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The Role of NO and cGMP in Antispasmodic Activity of *Ruta chalepensis* Leaf Extract on Rat Ileum

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Abstract: The aim of present study was to investigate the effect of hydroalcoholic extract of *Ruta chalepensis* (Rutaceae) leaves on rat ileum contractility and possible mechanism(s) involved. *Ruta chalepensis* extract was prepared by maceration method (ethanol 70%). Terminal portion of ileum (2 cm) was dissected out from male Wistar rats and mounted in an organ bath containing air bubbled Tyrode solution with 1 g initial tension and ileal contraction induced by KCl (60 mM) was recorded. The spasmolytic effect of the cumulative concentrations of extracts (0.01- 0.07 mg mL⁻¹) was reduced after tissue incubation with L-NAME (100 µM, 20 min). Methylene blue (30 µM) reduced the extracts (0.01-0.07 mg mL⁻¹) spasmolytic effect (p<0.001). Furthermore, it seems that the portion relaxatory effect of Rue extract on the rat ileum may be due to nitric oxide and the antispasmodic activity of the extract was mainly through a cGMP-dependent mechanism.

Key words: *Ruta chalepensis*, nitric oxide, ileum, antispasmodic

INTRODUCTION

Ruta features mainly shrubby plants, native to the Mediterranean region and present in traditional medicine of this region since Antiquity (Pollio *et al.*, 2008). Rue grows in the North of Boshehr (South of Iran) (Mozaffarian, 1999). *Ruta chalepensis* L. (Rutaceae) is a perennial herb widely used in folk medicine as an antirheumatic and antispasmodic (El-Sayed *et al.*, 2000) emmenagoges and abortifacients (Gonzales *et al.*, 2007). Phytochemical screening of the aerial parts of the plant showed the presence of alkaloids, flavonoids, coumarins, tannins, volatile oil, sterols, triterpenes (Al-Said *et al.*, 1990). Fenilpropanoids (anethol glycol), benzenoids (anisic acid), quinoline, acridone and terpenoids (Mancebo *et al.*, 2001).

It has been reported that hydroalcoholic extract of *Ruta chalepensis* inhibits rat ileum contraction induced by KCl (Moazedi *et al.*, 2008). Moreover, it has been shown that extract of the aerial parts of Rue on the Central Nervous System (CNS) induces depressant effects on the CNS (Gonzalez-Trujano *et al.*, 2006). Rue extract reduced LPS-induced (lypopolisaccharide), blood levels of nitrite, an indicator of nitric oxide production. Rue extract has an anticholinergic action on guinea pig small intestine then, the relaxatory and inhibitory effect of Rue extract on motility on small intestine has been also reported

by Molina *et al.* (1991). However, there is little information regarding the underlying mechanism that mediated its spasmolytic action. The aim of the present study was therefore, to investigate the possible mechanism that mediated the effects of *Ruta chalepensis* leaf hydroalcoholic extract (RCE) on rat ileum.

MATERIALS AND METHODS

Plants material: *Ruta chalepensis* were collected (march 2007) from the North of Boshehr (South of Iran). The plants were identified by Boshehr Natural Resource Center. The leaves were dried under shade and powder was extracted by maceration method using 70% alcohol for 72 h at room temperature and mixed occasionally daily (Gharib-Naseri and Heidari, 2007). The mixture was then filtered (Whatman No. 1), filtrate was concentrated and dried at room temperature to obtain a dark green gummy paste (yield: 30%). The extract was stored at 4°C until being used and dissolved in bath solution before using in experiments.

Chemicals and reagents: Acetylcholine, N^o-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma (USA). Methylene blue and solutes of Tyrode solution were purchased from Merck (Germany).

Animals: All rats used in this study were treated in accordance with principals and guidelines on animal care of Shahid Chamran University. Male Wistar adult rats (200-250 g) were obtained from animal house of the same university and kept at 12 h light/dark cycle and at 20-24°C with free access to food and water. Rats were starved of food but not water for 24 h before experiment.

Ileum preparations: On the day of experiment the rats were sacrificed by sharp blow on the head. A piece (2 cm) was prepared from the terminal ileum (taken within a distance of 2-3 cm from caecum) and mounted in an organ bath containing Tyrode solution (10 mL) between two stainless steel hooks vertically. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer (Harvard transducer, UK) connected to a recorder (Harvard Universal Oscillograph, UK). The Tyrode solution (pH 7.4, 37°C) composition was (in mM): NaCl (139.9), KCl (2.68), CaCl₂ (1.8), MgCl₂ (1.05), NaHCO₃ (11.9), NaH₂PO₄ (0.42) and glucose (5.55) (Sadraei *et al.*, 2003) which continuously was bubbled with air. The initial tension was 1 g throughout the experiment and equilibrium period was 60 min during which, the bath solution was refreshed every 15 min. Ileum was contracted with 60 mM of KCl (Nasu *et al.*, 1994; Gharib-Naseri and Heidari, 2007). Also, cumulative concentration (0.01-0.07 mg mL⁻¹) of CRE was added to the organ bath when plateau of ileum contraction induced by KCl was achieved. The same procedures were carried out after tissue incubation with L-NAME (20 min, 100 µM) as nitric oxide synthase inhibitor (Gharib Naseri *et al.*, 2007) or after incubation with methylene blue (30 min, 30 µM) as a cyclic guanylate cyclase inhibitor (Ekblad and Sundler, 1997). Separate ileum preparations were used for each inhibitor. The volume of all chemicals added to the tissue bath was not more than 5% of organ bath volume.

Statistical analysis: The plateau of ileal contraction induced by KCl was regarded as 100% and percentage of the relaxation was calculated from changes in contraction. Results were expressed as Mean±SEM for n experiments (n indicates the number of tissue and coincides with number of animals). Statistical significance of differences between two means was assessed by Student's t test and multiple means were compared by analysis of variance (ANOVA). A p-value<0.05 were considered as significant.

RESULTS

Effect of CRE on KCl-induced contraction in the absence and presence of L-NAME: Cumulative concentration of CRE reduced the KCl-induced ileal contraction in a

concentration dependent manner. However, tissue incubation with L-NAME (20 min, 100 µM), caused a significant reduction (p<0.001, n = 7) in the antispasmodic activity of CRE as shown in Fig. 1. Representative traces of CRE spasmolytic effect on KCl-induced ileum contraction in the absence and presence of L-NAME is shown in Fig. 3a and b.

Effect of CRE on KCl-induced contraction in the absence and presence of methylene blue: Incubation of ileum preparation with (30 min, 30 µM, n = 8) as a cyclic guanylate cyclase inhibitor reduced (p<0.001) the extracts (0.01-0.07 mg mL⁻¹) spasmolytic effect on KCl-induced ileal contraction, as shown in Fig. 2. Representative trace

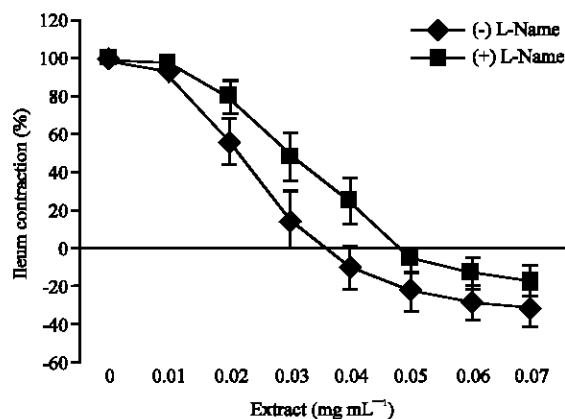


Fig. 1: Antispasmodic effect of *Ruta chalepensis* leaf extract on rat ileal contractions induced by KCl (60 mM, n = 7) before (-) and after (+) tissue incubation with L-NAME (20 min, 100 µM, n = 7). Inhibition nitric oxide synthase has altered the spasmolytic effect of the extract (***)p<0.001)

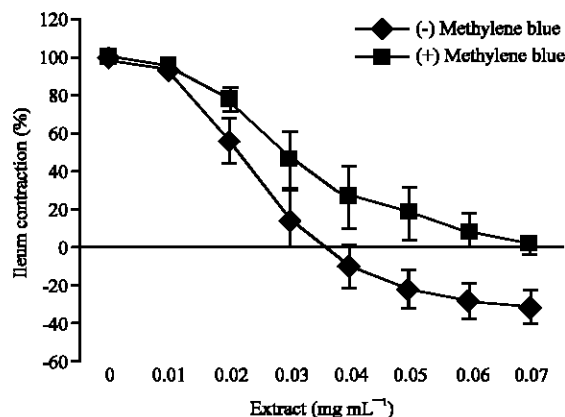


Fig. 2: Antispasmodic effect of RCE on rat ileal contractions induced by KCl (60 mM, n = 9) before (-) and after (+) tissue incubation with Methylene blue (30 min, 30 µM, n = 8)

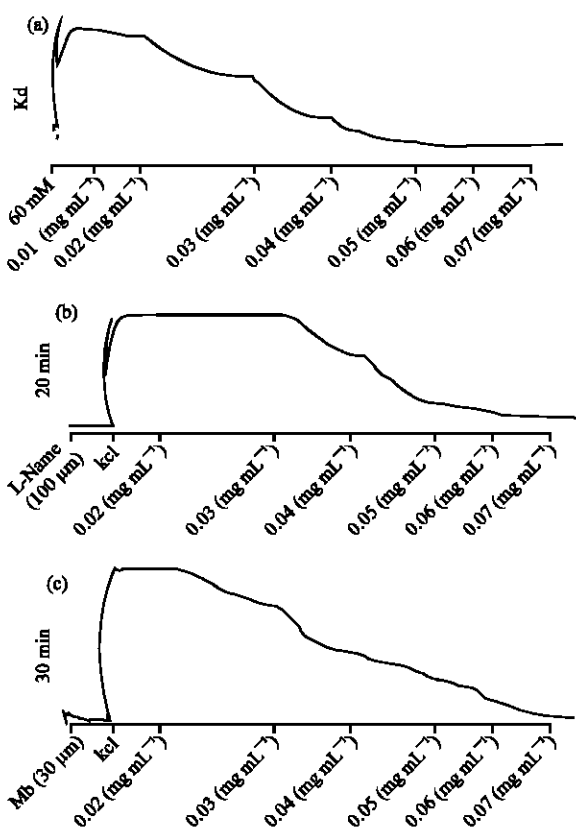


Fig. 3: Representative traces of cumulative concentration of *Ruta chalepensis* leaf extract on KCl-induced contraction ileum (a) in the absence, (b) in the presence of L-NAME and (c) methylene blue

of CRE spasmolytic effect on KCl-induced ileum contraction in the presence of methylene blue is shown in Fig. 3c.

DISCUSSION

The communication network of the Enteric Nervous System (ENS) involves many modulators such as acetylcholine, nitric oxide, ATP, VIP, opioid peptides, neuropeptide Y and 5HT (Holzer, 2004). The KCl is often used as a tool to bypass G-Protein Coupled Receptor (GPCR) stimulation and activate smooth muscle by changing the K^+ equilibrium potential and clamping membrane potential at some value above the resting level (Ratz *et al.*, 2005). The depolarization induced by high potassium concentration activates the L-type Voltage Dependent Calcium Channels (VDCCs) (Karaki *et al.*, 1997). It has been reported that CRE reduces the ileal contractions induced by KCl and acetylcholine (Moazedi *et al.*, 2008). Some reports indicate that Rue has

an anticholinergic action on the guinea pig small intestine and this effect has been reversible (Molina *et al.*, 1991). Influx of extracellular calcium, in part is through L-type calcium-channels (Aronsson and Holmgren, 2000). Furthermore, it has suggested that substances, which inhibit the Ach-induced contraction, act by blocking L-type Voltage Dependent Calcium Channel (VDCCs) (Gilani *et al.*, 2001) which they exist in the rat intestine (El-Bardai *et al.*, 2004). Certainly, the contractions induced by KCl are dependent on the entry of Ca^{2+} into the cells through voltage-dependent calcium channels, therefore a substance which can inhibit high K^+ -induced contraction is, considered to be a Ca^{2+} channel blocker (Cortes *et al.*, 2006). Although, the extract antispasmodic effect was clarified but to reveal other mechanisms in this action, the remaining experiments was carried out.

Nitric oxide (NO) is a major inhibitory nonadrenergic noncholinergic (NANC) neurotransmitter in the gastrointestinal tract (Michiru *et al.*, 2002). In the rat intestine, nitric oxide has been suggested to participate in nonadrenergic noncholinergic (NANC) relaxation of longitudinal muscle (Akiko *et al.*, 2005). The NO is synthesized from L-arginine by activation of nitric oxide synthase (Hamad *et al.*, 2003). In the present study, the cumulative concentration hydroalcoholic extract of *Ruta chalepensis* on KCl-induced contraction reduced in the presence nitric oxide synthase inhibitor (L-NAME). This effect indicated that at least, NO production was involved in the extract activity.

Nitric oxide relaxes ileum via increasing cGMP synthesis (Kanada *et al.*, 1992; Ijioma *et al.*, 1995). Recent evidence suggests that cGMP is also an important second messenger in airway smooth muscle with important relaxant effect (Hamad *et al.*, 2003). Since, in the present study the extract inhibitory effect was attenuated by methylene blue as a cyclic guanylate cyclase inhibitor, it suggests the extract act through cGMP pathway. It is possible to assume that antispasmodic effect of *Ruta chalepensis* leaf extract may be due to the presence of cGMP-dependent NO-mediated relaxation in tissue.

CONCLUSION

Our results suggest that the extract induces ileal relaxation mainly through blocking calcium influx and partly by elevating in NO and cGMP production.

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