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Spasmolytic Effect of *Petroselinum crispum* (Parsley) on Rat's Ileum at Different Calcium Chloride Concentrations

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Abstract: Traditional herbal medicines such as *Petroselinum crispum* (Parsley) have been used for treatment of intestinal disorders in Iran. To date no pharmacological evidence for their effectiveness has been reported. The aim of this study was to examine the relaxant effect of hydroalcoholic extract of *Petroselinum crispum* on isolated adult male Wistar rat's ileum contraction by KCl 60 (mM). The extract was prepared from parsley seed with 80% ethanol. A portion of ileum was removed and placed in an organ bath containing Tyrode solution (37°C, pH = 7.4) bubbled with air. From a resting tension of 1 g, isotonic Transducer and Harvard Universal Oscillograph were used for recording contraction of ileum after administration of 60 mM KCl. Various concentration of extract were added to the bath. This experiment showed that, the extract in a dose-dependent manner decreased the induced-KCl ileums contraction ($n = 7$, $p<0.001$). Also adding extract before influence of KCl, cause reduced effect of KCl ($n = 7$, $p<0.001$). In addition, the Tyrode solution without $CaCl_2$ and high concentration of K^+, various concentration of extract significantly ($n = 7$, $p<0.001$) decreased the $CaCl_2$-induced contraction. Therefore, it seems that the relaxation effect of extract alcoholic parsley seed on the contraction of ileum is performed by blocking of voltage-gated calcium channels.

Key words: Spasmolytic, alcoholic extract, ileum, chloride calcium, rat

INTRODUCTION

Petroselinum crispum (parsley), presumably native in the Mediterranean region, is nowadays cultivated throughout the world (Kreydiyyeh *et al*., 2001). It is belonging to the Apiaceae family (Fejes *et al*., 1998). A non-hairy biennial or short-lived perennial with much branched, green and cylindrical. The fruit is an ovate cremocrap, the isolated mericarps are curved and tapering (Damelio, 1999). Some scientists showed, that the essential oil obtained from the fruit of parsley has also strong action on the central nervous system. Characteristic constituents are: flavonoids (apiin, luteolin, apigenin and glycosides), essential oil (apiol, miriszticin), cumarines (bergapten, imperatorin) and Vitamin C (Fejes *et al*., 1998). The plant has been used in folk medicine as a diuretic, stomachic, anemmenagogue and abortifacient (Kreydiyyeh *et al*., 2001). Rats offered an aqueous parsley seed extract to drink, eliminated a significantly larger volume of urine per 24 h was compared to when they were drinking water. The mechanism of action of parsley seems to be mediated through an inhibition of the Na-K pump that would lead to adiuresis (Kreydiyyeh *et al*., 2002). Other results indicated that parsley to induce abortion (Ciganda and Laborde, 2003). Antioxidant and antibacterial activities of methanol and water extracts of parsley leaves and stems were determined (Wang and Kitts, 2006). An ethanolic extract of parsley, was tested for its ability to inhibit gastric secretion and to protect gastric mucosa against the injuries caused by pyloric ligation, hypothermic restraint stress, indomethacin and cytodestructive agents in rats (Al-Howiring *et al*., 2003). Parsley extract has a protective effect on against hepatotoxicity caused by diabetes (Ozsoy-Sacan *et al*., 2006). Parsley has been claimed in folk medicine to possess laxative properties attributed to the presence therein of some volatile oils that are more concentrated in seeds than in stems or leaves. This effect of parsley is expected an inhibitory effect of the herb on the activity of the Na-K ATPase from the rat colon. Some scientists report also the use of parsley seeds in the preparation of laxative and slimming teas. In addition, parsley used as a folk remedy to decrease blood glucose, has any anti diabetic effect on pancreatic B cells of rats

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(Yanardag *et al*., 2003). Parsley is a wild plant growing in some parts of Iran. Aerial parts of this plant have been used in Iranian traditional medicine for the treatment of diarrhea. To date no pharmacological evidence has been reported to support this claim. Although there are a number of ways to prevent diarrhea, the most effective anti-diarrheal drugs, such as antagonists of muscarinic receptors and opioid agonists, reduce contractile activity of the intestinal smooth muscle and thereby allow more time for water absorption (Sadraie *et al*., 2001). Gastrointestinal motility is an integrated process that includes myoelectrical activity, contractile activity, tone, compliance and transit. The smooth muscle of the intestine exhibits two distinct types of contractions: tonic and rhythmic phasic, which cause mixing and propulsive the two types of contractions differ in their sensitivity to calcium (Grasa *et al*., 2004). The objective of this research was to study the action of various extracts of *Petroselinum crispum* on contractile activity induced by one spasmogen in rat ileum to seek scientific evidence for the beneficial use of this herbal medicine in the treatment of gastrointestinal disorders.

MATERIALS AND METHODS

Plant extract: This protocol has been done in April, 2006 until May, 2007. Seeds of parsley were prepared by Jahad Research Center (Ahwaz) and identified by Department of Biology, Shahid Chamran University, Ahwaz, Iran. The samples were ground to powder, 1 g of powder was extracted using 10 mL of ethanol-distilled water (8:2 w/v), centrifuging for 15 min and the collecting the supernatants. This process was repeated three times. The ethanol was then removed by evaporation (Patumi *et al*., 1990; Seyyednejad *et al*., 2001).

Drugs and solution: The following drugs were used for the experiments: KCl (Merck), $CaCl_2$ (Merck), parsley (alcoholic extract). Chloride potassium (KCl) was made up as 60 mM (Nasu *et al*., 1994) stock solution in Tyrode. The plant extracts were made up 0.1 g/1 cc Tyrode as stock solution. Various concentration of $CaCl_2$ were made up as (0.225, 0.45, 0.9, 1.8 and 3.6 mM) stock, dilution in Tyrode (without $CaCl_2$ and high concentration of K^+). Tyrode's solution composed of (Mm): NaCl (139.9), KCl (2.68), $CaCl_2$ (1.8), $MgCl_2$ (1.05), $NaHCO_3$ (11.9), NaH_2PO_4 (0.42) and glucose (5.55), was made up in distilled water (Sadraie *et al*., 2001, 2003). All chemical were purchased from Merck.

Experimental procedure: Male Wistar rats (225±25 g) were killed by cervical dislocation and a portion of ileum was isolated, removed and suspended in a 10 mL organ chamber containing Tyrode solution at 37°C. For the experiments done in Ca free solution we used Tyrodes solution without $CaCl_2$ and high concentration of potassium (60 mM). The connected tissue was carefully trimmed from the tissue and placed in Tyrode solution at 37°C and bubbled with air. Muscle preparations were set at a resting tension of 1 g and allowed to equilibrate for 1 h in tyrode solution. Isotonic contractions, elicited by KCl and $CaCl_2$ were recorded using a Harvard isotonic transducer and displayed on a Harvard Universal Oscillograph pen recorded device. Drugs were added directly to the organ bath in volumes usually not exceeding 5% of bath volume (10 mL organ bath). A contraction-response curve was also obtained by cumulative addition of drugs at 5 min intervals after addition of 60 mM KCl. Each drug concentration was at 5 min in contact with the tissue before their effects were evaluated.

Measurements and statistical analysis: Contractions were measured as maximum changes in tension from pre-drug baseline within the contact time or as the area under curve produced by tissue contraction at 5 min intervals just before addition of the next concentration of the test drug and expressed as percentage of control or maximum induced response for each tissue. Mean and standard error of mean (SEM) values were calculated for each group of results and significance between the means was calculated by two-tailed paired student's t-test or one-way analysis of variance (ANOVA). Values were considered to be significantly different from control when $p<0.05$.

RESULTS

The effect of extract on ileum's contraction-induced by KCl: Rats ileum suspended in Tyrodes solution shows irregular spontaneous contractile activity, which attenuates by changing of bath fluid. KCl 60 Mm (non-receptor stimulation for opening Ca channels) produced a sustained tonic contraction, which was maintained during the course of experiments. After administration of 60 mM KCl to the bath solution, various cumulative concentrations of extract (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg mL^{-1}) were added to the bath. The extract to the dose-dependent manner significantly ($n = 7$, $p<0.001$) inhibited the ileum contraction induced by 60 mM KCl (Fig. 1). These inhibitory effects of the parsley extract could be seen within 5 min of contact with the tissue and were maintained as long as it was present in the bath. As is shown in Fig. 2, administration of 0.8 mg mL^{-1} extract,

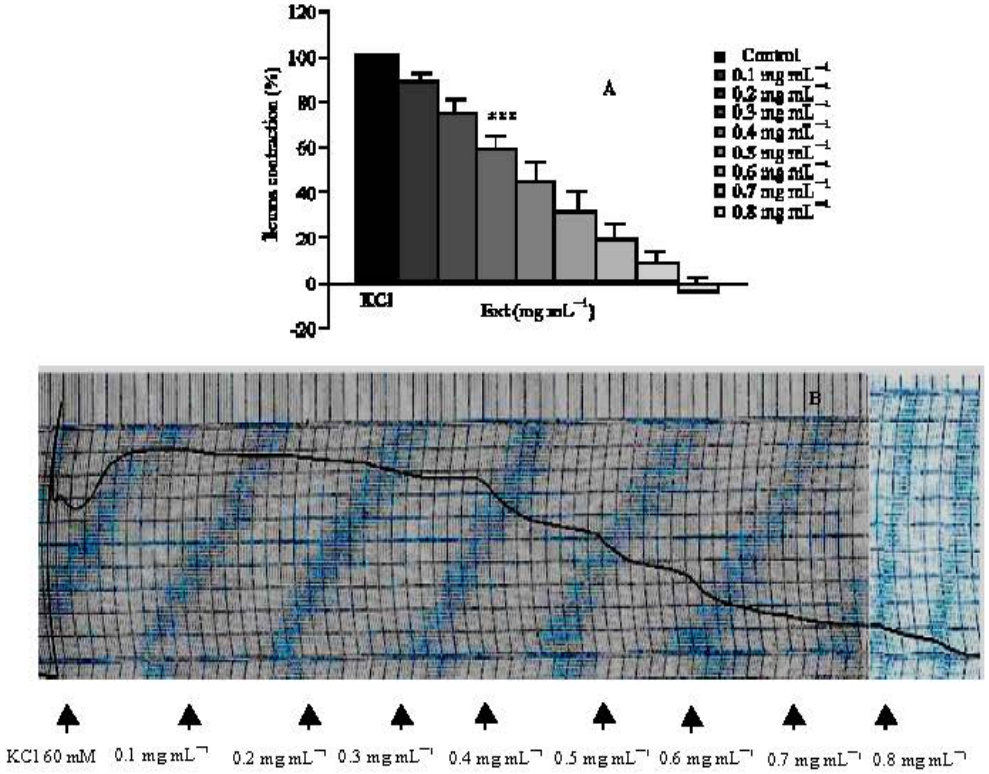

Fig. 1: The effect of cumulative concentration of alcoholic Parsley seed extract on KCl-induced contraction in the adult male rats ileum (n = 7). ***: p<0.001. A: Histogram, B: One sample of oscillogram

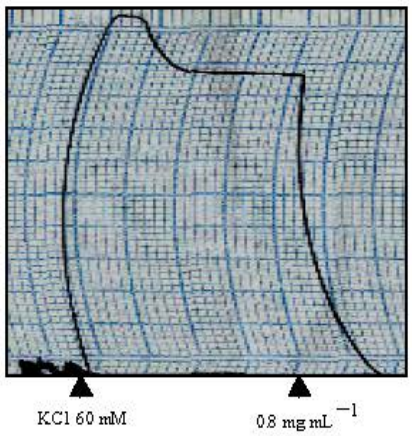

Fig. 2: The effect of 0.8 mg mL^{-1} extract on KCl-induced contraction in the adult male rat's ileum

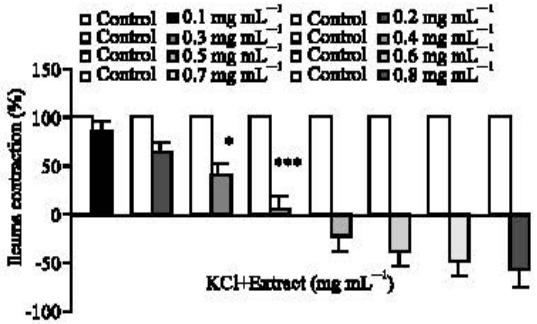

Fig. 3: The effect of non-cumulative concentration of alcoholic Parsley seed extract on KCl-induced contraction in the adult male rat's ileum (n = 7). *: p<0.05, ***: p<0.001

to become apparented maximum response relaxation in ileum but reduced density effect of it with pass a time. On the other hand, in another experiment KCl were added to the bath in present of each concentration of extract and them washing it for 15 min (non-cumulative). These results showed that, utilization from KCl could contractions on ileum for several time which were no significant difference between them (n = 7, p<0.001) (Fig. 3).

The effect of extract on ileum before effect of KCl: In this stage, ileums tissue incubation with various

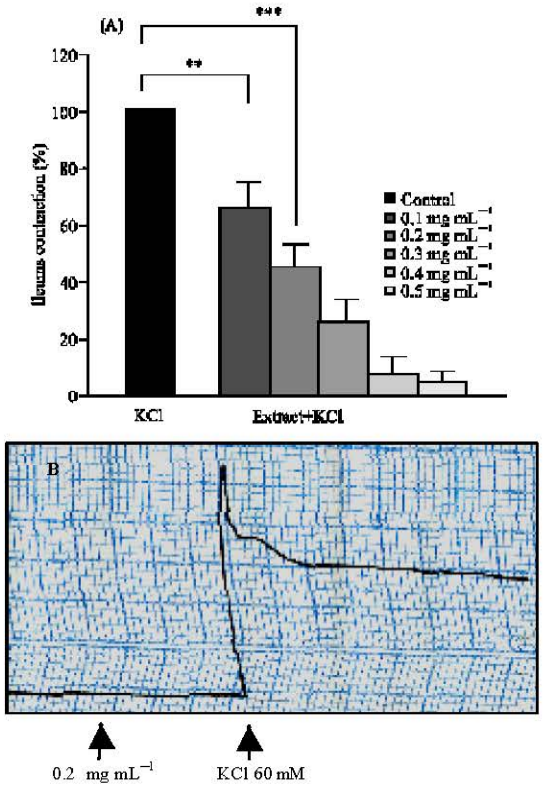

Fig. 4: The effect of various concentration of alcoholic parsley seed on adult male rat's ileum, before influence of KCl 60 mM (n = 7). **: p<0.01, ***: p<0.001; (A) Histogram and (B) One sample of oscillogram

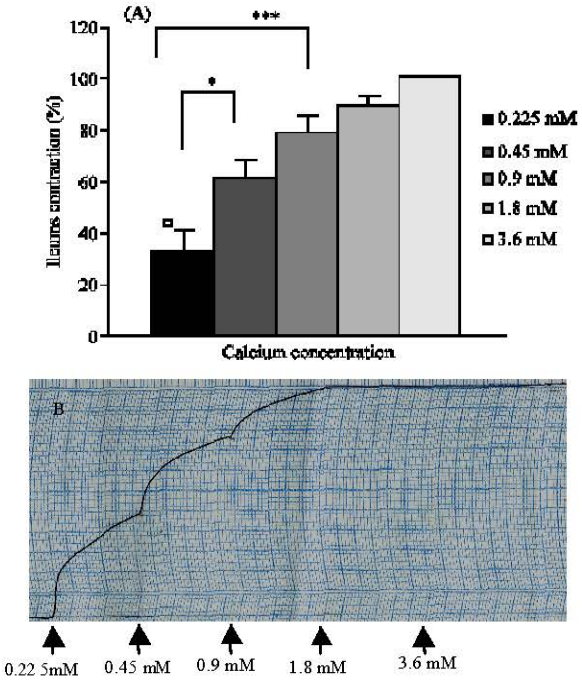

Fig. 5: The effect of various concentration of $CaCl_2$ on contraction of adult male rat's ileum at absent of extract (n = 7). *: p<0.05, ***: p<0.001; (A) Histogram and (B) One sample of oscillogram

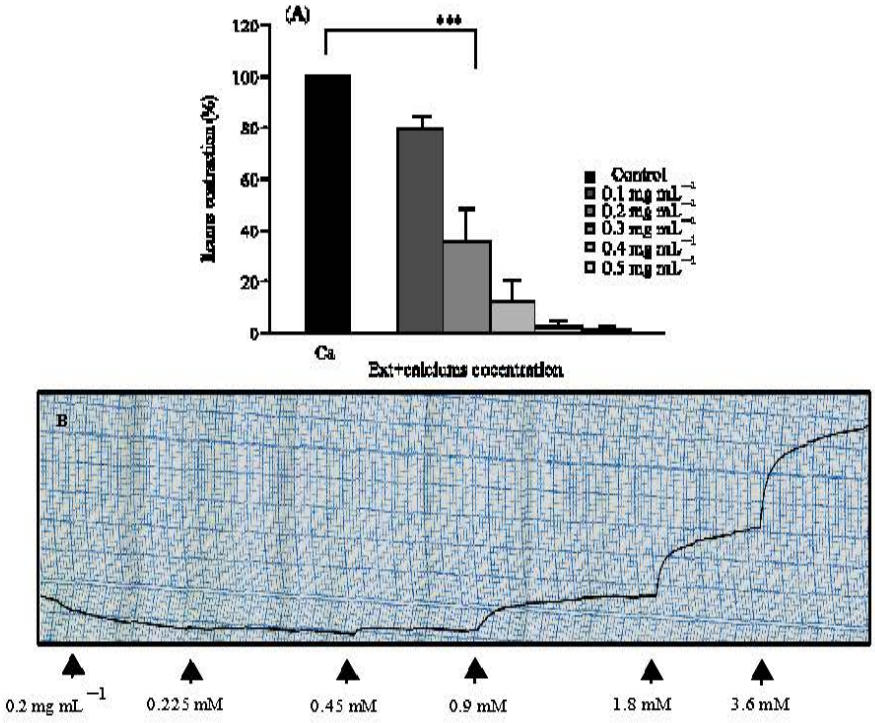

Fig. 6: The effect of various concentration of $CaCl_2$ on contraction of adult male rat's ileum at present of extract (n = 7); ***: p<0.001; (A) Histogram and (B) One sample of oscillogram

concentration of extract (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg mL^{-1}), then KCl 60 mM were added to the bath. The results indicated that the presence of alcoholic parsley seed extract, prevented from contraction-induced by KCl (n = 7, p<0.001) (Fig. 4).

The effect of various concentration of calcium on ileum's contraction in absent and present of extract: To investigate the possible mechanism responsible for the relaxation response induced by extract in the ileum, cumulative addition of various concentrations of $CaCl_2$ to the bath tissue that including high potassium and without $CaCl_2$, caused strong contraction (n = 7, p<0.001) (Fig. 5).The following this stage before adding $CaCl_2$, application different concentration of extract (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg mL^{-1}) at intervals of 5 min. The present results have provided evidence indicating that although in the low dose of extract, $CaCl_2$ caused contraction on ileum, but this effect were lesser than contractile response tissue in absent extract (n = 7, p<0.001) (Fig. 6).

DISCUSSION

This experiment showed that, alcoholic extract of *Petroselinum crispum* in a concentration-dependent manner inhibit rat ileum contraction induced by 60 mM KCl (Fig. 1). In addition, The inhibitory effects of all extracts were reversible after washing the tissue with fresh Tyrodes solution. These results confirm that the inhibitory effects observed, was not arising since fatigue smooth muscle ileum (Fig. 3). The parsley essential oil in current analysis characterized by the dominant presence of five Substance: myristicin, apiol, α-pinene, β-pinene and 1- ally 1, 2, 3, 4, 5-tetramethoxy-benzene (Hui *et al*., 2006). Essential oil of *Alpinia speciosa schum* (EOAS) possesses both relaxant and antispasmodic actions in ileum (Bezerra *et al*., 2000). Some reports indicated that the effects of the *Plectrantus barbatus* essential oil (PBEO) decreased the basal tonus of the ileum with a maximal response compared with inhibition achieved with α-pinene. They show that PBEO has intestinal relaxant and antispasmodic activity and suggest that it seems to occur down stream of calcium entry or release from internal stores. The main active principle for its relaxant and spasmolytic activity seems to be α-pinene (Camara *et al*., 2003). α-pinene and β-pinene two components of the *Ferula gummosa* essential oil (EGEO) and the hydroalcoholic extract had clear inhibitory effect on tonic contraction induced by KCl (Sadraie *et al*., 2001). The inhibitory effect of *Teucrium polium* essential oil on smooth muscle is due to an effect on calcium channels

(Aqel and Ghavaibeh, 1990). The inhibitory effects of essential oil from various plants with different components are qualitatively similar, they may all have a similar mechanism of action (Sadraie *et al*., 2001). The aqueous extract of *Pavetta crassipes* leaves exerted, a concentration-dependent inhibition of the spontaneous motility or elevated tone in these preparations. Their results indicate the presence of biologically active substance whose action might be mediated through calcium channels. A preliminary phytochemical screening of the leaf extract of *P. crassipes* revealed the presence of flavonoids, tannins an anthraquinones as possible candidates for such inhibition substances (Amos and Okwuasaba, 1998). The inhibitory effect of quercetin on the guinea-pig ileum contractile response, at least partially, due to blockade of calcium concentration by KCl. Quercetin inhibits the contractile response of ileum, decreasing the calcium concentration available for contractile machine (Galvez *et al*., 1996). There was a report that hydroalcoholic extract of *Pycnocycla spinosa* was shown to have spasmolytic action *in vitro* and antidiarroheal effect *in vivo*. The hydroalcoholic extract of *P. spinosa* is composed of alkaloid, flavonoid and saponin-rich components. Antispasmodic action of hydroalcolic extract of *P. spinosa* could be due to components in the alkaloid-rich fraction and to a lesser extract, to its flavonoid-rich fraction (Sadraie *et al*., 2003). Many scientists in their investigations have been shown that vasorelaxant action of the aqueous leaf extract of *Persea americana* on isolated rat aorta. The extract reduced vasoconstriction probably by inhibiting calcium influx through calcium channels (Owolabi *et al*., 2005). However, it seems that relaxation effect observed on rat isolated ileum contractions, was induced by present components (essential oil, α-pinene, β-pinene) of hydroalcoholic extract of *Petroselinum crispum.*

In order to survey mechanism the action of extract, the role of calcium on contraction induced by KCl was investigated. Also our results showed that various concentration of *Petroselinum* extract decreased the KCl-induced contraction in Tyrode solution, which including high K^+ (60 mM) and without $CaCl_2$ (Fig. 5). This result is similar as the result of many other investigators. For instance, as shown by Gharib Naseri *et al*. (2006), spasmogenic effect of cumulative concentration of calcium on rat colon in Ca-free with rich potassium Tyrode solution, was reduced by the hydroalcoholic extract of the grape leaf.

The contractile activity of smooth muscle can be controlled by numerous factors, including hormones, autonomic nerves, pacemaker activity and variety of drug. Like skeletal muscle or cardiac muscle, the contraction of smooth muscle is calcium-dependent and the agents listed above cause smooth muscle contraction by increasing intracellular calcium concentration. Smooth muscle also has an intracellular membrane network of Sarcoplasmic Reticulum (SR) that serves as an intracellular reservoir for calcium. Calcium can be released from the SR into the myoplasma when stimulatory neurotransmitters, hormones or drugs bind to receptors on the sarcolemma (Watras, 2004). Ca is a fundamental second messenger in smooth muscle cells. An increase in cytoplasmic Ca concentration and binding to calmodulin and the activation of myosin light chain kinase is the primary stimulus for contraction. The Ca used in the activation of the contractile apparatus enters the cytoplasmic compartment during periods of membrane depolarization, mechanical distortion, or stimulation by agonists. Contraction of smooth muscle is regulated by cytosolic Ca levels and sensitivity to Ca of the contractile elements in response to changes in the cell (Grasa *et al*., 2004; Ratz *et al*., 2005). There is evidence that there can be significant variation in the degree of participation of extracellular and intracellular Ca in smooth muscle contraction. Other results indicate that extracellular Ca participates in spontaneous activity and enters cytosol by L-type voltage-dependent Ca channels (Grasa *et al*., 2004). Calmodulin (CaM) plays important roles for contractile activity in smooth muscles. In gastrointestinal smooth muscles (GISM), CaM functions as the regular of contractile behavior and electrical rhythmicity. In GISM, contraction is mediated by the initial [Ca]i transient, resulting in Ca/Calmodulin-dependent activation of myosin light chain (MLC) kinase, phosphorylation of MLC20 and interaction of actin and myosin, whereas relaxation is mediated by cAMP-and or cGMP-dependent protein kinase which inhibit the initial [Ca]i transient and reduced the sensitivity of MLC kinase to [ca]i (Ohya and Horowitz, 2002). Studies done in 1984, with the use of intact smooth muscle tissue, showed that GPCR agonist (G protein-coupled receptors) can produce greater increase in force for a given increase in [ca]i than KCl. KCl-induced contraction has long been known to be due to membrane depolarization causing Ca entry through voltage-operated Ca channels (VOCCs), activation of Ca-dependent MLC kinase and increase in MLC phosphorylation (Ratz *et al*., 2005). Also there is evidence for that in guinea pig ileum and Taenia coli, high K^+ elicits an increase in [Ca]i and transient contractions. The spontaneous movements in longitudinal muscle of sheep duodenum persist in Ca-free solutions with EDTA for 20 min. Verapamil, nifedipine and diltiazem all of which are Ca antagonists, reduce the mechanical activity of rabbit ileum, rat duodenum, sheep duodenum and canine ileum. Results demonstrated that in the spontaneous contractions of rabbit intestinal muscle extra cellular Ca plays principles role in longitudinal and circular muscle, whereas both extra cellular and intra cellular Ca participate

in the Ach and KCl induced contractions in rabbit small intestine (Grasa *et al*., 2004). However, in order to suggest which the inhibitory effect on the contraction of ileum produced by the alcoholic extract of the parsley seeds results from blocking voltage-gated calcium channels. Although the relaxation role of parsley seed has been demonstrated, still the chemical nature and identity of the active ingredient(s) are unknown and will be investigated in a follow-up project.

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