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Antispasmodic effect of *Physalis alkekengi* fruit extract on rat uterus

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Abstract

Background: Studies have shown that *Physalis alkekengi* reduces implantation and induces antifertility in rat. In Iranian traditional medicine it is believed that this plant has abortifacient and antifertility activities.

Objective: The goal of this study was to evaluate the effect of *Physalis alkekengi* ripe fruit hydroalcoholic extract (PFE) on uterine contractility and its possible mechanism(s).

Materials and Methods: Extraction of *Physalis alkekengi* fruit was carried out by maceration method (70% alcohol). Uterus was dissected out from adult non-pregnant rat (Wistar) and contracted by KCl (60mM) or oxytocin (10mU/ml) in an organ bath containing De Jalon solution and the effect of PFE on the uterine contractions was investigated. Furthermore, the role of α- and β-adrenoceptors, opioid receptors, nitric oxide and cyclic guanosine monophosphate synthesis inhibitors on the extract effects were evaluated.

Results: KCl- and oxytocin-induced uterine contractions were inhibited ($p<0.001$) by the cumulative concentrations of the extract in a concentration dependent manner. Incubation of uterus with propranolol (1μM) and L-NAME (100μM) attenuated the PFE antispasmodic effect ($p<0.05$). But the PFE effect was unaffected by phentolamine (1μM), naloxone (1μM) or methylene blue (10μM). In Ca^{2+}-free with high potassium (60mM) De Jalon solution, cumulative concentrations of $CaCl_2$ (0.1-0.5mM) induced uterine contraction concentration-dependently ($p<0.001$). Uterus incubation (5min) with PFE (0.25-1.75mg/ml) attenuated the $CaCl_2$–induced contractions ($p<0.05$).

Conclusion: It seems that the extract induced antispasmodic effect mainly via calcium influx blockade and partially through blocking β-adrenoceptors and nitric oxide (NO) synthesis. However, neither α-adrenoceptors nor opioid receptors or cGMP synthesis were involved.

Key words: *Rat, Uterus, Physalis alkekengi, Antispasmodic*.

Introduction

Physalis alkekengi (locally called Kakenge), winter cherry or Chinese lantern (from Solanaceae) with an edible fruit is an indigenous herb in Iran (1). It has been reported that winter cherry traditionally has been used for difficult urination, inflammation, kidney and bladder stone, arthritis and rheumatism (1, 2). Whole plant extract of *Physalis alkekengi* has shown anti-bacterial activity (3). In the fruit, a major steroid glycoside, a free sugar and a glycoalkaloid have been identified with antiestrogenic activity (4) which probably is due to its steroid glycosides (5, 6). Chromatography analysis has shown that sepal in *Physalis alkekengi* contains zeaxanthin and beta-cryptoxanthin esters or carotenoid esters which can be used as food additives or nutraceuticals (7).

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Recently, four new steroids compounds; physalin Y, physalin Z, phyalin I and physalin II were isolated from its calyces (8). *Physalis alkekengi* has been used as an abortive and contraceptive plant in Iranian traditional medicine for many years (9) and it has been reported that intraperitoneally administration of *Physalis alkekengi* fruit produces 100% diestrus in female rats which was a reversible effect in addition to reduce plasma progesterone level (10).

Recently it has been also shown that the same administration of the extract in 1-5 days pregnant rats causes reduction in number of implantation sites, number of neonates and their weights (11). This plant contains physalin, citric acid and vitamin C (2, 9). The antispasmodic effect of *Physalis alkekengi* leaf extract on ileum contractility has been shown (12), but regarding to the mentioned effects on uterus, there is no documented evidence referring to the fruit extract on uterine contractility. Thus the present study was an attempt to investigate the effect of *Physalis alkekengi* ripe fruit hydroalcoholic extract (PFE) on uterine contraction and its possible mechanism(s).

Materials and methods

Plant material and extraction

Physalis alkekengi fruit was purchased from local herbal shop (October 2007) in Ahwaz and authenticated by Dr. Sedighi Dehkordi from the Department of Horticultural Science, Ahwaz Shahid Chamran University. A voucher specimen was deposited at Herbarium of the same department for further references. The fruit was powdered by an electrical grinder and the powder was extracted by macerating method using 70% alcohol for 72 h at room temperature and mixed occasionally daily. The mixture was then filtered (Whatman No.1) and the filtrate was concentrated in rotary evaporator and dried at room temperature to obtain a reddish powder (yield: 17.34%). The PFE powder was stored at 4°C until being used.

Drugs

Propranolol and N^{ω}-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma (USA) and phentolamine from Novartis (USA). Naloxone, oxytocin and estradiol valerate were purchased from Tolidaru, Minoo and Aboraihan Companies respectively (Iran). Methylene blue and other chemicals were purchased from Merck Company (Germany).

Animals and uterus tissue preparation

In this in vitro study, animals were treated in accordance with principals and guidelines on animals care of Ahwaz Jundishapur University of Medical Sciences (AJUMS). Adult female Wistar rats (200.9±3.4 g) were obtained from AJUMS Animal House and kept at 12-h light/dark cycle and at 20-24 °C with free access to food and water.

On the day of experiment the rats were sacrificed by a sharp blow on the neck. After laparotomy, from the cervical portion of each uterus horn a piece (1-1.5 cm) was dissected out and mounted in an organ bath containing De Jalon 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 isometric transducer (UF1 Harvard transducer, UK) connected to a recorder (Harvard Universal Oscillograph, UK). The De Jalon solution composition (pH 7.4 and 29 °C) was (in mM): NaCl (154), KCl (5.6), $CaCl_2$ (0.3), $NaHCO_3$ (1.7), $MgCl_2$ (1.4) and glucose (5.55) which was continuously 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 uterus was contracted by KCl (60 mM) and once the plateau was achieved, the PFE (0.25, 0.5, 0.75, 1, 1.25, 1.5 and 1.75 mg/ml) was added cumulatively to the organ bath. A group of animals were received estradiol valerate (0.5 mg/kg, SC) 24 hours prior to the experiment. The uterus of these rats was pretreated with the same concentrations of the PFE for 2 min (non-cumulatively) and then tissue preparation was contracted by oxytocin (OT, 10 mU/ml).The extract effect was also studied on separate tissues after 30 min incubations with 1 μM of phentolamine, propranolol or naloxone as non-selective α- and β-adrenoceptors and opioid receptors antagonists respectively. In addition, the extract activity was examined after tissue incubation (20 min) with 100 μM of L-NAME or methylene blue (10 μM, 15 min) as a nitric oxide synthase and guanylyl cyclase inhibitor respectively. Each tissue preparation was used only for one of the spasmogens and antagonists. To study the role of extracellular calcium, in Ca^{2+}-free and rich KCl (60 mM) De Jalon solution, $CaCl_2$ was added to the organ bath cumulatively (0.1-0.5 mM) before and after tissue incubation (5 min) with PFE (0.25-1.75 mg/ml). All chemicals were dissolved in the De Jalon solution and the total volume of added solutions did not exceed more than 5% of the bath volume. Seven to 9 animals were used for each protocol as indicated in result section.

Statistical analysis

The plateau of contractions induced by KCl and the contraction peak induced by OT were regarded as 100% and the changes in contractions induced by the extract were calculated and expressed as mean±SEM. Statistical comparisons were made by one and two way ANOVA and p values less than 0.05 were considered significant.

Results

Effect of PFE on the KCl- and oxytocin-induced uterus contractions

Physalis alkekengi fruit hydroalcoholic extract (PFE) reduced the uterus contractions induced by KCl (60 mM) and oxytocin (10 mU/ml) significantly (one-way ANOVA, $p<0.001$) and in a concentration dependent manner as shown in Table I. In both studies, at the end of experiment, the control (PFE at 0.0 mg/ml) was recorded again to show the disappearance of extract effect. In the KCl-induced contraction, in contrast to oxytocin study, the extract activity at higher doses was almost constant. The applied concentrations of the extract on KCl- and oxytocin-induced contractions were cumulative and non-cumulative respectively.

PFE activity after tissue incubation with adrenergic antagonists

Figure 1 shows that the antispasmodic effect of PFE on KCl-induced uterus contraction was unaffected by tissue incubation with phentolamine (as an α-adrenoceptor antagonist, 1 μM). However, tissue incubation with propranolol (1 μM, as a β-adrenoceptor antagonist) attenuated the extract antispasmodic effect (two way ANOVA, $p<0.05$).

PFE activity after tissue incubation with naloxone, L-NAME or methylene blue

As figure 2 shows, the PFE antispasmodic effect on the KCl-induced uterine contractions was not affected by tissue incubation with naloxone (1 μM), as a non-selective opioid receptors antagonist, and with methylene blue (10 μM), as a guanylyl cyclase inhibitor. But, tissue incubation with L-NAME (100 μM), as a nitric oxide synthase inhibitor attenuated the PFE antispasmodic effect (two way ANOVA, $p<0.05$).

Effect of PFE on the uterine contraction induced by $CaCl_2$

In the Ca^{2+}-free with high K^+ (60 mM) De Jalon solution, applying cumulative concentrations of $CaCl_2$ (0.1-0.5 mM) contracted the uterus in a concentration - dependent manner (one way ANOVA, $p<0.001$) as shown in figure 3. Tissue incubation (5 min) with PFE (0.25, 1 and 1.75 mg/ml) reduced the $CaCl_2$-induced contractions concentration- dependently (at 1 mg/ml, two way ANOVA, $p<0.01$).

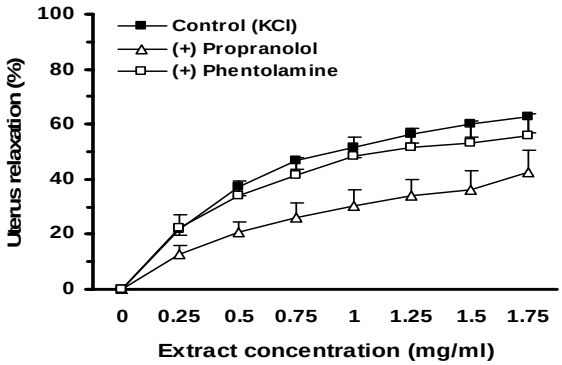

Figure 1. The antispasmodic effect of PFE on uterine contractions induced by KCl (60 mM) before (control, n=7) and after incubation (30 min) with phentolamine (1 μM, n=8) or propranolol (1 μM, n=8).

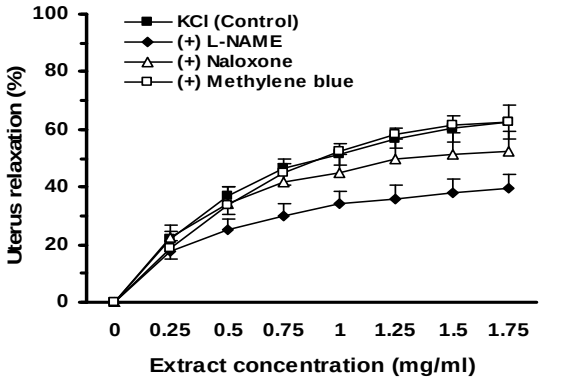

Figure 2. The antispasmodic effect PFE on uterine contractions induced by KCl (60 mM, n=7) before (Control) and after incubation with naloxone (1 μM, n=9), L-NAME (100 μM, n=9) or with methylene blue (10 μM, n=7).

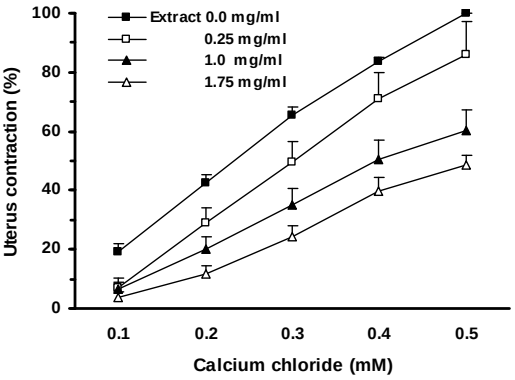

Figure 3. Spasmogenic effect of $CaCl_2$ in De Jalon Ca^{2+}-free but with high K^+ (60 mM) on uterus before and after 5 min tissue incubation with PFE (n=7). The effects of PFE at 0.0 and 1 mg/ml were significantly different ($p<0.01$).

Table I. PFE antispasmodic effects (percentage) on uterine contractions induced by KCl (60 mM, n=7) or oxytocin (10 mU/ml, n=7).

	***Physalis alkekengi* fruit extract (mg/ml)**							
Spasmogen	0.25	0.5	0.75	1	1.25	1.5	1.75	0
KCl	29.7% (±6.6)	48.4% (±11.2)	67.4% (±11.6)	70.1% (±11.7)	75.2% (±11.4)	80.1% (±11.7)	82.4% (±11.9)	0%
Oxytocin	0.96% (±0.7)	3.1% (±1.4)	5.8% (±2.8)	17.8% (±5.7)	37.6% (±10.4)	80.7% (±8.6)	98.6% (±0.7)	5.3% (±2.3)

Discussion

This study showed that hydroalcoholic extract of *Physalis alkekengi* fruit extract induces antispasmodic effect on the rat uterine contraction caused by KCl or oxytocin. In this study, De Jalon solution with low calcium and potassium concentration and low temperature (29 °C) was used to reduce the spontaneous uterus contractions (13).

In addition, all used uterus preparations were dissected out from the cervical segment since different segments of the uterus vary in their responsiveness to stimulants (13). The observed antispasmodic effect of the PFE on KCl- and oxytocin induced contractions were completely and almost completely reversible respectively, since washing and refreshing the organ bath solution accompanied with abolishing the extract effect (100% for first control vs 94.73±2.25% for last control contraction induced by oxytocin). It may suggest that, therefore, the observed antispasmodic effect is occurred on the surface of the uterine smooth muscle cells rather than inside the cells. Extracellular high potassium concentration , as a non receptor spasmogen, depolarizes the smooth muscle followed by contraction (14) in which, the voltage dependent calcium channels (VDCCs) are involved (15) and the existence of L-type VDCCs in rat uterus has been documented (16, 17). It has been suggested that those substances that inhibit the KCl-induced contractions act through blocking the VDCCs (18). On the other hand, oxytocin elevates $[Ca^{2+}]_i$ by activating the L-type VDCCs (19) and also by activating phospholipase C and increasing inositol triphosphate (IP_3) production (20, 21) followed by releasing calcium from intracellular pools such as sarcoplasmic reticulum (21). In the absence of external Ca^{2+}, however, oxytocin releases Ca^{2+} from the sarcoplasmic reticulum (SR) through IP_3 but produces only a small increase in force , demonstrating a requirement for Ca^{2+} entry as part of the mechanism of agonist action (22). IP_3 triggers Ca^{2+} influx from extracellular space further. Increased $[Ca^{2+}]_i$ binds with calmodulin to activate myosin light chain kinase which phosphorylates myosin light chain to trigger contractile machinery of the myocytes (23). Although the cumulative and non-cumulative methods of applying the extract were used for KCl and oxytocin inducing uterine contractions, however, the comparison of the PFE antispasmodic effect on these spasmogens still shows that the effect of the extract on oxytocin-induced contraction is more potent than on KCl-induced contraction. This point indicates that probably the oxytocin receptors are more involved in the extract activity. Furthermore, these spasmogens are similar in acting through VDCCs and therefore, it may conclude that Ca^{2+} influx was involved in the extract activity. To support this hypothesis, the Ca^{2+}-free with high potassium De Jalon solution was used. This solution depolarizes uterus but applying calcium was necessary to induce contraction (14, 24). As mentioned previously, the uterine contractions induced by $CaCl_2$ were attenuated by PFE. Since, after depolarization, the main route of increasing $[Ca^{2+}]_i$ is influx of Ca^{2+} from extracellular fluid (14), therefore, it seems that PFE has inhibited the Ca^{2+} influx as shown in the KCl and oxytocin results. β-adrenoceptor activation relaxes uterus (25). Reducing the PFE antispasmodic effect by propranolol indicated that extract has carried out its action (in part) via these receptors. Ineffectiveness of phentolamine to reduce the PFE activity showed that α-adrenoceptors were not involved. Opioid receptors activation relaxes uterus (26). In this study, the PFE activity was unaffected by naloxone, as a non-selective opioid receptors antagonist, which suggests that extract activity was not mediated via these receptors. Nitric oxide (NO) relaxes uterus (27). Deceasing the relaxatory effect of PFE by L-NAME indicated the possible involvement of NO. While, the PFE relaxatory effect was unaffected by methylene blue, as a guanylyl cyclase inhibitor, which indicated that cGMP synthesis was not involved. Although it is well documented that NO

induces relaxation through cGMP pathway, however, it has been reported that NO-induced relaxation in non-pregnant uterus, increased $[cGMP]_i$ do not appear to be required (28, 29).

Estrogens (mainly estradiol) oppose the relaxatory actions of progesterone and augment myometrial contractility and excitability. The balance between the relaxatory actions of progesterone and the stimulatory actions of estrogens is pivotal in determining the contractile state of the pregnancy myometrium and the timing and process of parturition (30) and estrogens increase the uterine contractility (31). Furthermore, estrogen perfusion associated with an increase in intrauterine pressure (32).

On the other hand, it has been reported that *Physalis alkekengi* fruit extract has estrogen antagonist (6) and anti-fertility activities (11), therefore, the PFE antispasmodic effect probably is due to presence the estrogen antagonist. To our knowledge, the relaxatory effect of *Physalis alkekengi* fruit on the isolated pregnant or non-pregnant uterine smooth muscle has not been studied, therefore, the comparison of our results with other studies were impossible. Recently it has been reported that calyces of this plant has ingredients with inhibitory effect on releasing NO from macrophages (8). This report does not appear to agree with our results which indicated that the fruit extract induces antispasmodic activity, in part, via NO.

In addition, most of extracts with abortifacient activity induce uterus contraction, however, it has been reported that some extracts such as rosemary with antispasmodic effect has abortive activity (33). Therefore, some researcher has doubt about using uterine muscle relaxant drugs for women with threatened miscarriage (34). Although more studies are needed but possibly *Physalis alkekengi* fruit extract acts though the same mechanism as rosemary extract.

Conclusion

Our results indicated that PFE induces antispasmodic effect on rat uterus mainly through blockage of the Ca^{2+} and partly by inhibiting NO synthesis and antagonizing the β-adrenoceptors. Although the abortifacient and anti-implantation effects of *Physalis alkekengi* has been reported, however, the correlation between in vivo activities and the antispasmodic effect is not evaluated yet. Therefore it seems necessary to examine the effect of the extract on pregnant uterus.

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