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The Role of Opioid and α -adrenergic Receptors in the Ileal Antispasmodic Activity of *Ruta chalepensis* Extract

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Abstract: The aim of present study was to investigate the role of opioid and α -adrenergic receptors in antispasmodic activity of *Ruta chalepensis* (L.) hydroalcoholic extract (RCHE) in rat ileum contractions induced by KCl. A piece of ileum (2 cm) was dissected out from male Wistar rats and mounted in an organ bath containing air bubbled Tyrode solution (37°C). Isotonic Transducer is exceptionally sensitive and stable and connected to Harvard Universal Oscillograph (UK). The tissue was under 1 g initial tension and contractions were recorded isotonicly. The RCHE was prepared by macerated with alcohol (70%). Ileum was contracted by KCl (60 mM) and cumulative concentrations of RCHE were applied (0.01-0.07 mg mL⁻¹) before and after tissue incubation (30 min) with naloxon (1 μ M) or phentolamine (1 μ M). RCHE reduced KCl-induced contraction in a concentration dependent manner (p<0.001). However, tissue incubation with naloxone and phentolamine attenuated the spasmolytic effect of RCHE (p<0.001). We conclude that in the antispasmodic activity of RCHE, at least in part, the opioid and α -adrenergic receptors are involved.

Key words: Antispasmodic, *Ruta chalepensis*, opioid receptors, α -adrenergic receptors, rat, Ileum

INTRODUCTION

Ruta is a genus of Rutaceae family (Pollio *et al.*, 2008), with sixty species is native to the Mediterranean and western Asia and belongs to Rutaceae family. Rue is well known since ancient times and frequently mentioned in the literature (Al-Sagair, 2004). *Ruta chalepensis* is a rich source of important secondary metabolites (Gunaydin and Savci, 2005). Photochemical of *Ruta chalepensis* (L.) study showed the presence of alkaloids, flavonoids, coumarins, tannins, volatile oil, sterols and/or triterpenes (Al-Said *et al.*, 1990), two quinoline alkaloids (El-Sayed *et al.*, 2000) and rich source of furanocoumarins (Gunaydin and Savci, 2005). *Ruta chalepensis* induces sedative-hypnotic potentiation, anxiolytic, anticonvulsant and antinociceptive effects (Gonzalez-Trujano *et al.*, 2006). Traditionally is believed that rue is useful for rheumatic pain, amenorrhoea, hysteria (Al-Bakri and Afifi, 2007) and anti-inflammatory effect (Iauk *et al.*, 2004). It has been reported that hydroalcoholic extract of *Ruta chalepensis* in a concentration-dependent manner inhibits rat ileum contraction induced by KCl (Moazedi *et al.*, 2008). Rue extract has an anticholinergic action on the small intestine of male guinea pigs (Molina *et al.*, 1991). Despite the traditional use and some scientific studies on this herb, certain its properties still have not been carried out. The aim of the present study, therefore, was

to investigate the role of opioid and α -adrenergic receptors involvement in the spasmolytic effect of hydroalcoholic *Ruta chalepensis* leaf extract on isolated rat ileum.

MATERIALS AND METHODS

Chemicals: Naloxone and phentolamine were purchased from Tolidaro Company (Iran) and Sigma (USA) respectively. All chemicals for Tyrode solution were purchased from Merck (Germany). Tyrode solution composition was (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). Extract, phentolamine and naloxone were dissolved in Tyrode solution and sum of volumes which added to tissue bath was not more than 0.5 mL.

Preparation of extract: *Ruta chalepensis* was collected in March 2006 from the north of Boshehr (South of Iran). The plant was identified by academic staff of Boshehr Natural Resource Center. The herb was dried under shade and leaves were powdered by electric grinder and powder was extracted by maceration using 70% ethanol for 72 h at room temperature (Naseri and Heidari, 2007). After filtration (Whatman No.1), solvent was evaporated. The extract was stored at 4°C until further use. A dark green

sticky extract was obtained (yield 30% W/W with respect to the starting crude material). All the concentrations are the final concentrations in the organ bath.

Animals and tissue preparation: This study has been done from April 2007 to May 2008 Male Wistar rats (200-250 g) were obtained from Animal Facility of Jundishapur Ahwaz University of Medical Sciences and housed at 20-24°C under 12/12 h light/dark cycle and had free access to food and water. Rats were starved but not banned from water for 24 h before experiment. All experiments procedures describe below were approved by Ethical Committee of Ahwaz Shahid Chamran University. Rats were sacrificed by a sharp blow on the head and a segment (2 cm) was dissected from the terminal ileum and rinsed intraluminally with cold oxygenated Tyrode solution. The ileum was suspended in an organ bath (10 mL) containing Tyrode solution (37°C, pH = 7.4) between two stainless steel hooks and solution was bubbled with air continuously. The lower hook was fixed at the bottom of the organ bath and the upper one was connected to an isotonic transducer (Harvard transducer, UK). The ileal contractions were recorded (Universal Harvard Oscillograph, UK) under 1 g resting tension, after 60 min equilibrium period during which, the tissue bath solution was refreshed every 15 min. Each ileal preparation was contracted by KCl 60 mM (Nasu *et al.*, 1994) until stable responses were observed. Once the plateau was achieved, the extract applied cumulatively (0.01-0.07 mg mL⁻¹) and the result was accepted as control. In separate experiments the RCHE was studied after 30 min tissue incubations with 1 µM naloxone or with 1 µM phentolamine (Naseri and Heidari, 2007) as opioid receptor antagonist and α-adrenoceptor antagonist, respectively.

Statistical analysis: Plateau of ileal contraction induced by KCl was assumed as 100% and reductions induced by extract calculated. Percentage of ileal relaxation was expressed as Mean±SEM. Results were analyzed using one way and two ways Analysis of Variance (ANOVA) and differences between mean accepted significant at 0.05.

RESULTS

Effect naloxone on RCHE spasmolytic activity in rat ileum: Cumulative concentration of RCHE (0.01-0.07 mg mL⁻¹) attenuated the ileal contraction in a concentration dependent manner (n = 7, one way ANOVA, p<0.001). After tissue incubation with naloxone, however, the extract reduced the KCl-induced ileal contraction (n = 7, one-way ANOVA, p<0.001). The comparison these two responses indicated that the

presence of naloxone has reduced the relaxatory potency of the extract (n = 7, two-ways ANOVA, p<0.001). The results are presented in Fig. 1. Actual recording are presented in Fig. 3a and b.

Effect of phentolamine on RCHE antispasmodic effect in rat ileum: As it has been shown in Fig. 2, after tissue

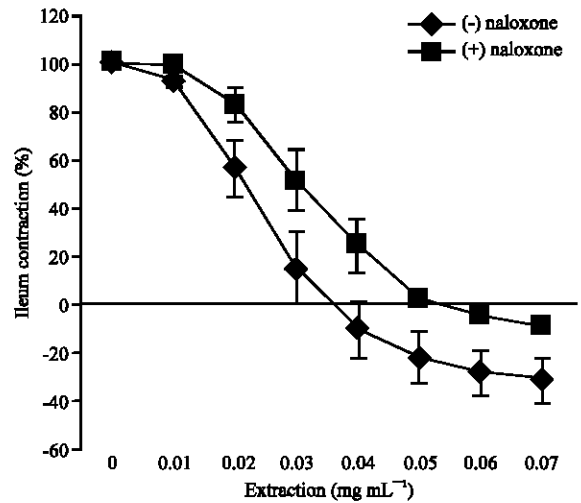


Fig. 1: Effect of cumulative concentration of *Ruta chalepensis* leaf extract on KCl (60 mM)-induced rat ileal contraction before (-) and after (+) tissue incubation with naloxone (30 min, 1 µM, n = 7). Two-ways ANOVA analysis has shown that naloxone attenuated (p<0.001) the antispasmodic effect of the extract

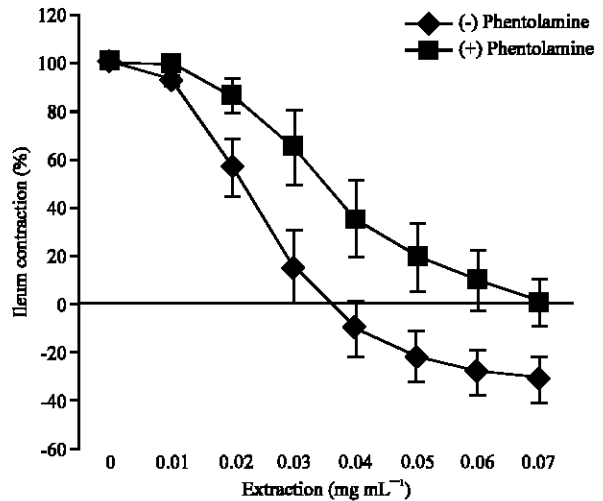


Fig. 2: Antispasmodic effect of *Ruta chalepensis* (L.) leaf extract on rat ileal contractions induced by KCl (60 mM, n = 7) before (-) and after (+) tissue incubation with phentolamine (30 min, 1 µM, n = 7). Phentolamine reduced the antispasmodic effect the extract (two-way ANOVA, p<0.001)

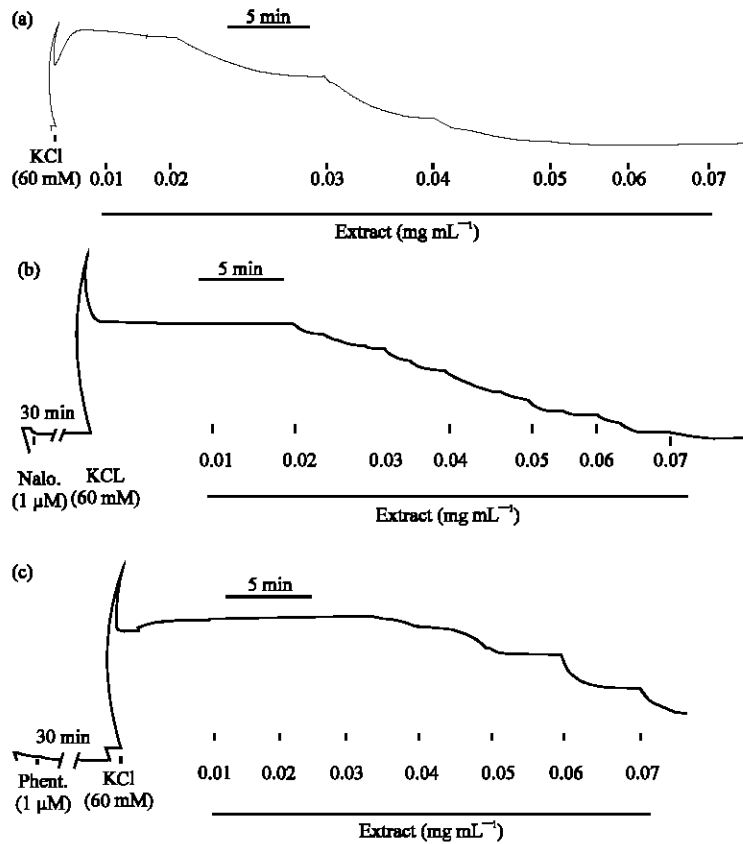


Fig. 3: Representative traces of cumulative concentration of *Ruta chalepensis* (L.) leaf extract on KCl-induced contraction in rat ileum in the (a) absence in (b) the presence of naloxone and (c) phentolamine

incubation with phentolamine (30 min, 1 μM , n = 7) cumulative concentrations (0.01-0.07 mg mL^{-1}) of *Ruta chalepensis* leaf hydroalcoholic extract attenuated (one-way ANOVA, $p < 0.001$) the KCl-induced ileal contraction. The antispasmodic activity of the extract, however, has been reduced as compared with its effect when phentolamine was absent in the media (two-ways ANOVA, $p < 0.001$). Representative trace of RCHE spasmolytic effect on KCl-induced ileum contraction in the presence of phentolamine is shown in Fig. 3c.

DISCUSSION

The results of the present study demonstrated that *Ruta chalepensis* (L.) leaf extract spasmolytic activity was reduced by rat ileal preparation incubation with naloxone and phentolamine.

The alimentary canal is equipped with the largest collection of neurons outside the Central Nervous System (CNS), the gut can rightly be considered as a neurological organ (Holzer, 2004). The communication network of the Enteric Nervous System (ENS) involves acetylcholine,

tachykinins (substance P, neurokinin A), Nitric Oxide (NO), adenosine triphosphate, Vasoactive Intestinal Polypeptide (VIP), opioid peptides, neuropeptide Y and 5-hydroxytryptamine (5-HT) as major transmitters (Holzer, 2004).

High extracellular potassium has long been used as a convenient stimulus to activate smooth muscle cells by a highly reproducible and relatively simple mechanism involving activation of voltage-operated Ca^{2+} channels that leads to increase the cytosolic free Ca^{2+} (Ratz *et al.*, 2005). Rue leaf hydroalcoholic extract is a potent relaxant for contractions induced by acetylcholine (receptor operating agonist) and KCl (non-receptor operating agonist) in rat ileum (Moazedi *et al.*, 2008). Administration of Rue extract (20%) before ACh in different segments of small intestine from male guinea pig inhibited the contraction induced by ACh (Molina *et al.*, 1991). Furthermore, following washing, the inhibitory activity was totally reversible and tissue responded to KCl, suggesting that these effects are membrane mediated. Other reports have also shown that rue extract antagonizes acetylcholine effect on different segments of

guinea pig small intestine which could be reversed by washing (Molina *et al.*, 1991). This report however is consistent with our results.

The antispasmodic effect of RCHE on KCl-induced contractions has been shown in our previous study and the role of voltage operated calcium channel was postulated (Maozedi *et al.*, 2008) however, the present study revealing the possible roles of opioid and α -adrenergic receptors.

The L-type Ca^{2+} channels require relatively strong depolarization for activation (Beech, 1993). 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).

The opioid receptor system consists of three types of heterogeneous, G-protein-coupled, opioid receptors mu (μ), delta (δ) and kappa (κ), which have been pharmacologically characterized and cloned (Metcalf and Coop, 2005). Immunohistochemical studies have revealed that all opioid receptor subtypes are present in neural tissue of rat Enteric Nervous System (ENS), but not in smooth muscle cells (Gray *et al.*, 2005). With respect to transit in rats, in vivo studies employing the charcoal meal method have indicated that μ and δ receptor activation causes transit slowing, but κ receptor activation has little or no effect. In apparent contrast, an in vitro study indicated that both μ and δ receptor activation had an inhibitory influence on the peristaltic reflex of the rat ileum (Gray *et al.*, 2005). It has been reported that μ -opioid receptors are located on myenteric plexus neurons (Fichna *et al.*, 2007). In the rat, μ -opioid receptors has been observed in neurons of both the submucosal and myenteric plexus and in fibers distributed to the muscle, vasculature and mucosa, as well as in presumed interstitial cells of Cajal in the myenteric plexus and deep muscular plexus (Sternini, 2001). Endogenous opioid peptides were identified with a gastrointestinal bioassay in which opioid receptor agonists are quantified by their ability to inhibit electrically evoked contractions of the guinea-pig ileum (Holzer, 2004). Endogenous opioids are known to circulate in low levels in the plasma of many mammals, including humans and rats (Dehpour *et al.*, 2000). Morphine inhibited gastrointestinal functions, transit and gastric emptying, in a naloxone-reversible manner (Fichna *et al.*, 2007). Comparison of inhibitory effect of *Ruta chalepensis* extract in absent and presence of naloxone as a non-selective opioid receptors antagonist showed a significantly reduction in the spasmolytic activity of

RCHE (Fig. 1). We may conclude therefore, that some part of antispasmodic effect of RCHE is carried out via opioid receptors. This result is consistent with a study that reports *Ruta chalepensis* induces antinociceptive activity in writhing test in mice (Gonzalez-Trujano *et al.*, 2006) and traditionally believed. Furthermore, it has been demonstrated that voltage-gated calcium channels are involved in ascending pain pathways (Zamponi *et al.*, 2009). Therefore, it may conclude that voltage-gated calcium channels are involved in both spasmolytic and antinociceptive activities of RCHE.

Gastrointestinal motility is also affected by adrenergic modulation and it is been shown that contractile activity in the rat ileum is mediated primarily by muscular β_3 , β_2 and α_1 -receptor mechanisms (Seiler *et al.*, 2005).

α -adrenoceptors were initially classified into α_1 and α_2 subtypes on the basis of obvious differences in location and function as well as differences in the affinities of agonists and antagonists (Liu and Coupar, 1997). adrenergic inhibitory motor mechanisms in rat jejunum and ileum occurring preferentially at the level of these smooth muscle cells rather than in the enteric nervous system (Seiler *et al.*, 2008). Postsynaptic α_1 -adrenoceptors involved in mediating relaxation in both muscle layers (Liu and Coupar, 1997). Generally, the role of α receptors seems to differ between species and anatomical regions of the gut. In rabbit jejunum, only α_1 but not α_2 receptors mediate inhibition and in canine jejunum, equine jejunum and rat colon, only α_2 but not α_1 inhibitory mechanisms have been described (Seiler *et al.*, 2008). Recently, it has been shown that the antispasmodic activity of RCHE on rat ileal contraction, the β -adrenoceptors were involved (Moazedi *et al.*, 2008, new article published in pakistan). Collectively, the present study the RCHE inhibitory effect was reduced by phentolamine (α -adrenoceptor antagonist) as shown in Fig. 2 which indicates that, at least in part, α -adrenoceptors were involved in the extract activity.

Collectively, the antispasmodic activity of *Ruta chalepensis* leaf extract possibly has been carried out mainly through voltage operated calcium channels and in part at least, via opioid and α -adrenergic receptors.

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