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Original Article

Antispasmodic Activity of Onion (*Allium cepa* L.) Peel Extract on Rat Ileum

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Abstract

Onion (*Allium cepa*) bulb from Liliaceae has antioxidant, spasmolytic and antihypertensive activities. The aim of present study was to investigate the antispasmodic effect of onion peel on rat ileum contractility. Onion peel powder was extracted by maceration in 70% alcohol for 72 h. A terminal portion of ileum from male Wistar rat was dissected and its contractions were recorded isotonically in an organ bath containing Tyrode solution (37 °C) under 1 g tension. Extract cumulative concentrations (0.1, 0.2 and 0.4 mg/ml) reduced the ileum contractions induced by KCl (60 mM) and carbachol (10 μM) dose-dependently ($P<0.0001$). The extract antispasmodic effect was not reduced by tissue incubation with propranolol (1 μM, 30 min), naloxone (1 μM, 30 min), L-NAME (100 μM, 20 min), glibenclamide (10 μM, 5 min) neither by tetraethylammonium (1 mM, 5 min). In Ca^{2+}-free with high K^+ (60 mM) Tyrode solution, extract reduced the ileum contractions induced by $CaCl_2$ dose-dependently ($P<0.05$, $P<0.01$). Onion peel extract inhibits ileum contractions without involving β-adrenoceptor, opioid receptor, nitric oxide production, and potassium channels activation. It is suggested that quercetin in onion peel extract induces spasmolytic effect via calcium channels.

Keywords: Spasmolytic; *Allium cepa* peel; Rat; Ileum.

Introduction

Onion (*Allium cepa*) bulb from Liliaceae has hypotensive (1), antioxidant (2) but boiling reduces the later property (3). The onion antioxidant effect was more potent than vitamin E (4) and its antioxidant activity is probably involves increased saving NO in L-NAME induced-hypertensive rats (5). Onion has a preventive activity against cancer (6, 7) and flavonoids extracted from onion peel improve male sexual function (2, 8). The antispasmodic activity of saponins from bulbs of red onion has been also reported (9) but this effect has not been shown on onion peel. Furthermore, red onion peel contains abundant flavonoids such as quercetin (2, 10) which is a calcium-antagonist (11).

Therefore, the aim of present study was to investigate the spasmolytic effect of red onion peel hydroalcoholic extract (OPE) on rat ileum contractility and the mechanism (s) involved.

Experimental

Extract preparation

Bulbs of red onion were obtained in a local market in March 2007. The voucher specimen of onion was identified by Dr. Aghel from Department of Pharmacognosy, Ahwaz Jundishapur University of Medical Sciences.

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The onions were peeled off and peels were then powdered and macerated with 70% ethanol (5% W/V) for 72 h at room temperature. After filtration, solvent was evaporated under vacuum. A dark red powder was obtained (yield 17.3% W/W with respect to the starting crude material).

Chemicals

Propranolol, L-NAME (anitric oxide synthase inhibor), glibenclamide (Glib) and tetraethylammonium (TEA) from Sigma (USA), naloxone from Tolidaru (Iran) and other chemicals from Merck (Germany) were purchased. All chemicals and extract were dissolved in the Tyrode solution and the total volume of all solutions were added to the organ bath was less than 5% of the bath volume. DMSO (50 µl) was added to glibenclamide and dilution was made by Tyrode solution. The final concentration of DMSO in the organ bath was 0.005% (W/W).

Animals

Male Wistar rats (178.5±5.2 g) were obtained from Ahwaz Jundishapur University of Medical Sciences animal house and kept at 12 h light/dark cycle and at 20-24 °C with free access to food and water. Rats were starved but not banned from water for 24 h before experiment. All experimental procedures described below were approved by the Ethical Committee of Ahwaz Jundishapur University of Medical Sciences.

Ileum tissue preparation

Rats were sacrificed by a sharp blow on the head and a segment (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 1 g initial tension. 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 Harvard Universal Oscillograph (UK). The Tyrode solution composition (pH 7.4 and 37 °C) was (in mM): NaCl (136); KCl (5); $CaCl_2$ (2); $NaHCO_3$ (11.9); $MgCl_2$ (0.98) NaH_2PO_4 (0.36) and glucose (5.55) which bubbled with air. The equilibrium period was 60 min and the bath solution was refreshed every 15 min. After equilibrium period, the ileum was contracted by KCl (60 mM) or carbachol (CCh, 10 µM) and once the plateau was achieved, the extract (0.05-0.8 mg/ml) applied cumulatively.

In separate experiments the OPE effect was studied after 30 min tissue incubations with 1 µM of propranolol or naloxone, non-selective β-adrenoceptors and opioid receptors antagonists respectively, or after 20 min incubation with 100 µM of L-NAME. Furthermore, in Ca^{2+}-free and rich KCl (60 mM) Tyrode solution, the tissue was contracted by cumulative concentrations of $CaCl_2$ (0.225-2.7 mM) before and after tissue incubation (3 min) with the extract (0.0125-0.1 mg/ml). In separate experiments, the antispasmodic effect of the extract was evaluated after tissue incubation (5 min) with glibenclamide (10 µM) or tetraethylammonium (1 mM) as K^+ channels blockers.

Statistical analysis

Ileal contractions induced by spasmogens (KCl and carbachol) were assumed as 100% and reductions induced by extract calculated. Percentage of ileal relaxation or contraction was expressed as mean±SEM. Results were analyzed using one and two way analysis of variance (ANOVA). Data was further subjected to LSD post hoc test and differences between means accepted significant at 0.05.

Results and Discussion

Onion peel extract (OPE) reduced the ileum contractions induced by KCl (60 mM) and CCh (10 µM) in a dose dependent manner (ANOVA, $P<0.0001$). The IC_{50} of KCl-induced contraction KCl was 0.161±0.016 mg/ml versus 0.314±0.053 for CCh-induced contraction which were significantly different ($P<0.01$). Two-way ANOVA test showed that these two response curves are significantly different ($P<0.001$). The results are shown in Figure 1. Incubation tissue preparation with propranolol (30 min, 1 µM), naloxone (30 min, 1 µM) did not attenuate the OPE antispasmodic effect but rather increased the extract antispasmodic effect (two-way ANOVA, $P<0.01$). Furthermore, tissue incubation with L-NAME (20 min, 100 µM) potentiated the extract spasmolytic effect (two-way ANOVA, $P<0.0001$). The

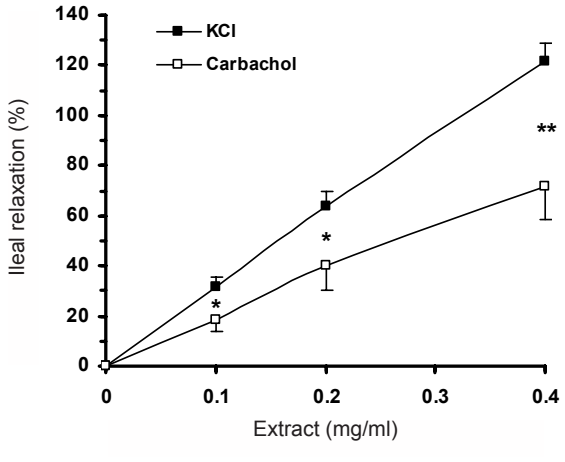

Figure 1. Antispasmodic effect of cumulative concentrations of OPE on rat ileal contractions induced by KCl (60 mM) and CCh (10 μM).* P<0.05, ** P<0.01, n=7 and 9 respectively.

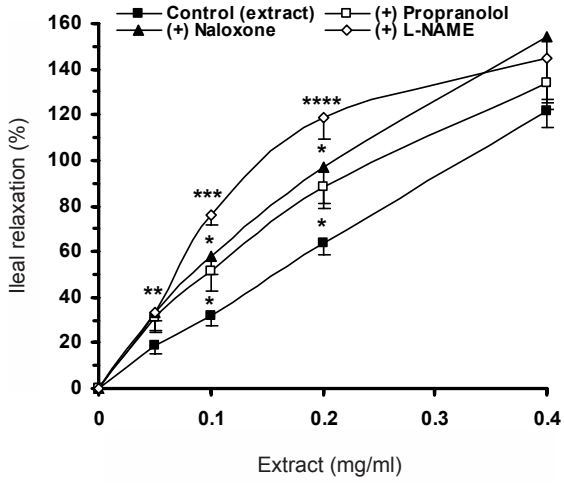

Figure 2. Spasmolytic effect of OPE on rat ileal contractions induced by KCl (60 mM) prior (control) and after tissue incubation with propranolol (30 min, 1 μM, n=7), naloxone (30 min, 1 μM, n=8) or L-NAME (20 min, 100 μM, n=9). Percentage of relaxation evoked by each extract concentration in the presence of propranolol, naloxone or L-NAME was compared with control condition (absence of these compounds). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

statistical comparisons of statistical analysis of each extract concentration for each condition are presented in Figure 2. In Ca^{2+}-free with high K^+ (60 mM) Tyrode solution, applying cumulative concentrations of $CaCl_2$ (0.225-2.7 mM) induced ileal contractions dose dependently (One-way ANOVA, P<0.0001) as seen in Figure 3. Ileum incubation (3 min) with OPE (0.0125, 0.025, 0.05 or 0.1 mg/ml) attenuated these contractions dose-dependently. The contractions in the absence and in the presence of extract (0.0125 mg/ml) were significantly different (Two-way ANOVA, P<0.0001, n=7). Incubation of ileum preparation (5 min) with glibenclamide (10 μM) or tetraethylammonium (1 mM) did not attenuated the OPE antispasmodic effect of OPE (0.1-0.4 mg/ml) on CCh-induced contraction but rather potentiated this effect (Two-way ANOVA, P<0.0001 and P<0.01 respectively). In Figure 4, percentage of evoked relaxation by each extract concentration in the presence of these K^+ channel blockers are compared with control condition (absence of these blockers).

The spasmolytic effect of OPE was not reversible completely, therefore, the spasmolytic effect was possibly accompany with binding to Ca^{2+} channels or/and entering to the smooth muscle cells. The reduction of KCl- and CCh-induced contractions by OPE was not result of the tissue tiredness since recording (25 min) contraction-induced by these spasmogens was not accompany with reduction in contraction. Intracellular Ca^{2+} elevation in smooth muscle is the main regulating factor of tension (12). In KCl-induced contractions, the voltage dependent calcium channels (VDCCs) are involved (13) and the existence of L-type VDCCs in rat ileum has been reported (14). It has been suggested that those substances that inhibit the KCl-induced contractions act via blocking these channels (15). On the other hand, CCh as a muscarinic receptor agonist (16) activates receptor-operated Ca^{2+} channel, elevates $[Ca^{2+}]_i$ and induces contraction (17) via M_2 and M_3 receptors (18). CCh also activates phospholipase C and enhances inositol triphosphate (IP_3) production (19) followed by promotion of Ca^{2+} release from intracellular Ca^{2+} pools (sarcoplasmic reticulum).

The spasmolytic effect of OPE on the KCl-induced contraction was more potent than on the CCh-induced contraction that may suggests, the extract inhibits the influx of Ca^{2+} without affecting the releasing calcium from intracellular pools by CCh. These two spasmogens are common in elevating $[Ca^{2+}]_i$ via calcium channels. Therefore, it is possible that the OPE reduced the ileum contraction via blocking these channels. The results of extract spasmolytic effect on $CaCl_2$-induced contractions support this possibility. In

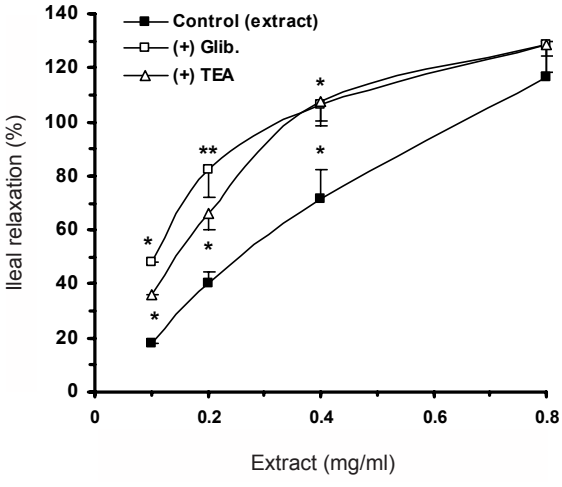

Figure 3. Spasmogenic effect of $CaCl_2$ on rat ileum in Ca^{2+}-free with high K^+ Tyrode solution prior (0.0 mg/ml) and after tissue incubation (3 min) with different concentrations of OPE (n=7). Ileal contraction induced by $CaCl_2$ (2.7 mM) was calculated as 100%. The statistical comparison was carried out between the extract concentrations at 0.0 and 0.0125 mg/ml. *P<0.05, **P<0.01.

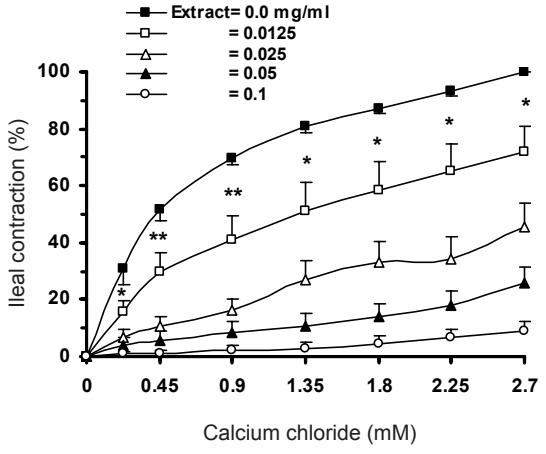

Figure 4. Spasmolytic effect of cumulative concentrations of OPE on rat ileal contractions induced by CCh (10 μM) prior (control, n=9) and after 5 min tissue incubation with glibenclamide (10 μM, n=9) or tetraethylammonium (1 mM, n=7). *P<0.05, **P<0.01.

Ca^{2+}-free and high K^+ Tyrode solution, the tissue has been depolarized by high K^+ (20) however, applying $CaCl_2$ to the organ bath was necessary for occurring contraction (17). The cholinergic antagonist receptor activity of extract was not supported since the extract inhibited both KCl- and CCh-induced contractions. Contractions induced by these spasmogens had similar amplitude (data not shown). If extract had induced evoked antispasmodic effect after entering into the cell and inhibiting Ca^{2+} release in addition to Ca^{2+} influx inhibiting, the inhibition of both contraction were similar in potency. Therefore, it seems that, inhibition of Ca^{2+} release is not occurred. The β-adrenoceptor (21) and opioid receptor activation (22) and nitric oxide (NO) relax ileum (23). But, the ineffectiveness of propranolol, naloxone and L-NAME to reduce the OPE spasmolytic effect indicated that neither these receptors nor NO were involved.

Results showed that the OPE antispasmodic effect was not reduced by glibenclamide as an ATP-sensitive K^+ channel blocker (24) and TEA as a non-selective K^+ channel blocker (24). Therefore, it may conclude that these channels were not involved neither. TEA has been also reported as a KCa channel inhibitor (25, 26). There is no explanation for potentiation of OPE spasmolytic activity after applying antagonists and inhibitors. The main point is that the OPE inhibitory effect has not been reduced in the presence of these antagonists and inhibitors which indicated that these receptors and K^+ channels were not involved. Quercetin is a major ingredient flavonoid of onion peel (8) with antispasmodic (27) and calcium-antagonistic properties (11) therefore, the OPE activity possibly was due to this flavonoid.

In conclusion, results suggest that calcium channels were involved in the onion peel hydroalcoholic extract spasmolytic effect on rat ileum.

Acknowledgment

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