Effect of Hydroalcoholic Extract of *Pycnocycla spinosa* on Rat Isolated Bladder Contraction

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**Abstract**

Hydroalcoholic extract of *Pycnocycla spinosa* is a relaxant of rat ileum and inhibits diarrhea in mice. As *P. spinosa* extract has spasmolytic activity on ileum, it could also affect other smooth muscles like bladder. Therefore, the aim of this study was to determine the effect of *P. spinosa* extract on rat bladder contraction.

In an in vitro study, the effects of *P. spinosa* extract, nifedipine and propantheline were tested on isolated rat bladder contractions induced by acetylcholine (ACh, 10 M) and KCl (80 mM).

*P. spinosa* extract, concentration-dependently, inhibited the bladder contractions induced by ACh with an IC50 of 265±28 μg/ml, and KCl with an IC50 of 518±86 μg/ml. The muscarinic cholinoceptor antagonist, propantheline, inhibited the response of ACh without affecting KCl response. Nifedipine, on the other hand, abolished the KCl response, while partially inhibiting the ACh contraction in rat bladder.

The antispasmodic effect of *P. spinosa* extract on bladder was observed at higher concentrations as compared to that of ileum. Therefore, it is unlikely that *P. spinosa* extract at anti-diarrheal doses affect the normal bladder emptying function.

**Keywords:** *Pycnocycla spinosa*; spasmolytic; bladder; ileum.

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**Introduction**

*Pycnocycla spinosa* Decne. Ex Boiss. var. spinosa (Fam. Umbelliferae) is a wild plant, growing in Iran (1, 2). Hydroalcoholic extract of *P. spinosa* is a potent relaxant of isolated ileum (3). In addition, *P. spinosa* extract was shown to have anti-diarrheal action at doses of 250 μg/kg to 1 mg/kg in mice (3). The anti-spasmodic action of *P. spinosa* extract is very similar to that of dicyclomine (4) and its anti-diarrheal dose on castor oil-induced diarrhea is very close to that of loperamide (5) and diphenoxylate (6). Therefore, *P. spinosa* extract could be an alternative remedy for the treatment of gastrointestinal spasm and diarrhea. The underlying mechanism of anti-diarrheal action of *P. spinosa* extract is most likely related to its gut motility inhibition. It has been shown that *P. spinosa* extract inhibits ileum contraction induced by KCl, acetylcholine (ACh) and serotonin (3) and therefore, *P. spinosa* extract may have an inhibitory effect on other smooth muscles, including bladder. Thus, the objective of this study was to investigate the effect of *P. spinosa* extract on rat isolated bladder contractions for the purpose of comparison with the ileum.

**Experimental**

**Plant material**

Aerial parts of *P. spinosa* were collected and prepared as described before (7) and hydroalcoholic extract was obtained by the percolation method (8).
Drugs and solutions

The following drugs were used for the experiments: Acetylcholine chloride (ACh, Sigma), *Pycnocycla spinosa* hydroalcoholic extract, nifedipine (Sigma), and propantheline bromide. ACh was made up as a 100 mM stock solution in distilled water and acidified with a drop of acetic acid. KCl was made up as a 2 M stock solution in distilled water. The hydroalcoholic extract was made up as a 10 mg/ml stock solution in 70% ethanol. Nifedipine was prepared as a 1 mM stock solution in dimethylsulphoxide (DMSO). Propantheline was dissolved in distilled water as a 10 mM stock solution. All dilutions were made in distilled water. All chemicals, unless otherwise stated, were purchased from Merck (Germany). Propantheline was a gift from Parsmino Pharmaceutical Company (Iran).

Experimental procedure

Male Wistar rats (bred in Isfahan), weighing between 200-250 g, were used in all experiments. Animals were killed by a blow on the head, followed by exsanguination. Whole urinary bladder was isolated and placed in oxygenated Tyrode's solution of the following composition (mM): NaCl 136.9, KCl 2.68, CaCl2 1.8, MgCl2 1.05, NaHCO3 11.9, NaH2PO4 0.42 and glucose 5.55. The connective tissue was carefully trimmed and the bladder was mounted in a 20 ml organ bath containing Tyrode's solution, which was maintained at 37°C and constantly aerated with oxygen (pH=7.4). Isotonic tension generated by KCl and ACh in the tissue was measured by a Harvard transducer and recorded on a Harvard Universal Oscillograph pen recorder device. The tissues were subjected to a resting tension of 1 g and allowed to equilibrate for 15 min during which time they were washed several times. The viability of each tissue was evaluated by examining the contractile response to KCl (80 mM). Drugs were added directly to the organ bath in volumes usually not exceeding 5% of the bath volume. A concentration-response curve was obtained by cumulative addition of the *P. spinosa* extract, nifedipine or propantheline at 15 min intervals, over KCl (80 mM) induced contraction. The effects of sub-maximal concentrations of ACh (10 μM) were studied, using a single dose regimen with a contact time of 30 s and time cycle of 3 min. The contact period of each concentration of the extract or drug was allowed at least for 10 min before the responses to KCl or ACh were evaluated. All experiments were conducted in parallel with time-matched controls, adding an equivalent volume of the vehicle.

Measurements and statistical analysis

Bladder contractions were measured as maximum changes in tension from pre-drug baseline within the contact time or as the area under the curve produced by tissue contraction at 5 min intervals just before the addition of next concentration of drug/extract (or vehicle) and the percentage inhibition of the initial response were calculated for each tissue. Mean and standard error of mean (SEM) values were calculated for each group of results and significance of differences between the means were calculated by the two-tailed paired Student's t-test and/or one way analysis of variance (ANOVA). Differences were considered statistically significant, when P<0.05. The Origin® computer program was used for fitting the non-linear curve and calculation of the IC50 values (IC50=drug concentration causing 50% of maximum response).

Results And Discussion

Rat bladder suspended in Tyrode's solution under 1 g tension, had a stable tension. KCl (80 mM) produced a sustained tonic contraction. ACh (10 μM) caused a rapid contraction, reaching its maximum within 30 s of contact. The hydroalcoholic extract of *P. spinosa* (80 μg/ml-1.28 mg/ml), in a concentration-dependent manner inhibited rat bladder contraction induced by KCl with an IC50 value of 518±86 μg/ml (n=5, Figure 1). At 1.28 mg/ml bath concentration, the response to KCl was almost abolished. The inhibitory effect of the extract was reversible, after washing the tissue with fresh Tyrode's solution. Relaxant effect of the extract was further examined on contraction induced by ACh (10 μM) and compared with
those of nifedipine and propantheline. \textit{P. spinosa} extract inhibited the tissue response to ACh in a concentration-dependent manner (IC50=265±28 µg/ml, n=6), with complete inhibition occurring at bath concentrations of 640 µg/ml (Figure 1). At this high concentration, \textit{P. spinosa} extract caused an initial tension that was not maintained and gradually subsided to baseline within 10 min.

Nifedipine (25 nM-64 µM) inhibited the tonic contraction to KCl (IC50=816 ±264 nM, n=6) and abolished the tonic response at a bath concentration of 6.4 µM (Figure 2). However, nifedipine partially inhibited the bladder response to ACh and still about 30% of the initial response remained at a concentration that completely inhibited the KCl response (Figure 2). Increasing the concentration of nifedipine had no further inhibitory effect on the contraction induced by ACh.

Propantheline (0.25 nM-16 nM) on the other hand, completely blocked the ACh response in a concentration-dependent manner (IC50=0.93±0.26 nM) (Figure 3), but had no effect on KCl induced contraction even at a concentration as high as 3.2 M (n=4). There was no significant reduction in ACh responses of the tissues treated with a comparable volume of the vehicle. However, the vehicle (4.5% ethanol), comparable to that available in the highest concentration of \textit{P. spinosa} extract (1.28mg/ml), caused a reduction in KCl tonic response. This was no significant reduction in the tonic contraction of the bladder in the other controls.

Bladder contraction is mediated by cholinergic, nonadrenergic and noncholinergic (NANC) mechanisms (9). Cholinergic stimulation is responsible for the major part of bladder contraction. ACh, released from postganglionic cholinergic nerves, activates postjunctional muscarinic receptors in the detrusor (9). Urinary bladder smooth muscle is enriched with muscarinic receptors, the majority of which are of M2 subtype, whereas the remaining minorities belong to the M3 subtype (10). Pharmacological characterization of muscarinic receptors mediating contraction of detrusor muscle in rat (11) and human bladder (12) suggest the involvement of M3 receptors. The role of dominant M2 receptor population is unclear, although its activation can contract the bladder indirectly, by reversing sympathetically mediated relaxation (10). Muscarinic M3 receptor stimulation has been shown to stimulate phosphoinositide hydrolysis in urinary bladder (13-15) and this is the most likely signaling mechanism of action of ACh used in these experiments. The contraction initiated by depolarization with high K+ was totally dependent on Ca2+ influx, since the addition of Ca2+ channel blocker "nifedipine", completely inhibited this contraction. Nifedipine also partially inhibited the ACh response. Therefore, our study shows that muscarinic excitation of rat

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Inhibitory effect of hydroalcoholic extract of \textit{P. spinosa} on tension development to KCl (n=5) and ACh (n=6) in rat isolated bladder. The effects of extract have been expressed as % inhibition from the initial tissue contraction before the addition of extract. The points are mean and the vertical bars show the SEM.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Inhibitory effect of nifedipine on tension development to KCl and ACh in rat isolated bladder. Drug effects are expressed as % inhibition from the initial tissue contraction before the addition of drug. The points are mean and the vertical bars show the SEM (n=6). **P<0.01, ***P<0.001 compared with the control group (Student's t-test).}
\end{figure}
bladder involves both the release of \( \text{Ca}^{2+} \) from intracellular stores and the influx of \( \text{Ca}^{2+} \). It has been reported that in detrusor smooth muscle, a rise in intracellular \( \text{Ca}^{2+} \) concentration, following activation of cholinergic receptors is independent of the membrane potential, but opening of the L-type \( \text{Ca}^{2+} \) channels, can be modulated by secondary effects on the membrane (16). Propantheline, a muscarinic receptor antagonist with clinical use for adult enuresis, urinary incontinence and gastrointestinal spasm, completely blocked the tissue response to \( \text{ACh} \) without affecting the \( \text{KCl} \) response. A similar effect was seen with another muscarinic receptor antagonist "atropine" on rat bladder (17). Hence, it is clear that \( \text{KCl} \) and \( \text{ACh} \) have distinct contractile mechanisms of action on rat bladder. Hydroalcoholic extract of \( \text{P. spinosa} \) abolished the contractions induced by \( \text{KCl} \) and \( \text{ACh} \). Nevertheless, there is a quantitative difference, as \( \text{P. spinosa} \) extract inhibited \( \text{ACh} \) response at lower concentrations. This difference is either due to the presence of different substances acting via different mechanisms or because \( \text{P. spinosa} \) extract has a more selective inhibitory effect on the contraction induced by \( \text{ACh} \). However, as propantheline has no effect on \( \text{KCl} \) induced contraction, even at concentrations higher than those that abolish \( \text{ACh} \) response, muscarinic receptor antagonism could not explain the inhibitory action of \( \text{P. spinosa} \) extract. Comparison of the relaxant effect of hydroalcoholic extract of \( \text{P. spinosa} \) on \( \text{KCl} \) contractions on ileum (IC50=40±7.3 \( \mu \text{g/ml} \)) with that of bladder (3), revealed that the extract was about ten times more potent on ileum. Of course there is a concern about the origin of the extract, as hydroalcoholic extracts are obtained from separate batches of plants. Therefore, in a pilot study, the effect of \( \text{P. spinosa} \) extract on ileum was also examined and the previously reported effect of the extract on ileum was reproduced. Therefore, \( \text{P. spinosa} \) extract, at concentrations that inhibit ileum spasm, had no inhibitory effect on bladder, thereby indicating its more selective action on ileum.

Thus, it is concluded that since the hydroalcoholic extract of \( \text{P. spinosa} \), at a concentration that inhibits ileum motility, does not exhibit spasmolytic effect on bladder smooth muscle, urine retention is not expected with anti-diarrheal doses of the compound.

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