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Antispasmodic Effect of *Piper nigrum* Fruit Hot Water Extract on Rat Ileum

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Abstract: The aim of this study was to investigate the effect of black pepper fruit hot water extract (BPE) on rat ileum contractility and the mechanism(s) of its action. The extract was prepared by adding black pepper powder to boiling distilled water followed by evaporated the solvent. Ileum was dissected from male adult rat (Wistar) and in Tyrode solution the tissue contractions were recorded by an isotonic transducer under 1 g tension. The cumulative concentrations of the BPE (0.0625-1 mg mL⁻¹) reduced the ileum contractions induced by KCl (60 mM) or carbachol (10 µM) concentration dependently ($p < 0.001$). In Ca²⁺-free Tyrode solution with high potassium (60 mM), BPE, (0.0625-1 mg mL⁻¹) attenuated the contractions induced by cumulative concentrations of CaCl₂ (0.225-2.7 mM) concentration dependently (ANOVA, $p < 0.05$). The incubation of the tissue preparation (20 or 30 min) with L-NAME (100 µM), naloxone (1 µM) or propranolol (1 µM) did not reduce the extract antispasmodic effect on KCl-induced ileum contraction. The extract spasmolytic effect was attenuated neither by glibenclamide (10 µM) nor by tetraethylammonium (1 mM). Present results suggest that the spasmolytic effect of the extract on rat ileum was possibly mediated via Ca²⁺ influx.

Key words: *Piper nigrum*, rat, ileum, spasmolytic

INTRODUCTION

Black pepper (*Piper nigrum*) from Piperaceae is the main spice food stuff and piperine is a pungent alkaloid of black pepper (Zargari, 1993). Black pepper stimulates rat gastric acid secretion which is weaker than red pepper effect (Vasudevan *et al.*, 2000) and piperine has a same effect (Ononiwu *et al.*, 2002) but piperine reduces mice small intestine secretions stimulated by castor oil (Capasso *et al.*, 2002). Black pepper increases rat pancreatic enzymes activity (Platel *et al.*, 2002) and piperine inhibits gastric emptying and gastrointestinal transit in rats and mice (Bajad *et al.*, 2001). Black pepper has antimicrobial (Dorman and Deans, 2000), antimutagenic (El-Hamss *et al.*, 2003), antioxidant and radical scavenging properties (Gulcin, 2005; Saxena *et al.*, 2007), reduces oxidative stress induced by high fat diet in rat (Vijayakumar *et al.*, 2004) and inhalation of black pepper oil improves the reflexive swallowing movement (Ebihara *et al.*, 2006). In Iranian folk medicine, black pepper is used for some gastrointestinal disorders and to relief menorrhagia in women. The effect of black pepper on ileal smooth muscle contractility has not been investigated. The aim of the present study therefore, was to study the effect of black pepper fruit hot water extract (BPE) on the rat ileum contractions and the possible mechanism(s) involved.

MATERIALS AND METHODS

Plant material and powder preparation: Black pepper fruit was purchased from local herbal shops (in October 2007) in Ahwaz (Khuzestan Province, Iran) and identified by botanists in Ahwaz Ramin University of Agriculture and Natural Sources. A voucher specimen was deposited at herbarium of our laboratory for further references. The method of the extract preparation was mainly as same as used traditionally. Therefore, black pepper fruit was powdered by an electrical grinder and the powder (10 g) was mixed with 200 mL boiling distilled water for 15 min. The mixture was then filtered through very fine cloth and thereafter the filtrate was centrifuged at 3500 rpm for 20 min. The supernatant was concentrated in rotary evaporator and then dried at room temperature to obtain a solid mass (1.3 g). The powder of Black Pepper Extract (BPE) was stored at 4°C until being used.

Chemicals and reagents: Propranolol, carbachol, N^o-nitro-L-arginine methyl ester (L-NAME), glibenclamide and tetraethylammonium (TEA) were purchased from Sigma (USA) and naloxone was purchased from Tolidaru (Iran). Other chemicals were purchased from Merck (Germany).

Animals: All rats used in this study were treated in accordance with principals and guidelines on animals care of Ahwaz Jundishapur University of Medical Sciences (AJUMS). Male adult Wistar rats (213 ± 5.6 g) were obtained from AJUMS animal house and kept at 12 h light/dark cycle and at $20-24^{\circ}\text{C}$ with free access to food and water. Rats were starved of food but not water for 24 h before experiment.

Ileum preparation: On the day of experiment a sharp blow on the head sacrificed the rats. Two or three segments (2 cm) were dissected out from the terminal ileum (taken within a distance of 2-3 cm from the 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 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 continuously was bubbled with air. The initial tension was 1 g throughout the experiment and equilibrium period was 60 min in which the bath solution was refreshed every 15 min. After equilibrium period, the ileum was contracted by 60 mM of KCl (Madeira *et al.*, 2002) and once the plateau was achieved, the extract ($0.0625-1$ mg mL^{-1}) was added cumulatively to the organ bath. The extract spasmolytic effect was also studied in separate tissues after 30 min incubations with 1 μM of propranolol (Storr *et al.*, 2000) or naloxone (Kaneda *et al.*, 2003) as a non-selective β -adrenoceptors and opioid receptors antagonists, respectively. The extract spasmolytic effect was also studied after 20 min tissue incubation with 100 μM of L-NAME (Ekblad and Sundler, 1997) as a nitric oxide synthase inhibitor. To evaluate the extract effect on CaCl_2 -induced ileum contraction, in Ca^{2+} -free and rich KCl (60 mM) Tyrode solution, CaCl_2 was applied cumulatively ($0.225-2.7$ mM) before and after tissue incubation (3 min) with extract ($0.0625-1$ mg mL^{-1}). In addition, the extract antispasmodic effect on carbachol (CCh, 10 μM)-induced contraction was evaluated after ileum incubation (5 min) with glibenclamide (10 μM) or TEA (1 mM) as ATP-dependent and non-selective potassium channel blocker respectively (Franck *et al.*, 1998). Separate ileum preparations were used for each spasmogen, antagonist or potassium channel blocker. The extract and all chemicals were dissolved in the Tyrode solution and the total volume of all solutions, which were added to the organ bath, did not exceed more than 5% of the bath volume.

Statistical analysis: The plateau of ileal contraction induced by KCl or CCh was regarded as 100% and percentage of relaxation was calculated from changes in the contraction. Results were expressed as mean \pm SEM. Statistical analysis was made by one- and two-way ANOVA. Data was further subjected to LSD post hoc test and a value of $p < 0.05$ was considered significant.

RESULTS

Effect of BPE on KCl and CCh-induced ileum contractions: Black pepper fruit hot water extract (BPE) attenuated the ileal contractions induced by KCl (60 mM, $n = 10$) or carbachol (CCh, 10 μM , $n = 9$) significantly (ANOVA, $p < 0.001$) and in a concentration-dependent manner. The two-way ANOVA indicated that these spasmolytic effects were not significantly different as shown in Fig. 1.

Effect of the BPE on CaCl_2 -induced ileum contractions: In Ca^{2+} -free with high K^+ (60 mM) Tyrode solution, applying cumulative concentrations of CaCl_2 (0.225 to 2.7 mM) induced ileum contractions in a concentration dependent manner ($p < 0.001$) as shown in Fig. 2. Three minutes incubation of tissue preparation with BPE ($0.0625-1$ mg mL^{-1}) reduced the contractions concentration-dependently. The CaCl_2 -induced contractions in the absence and in the presence of BPE (0.0625 mg mL^{-1}) were significantly different (two-way ANOVA, $p < 0.05$, $n = 8$).

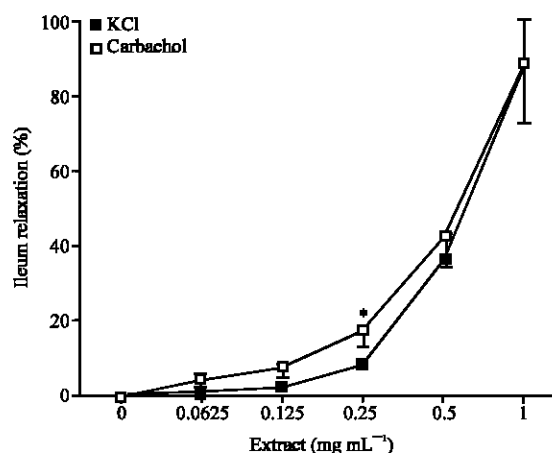


Fig. 1: The spasmolytic effect of black pepper fruit extract on the ileum contractions induced by KCl (60 mM, $n = 10$) or carbachol (10 μM , $n = 9$). The responses are different only at 0.25 mg mL^{-1} (* $p < 0.05$). However, two-way ANOVA indicated that these concentration-response curves are not different

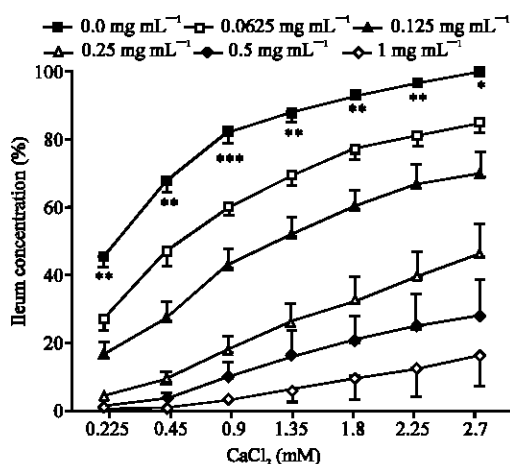


Fig. 2: The ileal contractions evoked by CaCl_2 before (0.0 mg mL^{-1}) and after incubation with black pepper fruit extract at different concentrations. The Tyrode solution was Ca^{2+} -free but with high K^+ (60 mM). Two-way ANOVA indicated that concentration-response curves of 0.0 and $0.0625 \text{ mg mL}^{-1}$ are different ($p < 0.05$, $n = 8$). The significance differences between spasmogenic effect of each CaCl_2 concentration in the absence and in the presence of extract ($0.0625 \text{ mg mL}^{-1}$) are also demonstrated (* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$)

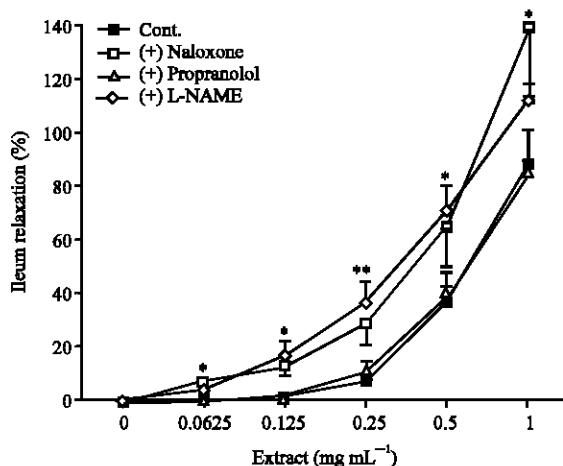


Fig. 3: The spasmolytic effect of black pepper fruit extract on the rat ileum contractions evoked by KCl (60 mM , $n = 10$) before (Cont.) and after 30 min tissue incubation with propranolol ($1 \text{ } \mu\text{M}$, $n = 9$), naloxone ($1 \text{ } \mu\text{M}$, $n = 7$) or 20 min incubation with L-NAME ($100 \text{ } \mu\text{M}$, $n = 7$). L-NAME potentiated the extract spasmolytic effect (two-way ANOVA, $p < 0.05$). The differences in the extract spasmolytic effects in the absence and in the presence of L-NAME are shown (* $p < 0.05$, ** $p < 0.01$)

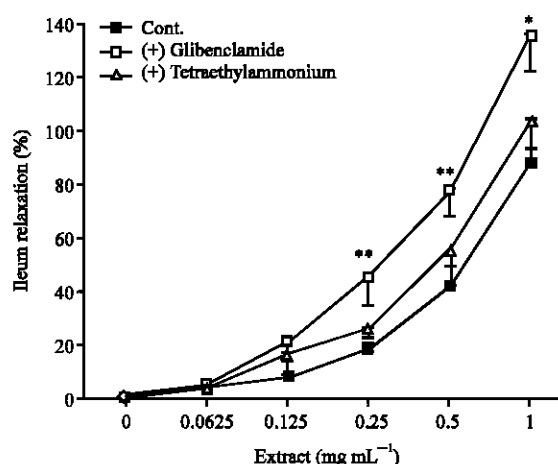


Fig. 4: Antispasmodic activity of black pepper fruit extract on the rat ileum precontracted by CCh ($10 \text{ } \mu\text{M}$, $n = 9$) before (Cont.) and after tissue incubation (5 min) with glibenclamide ($10 \text{ } \mu\text{M}$, $n = 9$) or with tetraethylammonium (1 mM , $n = 9$). The spasmolytic effects of the extract has been potentiated by glibenclamide (two-way ANOVA, $p < 0.05$). The differences in the extract spasmolytic effects in the absence and in the presence of glibenclamide are shown (* $p < 0.05$, ** $p < 0.01$)

Effect of BPE after ileum incubation with propranolol, naloxone or L-NAME: Ileum incubation (30 min) with propranolol ($1 \text{ } \mu\text{M}$, $n = 8$), naloxone ($1 \text{ } \mu\text{M}$, $n = 7$), or 20 min with of L-NAME ($100 \text{ } \mu\text{M}$, $n = 7$) did not reduce the spasmolytic effect of BPE on KCl -induced ileum contractions rather, in the presence of L-NAME, the BPE activity was potentiated (two-way ANOVA, $p < 0.05$) as shown in Fig. 3.

Spasmolytic effect of BPE in the presence of the potassium channel blockers: As Fig. 4 shows, incubation (5 min) of the ileum preparations with glibenclamide ($10 \text{ } \mu\text{M}$, $n = 9$) or with TEA (1 mM , $n = 9$) as ATP-operated and non-selective potassium channel blockers, respectively did not attenuate the BPE spasmolytic effect on CCh ($10 \text{ } \mu\text{M}$)-induced ileum contraction rather, glibenclamide potentiated the BPE activity (two-way ANOVA, $p < 0.05$).

DISCUSSION

The results of present study show that KCl -induced non-receptor-mediated and CCh -induced receptor-mediated ileal contractions are significantly reduced by black pepper fruit hot water extract (BPE). The extract used in this study has been prepared in same way as described in the Iranian folk medicine. The BPE

spasmolytic effect was reversible since tissue responsiveness to spasmogens was almost restored after refreshing the organ bath solution.

It is well known that the KCl-induced contraction in smooth muscle is due to an increase in Ca^{2+} influx through voltage-operated Ca^{2+} channels. On the other hand, CCh-induced contractile responses following receptor activation requires an increase intracellular Ca^{2+} which is provided by both Ca^{2+} influx through L-type Ca^{2+} channels and Ca^{2+} release from intracellular Ca^{2+} stores (Tanovic *et al.*, 2000; Zhang *et al.*, 2005). The similar reduction by the BPE in CCh- and KCl-induced contractions suggests that the BPE does not affect specific receptor-dependent mechanisms. It has been suggested that those substances that inhibit the KCl-induced contractions act through blocking the VDCCs (Gilani *et al.*, 2001). The BPE spasmolytic effect on the CaCl_2 -induced ileal contractions supported the possible involvement of Ca^{2+} influx (Fujimoto and Mori, 2004; Zhang *et al.*, 2005). The anticholinergic effect of BPE is unlikely since BPE should only inhibit the CCh-induced contraction.

The β -adrenoceptor activation relaxes ileum (Brown and Summers, 2001) but the ineffectiveness of propranolol indicated that the extract effect has not mediated via these receptors. Since a Nitric Oxide Synthase (NOS) is present in the myenteric plexus, Nitric Oxide (NO) has become a most likely candidate for mediating nonadrenergic-noncholinergic smooth muscle relaxation through the gastrointestinal tract (Takahashi, 2003). In our study, the extract activity was not reduced by L-NAME, indicates that the extract effect has not been mediated via the NO synthesis. Opioid receptors activation relaxes ileum (Gray *et al.*, 2005) and since naloxone did not reduce the extract activity therefore, opioid receptors have not been involved. The BPE spasmolytic effect neither reduced by glibenclamide nor by TEA, as an ATP-dependent and calcium-operated potassium channel blocker respectively (Nishida and Satoh, 2003; Kafali *et al.*, 2002). Therefore, the extract has not induced spasmolytic effect through activated these channels and hyperpolarization. The ileal contractility was unaffected by tissue incubation with applied antagonists or inhibitor. It has been demonstrated that black pepper reduces gastric emptying, gastrointestinal transit (Bajad *et al.*, 2001) and uterine contractions (Gharib Naseri and Yahyavi, 2007) and piperine inhibits the twitch response in guinea-pig ileum (Takaki *et al.*, 1990), which are consistent with the present results. The observed inhibitory effect probably is due to the piperine action.

CONCLUSION

Black pepper fruit extract contains spasmolytic constituents mediating their effect through blockade of Ca^{2+} influx, which may explain its traditional use in the treatment of some gastrointestinal disorders.

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