Antispasmodic effects of alcoholic extracts from polyherbal formulation “Prasaplai” on isolated rat uterine horn

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Abstract

This study investigated the spasmolytic effect of the alcoholic extracts from polyherbal formulation “Prasaplai” (PSP01 and PSP02), in comparison with the alcoholic extract from Zingiber cassumunar Roxb. (PSPoil), using in the in vitro model of isolated rat uterine horn. Our result indicated that all of the test materials significantly relaxed the muscle tension pretreated with oxytocin (2 nM) and KCl (50 mM) in concentration-dependent manner. The IC₅₀ (inhibition of force) of PSP01, PSP02 and PSPoil on oxytocin-induced contraction were 27.48±2.89, 30.26±4.44 and 32.51±3.05 µg/ml, respectively. The IC₅₀ of these extracts on KCl-induced contraction were 20.11±2.72, 19.67±2.99 and 27.80±4.62 µg/ml, respectively. This study demonstrated that the alcoholic extracts of polyherbal formulation “Prasaplai” contained spasmolytic activity on the uterus muscle. In addition, the prasaplai extracts were more potent than the extract of its major herbal ingredient.

Keywords: Uterine relaxation, Zingiber cassumunar, Prasaplai, polyherbal medicine

Introduction

Prasaplai preparation has been used orally in Thailand for a long time. Currently, it is on the list of the Thai Traditional Common Household Drug, announced by the Ministry of Public Health for relieving muscle pain, postpartum uterine involution and abnormal menstrual cycle (1). This preparation composes of ten medicinal plants and two minerals, with Zingiber cassumunar Roxb. or “plai” as a major ingredient (approximately 80%). Although there were certain reports of the spasmolytic activity of Prasaplai extract in the in vitro model of isolated rat uterus, there was uncertain whether the pharmacological actions of major herbal ingredient and the whole preparation would be equivalent. Thus, this study aimed to investigate the spasmolytic effect of the alcoholic extract of polyherbal formulation “Prasaplai” in comparison with the alcoholic extract of Zingiber cassumunar Roxb. (plai), in the in vitro model of isolated rat uterine horn.

Materials and methods

Chemicals and test materials

The alcoholic extracts of Prasaplai formulation were commercially available from two different sources (PSP01, PSP02) and the alcoholic extract of Zingiber cassumunar Roxb. (PSPoil). All the extracts were kindly provided by Thailand Institute of Scientific and Technological Research.

Tissue preparations

The animals were pretreated with estradiol-17-benzoate (1 mg/kg, i.p.) 24-48 hours before the experiments (2). On the day of experiment, the rat was sacrificed, and removed uterine horn. Then, the uterine horns were cut in to 4 segments (approximately 1.0-1.5 cm long), and suspended in Locke-Ringer solution at 37 °C gassed with carbogen (95% O₂ and
The contractile response was recorded isometrically with a force transducer UFI 1030 (ADInstruments, Australia) connected to PowerLab/4SP and computer equipped with program SCOPE CHART 5 V.2.0 (ADInstruments, Australia).

The study protocols were approved by the Ethics Committee on Animal Experiment, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Experimental protocol

Effects of the test materials on oxytocin and KCl-induced contraction

This experiment used oxytocin (2 nM) and KCl (50 mM) to induce uterine contraction. When the contraction reached plateau state, the test materials (10-50 µg/ml) were added cumulatively to the organ bath. The tension was recorded and expressed as percentage of the agonist-induced contraction. In separated experiments, the effects of DMSO were also determined as a control.

Statistical analysis

Results were expressed as mean±S.E.M. Statistical analysis was carried out by one-way ANOVA, followed by Dunnett’s test post hoc comparison. P<0.05 indicated statistical significance.

Results

Effects of the test materials on oxytocin and KCl-induced contraction

As shown in Figure 1, oxytocin (2 nM) caused rhythmic contractions of isolated rat uterus with the developed tension of 7.12±0.53 g and frequency of 7.85±0.48 stroke/10 min (n=16). The cumulative addition of the test materials (PSP01, PSP02 and PSPoil) 10-50 µg/ml significantly inhibited both force and frequency of oxytocin-induced uterine contraction in the concentration dependent manner (Figure 2) with the IC₅₀ (inhibition of force) were 27.48±2.89, 30.26±4.44 and 32.51±3.05 µg/ml, respectively.

Figure 1 Representative tracing showed effect of the test materials (PSP01) on oxytocin-induced contraction at cumulative concentrations 1=10, 2=20, 3=30, 4=40 and 5=50 µg/ml in Locke-Ringer solution.
Figure 2 Concentration-response curve of test extracts (10-50 µg/ml) on the contractile amplitudes (A) and frequency (B) of oxytocin-induced contraction on rat isolated uterine horns. Statistical comparison was performed by ANOVA analysis followed by Dunnett’s test, *p < 0.05 denotes statistically significant difference from DMSO.

The contractile response of uterus toward KCl (50mM) contain two phase including initial rapid, phasic contraction followed by a sustained tonic contraction (Figure 3). The baseline values of the contractile amplitude were 4.63±0.40 g (n=20). Cumulative addition of each alcoholic extracts (PSP01, PSP02 and PSPoil) 10-50 µg/ml in the sustained tonic contraction inhibited the concentration in dose dependent manner (Figure 4). The IC_{50} (inhibition of force) were 20.11±2.72, 19.67±2.99 and 27.80±4.62 µg/ml, respectively.

Figure 3 Representative tracing showed effect of the test materials (PSP02) on KCl-induced contraction at cumulative concentrations 1=10, 2=20, 3=30, 4=40 and 5=50 µg/ml in Locke-Ringer solution.

Figure 4 Concentration-response curve of the test extracts (10-50 µg/ml) on KCl-induced rat isolated uterine horns. Each point represents mean±S.E.M. of 4-6 experiments. Statistical comparison was performed by ANOVA analysis followed by Dunnett’s test. *p < 0.05 denotes statistically significant from DMSO. #p < 0.05 denotes statistically significant from PSPoil.
Discussions and Conclusions

Our finding showed that the alcoholic extract of Prasaplai formulation inhibited uterus contractions produced by oxytocin and KCl in a concentration-related manner. At the equivalent concentration, the spasmolytic action of the extracts of the whole prasaplai preparation was higher than the extract of the major ingredient. All of the test materials inhibited the contraction induced by either oxytocin or KCl. As known, potassium chloride induced contraction of smooth muscles via mechanism involved with an increase in Ca\(^{2+}\) influx through voltage-operated Ca\(^{2+}\) channels (3). In contrast, oxytocin causes myometrium contractions by acting on oxytocin receptors (4). Hence, our results suggested that the uterine relaxant effect of Prasaplai was probably mediated through a non-specific, spasmolytic mechanism. Further works would be in need to investigate the mechanisms involved.

Acknowledgement

This project was partly supported by Thailand Institute of Scientific and Technological Research.

References