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Effect of *Malus halliana* Koehne Polysaccharides on Functional Constipation

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Abstract: The effects of *Malus halliana* Koehne polysaccharides on functional constipation was investigated in this study. The rats were divided into six groups: normal group, model group, positive control group, *M. halliana* polysaccharides high dose groups 1200 mg/kg, medium dose groups 1000 mg/kg and low dose groups 800 mg/kg. The model of constipation was established by loperamide hydrochloride. Feces weight at 6 and 24 hours after treatment, Colon moisture content, in addition the levels of motilin (MTL), gastrin (Gas), somatostatin (SS), substance P (SP) in serum were used to evaluate the preventive effects of *M. halliana* polysaccharides on constipation. Compared with the model group, the positive control group, *M. halliana* polysaccharide high, medium and low dose group 6 h weight of feces, colon moisture content, the levels of motilin (MTL), gastrin (GAS) and substance P(SP) significantly ($p < 0.01$) increased, the levels of somatostatin (SS) significantly decreased. The results indicated that the high, middle and low dosage of *M. halliana* polysaccharide could effectively improve functional constipation. Amongst these doses, the low dose group was better than others.

Keywords: Functional constipation; Loperamide hydrochloride; *Malus halliana* Polysaccharides.

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Figure1: *Malus halliana* Koehne.

1 Introduction

Constipation is a common gastrointestinal disorder, which is a more complex pathogenesis, based on mainly gastrointestinal disturbance. The clinical manifestations of constipation are defecation difficulties, prolonged defecation time and so on. The classification of organic disease constipation can be divided into organic and functional [1]. Constipation has a tremendous impact on the life quality of patients, and even seriously threatens the lives of patients. The incidence of constipation in China is 6% and the overall prevalence is higher in females than in males [2-4].

Polysaccharides, as informative molecules, have been found to play an important role in growth and development, cell recognition, antiviral, anticoagulant, immune regulation and the maintenance of immune system balance [5-8]. Previous studies show that polysaccharides have some therapeutic effect on constipation such as Long C.X. et al. [9] found that *Dendrobium candidum* polysaccharide

could enhance the body immunity, regulate the balance of intestinal microbiota and intestinal enzyme activity, optimize the intestinal environment and improve the symptoms of spleen constipation. Luo D.D. et al. [10] studies show that the polysaccharide of *D. officinalis* has the laxative activity to constipated mice. Li H. et al. [11] used *Semen plantaginis* crude polysaccharides capsules to treat 93 cases of constipation patients, the results showed that *S. plantaginis* crude polysaccharides capsules could improve constipation. *S. plantaginis* polysaccharides and garlic polysaccharides extracts had laxative effect [12,13].

Malus halliana Koehne (Figure 1), belonging to the Rosaceae family, is distributed in Shanxi, Jiangsu, Anhui and Zhejiang Province in China [14]. *M. halliana*, taste slightly bitter, astringent, ping, belonging to the liver meridian. *M. halliana* has been used as a traditional Chinese medicine to treat metrorrhagia [15]. Pharmacological studies showed that *M. halliana* had the ability of protect liver, antioxidant, inhibition of α -glycosidase enzymes *in vitro* and antithrombotic effect *in vivo* [16-18]. Chemical composition research of *M. halliana* focused on flavonoids [19,20]. No studies on biological activity of polysaccharides have been reported. As a part of our continuous research for *M. halliana* flowers. In this study, the *M. halliana* flower's polysaccharides were extracted and investigated on functional constipation.

2 Materials and Methods

2.1 Animals

Male Sprague-Dawley/specific pathogen free (SD/SPF) rats weighting 200-220 g were purchased from Henan Animal Experiment Center (with License key of SCXK 2015-0004) (Zhengzhou, Henan, China). All animal studies are conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2 Chemicals and Plant Materials

Loperamide hydrochloride (batch number: 170421860) was obtained from Xian Janssen Pharmaceutical Ltd. (Xian, Shanxi, China). Maren pill (batch number: 20170203) was obtained from Wuhan Tai Fu Pharmaceutical Co., Ltd. (Wuhan, Hubei, China). Rat Motilin (MTL), Rat Gastrin (Gas), Rat Substance P (SP), Rat Somatostatin (SS), (Batch

numbers: 201709) were obtained from Senbeijia Nanjing Biotechnology Co., Ltd. (Nanjing, Jiangsu, China).

M. halliana flowers were collected in the Jinming campus of Henan University (Kaifeng, China) in March, 2017 (The voucher Number: 2017032601) and identified by Professor Changqin Li (Joint International Research Laboratory of Food & Medicine Resource Function, Henan Province). A voucher specimen was deposited in Joint International Research Laboratory of Food & Medicine Resource Function, Henan Province.

2.3 Extraction and isolation of polysaccharides

M. halliana flowers (960 g) were extracted by petroleum ether under 45°C three times for 2 h each time and the residue was extracted three times with 70% ethanol for 24 h each time. The solvents were filtered for 3 h each time, and the residue was extracted three times with distilled water of 1:24 (w/v) at 90°C. Then, the filtrate was filtered and concentrated under reduced pressure at 50°C, the precipitated with 95% ethanol (the final concentration was 80%) for 24 h. The precipitate was drip washed with 100% ethanol, acetone, petroleum ether each leaching 3 times, then re-dissolved in distilled water and concentrated under reduced pressure at 50°C. Finally, the polysaccharides solution was dehydrated by freeze-dried to obtain the crude polysaccharides.

2.4 Acute toxicity test

The results of the maximum dose test of *M. halliana* polysaccharides showed no behavioral abnormalities, apathy, convulsions, no change in skin and respiration, after the rats were observed for 3 days. The rat was dissected and its heart, liver and spleen were excised and no change was observed with the naked eye. So, *M. halliana* polysaccharides did not cause visible toxicity.

2.5 Experimental Design

2.5.1 Establishment of Constipation Model with loperamide hydrochloride

Forty eight SD rats were kept for a week (Room temperature 25±2°C, light for 12 h/d, relative humidity 40 ~ 45%). According to body weight, all rats were randomly divided into six groups each consisting of 8 animals: normal

group, model group, positive control group, *M. halliana* polysaccharides high dose group, medium dose group and low dose group, respectively. Three days before the model establishment, each group of rats were collected 24 h-feces, weighed wet weight, dried 8 h at 50°C. Wet and dry granulation rat weight was recorded before model establishment.

The normal group was orally administered with normal saline daily, the other groups were given intragastric administration with loperamide hydrochloride (3 mg / kg·d·2 mL) according to weight.

When the rats showed poor mental status, eating less, weight loss, stool grain size, dry and hard performance, the success of modeling for 14 d.

During the establishment of the rat constipation model, the rats were collected regularly for 24 h. The wet weight of the pellets was weighed, placed in an electric oven, dried at 50°C for 8 h, and the pellets were weighed. The rats were weighed one day apart and the data was recorded. Rats shrimp shape and rat activity in each group was observed.

2.5.2 Treatment with *M. halliana* polysaccharides

The rats in the normal group and the model group received 1 mL/100 g normal saline daily. Rats in the positive control group received Maren pill 0.6 g / kg, 1 mL / 100 g for gavage. Rats in the *M. halliana* polysaccharides high dose group, medium dose group and low dose group received 1200, 1000 and 800 (mg/kg), 1 mL/100 g weight of *M. halliana* polysaccharides, respectively.

During the treatment of rats, their 6 h shit were collected regularly every day, weighed and the data recorded. After 7 days, rats in each group were fasted for 12 h, then anesthesia with 10% chloral hydrate, and abdominal aorta blood was collected, 30 min at room temperature and centrifuged at 3000 r / min for 10 min to obtain rats serum samples were collected and the content of MTL, GAS, SS and SP were measured. The colon of the lower part of cecum was excised 2 cm, washed with tap water and saline, fixed with 4% paraformaldehyde for 24 h, 10 µm section, HE staining; 2 cm away from the cecum colon 2 cm, saline clean, filter paper dry, into the oven, 50°C drying 24 h.

2.6 Statistical analysis

All experimental results were expressed as mean ± standard deviation (SD). Statistical analysis was performed with

Table1: Rats defecation volume.

Group	24 h defecation volume(g)	6 h defecation volume(g)
normal group	52.06±2.99	14.75±1.17
model group	39.24±5.69***	8.75±0.54***###
positive control group	43.11±4.57***	15.35±1.17 ^{△△△}
high dose groups	41.77±5.62***	15.07±1.81 ^{△△△}
medium dose groups	41.78±3.90***	14.75±1.78 ^{△△△}
low dose groups	42.41±5.33***	20.37±3.18 ^{###△△△}

(n=8) Compare with the normal group: * $p < 0.05$, 0.01 < ** $p < 0.001$, *** $p < 0.001$; Compare with the model group: [△] $p < 0.05$, 0.01 < ^{△△} $p < 0.001$, ^{△△△} $p < 0.001$; Compare with the positive control group # $p < 0.05$, 0.01 < ### $p < 0.001$, ### $p < 0.001$

the SPSS19.0. Comparison between any two groups was evaluated using one-way analysis of variance (ANOVA).

3 Results

3.1 Effect on defecation in rats

Compared to the normal group, activities and diets of rats in each model group were decreased, furred coat color and easy hair removal, and the defecation of rats in model group decreased. In Table 1, compared to the normal group, the model group ($p < 0.001$) 24 h shrimp weight significantly decreased, indicating that constipation model was established.

In Table 1, compared to the normal group, the weight of the model group ($p < 0.001$) 6 h was significantly decreased than that of the treatment group and the normal group, which indicated that the constipation symptoms of the model group did not alleviate. Compared to the model group, the weight of the positive control group ($p < 0.001$), the high, medium and low ($p < 0.001$) doses of *M. halliana* polysaccharides significantly increased. Compared with the positive control group, the weight of *M. halliana* polysaccharide high, medium dose groups was similar and the effect was similar, the low dose group (0.01 < $p < 0.001$) significantly increased and the effect was better than that of the positive control group.

3.2 Effect on rat colonic moisture content

Compared to the normal group, the model group ($p < 0.001$) significantly decreased the water content of the

Table 2: The contents of colonic moisture.

Group	colonic moisture content(%)
normal group	72.69±2.39
model group	62.87±4.98 ^{***###}
positive control group	69.74±4.25 ^{ΔΔΔ}
high dose groups	68.86±2.07 ^{ΔΔ}
medium dose groups	68.49±3.87 ^{ΔΔ*}
low dose groups	71.52±3.37 ^{ΔΔΔ}

(n=8) Compare with the normal group: * $p<0.05$, 0.01<* $p<0.001$, *** $p<0.001$; Compare with the model group: $^{\Delta}p<0.05$, 0.01< $^{\Delta\Delta}p<0.001$, $^{\Delta\Delta\Delta}p<0.001$; Compare with the positive control group $^{\#}p<0.05$, 0.01< $^{\#\#}p<0.001$, $^{\#\#\#}p<0.001$.

colon, indicating that the model group did not reduce the symptoms of constipation. Compared with the model group, the water content of colon in the positive control group (0.001 < p <0.01) and the low of *M. halliana* polysaccharides (p <0.001) significantly increased and the colon water content significantly increased. Compared with the positive control group, the colon water content of the high, medium and low dose of *M. halliana* polysaccharide was similar to that of the positive control group.

3.3 The levels of motilin (MTL), gastrin (GAS), substance P(SP) and somatostatin (SS)

As shown in Table 3 and 4, compared with the normal group, the contents of GAS, MTL and SP in the model group were significantly decreased (p <0.001), and the SS content was significantly increased. Compared to the model group, the content of GAS and MTL in the positive control groups, high dose group, medium dose group and low dose group significantly increased (p <0.001). Compared to the model group, the contents of SP in the positive control groups, medium dose of *M. halliana* polysaccharides significantly increased, the levels in the low dose group significantly increased, and those in the high dose group were increased. Compared with the model group, the contents of SS in the positive control group, *M. halliana* polysaccharides high, medium and low dose group significantly decreased.

The results showed that the positive control group, *M. halliana* polysaccharides high, medium and low dose group had a therapeutic effect on constipation.

Table 3: Rat motilin (MTL), gastrin (GAS) contents.

Group	GAS(ng/mL)	MTL(ng/mL)
normal group	46.00±2.57	309.25±20.37
model group	37.23±2.79 ^{***}	223.39±19.21 ^{***}
positive control group	44.81±2.10 ^{ΔΔΔ}	295.14±16.17 ^{ΔΔΔ}
high dose groups	43.98±0.97 ^{ΔΔΔ}	263.74±10.35 ^{ΔΔ*#}
medium dose groups	44.65±1.03 ^{ΔΔΔ}	289.49±19.71 ^{ΔΔΔ}
low dose groups	46.24±2.20 ^{ΔΔΔ}	310.07±24.66 ^{ΔΔΔ}

Table 4: Rat substance P(SP) and somatostatin (SS) contents.

Group	SP(ng/mL)	SS(ng/mL)
normal group	69.88±8.48	182.32±2.68
model group	55.5±2.26 ^{***}	228.17±11.10 ^{***}
positive control group	65.17±3.61 ^{ΔΔ}	182.15±6.41 ^{ΔΔΔ}
high dose groups	62.54±3.16 ^{Δ*}	188.06±5.38 ^{ΔΔΔ}
medium dose groups	64.35±3.27 ^{ΔΔ}	171.65±6.82 ^{ΔΔΔ*#}
low dose groups	71.47±1.80 ^{ΔΔΔ}	172.69±2.50 ^{ΔΔΔ}

(n=8) Compare with the normal group: * $p<0.05$, 0.01<* $p<0.001$, *** $p<0.001$; Compare with the model group: $^{\Delta}p<0.05$, 0.01< $^{\Delta\Delta}p<0.001$, $^{\Delta\Delta\Delta}p<0.001$; Compare with the positive control group $^{\#}p<0.05$, 0.01< $^{\#\#}p<0.001$, $^{\#\#\#}p<0.001$

Figure 2a: Normal group.

3.4 Histological observations

From the histological sections HE staining, the normal group (Figure 2a) cells in normal morphology, cells arranged closely, and the model group (Figure 2b) increased cell matrix, irregular shape. Compared with the model group, the positive control group (Figure 2c), *M. halliana* polysaccharides high dose group (Figure 2d),

Figure 2b: Model group.

Figure 2e: Medium dose groups.

Figure 2c: Positive control group.

Figure 2f: Low dose groups.

Figure 2d: High dose groups.

medium dose group (Figure 2e), low dose group (Figure 2f), the cell morphology was restored, the cell matrix was reduced.

4 Discussion

Clinical manifestations of functional constipation mainly defecation effort, fecal dry knot, a sense of defecation and anorectal obstruction or obstruction can also be manifested as reduced the amount of defecation and frequency, which not only affects the appetite and intestinal absorption of patients, and can induce or aggravate colon cancer, cardiovascular and other diseases. This topic based on pre-experimental and reported in the literature [21,22], the choice of 3 mg / kg loperamide hydrochloride suspension twice daily gastric perfusion model, rats with poor mental

status, reduce eating, weight loss, stool grain size small, dry hard and other clinical manifestations of constipation. There is a variety of gastrointestinal hormones in the human gastrointestinal tract to regulate gastrointestinal motility, which has important physiological significance, such as motilin (MTL), gastrin (GAS) and somatostatin (SS) [23-25]. Intestinal neurotransmitters are also involved in the regulation of gastrointestinal motility, such as substance P (SP) [26]. Researches show that motilin (MTL) is an important neurotransmitter of gastrointestinal excitatory motor neurons, can speed up the gastrointestinal motility, improve gastrointestinal contractility and tension. Gastrin (GAS) mainly secreted by gastric antrum, duodenum and small intestinal mucosa open G cells, and stimulates gastric acid secretion and promotes gastrointestinal motility. Somatostatin (SS) inhibits smooth muscle contraction and the secretion of gastrin and pepsin. Substance P (SP) can inhibit gastrointestinal mucosal secretion, stimulate intestinal motility and contraction of smooth muscle movement. In patients with constipation, the levels of motilin (MTL), gastrin (GAS) and substance P (SP) were significantly decreased, and somatostatin SS content was significantly increased. The effect of *M. halliana* polysaccharides on functional constipation was evaluated by using six indexes of defecation, water content of colon, MTL, GAS, SS and SP. The results showed that the three doses of *M. halliana* polysaccharides could improve functional constipation in rats, low-dose group was the best than the other two dose groups.

Numerous studies have been reported that many kinds of polysaccharides, such as algae and plants, have the effect of improving constipation, such as *Enteromorpha* polysaccharides, *Inula japonica* polysaccharides, *Tremella* polysaccharides and *Flammulina velutipes* polysaccharides [27-30]. The mechanism may be related to the structure of polysaccharides and gut microflora, but the specific Mechanism needs to be further studied.

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