Alkaloids and flavonoid glycosides from the aerial parts of *Leonurus japonicus* and their opposite effects on uterine smooth muscle

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A B S T R A C T

The crude extract and some Chinese patented medicines of *Leonurus japonicus* Houtt. have been proven to affect the uterine smooth muscle. *L. japonicus* injection is widely used in obstetric departments in China for treating postpartum hemorrhage caused by uterine inertia. Bioassay-guided isolation of the 95% EtOH extract of *L. japonicus* yielded four cyclopeptides, nine alkaloids, and three flavonoid glycosides, including two previously undescribed cyclopeptides, namely, cycloeleonuripeptide G and cycloeleonuripeptide H. The structures of the cyclopeptides were elucidated to be cyclo-(L-Phe-L-Phe-Gly-L-Pro-Gly-L-Pro) and cyclo-(L-Phe-L-Ala-L-Pro-L-Ile-L-His-Gly-L-Ala-L-Pro), respectively, via spectroscopic and chemical methods. Cyclopeptides (cycloeleonuripeptides C and D) and alkaloids (imperialine-3β-D-glucoside and leonurine) promoted contraction of uterine smooth muscle strips isolated from normal rats. However, it was observed that flavonoid glycosides (spinosin, linarin, and apigenin-7-O-β-D-glucopyranoside) significantly inhibited contraction of the uterine smooth muscle strips.

1. Introduction

Postpartum hemorrhage (PPH) is a major cause of mortality among pregnant women. It accounts for more than 100,000 maternal deaths per year. It can also result in long-term uterine recovery difficulties after normal vaginal delivery or cesarean section (Khan et al., 2006). The main cause of PPH is uterine atony. Oxytocin, ergometrine, misoprostol, and other prostaglandins were recommended for preventing PPH in The World Health Organization (WHO) 2012 (WHO Guidelines Approved by the Guidelines Review Committee, 2012) and International Federation of Gynecology and Obstetrics (FIGO) 2012 guidelines (FIGO Safe Motherhood and Newborn Health Committee, 2012). In China, several Chinese patented medicines have been developed from the extract of *Leonurus japonicus* Houtt. (Labiatae) and are used in combination with oxytocin for treating PPH or uterine recovery difficulties. A number of clinical literature have indicated that *L. japonicus* injection has a notable curative effect on PPH and has gradually become a common drug for obstetric use in China (Gong et al., 2011; Lin et al., 2009; Liu et al., 2016; Su et al., 2016; Tan et al., 2017; Yuan and Chen, 2015).

The aerial parts of *L. japonicus*, a well-known traditional Chinese medicine, ranked as the top grade in “Sheng Nong Ben Cao Jing” and is called “Motherwort” or “Yi Mu Cao” (Chinese), meaning literally “beneficial herb for mothers”. It has been used to treat various gynecological blood disorders, particularly menstrual disturbances and PPH, for thousands of years (Peng, 2011). Modern pharmacological studies have demonstrated that the extract of *L. japonicus* promotes uterine muscle contraction in rats (Li et al., 2014b; Ma et al., 2000; Shang et al., 2014). Thus, *L. japonicus* is a good natural uterotonic.

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Our previous investigations on L. japonicus have led to the isolation of diterpenoids (Xiong et al., 2015a; 2015b), sesquiterpenoids (Xiong et al., 2013), and steroids (Zhou et al., 2015) with vasorelaxant, anti-platelet aggregative, or antibacterial activity. Based on a previous report that the total alkaloid extract of L. japonicus has a strong effect on the uterine muscle (Shang et al., 2014), we further carried out experiments to study the alkaloids via TLC and ultra-performance liquid chromatography-mass spectrometry. The results showed that alkaloids mainly exist in the n-butanol extract of L. japonicus. Furthermore, we have previously shown that the n-butanol extract has significant uterotonic effect on isolated rat uterine smooth muscle strips (Li et al., 2014a). Therefore, in the present study, we investigated the components of the n-butanol extract of L. japonicus that are responsible for the uterotonic effects of the extract.

2. Results and discussion

Sixteen chemical compounds, including four cyclopeptides (1–4), six steroidal alkaloid saponins (5–8), two guanidines (9 and 10), two spermidines (11 and 12), an isoquinoline (13), and three flavonoid glycosides (14–16) were isolated from the extract (Fig. 1). Interestingly, two cyclopeptides (3 and 4) and two alcohols (9 and 10) exhibited an excitatory effect on uterine smooth muscle strips, whereas three flavonoid glycosides (14–16) had an opposite effect.

Compound 1 was obtained as a white powder. Its molecular formula was determined to be C12H13N2O6 with 17 degrees of unsaturation based on positive HRESIMS data. The 1H and 13C NMR spectra showed characteristic signals for an imidazole ring [δH 12.25 (NH), 7.70 (s), 7.17 (s); δC 135.5, 134.5, 115.1] and a mono-substituted aromatic ring [δH 7.31 (2H, t, J = 7.2 Hz), 7.23 (2H, d, J = 7.2 Hz), 7.20 (1H, t, J = 7.2 Hz); δC 137.9, 129.1, 128.3, 126.5], which revealed the existence of a histidine (His) and a phenylalanine (Phe) in 1. Detailed analysis of 2D NMR data and acid hydrolysis further verified that 1 contained two alanines (Ala1 and Ala2), two prolines (Pro1 and Pro2), an isoleucine (Ile), and a glycine (Gly), in addition to the above His and Phe. Applying Marfey’s method, L. japonicus was configured to have all the amino acids (Fig. 1).

Using the same method as described for 1, the sequence of the amino acid units in 2 was determined using HMBC correlations of the exchangeable amide protons and α-amino protons (Fig. 2).

The remaining known compounds were identified as cyclopeptide C (3) (Morita et al., 1996), cycloleunoropine D (4) (Morita et al., 1997), hapepunine 3-O-a-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (5) (Kitajima et al., 1982), hapepunine 3-O-β-D-cellobioside (6) (Qian and Nohara, 1995), yibeinoside A (7) (Xu et al., 1990b), imperialine-3-D-glucoside (8) (Xu et al., 1990a), leonurine (9) (Yeung et al., 1977), 4-hydroxy-3,5-dimethoxybenzoic acid 4-guanidinopentyl ester (10) (Langford et al., 1980), N,N,N,N,N’-penta-p-(Z-Z)-coumaroylspermidine (11) (Jiang et al., 2008), N,N’,N’,N’-penta-p-(E-E)-coumaroylspermidine (12) (Jiang et al., 2008), juzirine (13) (Kimura et al., 1983), spinosin (14) (Lewis et al., 2000), linearin (15) (Quintin and Lewin, 2004), and apigenin-7-O-β-glucosidase (16) (He et al., 2014) by comparing their spectroscopic data with those reported in literature.

Since TCM preparations from L. japonicus are not administered orally but instead by injection for treating PPH, the isolates were evaluated for their ex vivo effect on uterine smooth muscle strips isolated from adult female Sprague-Dawley (SD) rats. However, compounds 6, 7, 10, and 13 were not examined for this effect due to their limited quantities. Using a cumulative dosing regimen, the effects of the isolates on contractile activity (entire area under the curve, AUC, g·mm/10 min), contractile tension (average force of contraction, g), and contractile frequency (numbers of contraction, n/10 min) of the uterine smooth muscle strips were evaluated. The difference values of contractile activity (ΔAUC), contractile tension (ΔT), and contractile frequency (ΔF) after and before each treatment were calculated for statistical analysis.