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Carthami Flos Depolarizes the Interstitial Cells of Cajal and Increases the Motility in Gastrointestinal Tract

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Abstract: Carthami Flos (CF) (safflower, *Carthamus tinctorius* Linne) belongs to the Compositae family and has been used in Asia as a natural medicine for treating the various diseases. However, the regulation of CF in gastrointestinal (GI) tract and the underlying mechanisms are unknown. In the present study, we investigated the effects of CF on the pacemaker activity of the Interstitial Cells of Cajal (ICCs) in murine small intestine and GI motility. Enzymatic digestion was used to dissociate ICCs from mouse small intestines. The whole-cell patch-clamp configuration was used to record pacemaker potentials from cultured ICCs clusters. *In vivo* effects of CF on GI motility were investigated by measuring the Intestinal Transit Rate (ITR) of Evans blue in normal and abnormal mice models. The CF depolarized resting membrane potentials in a concentration dependent manner but this action was blocked by Y25130 (a 5-HT_3 receptor antagonist) and RS39604 (a 5-HT_4 receptor antagonist). However, SB269970 (a 5-HT_7 receptor antagonist) did not. Pretreatment with Ca^{2+} free solution or thapsigargin (Ca^{2+} ATPase inhibitor in endoplasmic reticulum) abolished the generation of pacemaker potentials and suppressed CF-induced activity. *In vivo*, CF (0.01-1 g kg^{-1}, p.o.) not only significantly increased the ITR in normal mice but also ameliorated acetic acid-induced or STZ-induced diabetic GI motility retardation in a dose-dependent manner. So, CF regulates the pacemaker potentials in a dose dependent manner via external and internal Ca^{2+} regulations through 5-HT_3 or 5-HT_4 receptors. Also CF increases the ITRs in normal and acetic acid-induced or STZ-induced diabetic GI motility dysfunctions mice models. Therefore, CF might be a novel candidate for development as a prokinetic agent that may prevent or alleviate GI motility dysfunctions.

Key words: Carthami flos, gastrointestinal tract, interstitial cells of Cajal, pacemaker activity, GI motility

INTRODUCTION

Carthami Flos (CF) is the dried flower of safflower (*Carthamus tinctorius* L.) which has been grown for centuries in Asia for the orange-red dye (carthamin) and for its quality oil rich in polyunsaturated fatty acids. CF is known to have many medicinal properties for curing several chronic diseases in Korea (Li and Mundel, 1996). In traditional herbal medicine, CF is known as Honghua which has been widely used to alleviate pain, increase circulation and reduce blood-stasis syndrome and so on. Additionally, it can also be used for angina pectoris of coronary heart diseases, thromboangiitis obliterans, erythema multiiforme (Chen, 2006) and antitumour activity in certain cancers (Wu *et al*., 2013).

Interstitial Cells of Cajal (ICCs) are the pacemaker cells of the gastrointestinal (GI) system. Absence or reduced numbers of ICCs causes abnormally slow electrical waves and reduces smooth muscle cell contractility and intestinal transit (Hou *et al*., 2005; Huizinga *et al*., 1995; Ward *et al*., 1994). In addition, the loss of ICC is implicated in several motility disorders which suggests that ICC play an important role in the regulation of GI motility (Kim *et al*., 2005). In addition, evidence indicates that endogenous agents, such as, neurotransmitters, hormones and paracrine substances, modulate GI tract motility by influencing ICC (Lee *et al*., 2013). Therefore, in GI motility research, ICCs are the major tools used to study GI motility.

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In the present study, the effect of CF extract on the pacemaker potentials of cultured ICCs clusters were investigated. In addition, the effects of CF extract on GI motility functions in normal and in AA and diabetic conditions by measuring the Intestinal Transit Rates (ITR) of Evans blue *in vivo* were also investigated.

MATERIALS AND METHODS

Preparation of CF extract: Standardized aqueous extract of Carthami Flos (*Carthamus tinctorius* L.; catalog number: CW04-081) was obtained from the plantextract bank at KRIBB (Korea Research Institute of Bioscienceand Biotechnology), Daejeon, Korea. HPLC was performed using an ACQUITY UPLCTM system (Waters Corporation, Milford, MA, USA) which was equipped with a photodiode array detector. Chromatographic separations were performed on a 2.1×100 mm, 1.7 μm ACQUITY BEH C18 (Waters Corporation) chromatography column. The column temperature was maintained at 35°C and the mobile phases A and B were water with 0.1% formic acid and acetonitrile with 0.1% formic acid, respectively. Optimized UPLC elution conditions were as follows: 0-1 min, 5% B; 1-10.5 min, 5-28% B; wash to 13.5 min with 100% B and a 1.5 min recycle time. The flow rate was 0.4 mL min^{-1}. The aqua extract of CF was filtered on membrane filters with a pore size 0.20 mm (Millipore) and the injection volume was 3 μL. The preparation and quality analysis of some active fractions were reported in the previous study (Kim *et al*., 2014).

Preparation of cells and cell cultures: Animal care and experiments were conducted in accordance with the principles issued by the ethics committee of Pusan National University (Republic of Korea). The ICR mice were used throughout the study. Small intestines (from 1 cm below the pyloric ring to the cecum) were removed and opened along the mesenteric border. Luminal contents were removed using Krebs-Ringer bicarbonate solution and the tissues obtained were pinned to the bases of Sylgard dishes. Mucosa was removed by sharp dissection and small tissue strips of intestine muscle (consisting of both circular and longitudinal muscles) were equilibrated for 30 min in Ca^{2+} free Hank's solution (containing (in mM); KCl 5.36, NaCl 125, NaOH 0.34, Na_2HCO_3 0.44, glucose 10, sucrose 2.9 and HEPES 11; pH 7.4). Cells were then dispersed in an enzyme solution containing collagenase (Worthington Biochemical, Lakewood, NJ, USA, 1.3 mg mL^{-1}), bovine serum albumin (BSA, Sigma-Aldrich, St Louis, MO, USA, 2 mg mL^{-1}), trypsin inhibitor (Sigma-Aldrich, 2 mg mL^{-1}) and ATP (0.27 mg mL^{-1}) and plated onto sterile glass coverslips coated with murine collagen (2.5 μg mL^{-1}; Falcon/BD, Franklin Lakes, NJ, USA) in 35 mm culture dishes. Cells were then cultured at 37°C in a 95% O_2-5% CO_2 incubator in smooth muscle growth medium (SMGM; Clonetics, San Diego, CA, USA) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, USA) and murine stem cell factor (SCF; 5 ng mL^{-1}; Sigma-Aldrich). All experiments were performed on cells cultured for 1 day. ICCs were identified immunologically using anti-c-kit antibody (phycoerythrin (PE)-conjugated rat anti-mouse c-kit monoclonal antibody; eBioscience, San Diego, CA) at a dilution of 1:50 for 20 min.

Patch-clamp experiments: The physiological salt solution used to bath cultured ICC clusters (Na^+-Tyrode) contained (in mM): KCl 5, NaCl 135, $CaCl_2$ 2, glucose 10, $MgCl_2$ 1.2 and HEPES 10 (adjusted to pH 7.4 with NaOH). The pipette solution used to examine pacemaking activity contained (in mM): KCl 140, $MgCl_2$ 5, K_2ATP 2.7, NaGTP 0.1, creatine phosphate disodium 2.5, HEPES 5 and EGTA 0.1 (adjusted to pH 7.2 with KOH). Patch-clamp techniques were conducted in whole-cell configuration to record membrane potentials (current clamp) from cultured ICCs using Axopatch I-D and Axopatch 200B amplifiers (Axon Instruments, Foster, CA). Command pulses were applied using an IBM-compatible personal computer and pClamp software (version 6.1 and version 10.0; Axon Instruments). Data were filtered at 5 kHz and displayed on an oscilloscope, a computer monitor and/or on a pen recorder (Gould 2200; Gould, Valley View, OH, USA). Results were analyzed using pClamp and Origin software (version 6.0, Microcal, USA). All experiments were performed at 30-33°C.

Animals: All animals were obtained from Samtako Bio Korea Co., Ltd. Male ICR mice weighing 23-30 g were used in the study. Animals were maintained under controlled conditions (21±3°C, humidity 50±6% and illumination light on 6 am to 6 pm). Animal care and experiments were conducted in accordance with the principles issued by the ethics committee of Pusan National University (Republic of Korea). Animals were allowed free access to a commercial diet and tap water but were deprived of food with free access to tap water for 24 h before experiments. All experiments were conducted between 10 am and 6 pm.

Single oral dose toxicity of CF extract in mice: The CF extract was administered intragastrically to mice through an orogastric tube at different doses (0, 0.5, 1, 2 or 5 g kg^{-1} of CF extract delivered at 10 mL kg^{-1}). Six mice were tested for each dose and gender combination and thus, a total of sixty mice were used. Each group was

carefully observed for overt clinical signs and mortality at hourly intervals for 5 h after administration and then on a daily basis for 14 days. Individual body weights were measured before dosing and on days 1, 3, 7 and 14 after administration. On day 14, the last day of observation, all animals were euthanized under ether anesthesia and necropsied.

Measurement of ITR of Evans blue: The effect of CF extract on intestinal propulsion was assessed by measuring the intestinal transit distances of Evans blue solution (5% w/v, in DW). At 30 min after the intragastrically (i.g.) administration of CF extract to normal mice, Evans blue solution was administered i.g., through an orogastric tube at a volume of 0.1 mL kg^{-1} of body weight. Animals were sacrificed 30 min after this administration and the intestinal transit of Evans blue during the 30 min period was determined by measuring the distance the Evans blue had migrated in the intestine from the pylorus to the most distal point of the intestine. Intestinal transit was expressed as Intestinal Transit Rate (ITR) (%) which was defined as the distance traveled by Evans blue expressed as a percentage of total small intestine length (from the pylorus to the ileal end). In order to minimize possible inter-day variations in ITR measurements, they were performed in normal and GI dysfunction model mice on the same day.

Mouse model of peritoneal irritation by acetic acid: Peritoneal irritation was induced using Acetic Acid (AA) in mice 30 min after the i.g., administration of SC extract (or DW as vehicle). PIA was induced by an intraperitoneal injection of acetic acid (0.6% w/v, in saline) at a dose of 10 mL kg^{-1}, as previously described (Friese *et al*., 1997; Lee *et al*., 2005; Lyu and Lee, 2013). After injecting acetic acid, mice were placed in individual cages and allowed to recover for 30 min.

STZ-induced diabetic mouse model: Male ICR mice (aged 5 weeks) were used in this experiment, the protocol of which was approved by the Animal Care and Use Committee of Pusan National University College of Korean Medicine. Mice were randomly allocated to two groups, that is, to the control group or the diabetic group. To produce diabetes, mice were fasted overnight and the next day, STZ (Sigma-Aldrich, St. Louis, MO) solution was administered intraperitoneally. STZ was prepared freshly in 0.1 M/L ice-cold citrate buffer (pH = 4.0) and administered at a dose of 200 mg kg^{-1} body weight. Control mice were intraperitoneally administered the same volume of 0.1 M/L citrate buffer. Animals had free access to food and water and were maintained under standard housing conditions (24-27°C, RH 60-65%) under a 12 h light/dark cycle. After two months, blood was withdrawn from a mice tail vein after an 8 h fast and blood glucose concentrations were measured using a one-touch blood glucose monitoring system (Johnson and Johnson). Diabetes was defined as a blood glucose level >16 mM.

Drugs: All agents were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical analysis: Data was expressed as Means±Standard errors. The significances of differences were evaluated using the Student's t-test. The p-values of <0.05 were deemed significant. The n values reported in the text refer to the number of cells used in patch-clamp experiments.

RESULTS

Effect of CF extract on pacemaker potentials in cultured ICCs clusters: The effect of CF extract on the pacemaking activity in the cultured ICCs clusters in the GI tract were examined. Recordings from cultured ICCs clusters under current clamp mode (I = 0) showed spontaneous pacemaker potentials (Fig. 1). Mean resting membrane potential was -52.3±1.2 mV and its mean amplitude was 20.7±3 mV. In the presence of CF extract (5-30 μg mL^{-1}), membrane potentials were depolarized to 4.3±1.4 mV at 5 μg mL^{-1}, 11.6±1.2 mV at 10 μg mL^{-1} and 21.5±1.3 mV at 30 μg mL^{-1} (Fig. 1a-d). Frequency was decreased to 19.1±1.4 mV at 5 μg mL^{-1}, 19.5±1.3 mV at 10 μg mL^{-1} and 3.2±1.4 mV at 30 μg mL^{-1} (Fig. 1a-c and e). Summarized values and a bar graph of the effects of CF extract on pacemaker potentials are provided in Fig. 1d and e (n = 7). These results show that CF extract has a membrane depolarization effect on ICCs clusters.

Identification of CF extract receptor subtypes in cultured ICCs clusters: To investigate the relationship between CF extract and its receptors, we studied about the 5-HT receptors because 5-HT receptors are known to mediate the motility of GI tract and is of particular interest due to its strong association with potent prokinetic activity, especially the 5-HT receptor subtype 4 (5-HT_4R) (Gershon and Tack, 2007). Therefore, we investigated whether the prokinetic action of CF extract involves 5-HT receptors. Previous studies have shown that 5-HT interacts with seven different 5-HT receptor subtypes, but in another study only three (5-HT_3R, 5-HT_4R and 5-HT_7R) were found in the ICCs in the murine small intestine (Liu *et al*., 2011; Shahi *et al*., 2011). To identify the receptor subtypes of 5-HT involved in the effects of CF extract, ICCs were pretreated with various 5-HT receptor antagonists and then treated with CF extract. The Y25130

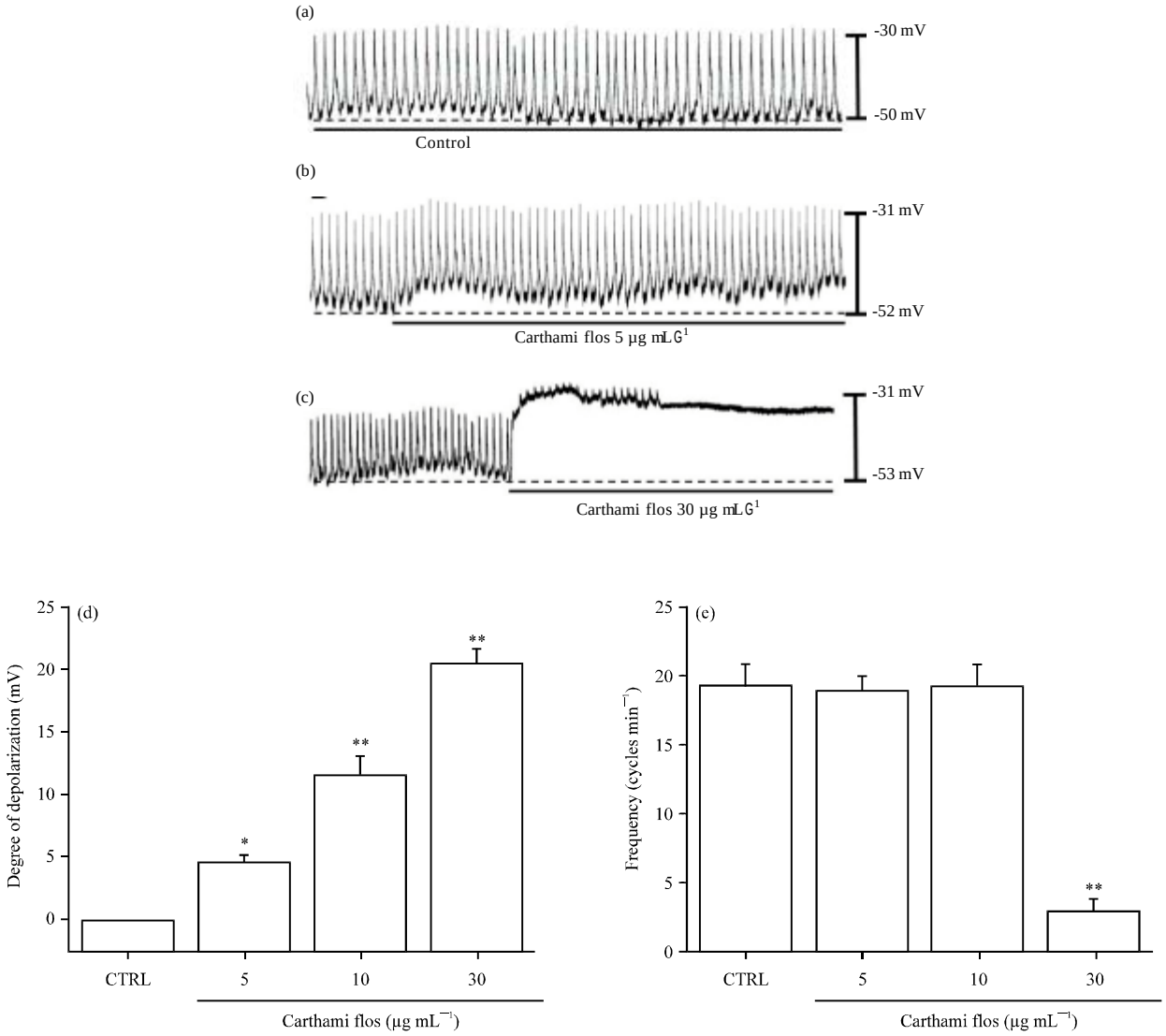

Fig. 1(a-e): Effects of CF extract on pacemaker potentials in cultured ICCs clusters from GI tract. (a-c) It shows the pacemaker potentials of ICCs exposed to CF extract (5-30 μg mL^{-1}) in current clamp mode (I = 0). The changes of (d) Depolarization and (e) Frequency caused by CF extract are also summarized in D and E. Bars represent Mean±SEs. *,**Significantly different from untreated controls at $p<0.05$, $p<0.01$, respectively. CTRL: Control

(a 5-HT$_3$ receptor antagonist), RS39604 (a 5-HT$_4$ receptor antagonist) and SB269970 (a 5-HT$_7$ receptor antagonist) were all pretreated at a concentration of 10 μM for 5 min and CF extract was added (Fig. 2). After pre-treatment with Y25130, membrane depolarization by CF was found to be blocked (Fig. 2a); membrane depolarization produced in the presence of Y25130 by CF extract was 0.8±0.9 mV (n = 5; Fig. 2d). RS39604 also blocked CF extract-induced membrane depolarization and the membrane depolarization produced in the presence of RS39604 by CF extract was 0.8±0.8 mV (n = 5; Fig. 2b and d). However, pretreatment with SB269970 did not block the effect of CF extract (n = 5; Fig. 2c and d). These results show that CF extract modulates pacemaker potentials through 5-HT$_3$ and 5-HT$_4$ receptor-mediated pathways.

Effects of external Ca^{2+} free solution and Ca^{2+} ATPase inhibitor in the endoplasmic reticulum on CF extract-induced pacemaker potentials in cultured ICCs clusters: External Ca^{2+} influx have an important role in GI smooth muscle contractions and is necessary for the generation of pacemaker potentials by ICCs. The

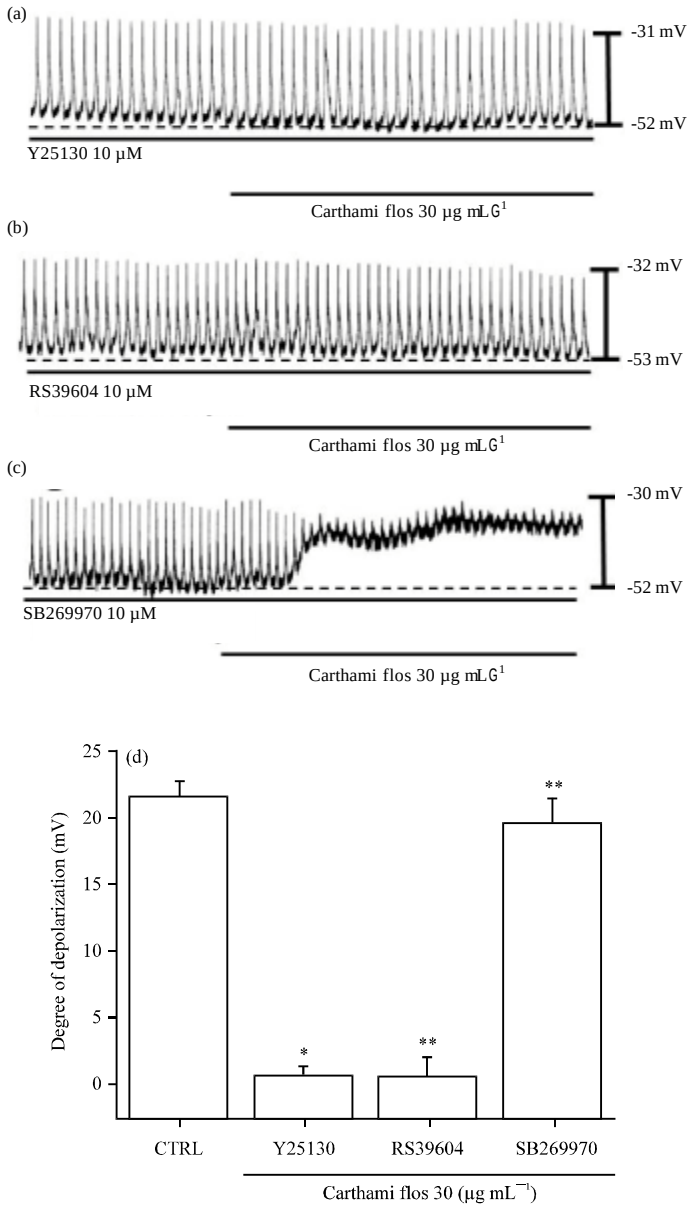

Fig. 2(a-d): Effects of 5-HT receptor subtype antagonists on CF-induced pacemaker potential responses in cultured ICCs clusters. (a) Pacemaker potentials of ICCs exposed to CF (30 μg mL^{-1}) in the presence of 5-HT$_3$ receptor antagonist (Y25130; 10 μM). Y25130 was pretreated for 5 min and then SHXXT was added. After pretreatment, membrane depolarization by SHXXT was found to be blocked, (b) Pacemaker potentials of ICCs exposed to CF in the presence of 5-HT$_4$ receptor antagonist (RS39604; 10 μM). RS39604 was pretreated for 5 min and then SHXXT was added. After pretreatment, membrane depolarization by SHXXT was found to be blocked, (c) Pacemaker potentials of ICCs exposed to CF in the presence of 5-HT$_7$ receptor antagonist SB269970 (10 μM). Pretreatment with SB269970 did not block the effect of SHXXT and (d) Responses to CF in presence of different receptor antagonists. Bars represent Mean±SEs. **Significantly different from untreated controls at $p<0.01$. CTRL: Control

generation of pacemaker currents is known to be dependent on intracellular Ca^{2+} oscillations (Ward *et al*., 2000). To investigate the roles of external and of internal Ca^{2+}, CF extract was examined under external Ca^{2+} free

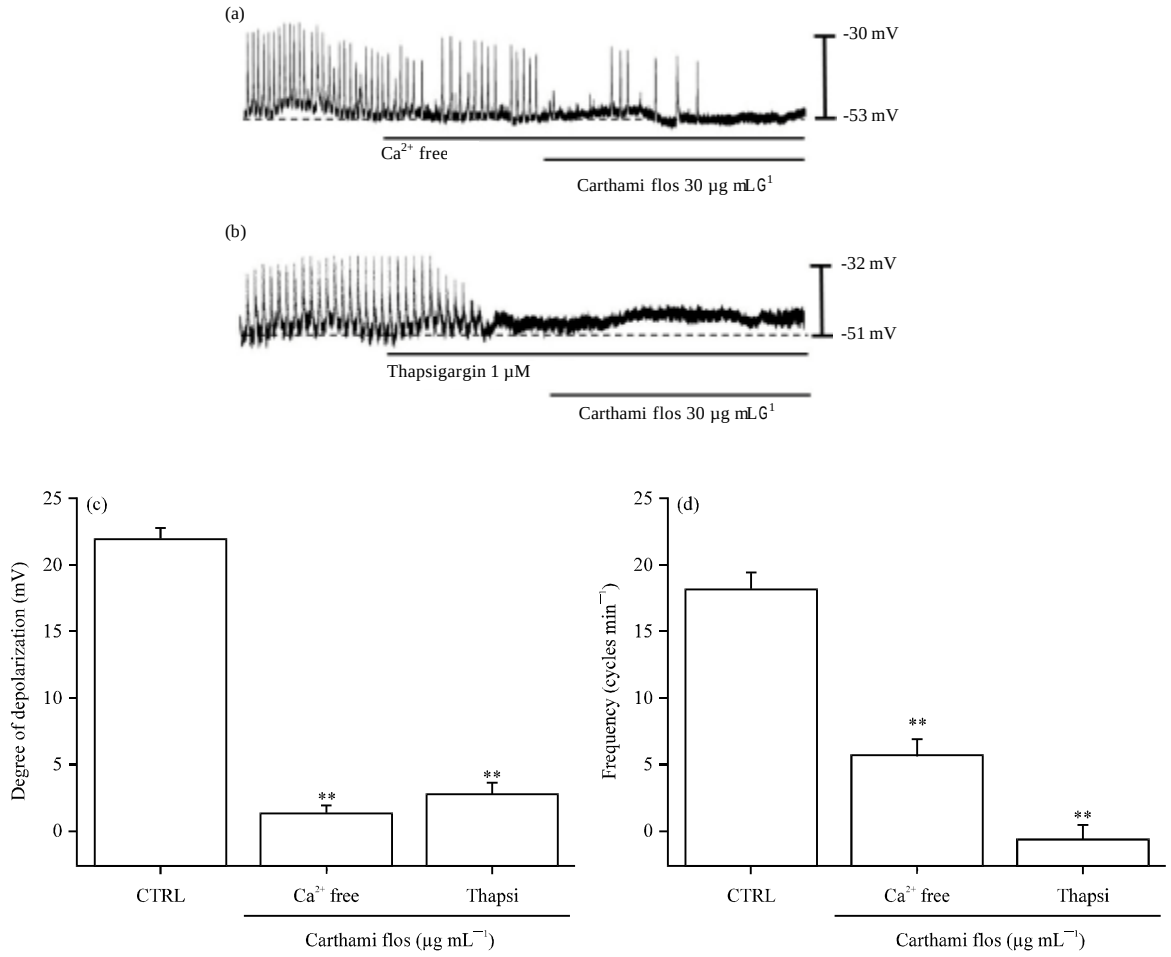

Fig. 3(a-d): Effects of an external Ca^{2+} free solution and thapsigargin on CF extract-induced membrane depolarizations in cultured ICCs clusters. (a) External Ca^{2+} free solution abolished the generation of pacemaker potentials and CF extract did not induce the membrane depolarizations, (b) Thapsigargin (an inhibitor of Ca^{2+} ATPase in the endoplasmic reticulum) abolished the generation of pacemaker potentials and CF extract did not induce the membrane depolarizations and (c-d) CF extract-induced actions. Bars represent Mean±SEs. **Significantly different from untreated controls at $p<0.01$. CTRL: Control

conditions and in the presence of thapsigargin, an inhibitor of Ca^{2+} ATPase in the endoplasmic reticulum (Choi *et al*., 2009; Koh *et al*., 2002). Pretreatment with an external Ca^{2+} free solution abolished the pacemaker potentials and under this condition, CF extract did not induce membrane depolarizations (n = 5; Fig. 3a and c). Additionally, CF extract-induced membrane depolarizations were inhibited by thapsigargin pretreatment (n = 5; Fig. 3b and d). These results show that CF extract-induced responses are dependent on the internal and external Ca^{2+} regulations.

Acute toxicity of SC extract in normal mice: The membrane depolarizations of ICCs initiate contraction of the GI tract. To examine the effect of CF extract on GI motility, the ICR normal mice were used. Of sixty mice tested, no fatality occurred during the 2 weeks even at a dose of 5 g kg^{-1}, indicating that the minimal lethal dose of CF extract in normal mice exceeds 5 g kg^{-1}. Furthermore, no abnormal clinical signs were observed and a nearly identical increase in body weight was observed for all the mice. Furthermore, no abnormal findings were evident at necropsy after 14 days. Accordingly, CF extract appeared to be safe which is consistent with its widespread use in traditional Korean medicine at single doses of 9-12 g in man (Kim *et al*., 1997).

Effects of CF extract on ITR in normal mice: Mean ITR (%) values for Evans blue during 30 min in normal mice are shown in Fig. 4. The mean ITR for non-treated normal

mice (control) was 61.5±2.1%. On the other hand, the ITR values for CF extract at 0.01, 0.1 and 1 g kg^{-1} were 64.1±2.5, 73.2±4.2 and 89.2±1.8%, respectively (*p<0.05; **p<0.01; Fig. 4). These show that CF extract increased ITR (%).

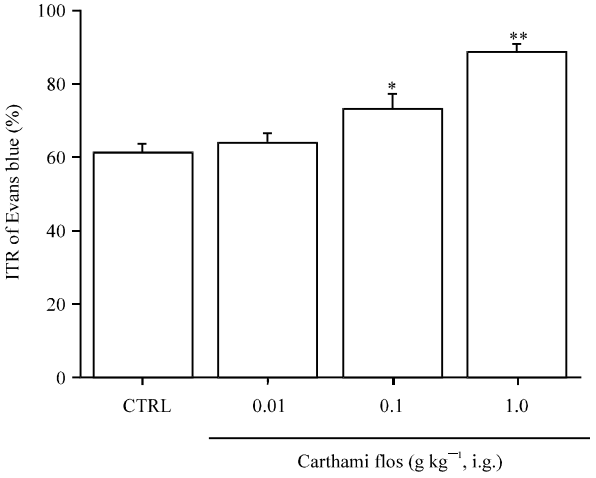

Fig. 4: Effects of CF extract on ITR (%) in normal mice. ITR (%) values determined using Evans blue over 30 min following the i.g., administration of an Evans blue solution 30 min pretreating normal mice (n = 6 for each bar, except for control (n = 15)) with CF extract. Bars represent Mean±SEs. *,**Significantly different from untreated controls at p<0.05, p<0.01, respectively. CTRL: Control

Effects of CF extract on ITR in mice with GI motility dysfunction: To further examine the effect of CF extract on GI motility, the AA mouse and STZ-induced diabetic mouse models were used with experimental GI motility dysfunction. As was expected, a significant retardation in ITR (%) was observed in the AA mouse model (37.6±1.7%; Fig. 5). On the other hand, a significant inhibition of this retardation was observed when CF extract was administered at 0.01, 0.1 or 1 g kg^{-1} i.g., in the AA mouse model (40.4±1.3%, 46.2±3.4% (p<0.05) and 64.3±4.4% (p<0.01), respectively; Fig. 5). No abnormal clinical signs or changes were observed in AA mice model after i.g., administration. Furthermore, a significant retardation in ITR (%) was observed in STZ-induced diabetic mice (52.1±2.6%; Fig. 6). On the other hand, significant inhibition of this retardation was observed after CF extract was administered at 0.01, 0.1 or 1 g kg^{-1} i.g., to STZ-induced diabetic mice (53.2±1.1, 59.5±1.3 and 69.5±2.4% (p<0.01), respectively; Fig. 6). No abnormal clinical signs or changes were observed in STZ-induced diabetic mice after administering CF extract at any i.g., dose.

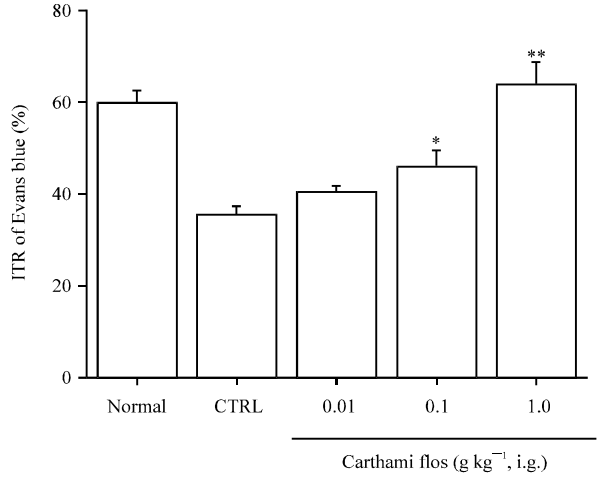

Fig. 5: Effects of CF extract on ITR (%) in acetic acid (AA) mice. The ITR (%) values over 30 min following the i.g., administration of Evans blue 30 min after the induction of AA in mice (n = 6 for each bar). Bars represent Mean±SEs. *,**Significantly different from AA control at p<0.05, p<0.01 level, respectively. CTRL: Control

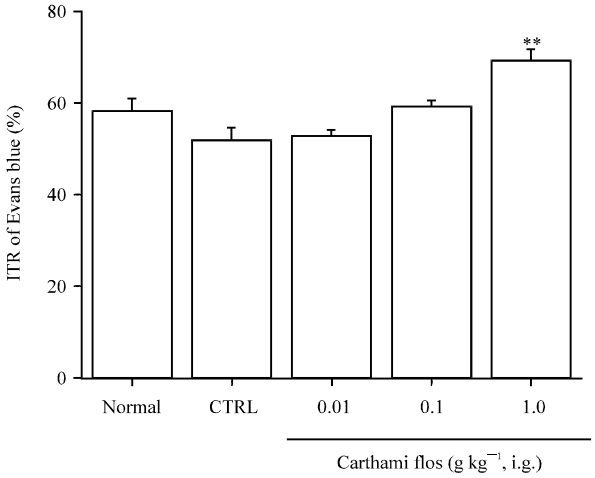

Fig. 6: Effect of CF extracts on ITR (%) values in STZ-induced diabetic mice. The ITR (%) values over 30 min following the i.g., administration of Evans blue 2 months after the induction of STZ in mice (n = 6 for each bar). Bars represent Mean±SEs. **Significantly different from STZ-induced diabetic controls at p<0.01. CTRL: Control

DISCUSSION

The CF is used in traditional Asian medicine to show the antioxidant activity (Hiramatsu *et al*., 1998; Salem *et al*., 2011), anti-inflammatory activity (Bak *et al*., 2011; He *et al*., 2011; Wang *et al*., 2011), anti-lipase property (Zheng *et al*., 2010), anti-myocardial ischemia effect (Han *et al*., 2009) and activation of blood flow (Lu *et al*., 2008). However, uptil now, there is no report about the GI motility.

The ICCs serve as the pacemaker cells of the GI tract by generating spontaneous pacemaker potentials and conducting slow waves into smooth muscle syncytium, because they are electrically coupled to neighboring smooth muscle cells (Huizinga *et al*., 1995; Sanders *et al*., 2012a; Ward *et al*., 1994). Smooth muscle cells respond to slow wave depolarization by activating L-type Ca^{2+} channels (Sanders *et al*., 2012a). In addition, smooth muscle response is regulated by neural inputs and both excitatory and inhibitory enteric motor neurons are closely associated with ICCs (Lee *et al*., 2013). Thus, ICCs play an important role in the determination and regulation of GI motility.

In the present study, we found that CF extract depolarized resting membrane potentials in ICCs via internal or external Ca^{2+} dependence through 5-HT_3 or 5-HT_4 receptors. Furthermore, CF extract stimulated *in vivo* GI contractility, indicating that CF extract could exert its effects on the GI system.

The pacemaker activities of ICCs in the murine small intestine are mainly due to periodic activations of nonselective cation channels (NSCCs) (Kim *et al*., 2006; Koh *et al*., 2002) or Cl^- channels (Huizinga *et al*., 2002; Park *et al*., 2005; Parsons *et al*., 2012; Zhu *et al*., 2005, 2009). Kim *et al*. (2005) suggested that Transient Receptor Potential Melastatin (TRPM) 7 is required for ICCs pacemaker activity in the murine small intestine and Hwang *et al*. (2009) suggested that a Ca^{2+} activated Cl^- channel (CaCC) is involved in slow wave generation in ICCs. This channel is the transmembrane protein 16A (Tmem16A). Tmem16A encodes the anoctamin 1 (ANO1) channel (Hwang *et al*., 2009; Namkung *et al*., 2011; Sanders *et al*., 2012b; Yang *et al*., 2008; Zhu *et al*., 2009). Therefore, it may be important to examine the effect of CF extract on TRPM7 and ANO1 channels. CF extract might modulate the ion channel in ICCs cell membrane and regulate the pacemaker activity.

Asian traditional medicine systems have identified several herbs and spices that can be used to treat GI tract disorders (Borrelli and Izzo, 2000; Langmead and Rampton, 2006; Sengupta *et al*., 2004). Poncirus Fructus (PF) extracts were reported to increase GI transit time for charcoal and accelerate small bowel transit in the rat (Lee *et al*., 2005). In addition, PF extract had been reported to stimulate rat distal colon motility (Choi *et al*., 2010). Therefore, PF extracts appear to be potential prokinetic agents in the GI tract (Lee *et al*., 2005). Furthermore, *Citrus unshiu* peels have been reported to function as a gastroprokinetic agent and GI motility was significantly increased by *Citrus unshiu* peels (Lyu and Lee, 2013). Additionally, schisandra chinensis (Turcz.) Baill. (SC) modulated the pacemaking activity in ICCs and increased the GI motility in mice (unpublished data). Therefore, traditional medicines have played an important role in health care and despite the great advances made in modern medicine, traditional medicine is still the primary form of health care in many countries (Bai, 1993).

Taken together, CF extract depolarized the membrane potentials of ICCs in a dose dependent manner through 5-HT_3 and 5-HT_4 receptors via internal or external Ca^{2+} modulation. Additionally, in *in vivo* experiments, CF extract accelerated ITR in normal and in GI dysfunctions mice (AA and STZ-induced diabetic mice), respectively. In conclusion, CF extract could modulate the pacemaker activity of ICCs and might have potentials as a treatment for GI motility disorders and be one of the candidates for the development of agents that target the retardation of GI activity.

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