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Carthami flos regulates gastrointestinal motility functions

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ABSTRACT

Background: Gastrointestinal (GI) symptoms are common in the general population. This investigation studied the effects of *Carthami flos* (CF), a natural product, on GI motility.
Methods: We checked the intestinal transit rates (ITRs) or gastric emptying in normal and in GI-motility-dysfunction (GMD) mice *in vivo*. The GMD mice were made by acetic acid or streptozotocin.
Results: Both ITRs and gastric emptying were increased by CF (0.0025–0.25 g/kg) dose dependently. Also, in the GMD mice models, acetic-acid-induced peritoneal irritation, and streptozotocin-induced diabetic mice, the ITRs were decreased compared to normal mice, and these decreases were inhibited by CF.
Conclusion: These results suggest that CF is one of the good candidates for the development of a prokinetic agent that may regulate GI-motility functions.

1. Introduction

Gastrointestinal (GI) motility disorders, which are common in the general population, lead to a substantial decrease in quality of life. Frequent GI-motility-related symptoms include constipation, abdominal pain, bloating, vomiting, diarrhea, and incomplete evacuation.[1] These symptoms may be linked to motility disturbances caused by either prolonged/accelerated GI transit or peristaltic dyscoordination.[2]

Traditional Chinese medicine has a crucial role in the treatment of various diseases. *Carthami flos* (CF, also known as *Carthamus tinctorius* L.) is an annual safflower belonging to the family Compositae. The major constituents of CF are glycerides, linoleic acid, oleic acid, and so on.[3] CF has been used for centuries in Asia to treat cervical disk herniation,[4] shoulder pain,[5] degenerative knee arthritis,[6] urological diseases,[7] and lung inflammation.[8] In addition, CF is used for blood stasis caused by sprains or accidents, and to treat both baldness and pain caused by kidney deficiency.[9] In the small intestinal interstitial cells of Cajal (ICC) of mice, CF depolarized pacemaker potentials.[10] However, few animal studies have assessed the effects of CF extract on GI-motility functions. Therefore, this investigation studied the effects of CF extract on GI motor functions by measuring the intestinal transit rates (ITRs) and gastric emptying (GE) in an *in vivo* mouse model.

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2. Methods

2.1. Ethics

Animal care and experiments were conducted in accordance with the guidelines issued by the ethics committee of Pusan National University (Busan, Korea; approval number PNU-2016-1370) and those issued by the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

2.2. CF preparation

The powder of an aqueous extract of CF (*C. tinctorius* L.; CW04-082) was obtained from the plant-extract bank at the Korea Research Institute of Bioscience & Biotechnology (Daejeon, Republic of Korea). The powder was immersed in distilled water (DW) and extracted for 2.5 hours. After that, it was evaporated under reduced pressure using a DW-290 (Daewoong, Seoul, Republic of Korea) at 100 °C. The extract was then lyophilized using a Clean-vac 12 dryer (Biotron Electronics Corporation, Calgary, Alberta, Canada) for 24 hours. Next, CF was dissolved in DW at a concentration of 20 mg/mL, and stored at 4 °C as a stock solution.

2.3. Animals

To check the *in vivo* effects of the CF extract on the GI tract, male Institute of Cancer Research (ICR) mice were used (Samtako BioKorea Co., Ltd., Osan, Republic of Korea). The animals were maintained under controlled conditions (weighing 22–24 g, 21 ± 2 °C, relative humidity 51 ± 6%, lights on 7:00 am to 7:00 pm). The animals were deprived of food for 24 hours prior to the experiments.

2.4. Measurement of ITR using Evans blue

After administering CF extract to normal ICR mice intragastrically, 5% Evans-blue solution was administered (0.1 mL/kg) through an orogastric tube. The animals were sacrificed after Evans-blue administration, and we checked the dye distance in the intestine from the pylorus to its most distal point.

2.5. GI-motility-dysfunction mice model

Two experimental GI-motility-dysfunction (GMD) models were made: a peritoneal-irritation model by injecting 0.6% acetic acid (AA) intraperitoneally at 10 mL/kg,[11,12] and a diabetic model by streptozotocin (STZ). For the STZ-induced diabetic model, male ICR mice (aged 4 weeks) were used. To produce diabetes, the mice were administered with STZ (Sigma-Aldrich, St. Louis, MO, USA) solution. Fresh STZ was prepared in citrate buffer (0.1 M/L; pH = 4.0), and administered at 200 mg/kg body weight.[13] Two months after STZ injection, blood was withdrawn from a tail vein and blood-glucose concentrations were measured using a ONETOUCH SelectSimple kit (Johnson & Johnson Medical Company, New Brunswick, USA). Diabetes was defined as a blood-glucose level of >16 mM.

2.6. GE evaluation

After treatment with CF extract, a 0.05% phenol-red solution was administered. Twenty minutes later, the mice were sacrificed and stomachs were removed. The removed stomachs were cut into several pieces in 0.01N NaOH (5 mL) and treated with 20% trichloroacetic acid (0.2 mL). Next, the mixtures were centrifuged for 10 minutes (1050×*g*), and after that, the 0.05 mL supernatants were added to 0.2 mL NaOH (0.5N). The absorbances were subsequently measured using a spectrometer at 560 nm. The GE value (%) was calculated by 100–(A/B) × 100, where A was the test stomach absorbance, and B was the control stomach absorbance.

2.7. Drugs

The dried immature fruit of *Poncirus trifoliata* Raf. (PF) was prepared as previously described,[14,15] and its prokinetic activities were compared with those of the CF extract. This PF was selected because it is one of the most popular traditional folk medicines in Korea and exerted potent prokinetic activities in animals.[16] All drugs were purchased from Sigma-Aldrich.

2.8. Statistical analysis

The results are expressed as the means ± standard errors. Statistical analysis was performed using Student *t* test or by analysis of variance followed by Tukey's multiple-comparison test, as appropriate. The statistical significance was accepted for $p < 0.05$.

3. Results

3.1. CF regulates the ITR in normal mice

The mean ITR (%) in normal mice was 54.2 ± 2.2% (Fig. 1). Treatment with PF (1 g/kg) increased the ITR [72.3 ± 5.0% ($p < 0.01$)]. Treatment with the CF extract also increased the ITR (%) in a dose-dependent manner, with ITR values at 0.0025 g/kg, 0.025 g/kg, and 0.25 g/kg of 55.4 ± 2.6%, 65.7 ± 2.5% ($p < 0.05$), and 74.2 ± 2.2% ($p < 0.01$), respectively, being observed (Fig. 1).

3.2. CF regulates the ITR in mice with GMD

To investigate the effects of the CF extract on the GI-motility functions, we used an AA- and an STZ-induced experimental GMD models. As mentioned earlier, the AA model showed a significant inhibition of ITR (%) [29.9 ± 4.5% ($p < 0.01$ vs. normal); Fig. 2]. However, a significant retardation of this inhibition was observed when the CF extract was administered at 0.0025 g/kg, 0.025 g/kg, or 0.25 g/kg intragastrically [33.4 ± 2.6%, 40.1 ± 1.9%, and 64.5 ± 3.9% ($p < 0.01$), respectively; Fig. 2]. Also, the STZ-induced diabetic models also showed a significant ITR (%) retardation (42.5 ± 2.6%; Fig. 3), which was also significantly inhibited by the CF extract at 0.0025 g/kg, 0.025 g/kg, or 0.25 g/kg [44.8 ± 1.9% ($p < 0.01$), 52.6 ± 1.8% ($p < 0.01$), and 61.1 ± 2.9% ($p < 0.01$), respectively; Fig. 3]. No abnormal clinical signs or changes were observed in the experimental GMD

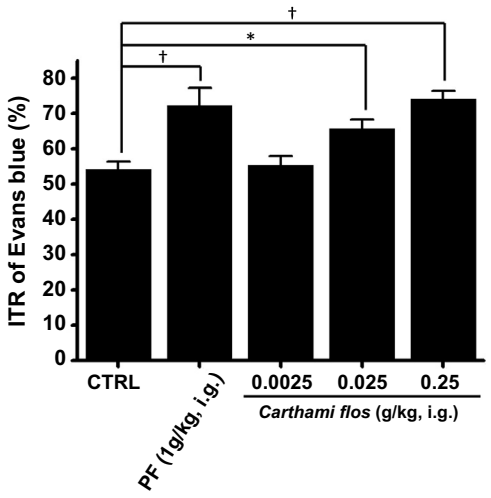

Fig. 1 – Effect of aqueous extract of CF on ITR (%) in mice. The mice were treated with CF extract, and then ITR were determined 30 minutes after Evans-blue administration. Bars represent means ± SEs.
***$p < 0.05$.**
†$p < 0.01$.
CF, *Carthami flos*; CTRL, control; ITR, intestinal transit rate; PF, *Poncirus trifoliata* Raf.; SE, standard error; i.g., intragastric.

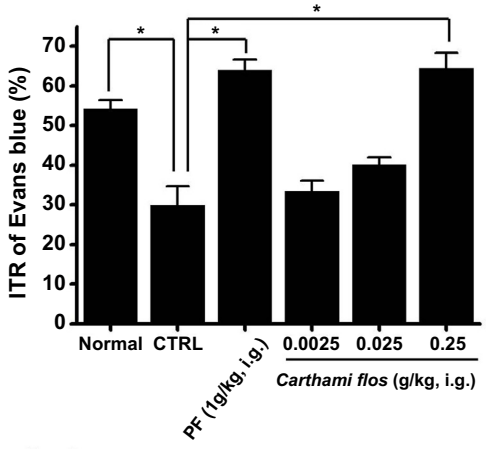

Fig. 2 – Effect of aqueous extract of CF on ITR in AA-treated mice. After the AA-treated mice were made, the mice were treated with CF extract, and then the ITRs were determined 30 minutes after Evans-blue administration. Bars represent the means ± SEs.
***$p < 0.01$.**
AA, acetic acid; CF, *Carthami flos*; CTRL, control; ITR, intestinal transit rate; PF, *Poncirus trifoliata* Raf.; SE, standard error; i.g., intragastric.

models. These results indicate that the CF extract increased the ITR in the GMD mice.

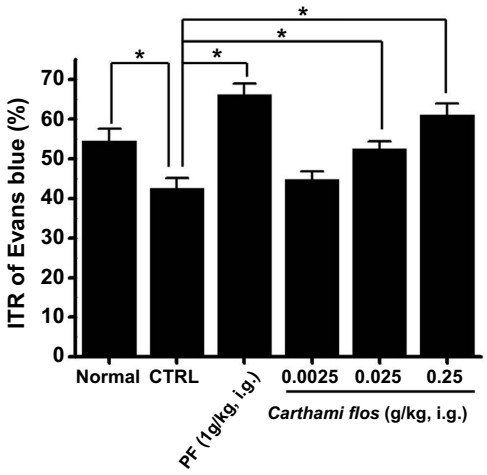

Fig. 3 – Effect of aqueous extract of CF on ITR in STZ-induced diabetic mice. Two months after administering STZ, the mice were treated with the CF extract, and then ITRs were determined 30 minutes after Evans-blue administration. Bars represent means ± SEs.
***$p < 0.01$.**
CF, *Carthami flos*; CTRL, control; ITR, intestinal transit rate; PF, *Poncirus trifoliata* Raf.; SE, standard error; STZ, streptozotocin; i.g., intragastric.

3.3. *CF regulates GE in normal mice*

The CF extract (0.0025 g/kg, 0.025 g/kg, and 0.25 g/kg)-treated groups showed significantly enhanced GE (%) values when compared to the normal group [GE values at CF extract 0.0025 g/kg, 0.025 g/kg, and 0.25 g/kg were 54.5 ± 1.5%, 55.3 ± 1.2%, and 64.7 ± 1.1% ($p < 0.01$), respectively; Fig. 4]. The 0.25 g/kg CF extract had effects similar to those of mosapride at 5 mg/kg [67.7 ± 2.2% ($p < 0.01$)] and domperidone at 5 mg/kg [64.1 ± 1.7% ($p < 0.01$)] (Fig. 4).

4. Discussion

GI-motility disorders have been diagnosed with increased frequency. Although the initial medical management is the usual course, surgical procedures are increasingly being applied to treat these disorders now.[17] GI-motility disorders occur when the gut has lost its ability to coordinate smooth muscle–ICC–neuron regulation by endogenous or exogenous agents.[18,19] GI-motility disorders are of clinical importance in many bowel disorders.[20] GI-motility-modulating drugs include all compounds that have pharmacological activity of modulating (stimulating or inhibiting) GI motility, which are mainly used for the treatment of functional GI diseases. Moreover, many new GI-motility-modulating drugs are currently under investigation.[21]

We previously investigated the effects of CF on ICC in the mouse small intestine.[10] CF did not show cytotoxic activity and induced antioxidant activity on ICC. Moreover, CF depolarized the pacemaker potentials of ICC in a concentration-dependent manner.[10] Therefore, we believe that the GI tract can be a target of CF, and their inter-

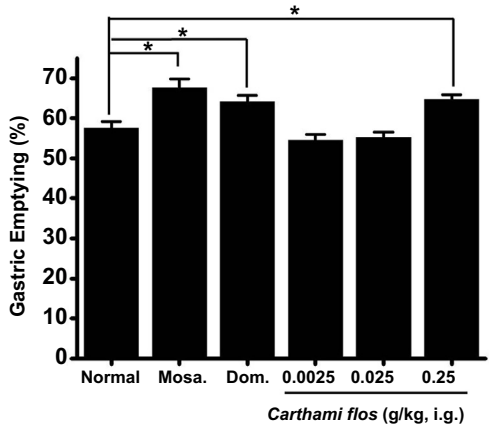

Fig. 4 – Effect of aqueous extract of CF on GE. After a 24-hour fast, the mice were orally administered with the indicated dosages of CF extract, 5 mg/kg mosapride (a 5-HT$_4$ receptor agonist), 5 mg/kg domperidone (a dopamine receptor antagonist), or DW (control). Bars represent the means ± SEs.
***$p < 0.01$.**
CF, *Carthami flos*; CTRL, control; Dom., domperidone; DW, distilled water; GE, gastric emptying; Mosa., mosapride; SE, standard error; 5-HT$_4$, 5-hydroxytryptamine receptor 4; i.g., intragastric.

action could affect intestinal motility. In addition, many authors have studied the effects of CF. CF has been used to improve blood circulation by lowering the levels of blood lipids,[22] and can modulate neutrophilic lung inflammation.[8] Additionally, it is known that CF activates Nrf2; inhibits NF-kappaB activation;[23] and suppresses lipopolysaccharide-induced expression of interleukin-1 beta, inducible nitric oxide synthase, and cyclooxygenase-2 in RAW 264.7.[24] CF also has antioxidant and hepatoprotective effects in rats.[25] However, although the considerable use of CF, little is known about its *in vivo* effects on GI motility. In this study, the CF extract significantly and dose-dependently accelerated the ITR (Fig. 1). In the experimental GMD models, AA mouse, and STZ-induced mouse, the CF extract significantly inhibited GMD-induced retardation (Figs. 2 and 3), and the CF-treated mice had significantly greater GE values, with 0.25 g/kg having effects similar to those of domperidone and mosapride (Fig. 4).

CF induced the pacemaking-activity depolarizations of ICC.[10] However, CF did not regulate the pacemaking-activity frequency.[10] Generally speaking, the pacemaking-activity depolarizations induced the decrease of frequency.[26] Unusually, CF-induced pacemaking-activity frequency had no change.[10] Therefore, we think that CF increased the GI motility because of not ICCs pacemaking activity frequency but depolarization regulations. CF induced the pacemaking-activity depolarizations of ICC, and this may induce depolarizations of smooth muscle cells. Therefore, CF might induce the increase of GI motility.

Korean or Chinese Oriental medicine may be an attractive alternative based on the perception of their "natural" approach and their low risk of side effects in GI disease. There are many commercially available herbal supplements and preparations. Indeed, the Food and Drug Administration estimates that around 29,000 herbal, vitamins, or supplements are available,[27] and approximately 11–43% of patients with GI disorders use alternative or complementary techniques.[28–30] Therefore, we believe that CF may be a good gastroprokinetic agent and that future studies to investigate its side effects are warranted. In summary, both the ITR and GE values were dose-dependently increased by the CF extract. Furthermore, the ITRs of GMD mice were significantly lower than those of normal mice, and these inhibitions were significantly inhibited by the CF extract. Taken together, our results suggest that CF may be one of the good candidates for the development of a gastroprokinetic agent.

Conflicts of interest

The authors have no potential conflict of interest to declare.

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