidant activities were stronger when concentration of Taurine-Galactose was increased, although its antioxidant activity against DPPH did not show strong ability.

As a chronic gastrointestinal disorder, constipation is affecting as high as 20% of adults [3, 20]. Loperamide is mostly used to induce constipation in animal model. It can slow down movement of the stool and prolong stool drainage period [5]. Current constipation management strategies require a phased approach to reduce symptoms by using laxatives. Therefore, we studied the laxative effect of Taurine-Galactose on

loperamide-induced constipated rats. In this study, final body weight and weight gain of the CON group were significantly lower than those of the NOR group. However, during the experimental period, final body weight, weight gain, food intake, and FER were similar among experimental groups. This indicates that loperamide, Taurine-Galactose, or Dulcolax S does not affect organ weight, food intake, or FER.

In constipation, the number of fecal pellets has been used as an indicator of laxative effects [21-23]. In the present study, the number, weight, and water content of fecal pellets in the CON group were significantly lower than those in the NOR group. On the contrary, the number, weight, and water content of fecal pellets were significantly elevated by Taurine-Galactose supplementation. These results indicate that Taurine-Galactose can improve loperamide-induced constipation rats.

In this study, carmine was used as a marker to estimate gastrointestinal transit ratio in constipated rats induced by loperamide. It has been known that passing through the gastrointestinal tract reflects the overall gastrointestinal activity. Therefore, measuring gastrointestinal transit ratio is useful for diagnosing constipation [23]. The CON group showed significant decreases in transit distance and gastrointestinal transit ratio compared to the NOR group. On the contrary, the T-G group showed significant increases of transit distance and gastrointestinal transit ratio than the NOR group.

Results of this study showed that serum levels of gastrin and motilin were significantly increased by Taurine-Galactose. Thus, Taurine-Galactose can enhance colonic motility. Serum concentration of somatostatin in the CON group was significantly higher than that in other groups. However, T-G did not affect serum concentration of CGRP in loperamide-induced constipated rats. These results indicate that Taurine-Galactose can be effective and useful in the treatment of constipation. However, more studies are needed to ensure the laxative effect of Taurine-Galactose.

CONCLUSIONS

In conclusion, Taurine-Galactose has antioxidant activities. In addition, constipation induced by loperamide in rats could be alleviated by Taurine-Galactose. Taurine-Galactose treatment improved several fecal parameters, including the number, weight, and water content of fecal pellets and gastrointestinal transit ratio. It also improved serum levels of GAS, MTL and SS. Taken together, our results suggest that Taurine-Galactose can alleviate symptoms of constipation induced by loperamide.

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