

Antispasmodic effects of *Pycnocycla spinosa* seed and aerial part extracts on rat ileum and uterus smooth muscle contractions

¹Sadraei H., ²Asghari Gh., ¹Andishah M.

¹Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, ²Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

Background and the purpose of study: Hydroalcoholic extract of *Pycnocycla spinosa* has a relaxant effect on ileum and inhibits castor oil induced diarrhoea in mice. However, effects of *P. spinosa* seed extracts on ileum and uterus hasn't been investigated. The aim of this study was to investigate effect of *P. spinosa* seed and extracts of the aerial part on rat ileum and uterus smooth muscle contraction.

Methods: A 70% ethanol extract of seed and aerial parts of *P. spinosa* was prepared by a percolation method. Uterine horns or ileum were dissected from non-pregnant female Wistar rats (200-230g) and cut into longitudinal strips and mounted for isotonic recording under 1g tension in Tyrode's solution. Effects of the extracts were examined on tonic contractions induced by KCl (80mM) on both tissues and on phasic spasm induced by oxytocin (0.002iu/ml) on the uterus.

Results: The aerial part extract inhibited rat ileum contractions induced by 80mM KCl ($IC_{50}=42\pm3.4\mu\text{g/ml}$) in a concentration dependent manner and it also inhibited rat uterus contraction induced by 80 mM KCl. However, its inhibitory effects were observed with higher concentration of the extract ($IC_{50}=420\pm90\mu\text{g/ml}$) and at concentration of 1.28mg/ml of the extract in the bath the response was $19\pm7\%$.

The aerial part extract (40-640 $\mu\text{g/ml}$) also reduced the evoked phasic response of uterus by oxytocin ($IC_{50}=71\pm17.3\mu\text{g/ml}$). The seed extract reduced the uterus response to oxytocin in a concentration-dependent manner, and inhibited tissue response completely at 160 $\mu\text{g/ml}$ ($IC_{50}=27\pm4\mu\text{g/ml}$).

Major conclusion: From this study it was concluded that the seed extract of *P. spinosa* have similar inhibitory properties on rat isolated uterus and ileum contractions, while the extract of the aerial part of *P. spinosa* is more selective inhibitor of ileum contraction, and at higher concentrations it also inhibits uterus spasm.

Keywords: *Pycnocycla spinosa*; Spasmolytic; Uterus; Ileum; Seed, Aerial parts

INTRODUCTION

Pycnocycla spinosa Decne. exBoiss. var. *spinosa* (Fam. Umbelliferae) is a wild plant growing in Iran (1-3). Hydroalcoholic extract of *P. spinosa* (from the aerial part of the plant) is a potent relaxant of isolated ileum and it inhibits ileum contraction induced by KCl, acetylcholine and 5-HT (4-5). In addition, *P. spinosa* extract has antidiarrhoeal action at doses of 250 $\mu\text{g/kg}$ to 1mg/kg in mice (4). The hydroalcoholic extract contains alkaloids, flavonoids and saponins components (5). The antispasmodic action of *P. spinosa* extract is partly due to alkaloid and flavonoid fractions (5). The underlying mechanism of anti-diarrhoeal action of *P. spinosa* extract is most likely related to inhibition of the gut motility (4). Accordingly it may be assumed

that *P. spinosa* extract may have inhibitory effects on other smooth muscles. It has been reported that *P. spinosa* extract has a more selective pharmacological activity on ileum in comparison with bladder (6). The objective of this research was to investigate the effect of *P. spinosa* extract on rat isolated uterus contractions. Furthermore, pharmacological activity of extracts obtained from plant seed were compared with the extract from aerial part of the plant.

METHODS

Drugs and solutions

Tyrode's solution composed of (mM): NaCl, 136.9; KCl, 2.68; CaCl₂, 1.8; MgCl₂, 1.05; NaHCO₃, 11.9; NaH₂PO₄, 0.42 and glucose 5.55

made up in distilled water. All chemical were purchased from Merck. KCl was made up as 2M solution in distilled water. Oxytocin (10 units/ml, Iran-Rasht) was diluted in distilled water. The extract from aerial part was made up as 10mg/ml of the stock solution in distilled water, while the seed extract was made up as 10mg/ml of the stock solution in 50% ethanol. Further dilutions were made in distilled water or Tyrode's solution accordingly.

Extract preparation

P. spinosa was collected from Isfahan University garden which is located at the base of Sofah mountain in Isfahan (Iran). The aerial part of *P. spinosa* was collected in July when the plant was in flowering period and the seed were collected in August. A voucher specimen was authenticated and deposited in the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences (Isfahan, Iran). The plant was dried in shade and a 70% ethanolic extract was prepared by a percolation method (7). After evaporation of the ethanol, the amount of dry crude extract was determined (w/w).

Pharmacological studies

Non-pregnant female Wistar rats (bred in Isfahan) weighing between 200-230g, were killed by a blow on the head followed by exsanguination. Uterine horns or ileum were cut into longitudinal strips and mounted in 20ml organ bath containing Tyrode's solution, which was maintained at 37°C and constantly aerated with oxygen. In the case of uterus, the rats were pretreated a day before the experiment with 17-β-estradiol (100μg/kg; s.c.). The tissues were subjected to a resting tension of 1g and allowed to equilibrate for 15 minutes and during this period of time were washed several times. Isotonic contraction was measured by a Harvard transducer and recorded on a Harvard Universal Oscillograph pen recorder device. Relaxant effect of the extracts was examined on tonic contraction induced by KCl (80mM) on both tissues and on the rhythmic spasm of uterus induced by oxytocin (0.002iu/ml). After equilibration, the extract was added in a cumulative manner to the bath at 15min intervals for KCl and 10min intervals for oxytocin. All experiments were conducted in parallel with time-matched controls by addition of an equivalent volume of the vehicle.

Measurements and statistical analysis

Contractions were measured as area under the curve from baseline, produced by tissue contraction at 5min intervals just before addition of each concentration of the extract and expressed as % of control response for each tissue. Values

are quoted as mean±SEM. The significance of differences between the means was calculated by two tailed paired Student's t-test or by one-way analysis of variance (ANOVA), followed by post Hoc test, as appropriate. Origin computer program was used for plotting the graphs and calculation of inhibitory concentration causing 50% of maximum response (IC₅₀).

RESULTS

While the yield of aerial extract was 12% (W/W) and had no specific smell, the yield of seed extract was 4% (W/W) and it had a strong smell. Both extracts had greenish-brown colour.

KCl (80mM) induced a sustained tonic contraction in both tissues. Oxytocin (0.002IU/ml) induced a rhythmic contraction in the uterus. The rhythmic responses had tendency to subside down gradually in long period of time.

Both seeds and aerial part extracts of *P. spinosa* inhibited rat ileum contractions induced by 80 mM KCl in a concentration-dependent manner (Figure 1). Relaxation of the ileum with aerial part began with 10μg/ml of the extract in the bath, reduced to 10±5% with 80μg/ml and inhibited completely at 160μg/ml of the bath concentration. Relaxation of the ileum with the seed extract began at 5μg/ml of the bath concentration and complete inhibition was achieved with 80μg/ml of the extract in the bath (Figure 1). No significant changes were observed in the KCl time-matched control tissues treated with the vehicle (Figure 1). In the case of rat uterus, the inhibitory effect of aerial extract on KCl induced contraction began at 80μg/ml of the bath concentration and by increasing the concentration to as high as 1.28mg/ml 19±7% of the initial response was still remained (Figure 2). Seed extracts also concentration-dependently (10-160μg/ml, n=7) inhibited the uterus contractions induced by KCl (80mM). With concentration of 160μg/ml of *P. spinosa* in the bath, response to KCl was abolished (Figure 2). No significant changes were observed in the KCl time matched control tissues treated with the vehicle (Figure 2).

Relaxant effects of the *P. spinosa* extracts on rat uterus were further examined on oxytocin-induced contractions. Seed extracts reduced the tissue response to oxytocin in a concentration-dependent manner (2.5-160μg/ml) and completely inhibited uterus contraction at 160μg/ml of the bath concentration (see Figure 3). The aerial part extract of *P. spinosa* also concentration-dependently reduced oxytocin evoked rhythmic response of the uterus. When concentration of the extract in the bath was 640μg/ml, response to

Table 1. Comparison of IC₅₀ values of extracts of the aerial part and the seed of *P. spinosa* on rat isolated ileum and uterus contractions induced by KCl (80mM) or oxytocin (0.002iu/ml). * indicate statistical significant differences between aerial and seed extracts (*P<0.05, ***P<0.001, ANOVA).

<i>P. spinosa</i>	IC ₅₀ (concentration causing 50% of maximum inhibition)		
	ileum (KCl)	uterus (KCl)	uterus (oxytocin)
aerial extract	42±3.4µg/ml (n=6)	420±90µg/ml (n=5)	71±17.3µg/ml (n=6)
seed extract	26±6.1µg/ml (n=4)	38±4.8µg/ml (n=7)***	27±4.0µg/ml (n=6)*

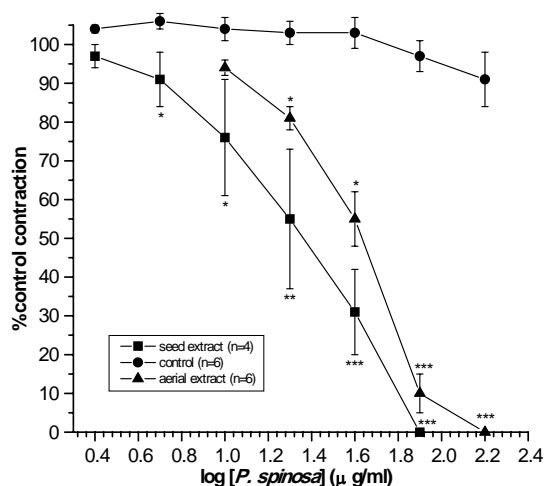


Figure 1. Concentration-response effects of the extracts of seed and aerial part of *P. spinosa* on tension which was induced by KCl (80mM) in rat isolated ileum (n=6). The points are mean and the vertical bars show the SEM. *P<0.05, **P<0.01, ***P<0.001 compared with corresponding point in the vehicle treated time-matched control tissues (Student's t-test).

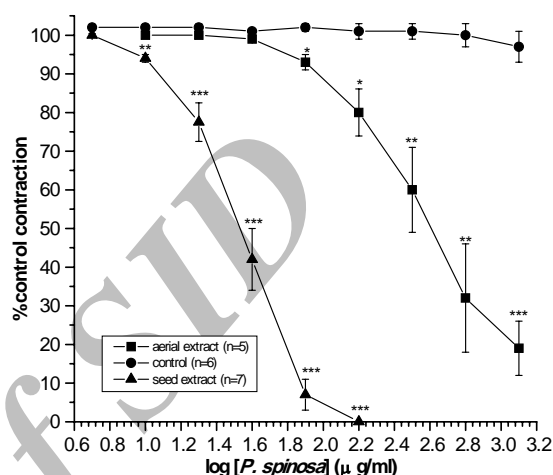


Figure 2. Concentration-response effects of the extracts of seed and aerial part of *P. spinosa* on tension induced by KCl (80mM) in rat isolated uterus. The points are mean and the vertical bars show the SEM. *P<0.05, **P<0.01, ***P<0.001 compared with corresponding point in the vehicle treated time-matched control tissues (Student's t-test).

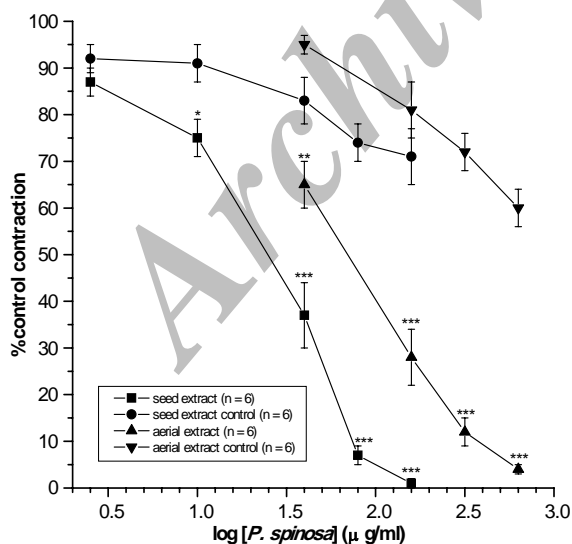


Figure 3. Concentration-response effects of the extract of seed and aerial part of *P. spinosa* on tension induced by oxytocin (0.002iu/ml) in rat isolated uterus. The points are mean and the vertical bars show the SEM. *P<0.05, **P<0.01, ***P<0.001 compared with corresponding point in the vehicle treated time-matched control tissues (Student's t-test).

oxytocin was reduced to 4±1% (Figure 3). However, in the time matched control tissues, smaller but significant reduction occurred in the oxytocin induced uterus contraction over the course of study (P<0.05). Therefore, the actual inhibitory effect on phasic contractile response of the uterus is less than that which was observed (see Figure 3). Table 1 summarizes the IC₅₀ values of seed and aerial part extracts on rat ileum and uterus contractions. Comparison of IC₅₀ values, indicate no statistically significant difference between antispasmodic activity of seed and aerial part extracts on ileum. On the other hand, there are statistically significant differences between inhibitory action of the seed and aerial part extracts on rat uterus contractions induced by KCl (P<0.001) or oxytocin (P<0.05). In another words, seed and aerial part extracts of *P. spinosa* are equipotent on inhibition of the rat ileum contractions while the seed extract is more potent on rat uterus (see Table 1).

DISCUSSION

Hydroalcoholic extract from aerial part of *P. spinosa* inhibited KCl induced contraction of

ileum at concentration ranges similar to the previous studies (4-6). Therefore, there wasn't any difference in pharmacological activity of the plant extract that were collected on three successive years from the same area. The extract of the aerial part of *P. spinosa* inhibited rat isolated uterus contractions induced by KCl and oxytocin. Nevertheless, there was a quantitative difference as the aerial part extract inhibited oxytocin response at lower concentrations (see Figure 3). This difference is either due to the presence of different substances acting via different mechanisms or *P. spinosa* extract has more selective inhibitory effects on contraction induced by oxytocin, which requires further investigation. A comparison of the relaxant effect of the aerial part extract of *P. spinosa* on KCl contractions on ileum with that of uterus at IC₅₀ levels (see Table 1) revealed that the aerial part extract was about ten times more potent on the ileum (see Figure 1). From pharmacological point of view this difference shows a great selectivity of the aerial part extract towards ileum contractions. In a separate study it has been shown that aerial part extract of *P. spinosa* in concentration of 80µg/ml to 1.28mg/ml in the bath inhibits rat bladder contraction induced by KCl (6). This result is very close to the inhibitory response of the aerial extract on the rat uterus of this study. Therefore, the aerial part extract of *P. spinosa* at concentrations that inhibit ileum spasm had no or

just small inhibitory effect on rat uterus and bladder smooth muscle contraction, indicating its selective relaxant action on the ileum.

Seed extracts of *P. spinosa* was also a potent relaxant on rat isolated ileum. However, the concentration required for relaxant effect of the seed extract were nearly equal on both ileum and uterus smooth muscles. Furthermore, seed extract at similar concentrations inhibited oxytocin and KCl induced contraction on rat uterus (Figures 2 & 3). Although both seed and aerial part extracts are potent inhibitor of ileum contraction, the aerial extract is less potent on uterus contraction. The difference between seed and aerial part extracts on uterus contractions may indicate presence of additional components in the seed extract, which are more active on uterus smooth muscle. The presence of extra components in the seed extract also can explain the higher activity of the seed extracts on oxytocin induced uterus contractions (Figure 3).

From this study it may be concluded that the seed extract of *P. spinosa* is more active for prevention of uterus spasm, while the aerial part extract might be a more suitable remedy for ileum spasm.

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