Original Article

Antispasmodic Effects of Aqueous and Hydroalcoholic *Punica granatum* Flower Extracts on the Uterus of Non-pregnant Rats

Akram Ahangarpour *, Razieh Heidari, Mahsa Abdolahzadeh, Ali Akbar Oroojan

- *Department of Physiology, Physiology and Diabetes Research Centers, Jundishapur University of Medical Sciences, Ahvaz, Iran*

Abstract

Background: *Punica granatum Linn.* (PG) is native to the Mediterranean region. Its flower exhibited antioxidant activity. The present study attempt to investigate the effect of these extract on uterine contraction and its possible mechanism(s).

Methods: Thirty five female Wistar rats (200–300 *g*) at estrous phases of cycle was examined in this study; pieces of virgin adult rat uterus (1.5 *cm*) were suspended in an organ bath containing 10 *ml* of De Jalon solution at 29 *°C*. Tissue contractility was isometrically recorded. KCl (60 *mM*), $BaCl_2$ (4 *mM*) and oxytocin (10 *mU/ml*) were applied to the tissue in the presence and absence of aqueous and hydroalcoholic extracts of the plant (0.05, 0.1, 0.2, 0.4 and 0.8 *mg/ml*). Propranolol (1 *μM*) and naloxane (1 *μM*) were added in KCl induced contractions. The results were analyzed by ANOVA and $p<0.05$ were considered as significant.

Results: Cumulative concentration of extracts reduced uterine contractions induced by KCl dose-dependently ($p<0.01$). Extracts in a dose dependent ($p<0.05$) reduced uterine contractions decreased dose-dependently after of addition oxytocin. The extracts added cumulatively to the organ bath reduced contractions but they did not affect uterine contractions induced by $BaCl_2$ except the last dose. Spasmolytic effects of the extracts were not affected by propranolol or naloxane in KCl induced contractions.

Conclusion: Extracts diminished K^+-induced contraction in uterus, therefore it seems that substances that decrease K^+-induced contraction can also block voltage dependent calcium channel. The extracts did not have any effect on β-adrenoceptors or potassium channels.

* *Corresponding Author:* Akram Ahangarpour, Department of Physiology, Physiology and Diabetes Research Centers, School of Medicine, Jundishapur University of Medical Sciences, Ahvaz, Iran
E-mail: ahang1002002@yahoo.com

Received: Sep. 26, 2011
Accepted: Dec. 24, 2011

Keywords: Naloxane, Oxytocin, Propranolol, *Punica granatum* flower, Uterus.
To cite this article: Ahangarpour A, Heidari R, Abdolahzadeh M, Oroojan AA. Antispasmodic Effects of Aqueous and Hydroalcoholic *Punica granatum* Flower Extracts on the Uterus of Non-pregnant Rats. J Reprod Infertil. 2012;13(2):138-142.

Introduction

Punicaceae plant, *Punica granatum Linn* (Pomegranate in English), is widely distributed in the Middel Eastern countries, including Iran, extending throughout the Mediterranean region (1). Some of its biological activities such as antitumour (2), antibacterial (3), antidiarrhoeal (4), antifungal (5), antiulcer (6) and antifertility (7) effects, have been reported with various extracts from different parts of this plant. Trees compartments include seeds, juice, peel, leaves, flowers, bark and roots. There is a conception that a mixture of pomegranate seeds, juice, and peel could prevent abortion (1). Hayouni et al. have reported *Punica granatum L.* peels' wound healing potential *in vivo* (8). Oil, juice and peel of this plant have weak estrogenic effects for the treatment of menopausal symptoms. Moreover, juice and peel of this plant have potent antioxidant properties (9). Hassanpour Fard et al. indicated that the whole fruit extract of pomegranate has

cardioprotective effect against doxorubicin-induced cardiotoxicity in rats (10). Punicic acid, the main constituent of pomegranate seed (70%–80%), exhibited potent growth inhibitory activities in androgen-dependent LNCaP cells, which appear to be mediated by both antiandrogenic and pro-apoptotic mechanisms (11). Its juice, peel and oil also showed to have anticancer activities, including interference with invasion, cell cycle, tumor cell proliferation and angiogenesis, that may be associated with anti-inflammatory, pharmacological and phytochemical actions of all *Punica granatum* components (9). Flavonoids of the fruit's juice have preventing effects on low-density lipoprotein oxidation; therefore, this part of the plant is antiarthrogenic (12). *Punica granatum* flowers have used for the treatment of diabetes mellitus in Greek medicine (13). The antioxidant anti-inflammatory and anti-diabetic effects of *Punica granatum* extract have been shown on rat's uterus (14), but regarding the aforesaid effects on uterus, there seemed to be no document on *P. granatum* flower extract that exhibits contractile effects on uterine. Thus, the present study was an attempt to investigate the effects of *Punica granatum* flower extract on uterine contraction and its possible mechanism(s).

Methods

Plant extraction: Dry *Punica granatum* flower were purchased from Ahvaz green-grocery in 2009, before it was powdered by a strainer.

Aqueous extract: Subsequently 50 *g* of *Punica granatum* flower powder was mixed with 200 *ml* distilled water in 30 *min* condition in hot water. The mixture was filtered with a Whatman grade No 1 filter paper and centrifuged for 20 *min* at 3500 *g* per minute. The solvent was evaporated at ambient temperature and extract powder was kept at 4 ^{o}C until used (15).

Hydro-alcoholic extract: The powder was extracted with 70% ethanol for 72 *hr* using macerated method. The mixer was filtered with Whatman grade No. 1 filter paper. The solvent of the filtrate was evaporated at ambient temperature and the extracted powder was kept at 4 ^{o}C until used (16).

Animals and uterus tissue preparation: 35 female Wistar rats used in this study were treated in accordance with the principles and guidelines on animals care of Ahwaz Jundishapur University of Medical Sciences. Adult female Wistar rats (200–250 *g*) were kept at 12 *hr* light/dark cycle and at 20–24 ℃ with free access to water and food. subsequently vaginal smears were prepared and the stage of estrous cycle was assessed. All experiments were carried out in the afternoon on the day of pro-estrus, for we noted that uterine contractions started only late in the morning of pro-estrus, when a precipitous fall in estradiol concentration occurs (17).

Rats were anaesthetized by ether, each uterus was rapidly removed after laparotomy and it was washed with cold oxygenated De Jalon solution. 10 *mm*-long muscle rings were sliced from the uterine horns and mounted vertically in an organ bath containing 10 *ml* of De Jalon solution composed of $CaCl_2$ (0.3 *mM*), NaCl (154 *mM*), KCl (5.6 *mM*), $MgCl_2$ (1.4 *mM*), NaHCO (1.7 *mM*) and glucose (5.5 *mM*). The organ bath was maintained at 29 *°C* and air was bubbled through it (18). The tension of the myometrial rings was measured with a gauge transducer.

Uterus was suspended between two stainless steel hocks; one of the hocks was fixed to the chamber wall while the other was attached to an isometric force transducer (UF1 Harvard transducer, UK) and to an ink-writing curvilinear polygraph (Universal Harvard Oscillogragh, UK). The rings were equilibrated for about 1 *hr* before the experiments were done with a solution change in every 15 *min*. The initial tension of the preparation was set to about 1 *g*.

Drugs: Propranolol was purchased from Sigma, USA and naloxone and oxytocin were purchased from Toliddaru and Aboraihan companies in Iran. Other chemicals were purchased from Merck, Germany. To prevent changes in electrolyte composition of the organ bath solution, all chemicals were dissolved in De Jalon solution and the total volume of all solutions added to the organ bath did not exceed more than 5% of the bath volume.

Experimental protocols: After the equilibrium period, the uterus was contracted by 60 *mM* of KCl (15) and at the first plateau of KCl induced contractions; the extracts (0.05, 0.1, 0.2, 0.4, 0.8 *mg/ml*) were added cumulatively to the organ bath. This protocol applied on the contraction induced by $BaCl_2$ (4 *mM*). The uterus of the rats was contracted for 3 *min* by 10 *mU/ml* of oxytocin (15) before the tissues were relaxed by different concentrations of the extract (0.05, 0.1, 0.2, 0.4 and 0.8 *mg/ml*). The extract's spasmolytic effect was also studied on separate tissues after 30 *min* of incubations with propranolol and naloxone

(18) as non-selective α- and β-adrenoceptors and opioid receptors antagonists, respectively. These protocols were repeated for 7 rats in each group.

Statistical analysis: The results were statistically analyzed by ANOVA and post hoc LSD tests and $p<0.05$ were considered as significant. The (n) represents the number of animals used in each protocol.

Results

Effect of *Punica granatum* flower aqueous and hydroalcoholic extracts on the KCl and oxytocin induced uterus contractions.

At first during of 3 *min*, possible repeat of contractions that induced by KCl and oxytocin was achieved to showed health tissue insurance (19). Cumulative concentrations of the extracts (0.05, 0.1, 0.2, 0.4, and 0.8 *mg/ml*) reduced uterine contractions induced by KCl (60 *mM*) and oxytocin (10 *mU/ml*) significantly and in a dose-dependent manner. KCl (60 *mM*) and oxytocin (10 *mU/ml*) had significant difference with all of the hydroalcoholic, ($p<0.05$), and aqueous, respectively $p<0.001$ and $p<0.05$ concentrations of the extracts. The comparison of these inhibitory effects indicates that the spasmolytic effect of the extracts on oxytocin-induced contractions is greater than for KCl–induced contractions (Table 1).

Effects of aqueous and hydroalcoholic extracts of *Punica granatum* flower on KCl-induced contractions in the presence of adrenergic antagonists.

The spasmolytic effect of aqueous and hydroalcoholic extracts of the flowers on KCl- induced uterus contractions did not decrease in the presence of propranolol (1 μM), as a β-adrenoceptor antagonist. There were significant differences between different concentrations of propranolol (1 μM and 0.05 *mg/ml,* $p<0.05$) and different concentrations of the extracts (0.1, 0.2, 0.4, and 0.8 *mg/ml*, $p<0.001$), (Table 1).

Effects of aqueous and hydroalcoholic extracts of *Punica granatum* flower on KCl-induced contractions in the presence of opioid reseptor antagonists.

The antispasmodic effect of aqueous and hydroalcoholic extracts of the flowers on KCl-induced uterine contractions was not reduced by tissue incubation with naloxone (1 μM), as a non-selective opioid receptors antagonist. The results showed a significant difference in antispasmodic activity between the naloxone (1 μM) group and of aqueous and hydroalcoholic concentrations (0.1, 0.2, 0.4, 0.8 *mg/ml*) of the extracts in the presence of naloxone, ($p<0.001$) (Table 1).

Effects of aqueous and hydroalcoholic extracts of *Punica granatum* flower on the $BaCl_2$ induced uterus contractions.

Applying $BaCl_2$ to the organ bath induced a continuous contraction in rat uterus which the extracts could not reduce significantly except the highest dose of the hydroalcoholic form ($p<0.001$), (Table 1).

Table 1. Effect of different amounts of PGE on uterine contractions induced by KCl (60 *mM*), oxytocin (10 *mU/ml*), Kcl+propranolol, Kcl+naloxane, and barium (4 *mM*)

Ecbolic agents	Type of extract	Uterine contraction (Mean±SD)/ PGE extract *				
		0.05 *mg/ml*	0.1 *mg/ml*	0.2 *mg/ml*	0.4 *mg/ml*	0.8 *mg/ml*
Kcl						
	hydroalcoholic	81.43±6^{a}	70.59±6.9^{a,b}	57.92±6.4^{b}	42.22±6.9^{c}	17.87±9^{d}
	aqueous	78±13^{a}	62.28±18^{b}	45.94±6.3^{c}	26.14±4^{d}	10.37±4.4^{e}
Oxytocin						
	hydroalcoholic	72.45±13^{a}	42.77±16^{b}	11.53±7.84^{c}	0±0^{c}	0±0^{c}
	aqueous	68±13^{a}	39.92±18^{b}	4.94±3.87^{c}	0±0^{c}	0±0^{c}
Kcl+ propranolol						
	hydroalcoholic	78.14±6^{a}	68.38±7.35^{a,b}	54.82±8.77^{b,c}	39.79±10^{c,d}	20.28±9.61^{d}
	aqueous	70.3±6^{a}	58±7.48^{a,b}	44.97±9.12^{b,c}	33.23±10.3^{c,d}	17.61±9.64^{d}
Kcl+ naloxane						
	hydroalcoholic	87.68±4^{a}	72.56±8.26^{b}	52.91±7.51^{c}	36.24±7^{d}	16.42±4.81^{e}
	aqueous	86.1±4.6^{a}	69±8.47^{a,b}	56.7±10.15^{b,c}	40.79±10.7^{c}	20.29±7.7^{d}
Barium						
	hydroalcoholic	99.2±0.8^{a}	99.2±0.8^{a}	97.61±2.38^{a}	96±3.96^{a}	75.44±6.41^{b}
	aqueous	98.8±0.8^{a}	97.43±1.64^{a}	95.6±2.78^{a}	93±4.4^{a}	92.91±5^{a}

a-e: Numbers with different superscript letters in the same row differ significantly ($p<0.05$)

* The uterine contraction is proportional to 100% contraction induced by ecbolic agents under the influence of different amounts of PGE

Discussion

The results of this study showed that aqueous and hydroalcoholic extracts of *Punica granatum* flower could induce spasmolytic effects on uterine muscle contractions caused by KCl, barium chloride or oxytocin. The highest dose of both extracts had the highest antispasmodic effect on uterine contractions.

It has been reported that in KCl-induced contractions, Voltage Dependent Calcium Channels (VDCCs) are involved and the existence of L-type VDCCs in rat uterus has been documented. Therefore, it is suggested that those substances which decrease K^+-induced contraction can also block the VDCCs (20).

In next step of this study, the effect of PGE on oxytocin-induced uterus contraction was investigated in De Jalon solution. Oxytocin binds to its receptors and increases inositol triphosphate (IP_3) production. Oxytocin also activates the L-type VDCCs (21). Although the extracts were used for KCl- and oxytocin- induced uterine contraction, however, the comparison of PGE antispasmodic effect on these spasmogens shows that the effects of the extracts on oxytocin-induced contractions is more potent than on KCl-induced contraction. This results indicate that probably oxytocin receptors are more involved in the extract activity. Furthermore, these spasmogens are similar in acting through VDCCs; therefore, it may be conclude that Ca^{2+} influx could be involved in the extract activity.

Adrenoceptors are important in uterine contractility but propranolol was unable to reduce spasmolytic effects of PGE by antagonizing the β-adrenoceptors. The role of β-adrenoceptors is relaxation of uterus (22). Therefore, inability of propranolol to induce antispasmodic effects of PGE indicated that the extract had not delivered its action via these receptors.

Opioid system has been observed in the endometrial and myometrial regions of the uterus and opioid receptors activation prevents uterine contractions (23, 24). However, naloxone, as a non-selective opioid receptors antagonist, was unable to reduce PGE spasmolytic effects. This result indicates that these receptors could not have been involved. One study showed that barium chloride could induce smooth muscle contraction by blocking potassium channels (25), and it has been reported that Ba^{2+} could increase Ca^{2+} release from intracellular pools in smooth muscles and uterus (26). PGE could not reduce uterine contractions induced by $BaCl_2$ except the highest dose (0.8 *mg/ml*) of its hydroalcoholic extract. Therefore, it could be concluded that the highest dose of this extract could probably prevent release of calcium or calcium efflux. Furthermore our results demonstrated the involvement of calcium channels in spasmolytic effects of *Punica granatum* flower in rat uterus, which may support the use of this plant in traditional medicine to relieve dysmenorrhea.

Conclusion

In conclusion, our result indicated that aqueous and hydroalcoholic extracts of *Punica granatum* flower could induce relaxant effects on uterus of virgin rat and uterine contractions were decreased without involvement of β-adrenoceptors or opioid receptors. These results indicate that aqueous extracts of *punica granatum* flower could induce spasmolytic effect on rat uterus through blockage of VDCCs. These results support the clinical efficacy and use of *Punica granatum* flower in the treatment of dysmenorrhoea and other uterine spasmodic disorders. This process appears to be the most relevant physiological process and should be the target of future research.

Acknowledgement

This research was financially supported by the Student Research Center of Ahvaz Jundishapour Medical Sciences University, Ahvaz, Iran.

Conflict of Interest

Authors did not have any conflicts of interests regarding the contents of this paper.

References

1. Xie Y, Morikawa T, Ninomiya K, Imura K, Muraoka O, Yuan D, et al. Medicinal flowers. XXIII. New taraxastane-type triterpene, punicanolic acid, with tumor necrosis factor-alpha inhibitory activity from the flowers of Punica granatum. Chem Pharm Bull. 2008;56(11):1628-31.
2. Huang TH, Peng G, Kota BP, Li GQ, Yamahara J, Roufogalis BD, et al. Anti-diabetic action of Punica granatum flower extract: activation of PPAR-gamma and identification of an active component. Toxicol Appl Pharmacol. 2005;207(2):160-9.
3. Prashanth D, Asha MK, Amit A. Antibacterial activity of Punica granatum. Fitoterapia. 2001;72(2):171-3.
4. Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP, et al. Studies on antidiarrhoeal activity of Punica granatum seed extract in rats. J Ethnopharmacol. 1999;68(1-3):205-8.

5. Dutta BK, Rahman I, Das TK. Antifungal activity of Indian plant extracts. Mycoses. 1998;41(11-12):535-6.
6. Gharzouli K, Khennouf S, Amira S, Gharzouli A. Effects of aqueous extracts from Quercus ilex L. root bark, Punica granatum L. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. Phytother Res. 1999;13(1): 42-5.
7. Gujral ML, Varma DR, Sareen KN. Oral contraceptives. Part I. Preliminary observations on the antifertility effect of some indigenous drugs. Indian J Med Res. 1960;48:46-51.
8. Hayouni EA, Miled K, Boubaker S, Bellasfar Z, Abedrabba M, Iwaski H, et al. Hydroalcoholic extract based-ointment from Punica granatum L. peels with enhanced in vivo healing potential on dermal wounds. Phytomedicine. 2011;18(11):976-84.
9. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol. 2007;109(2):177-206.
10. Hassanpour Fard M, Ghule AE, Bodhankar SL, Dikshit M. Cardioprotective effect of whole fruit extract of pomegranate on doxorubicin-induced toxicity in rat. Pharm Biol. 2011;49(4):377-82.
11. Gasmi J, Sanderson JT. Growth Inhibitory, Antiandrogenic, and Pro-apoptotic Effects of Punicic Acid in LNCaP Human Prostate Cancer Cells. J Agric Food Chem. 2010 Nov 10. [Epub ahead of print]
12. Aviram M, Dornfeld L, Kaplan M, Coleman R, Gaitini D, Nitecki S, et al. Pomegranate juice flavornoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drugs Exp Clin Res. 2002;28(2-3):49-62.
13. Jafri MA, Aslam M, Javed K, Singh S. Effect of Punica granatum Linn. (flowers) on blood glucose level in normal and alloxan-induced diabetic rats. J Ethnopharmacol. 2000;70(3):309-14.
14. Zargari A. Medicinal plants. 5th ed. Tehan: Tehran University Press; 1992. p. 344.
15. Ahangarpour A, Oroojan AA. The effects of Cassia italica leaves aqueous extract on non-pregnant uterus contraction in rats. Iran J Reprod Med. 2010;8(4):179-84.
16. Naseri MK, Arabian M, Badavi M, Ahangarpour A. Vasorelaxant and hypotensive effects of Allium cepa peel hydroalcoholic extract in rat. Pak J Biol Sci. 2008;11(12):1569-75.
17. Acritopoulou-Fourcroy S, Marçais-Collado H. Involvement of alpha-adrenoceptors in myometrial responses in the pro-oestral rat. Br J Pharmacol. 1988;93(1):185-91.
18. Gharib Naseri MK, Mohammadian M, Gharib Naseri Z. Antispasmodic effect of Physalis alkekengi fruit extract. Iran J Reprod Med. 2008;6(4):193-8.
19. Oropeza MV, Ponce-Monter H, Villanueva-Tello T, Palma-Aguirre JA, Campos MG. Anatomical differences in uterine sensitivity to prostaglandin F(2alpha) and serotonin in non-pregnant rats. Eur J Pharmacol. 2002;446(1-3):161-6.
20. Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K, et al. Calcium movements, distribution, and functions in smooth muscle. Pharmacol Rev. 1997;49(2):157-230.
21. Zhou XB, Lutz S, Steffens F, Korth M, Wieland T. Oxytocin receptors differentially signal via Gq and Gi proteins in pregnant and nonpregnant rat uterine myocytes: implications for myometrial contractility. Mol Endocrinol. 2007;21(3):740-52.
22. Tolszczuk M, Pelletier G. Autoradiographic localization of beta-adrenoreceptors in rat uterus. J Histochem Cytochem. 1988;36(12):1475-9.
23. Zhu Y, Pintar JE. Expression of opioid receptors and ligands in pregnant mouse uterus and placenta. Biol Reprod. 1998;59(4):925-32.
24. Ohia SE, Laniyonu AA. Naloxone-insensitive inhibitory and excitatory effects of opioid agonists in the rat isolated uterus. J Pharm Pharmacol. 1989;41 (3):168-72.
25. Huang Y. BaCl2- and 4-aminopyridine-evoked phasic contractions in the rat vas deferens. Br J Pharmacol. 1995;115(5):845-51.
26. Gharib Naseri MK, Mazlomi H, Goshaiesh M, Vakilzadeh G, Heidari A. Antispasmodic effect of Zataria multiflora boiss. Leaf extract on the rat uterus. Iran J Pharm Res. 2006;5(2):131-6.