Active pharmaceutical ingredients and mechanisms underlying phasic myometrial contractions stimulated with the saponin extract from Paris polyphylla Sm. var. yunnanensis used for abnormal uterine bleeding

L. Guo1,†, J. Su1,†, B.W. Deng1,†, Z.Y. Yu1, L.P. Kang2, Z.H. Zhao1, Y.J. Shan1, J.P. Chen1, B.P. Ma2 and Y.W. Cong1,3

1Department of Pathophysiology, Beijing Institute of Radiation Medicine, No. 27 Taiping Road, Beijing 100850, China; 2Department of Biotechnology, Beijing Institute of Radiation Medicine, Beijing 100850, China
3Correspondence address. Tel: +86-10-68210077 ext. 931223; Fax: +86-10-68214653; E-mail: congyw@nic.bmi.ac.cn

BACKGROUND: Total steroidal saponins of Paris polyphylla Sm. var. yunnanensis (TSSP) have been widely used in China for the treatment of abnormal uterine bleeding (AUB). But until now, the main active constituents and the mechanisms underlying the pharmacological actions on uterine activity have not been described. METHODS: Total steroidal saponins were extracted with EtOH and purified by chromatography. In vitro isometric contraction studies were performed using myometrial strips from estrogen-primed or pregnant rats. Intracellular calcium was monitored under a confocal microscope using Fluo-3 AM-loaded myometrial cells. RESULTS: TSSP dose-dependently induced phasic myometrial contractions in vitro. Experiments with calcium channel blockers or kinase inhibitors demonstrated that the TSSP-stimulated myometrial contraction was mediated by an increase in $[\text{Ca}^{2+}]_i$ via influx of extracellular calcium and release of intracellular calcium. Through bioassay-guided separation, it was found that total spirostanol saponins exhibited contractile activity in myometrium and Pennogenin-3-O-$\alpha$-L-ara-binofuranosyl(1$\rightarrow$4)$[\alpha$-L-rhamnopyranosyl(1$\rightarrow$2)]-$\beta$-D-glucopyranoside (PARG) was identified as the active ingredient of TSSP. Furthermore, the contractile response of rat myometrium to PARG was significantly enhanced with advancing pregnancy. CONCLUSIONS: These data provide evidence that myometrial contractility stimulated by TSSP results from $[\text{Ca}^{2+}]_i$ increase and supports the possibility that some spirostanol glycosides may represent a new type of contractile agonist for the uterus.

Keywords: steroidal saponins; Paris polyphylla Sm. var. yunnanensis; myometrial contractility; abnormal uterine bleeding; active pharmaceutical ingredients

Introduction

Abnormal uterine bleeding (AUB) is one of the most common disorders encountered by the gynecologist. About one-third of gynecological consultations are carried out for AUB and this ratio rises to 70% in women premenopause and postmenopause (Oehler et al., 2003). AUB includes both dysfunctional uterine bleeding, which can be anovulatory or ovulatory, and bleeding due to structural causes, including fibroids, polyps, endometrial carcinoma and pregnancy complications (Ely et al., 2006). In addition, AUB can also result from contraception (Schrager, 2002). In view of the high morbidity, treatment of AUB is quite important for women’s healthcare.

Although $\sim$35% of women with AUB will subsequently undergo a hysterectomy, which is the definitive cure for menorrhagia, medical therapy has enormous potential for most women, especially those with dysfunctional uterine bleeding (Banu and Manyonda, 2005). Several drugs have been demonstrated to decrease menstrual bleeding in patients with AUB; these include progestins, combinations of estrogen and progestin, prostaglandin synthetase inhibitors and plasminogen inhibitors, such as tranexamic acid. However, side effects often make them unsuitable for long-term use (Banu and Manyonda, 2005; Showstack et al., 2006).

The rhizome of Paris polyphylla Sm. var. yunnanensis is a well-known traditional Chinese medicine used for the treatment of various diseases, especially in therapy of traumatic bleeding. Tian et al. (1986) first reported the uterine contractile effects of the rhizome of Paris polyphylla Sm. var. yunnanensis by animal experiments and excellent clinical results of its ethanol preparation in treating 300 cases of uterine hemorrhage...
of various etiology. On the basis of these findings, a saponin extract from *Paris polyphylla* Sm. var. *yunnanensis* has been developed into a drug for the treatment of AUB; this drug was named GongXueNing (GXN) Capsule in China market (Zhao and Shi, 2005). In recent years, many steroidal saponins have been isolated from the rhizome of *Paris polyphylla* Sm. var. *yunnanensis*, and some were proposed to be responsible for its uterine contractile activity (Zhou, 1991). But until now, the active pharmaceutical ingredients of the rhizome of *Paris polyphylla* Sm. var. *yunnanensis*, and the mechanisms of its pharmacological actions on uterine contractions have not been described. Therefore, the aim of this study was to investigate mechanisms underlying the phasic myometrial contractions induced by the total steroidal saponins extracted from *Paris polyphylla* Sm. var. *yunnanensis* (TSSP), and then, by bioassay-guided separation, to identify the active chemical constituents of TSSP responsible for the myometrial contractions.

**Materials and Methods**

**Chemicals**

Chemicals used in the current experiments were nitrendipine, 2-nitro-4-carboxyphenyl-N, N-diphenylcarbamate (NCDC), 2-aminoethoxydiphenyl borate (2-APB), wortmannin and ruthenium red, which were purchased from Sigma (St. Louis, MO). Stock solutions were prepared in DMSO. Estrotilben was obtained from Beijing Yimen Co. Ltd and dissolved in 0.9% sodium chloride. All drugs were added to the bath in volumes of 5 μl.

**Extraction and purification of steroidal saponins**

The rhizome of *Paris polyphylla* Sm. var. *yunnanensis* (Franch) Hand Mazz. (7.8 kg) was extracted with hot EtOH (95%). The EtOH extract was concentrated under reduced pressure and re-extracted with an equal volume of *n*-BuOH to give a crude material [total saponins of *Paris polyphylla* Sm. var. *yunnanensis* (TSSP)]. Through bioassay-guided separation, active compounds were further isolated and the purification procedure is presented as supplementary data (Miyamura et al., 1982).

**Animal preparation**

Virgin female Wistar rats, weighing 240–280 g, were purchased from the Laboratory Animal Center, Chinese Academy of Medical Sciences, and housed with food and water available *ad libitum*. The animals were pretreated intraperitoneally with estrotilben (0.1 mg/kg) at 48 h before the experiments (Yanagita et al., 2000). Timed-pregnant Wistar rats were prepared as described previously (Crankshaw and Gaspar, 1992). Virgin female rats were placed in separate cages with one male each and left overnight. Pregnancy was dated by defining the morning of sperm positivity as Day 0 of gestation. Each rat was treated in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals.

**Myometrial contraction studies**

Bilateral uterine horns were excised from the rats that had been killed by cervical dislocation. After cleaning of adherences, the myometrial tissue was cut into 10 × 2 × 2 mm strips along the longitudinal axis of uterus. Strips were suspended vertically in 5-ml organ baths containing modified Kresbs’ solution (NaCl 136 mM, KCl 2.68 mM, CaCl$_2$ 1.8 mM, MgCl$_2$ 0.5 mM, NaHCO$_3$ 11.9 mM, NaHPO$_4$ 0.32 mM and 5.04 mM glucose pH 7.2), bubbled continuously with a mixture of 95% O$_2$/5% CO$_2$ and warmed to 37.2°C. Muscle tension was recorded isometrically with a tension transducer connected to a polygraph system (Pclab, Beijing Microsignalstar Technology Development Co. Ltd, Beijing, China). An initial resting tension of 1.0 g was applied. Each strip was first stimulated with 40 mM K$^+$ for 10 min to evaluate viability of the strip, and the recorded value was taken as the control (100%) (Shintani et al., 2000). A 30-min equilibration period was allowed before recording 10 min of spontaneous uterine contraction, which was taken as the basal value. Subsequently, TSSP, oxytocin or PGF-2α was added to the bath to stimulate myometrial contractions, and then calcium channel blockers or kinase inhibitors were added at 10-min intervals in an accumulative manner. Response curves of each tested uterine strips were plotted, and the contractions were measured as area under the curve (AUC) calculations. All recorded values subtracted the basal values, and were expressed as percentage of the control.

**Culture of dispersed myometrial cells**

Myometrial smooth muscle cells were collected from estrotilben primed rats. After the uteri were removed and dissected free of fat and endometrium, the tissues were cut into 1 × 1 × 1 mm$^3$ and placed in culture flasks containing RPMI-1640 medium supplemented with 30% bovine calf serum and maintained at 37°C in a 5% CO$_2$ atmosphere. Cells were subcultured every 3–4 days prior to reaching confluence. The semidispersed tissue was washed with PBS twice and trypsin (0.25% w/v) was added. When most of the cells were contracted and become round, the calf serum was added to neutralize the effect. The cells were then washed with PBS again, plated on glass coverslips and kept in a RPMI-1640 medium supplemented with 10% bovine calf serum for 24 h at 37°C in a 5% CO$_2$ atmosphere before the experiments.

**Intracellular Ca$^{2+}$ ([Ca$^{2+}$])$_i$ measurements**

To assess the [Ca$^{2+}$], the myometrial smooth muscle cells were loaded with Fluo-3 AM (10 μmol/l, Molecular Probes, Eugene OR) for 30 min at room temperature on the day of the experiment. Excess dye was removed by washing out with Hanks buffer (pH 7.4) and allowing 30 min for intracellular desterification of Fluo-3 AM (Lunardi et al., 2006). Changes in cytosolic free calcium concentration were monitored by detecting changes in fluorescence of single myometrial cells under a confocal scanning laser microscope (BioRad Alliance 2100 laser scanner) as described previously (Yamada et al., 1991). Fluo-3 AM was excited with the 488 nm line of an argon ion laser, and the emitted fluorescence was measured at 510 nm. A single myometrial cell was exposed to 50 μg/ml TSSP or 0.1% DMSO (vehicle control) for 60 s and the fluorescence was continuously recorded using time course software (Lasersharp 2000, Bio-rad, USA).

**Statistical analysis**

Results are expressed as means ± SEM. One strip obtained from one animal was used for each experiment, therefore the number of experiments (n value) also indicates the number of animals. Student’s *t*-test was applied for comparison of the means of two groups, and ANOVA was used for the means of multiple groups. Values of *P* < 0.05 were considered significant. Statistical analyses were performed using the SAS software package (SAS Institute, Cary, NC).

**Results**

**Effects of TSSP on the contractility of rat uterine preparations**

As a traditional Chinese drug preparation, the total steroid saponins extracted from the rhizomes of *Paris polyphylla*...
Sm. var. yunnanensis (TSSP) have been used for the treatment of AUB. To reveal the mechanism, the contractile activity of TSSP was first investigated. As shown in Fig. 1A, the application of TSSP in the modified Krebs’ solution typically produced an increase in frequency and intensity of phasic contractions of rat myometrial strips in a dose-dependent manner. To characterize the contractile activity of TSSP, the contractions were measured as the AUC at 10-min intervals and expressed as percentage of the response to 40 mM K+. On the basis of the dose–response curve (Fig. 1B), the maximal response of rat myometrium to TSSP was calculated as 23.19 ± 0.27% of the potassium response and the EC50 value of TSSP was 19.82 ± 0.42 µg/ml (n = 6). Under the same conditions, the maximal responses to oxytocin and PGF-2α were 51.09 ± 0.03% and 42.00 ± 0.05% of potassium response, respectively (data not shown, n = 3). These data indicated that TSSP has a relatively strong effect on rat myometrial contraction.

**Effects of TSSP on intracellular calcium in rat myometrial cells**

In common with smooth muscles, myometrial contraction relates to increases in intracellular calcium [Ca2+]i (Wray et al., 2001; Matthew et al., 2004a, b). Therefore, the changes of [Ca2+]i were measured in Fluo-3/AM-loaded myometrial cells. Flu-3 AM, a cell-permeable acetoxymethyl ester, exhibits a large fluorescence intensity increase on binding Ca2+. As shown in Fig. 2A, the myometrial cells loaded with Flu-3 AM exhibited a transient significant increase in fluorescence after stimulation with 50 µg/ml TSSP. As a control, cells treated with vehicle (0.1% DMSO) did not show any significant change in fluorescence (data not shown). In the same experiments, while continuously recording the fluorescence using a time course software, a rapid transient peak of fluorescence in myometrial cells was observed when challenged with TSSP (Fig. 2B), which indicated an increase in [Ca2+]i in rat myometrial cells. Furthermore, the increase of fluorescence was not reversed for at least 5 min, and retreatment with TSSP did not produce an additional peak in the fluorescence (data not shown).

An increase in [Ca2+]i results in the formation of a Ca2+-calmodulin complex in myometrial cells, which activates muscle myosin light chain kinase (MLCK), and then phosphorylates the regulatory myosin light chains, allowing them to rapidly bind to and detach from actin filaments and thus generate tension. Wortmannin, a specific MLCK inhibitor, caused significant inhibition to TSSP-induced contractions starting at a concentration of 0.5 µM and almost completely abolished the effects of the TSSP at 2.0 µM (n = 12). The inhibition by wortmannin involved reduction of both the potency and maximal effects of TSSP, suggesting MLCK activity is essential for TSSP-induced uterine force production (Fig. 2C). Therefore, these results proposed that TSSP-induced myometrial contractions may be mediated by the increase of [Ca2+]i.

**Role of extracellular calcium in TSSP-stimulated myometrial contractions**

The influx of extracellular calcium appears to be important during the maintenance of both cytosolic calcium oscillations and agonist-stimulated phasic smooth muscle contractions (Wray et al., 2001). As shown in Fig. 3A, spontaneous oscillatory uterine contractions were abolished in the absence of extracellular calcium, and exposure to TSSP resulted in one or two contraction waves only at high concentrations. Upon application of extracellular calcium to the organ bath, phasic myometrial contractions resumed. In contrast, oxytocin induced phasic contractions in the calcium-free bath, and the return of physiologic concentrations of extracellular calcium markedly enhanced the oxytocin-induced responses. These results supported the important contribution of extracellular calcium to the generation of phasic myometrial contractions induced by TSSP.

An involvement of L-type Ca2+ channels in agonist-stimulated myometrial contraction has previously been reported (Bae et al., 1999). The effect of a voltage-operated L-type calcium channel blocker, nitrendipine, on TSSP-induced myometrial contractions was determined in Fig. 3B (Lechner et al., 1989). It was showed that nitrendipine dose-dependently reduced myometrial contractions stimulated with 50 µg/ml of TSSP (n = 12). When the concentration of nitrendipine increased to 0.5 µM, oscillations of uterine contractions were completely abolished. Under the same conditions, 2.0 µM of nitrendipine was needed to completely inhibit myometrial contractions induced by 1 mU/ml of oxytocin (data not shown). These findings indicated that myometrial contraction stimulated with TSSP may rely to a greater extent
on L-type Ca$^{2+}$ channels than do those of oxytocin. Similar results were obtained with another L-type calcium channel blocker, verapamil (data not shown).

Involvement of the phosphatidylinositol-signaling pathway in TSSP-stimulated myometrial contractions

Previous reports have demonstrated that agonist-induced myometrial contractions are coupled to phosphoinositide-specific phospholipase C (PI-PLC) activation and inositol 1,4,5-trisphosphate (IP$_3$) production. IP$_3$ induces a rise in free cytosolic calcium via the release of intracellular calcium from IP$_3$-sensitive stores and the calcium-induced calcium release (Phillippe, 1994; Matthew et al., 2004a, b). 2-APB, a novel membrane-permeable IP$_3$-receptor inhibitor (Morales et al., 2005), significantly inhibited TSSP-stimulated phasic myometrial contractions ($n=12$) (Fig. 4A). As a control, a similar potency of 2-APB was observed to cause such inhibition in oxytocin-stimulated myometrial contractions (data not shown). Furthermore, inhibition of PI-PLC with NCDC, a specific inhibitor of PLC (Phillippe, 1994), was shown to have a significant inhibitory effect on TSSP-stimulated myometrial contractions, further supporting the key role of the IP$_3$ production in the contractile process ($n=12$) (Fig. 4B). In addition, ruthenium red, which inhibits calcium-induced calcium release from the ryanodine-sensitive intracellular calcium stores, also significantly inhibited TSSP-stimulated phasic contractions over a concentration range of 40–120 μM ($n=12$) (Fig. 4C). Above all, these results supported that the PI-signaling pathway plays an essential role in TSSP-stimulated phasic myometrial contractions.

Spirostanol-type pennogenin glycosides are active pharmaceutical ingredients of TSSP

The above results proposed that TSSP may contain some constituents which directly stimulate rat myometrial contractions 	extit{in vitro}. Then by bioassay-guided separation, total spirostanol- and furostanol-type steroid saponins of TSSP were first separated and their activities on myometrial contractions were examined, respectively. It was found that total spirostanol saponins, similar to TSSP, directly and more potentially stimulated rat myometrial contractions 	extit{in vitro}, while total furostanol saponins had little activity on myometrial contractions (data not shown). Through further purification from the total spirostanol saponins, one active compound was obtained and its structure was identified as Pennogenin-3-O-$\alpha$-L-arabinofuranosyl(1$\rightarrow$4)[$\alpha$-L-rhamnopyranosyl(1$\rightarrow$2)]-$\beta$-D-glucopyranoside (PARG) (Fig. 5A). As shown in Fig. 5B, PARG dose-dependently produced an increase in frequency and intensity of phasic contractions of rat myometrial strips, similar to, but stronger than, that of TSSP. Plotting the dose–response curves for PARG-stimulated myometrial contractions according to the procedure described above, it was found that
the maximal response to PARG was 27.62 ± 0.59% of potassium response and the EC$_{50}$ value was at 2.75 ± 0.13 mg/ml ($n = 3$) (Fig. 5C). As a control, its furostanol-type pennogenin glycosides, 26-O-β-D-glucopyranosyl-(25R,22)-hydroxy-5-ene-furostane-3β,17α,26-triol3-O-[α-L-arabinofuranos-(1→6)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, PARG (2) Penogenin-3-O-[α-L-arabinofuranos-(1→4)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, PARG (3) Pennogenin.

**Figure 3:** The effects of extracellular calcium on TSSP-induced myometrial contractions
(A) Representative recordings of the cumulative dose responses on myometrial strips induced by TSSP or oxytocin in calcium-free solution. After the stimulators were increased to the maximal effective dose, calcium was added to a final concentration of 1.8 mmol/L. (B) The inhibitory effect of nitrendipine on TSSP-induced myometrial contractions ($n = 12$). Data are expressed as means ± SEM. **$P < 0.01$ compared to control.

**Figure 4:** Involvement of the phosphatidylinositol-signaling pathway in TSSP-stimulated myometrial contractions. Inhibitory effects of cumulative doses of 2-APB (A), NCDC (B) and ruthenium red (C) on contractile responses after stimulation with 50 μg/ml TSSP in rat myometrial strips ($n = 12$). Data are expressed as means ± SEM. **$P < 0.01$ compared to control.

**Figure 5:** Contractile activity of steroidal saponins purified from TSSP in myometrial strips
(A) Chemical structures of one pennogenin glycoside with spirostanol structure (PARG), its furostanol-type saponin (FARG) and pennogenin. (B) Representative recordings of cumulative dose responses developed by PARG and vehicle in rat myometrial strips. (C) A dose-response curve for PARG-induced rat myometrial contractions ($n = 3$). Contraction was measured as AUC calculations and expressed as a percentage of 40 mM potassium response. The data are expressed as means ± SEM.

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In view of the role of $[Ca^{2+}]_i$ in TSSP-stimulated myometrial contractions, wortmannin, nitrendipine and 2-APB were then used to characterize the mechanisms of PARG-stimulated myometrial contractions. As shown in Fig. 6, treatment of myometrial strips with 5.6 μM PARG caused rhythmic contractions, similar to that stimulated with 50 μg/ml TSSP. Exposure to inhibitors with the same concentrations used above, nitrendipine, 2-APB and wortmannin all significantly inhibited PARG-induced myometrial contractions when compared with the control, suggesting that PARG and TSSP have similar mechanisms of stimulating myometrial contractions. Therefore, it is proposed that PARG is one of the active pharmaceutical ingredients of Paris polyphylla Sm. var. yunnanensis stimulating uterine contractile activity.

**Variation of PARG-stimulated rat myometrial contraction during pregnancy**

Rhythmic uterine contractions play an important role in parturition (Young, 2007). To uncover the role of PARG in a more physiological setting, the contractile activities of PARG was detected in rats during pregnancy. As shown in Fig. 7A, stimulation of isolated pregnant uterine strips with PARG revealed dose-response curves and maximal contraction was reached in the late period of pregnancy ($n = 3$). Changes in sensitivity and maximum developed forces of isolated pregnant uterine strips to PARG are represented in Fig. 7B. While the EC50-values showed little change across pregnancy, the Emax-values increased significantly with advancing pregnancy, obtaining the highest value before parturition. The 40 mmol/1 KCl-induced reference contraction was also increased in the late period of pregnancy.

**Discussion**

Steroidal saponins are widely distributed in the botanical kingdom and have many pharmacological actions and biological activities, such as anti-platelet, anti-tumor, anti-dementia, blood sugar-reducing, antibacterial, immuno-regulatory and estrogen-like effects (Rao and Gurfinkel, 2000; Sparg et al., 2004). To our knowledge, this is the first report that steroidal saponins from the rhizome of Paris polyphylla Sm. var. yunnanensis are strong contractile stimulators for the uterus. It was shown that total steroid saponins or its active ingredient PARG could induce rhythmic contractions of rat myometrium in a dose-dependent manner, very similar to the role of some inherent contractile agonists such as oxytocin and PGF2α.

Then, as determined by experiments with various calcium channel blockers (nifedipine, 2-APB and ruthenium red), kinase inhibitors (NCDC and wortmannin) and Ca$^{2+}$ indicator (Fluo-3 AM), we demonstrated that myometrial contractions induced by steroidal saponins are: (i) regulated by PLC, (ii) mediated by an increase in free cytosolic calcium from the influx of extracellular calcium via voltage-operated calcium channels, and the release of intracellular calcium from IP3-sensitive stores and the calcium-induced calcium channels and (iii) involved the activation of MLCK. Finally and importantly, the enhancement in the contractile response to PARG with advancing pregnancy further supported the possibility that some spirostanol glycosides may represent a new type of contractile agonist for the uterus, since it has been consistently found that an increases in uterine contractility to some inherent contractile agonists, such as oxytocin and PGF2α, occurs before the onset of labour in several species (Crankshaw and Gaspar, 1992; Kawarabayashi et al., 1997).

Steroidal saponin is an important class of natural products and is composed of a C-27 aglycone moiety and sugar chains of one or more monosaccharides. These compounds are classified as spirostanol saponins with a sugar chain at C3 position, and furostanol saponins with two sugar chains at both C3 and C26 positions, respectively (Rao and Gurfinkel, 2000; Sparg et al., 2004). As presented in this paper, we found that spirostanol-type pennogenin glycosides from Paris polyphylla Sm. var. yunnanensis have contractile activity for the rat uterus, while its furostanol-type pennogenin glycosides or pennogenin showed no activities. These findings suggested that the spirostanol skeleton and the 3-O-glycoside moiety are both essential for its uterine contractility. Similar to our findings, Matsuda et al. (2003) reported that the protective effects of pennogenin glycosides with spirostanol structure from Paris polyphylla Sm. var. yunnanensis, but not their furostanol-type saponins, on ethanol- or indomethacin-induced gastric mucosal lesions in rats.

AUB is one of the most frequent gynecological problems. The causes of AUB and morbidity in particular vary with the age of woman affected. During premenopause and perimenopause, the most frequent causes are hormonal (up to 90% of cases) as well as organic changes in the uterus such as myomas, adenomyosis uteri, or endometrial polyps (up to 70% of cases). Coagulation defects also cause AUB, particularly in girls and young women with no other recognizable...
cause (Bradley, 2005). Several drugs have been demonstrated to decrease menstrual bleeding in patients with AUB. Non-steroidal anti-inflammatory drugs will decrease bleeding by 30–50%. Oral contraceptives may be useful to stop acute bleeding and will decrease menstrual flow by ~50%. Tranexamic acid, a plasminogen inhibitor approved for the treatment of hemophilia, would also decrease flow by ~50% (Robins, 2001). However, side effects may make them unsuitable for long-term application. GXN, an ethanol-preparation extracted from the rhizome of *Paris polyphylla* Sm. var. *yunanensis*, ceased or remarkably reduced the amount of hemorrhage by ~95% in treatment of 300 cases of AUB, including 122 cases of dysfunctional uterine bleeding, 103 cases of menorrhagia and 75 cases of other causes (Tian et al., 1986). Because of its cheapness, convenience and few side effects, GXN has been widely used in China for treatment of AUB, with an average annual sales over 100 million RMB in recent years. As presented in this paper, spirostanol saponins, especially pennogenin glycosides, are found to be the active ingredients in stimulating myometrial contractions. Along with this study, we have also found that TSSP could dose-dependently decrease the total tail bleeding time of mice and enhance *ex vivo* rat platelet aggregations induced by ADP, and we have identified that the pennogenin glycosides with spirostanol structure, including PARG, are strong platelet agonists (Fu et al., 2007). As the main ingredients of GXN, it is proposed that the strengthening of uterine contraction and/or promotion of hemostasis *in vivo* by steroidal saponins may be responsible for the therapeutic effect of GXN on AUB. However at present, we have no data related to the effects of TSSP or its active ingredients on vascular or visceral smooth muscle other than the myometrium; this would be important for understanding the therapeutic effect of GXN and will be determined in our following study.

In summary, this study indicates that total steroidal saponins extracted from the rhizome of *Paris polyphylla* Sm. var. *yunanensis* possess direct uterotropic activity, which may justify their usage in the therapy of AUB in traditional Chinese medicine. Further mechanism studies demonstrated that myometrial contraction induced by TSSP is regulated by PLC activation and mediated by an increase in free cytosolic calcium from the influx of extracellular calcium and the release of intracellular calcium. Finally, via bioassay-guided separation, one spirostanol-type pennogenin glycoside was isolated from *Paris polyphylla* Sm. var. *yunanensis*, an was shown to exhibit a stronger effect on stimulating rat myometrial contractions. This is worthy of exploring as a lead for the drug discovery of AUB.

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Steroidal saponins induce myometrial contractions


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