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Full Paper

Protective effect of *Clinopodium chinense* (Benth.) O. Kuntze against abnormal uterine bleeding in female rats

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ABSTRACT

Objectives: To investigated the metrorrhagia volume-reduction activity, anti-inflammatory activity and repair-promoting activity of *Clinopodium chinense* (Benth.) O. Kuntze.
Methods: An abnormal uterine bleeding (AUB) model was induced via oral administration of mifepristone and misoprostol to pregnant rats, which were treated with the total extract of *C. chinense* (TEC). After 7 days, the metrorrhagia volume was measured, the levels of TXB2, 6-keto-PGF1α, IL-6 and TNF-α were measured by ELISA, the pathological changes and micro vessel density (MVD) of the endometrium were evaluated using HE and immunofluorescence staining, and the expression of VEGF, MMP-2/9 and TGF-β were assessed by Western blotting. Preliminary phytochemicals were screened and identified by UPLC-Q-TOF-MS.
Results: Eleven compounds in *C. chinense* were identified via comparison to standard substances. The results of animal experiment showed TEC could reduce metrorrhagia volume, alleviate pathological injury and increase MVD to promote recovery of the endometrium; TEC could also increase the levels of TXB2 and the expression of VEGF, TGF-β, decrease the levels of IL-6, TNF-α and the expression of MMP-2/9.
Conclusions: TEC showed beneficial effects on treating AUB by reducing metrorrhagia volume, inhibiting the inflammatory response and promoting the repair of the endometrium. Additionally, TEC also showed great haemostatic potential in AUB.

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1. Introduction

Abnormal uterine bleeding (AUB) is a common gynaecological disease in women of reproductive age with symptoms of irregular menstrual bleeding, prolonged time uterine bleeding or excessive vaginal blood loss.[1] At least 10% of women report suffering from pain associated to AUB.[2] AUB has negative effects on patients, including fatigue, anaemia, depression and financial burden.[3] Furthermore, women with heavy menstrual bleeding have more emergency room visits and higher hospitalization rates.[4] Previous research revealed that haemostasis disorders, endometrial damage and abnormalities in the inflammatory response in uterine tissues were the major pathological features of AUB.[5,6]

Non-surgical treatments including combined oral contraceptives, tranexamic acid, progestogens, and non-steroidal anti-inflammatory drugs are commonly used for the treatment of AUB,[7] but these treatments may cause side effects. For example, the endometrium will be thinner after the combined use of oral contraceptive pills, and tranexamic acid causes thrombosis and headache.[8] Surgery is recommended when the medical interventions fail, but patients suffer the risk of fertility loss.[9] As a result, in China, patients often resort to Traditional Chinese Medicine (TCM).[10]

Clinopodium chinense (Benth.) O. Kuntze (*C. chinense*, commonly named 'duanxueliu' in China) is a traditional folk herbal medicine that belongs to *Labiatae*, and it has been used for the treatment of metrorrhagia disease for hundreds of years according to ancient records.[11] Some clinical preparations are used in modern China. Previous research indicates the presence of flavonoids, phenylpropanoids, diterpenoids, triterpenoid saponins and volatile oil in *C. chinense*.[12,13] The pharmacological activity of *C. chinense* includes myocardial protection,[14] inhibition of the activity of a-Glucosidase

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and vascular endothelial cell protection.[15] However, the underlying therapeutic mechanisms in the treatment of AUB using *C. chinense* have been rarely reported.

TXB2 and 6-keto-PGF1α in the blood play important roles in the coagulation of organisms.[16] VEGF (vascular endothelial growth factor) is involved in angiogenesis and the repair of the endometrium.[17] TNF-α, IL-6 and TGF-β are commonly used to evaluate the inflammatory response.[18,19] MMP-2 and MMP-9 belong to matrix metalloproteinases (MMPs) that play important roles in degrading the extracellular matrix and these enzymes are involved in uterine bleeding.[20]

The symptoms of incomplete abortion induced by mifepristone and misoprostol are similar to AUB, and these drugs can be used as a model of AUB.[21] Leonurus Granules (LG) is one of the clinical applications of *Leonurus japonicus* Houtt. extract, which is widely used in China for its excellent therapeutic effect on regulating of postpartum haemorrhage and menstrual disturbance.[22] LG was selected as the positive drug in our research.

Above all, the aim of this study is to identify the chemical constituents and reveal the mechanisms of *C. chinense* in rats with AUB.

2. Materials and methods

2.1. Chemicals and reagents

C. chinense herbs were harvested in Huoshan County, Anhui Province in July 2018, and identified by Prof. *Nianjun Yu* (Resource Center of Traditional Chinese Medicine, Anhui University of Chinese Medicine).

Caffeic acid, hesperidin, linarin, luteolin, quercetin, naringenin, apigenin, kaempferol, buddlejasaponin IVb, sakosaponin b1 and acacia were purchased from Chengdu Must Biotechnology Co., Ltd. (Chengdu, China). Acetonitrile (LC/MS grade) was purchased from E. Merck (Darmstadt, Germany). Formic acid with a purity of 99% (UHPLC grade) was purchased from Anaqua Chemical Supply (ACS, Houston, USA).

Leonurus Granules (LG) was purchased from Qianjin Pharmaceutical Co. (Zhuzhou, Hunan, China). Mifepristone was obtained from Gedian Renfu Pharmaceutical Co. (Hubei, China). Misoprostol was obtained from Zizhu Pharmaceutical Co. (Peking, China). ELISA kits were purchased from MEIMIAN (China). Rabbit antibodies against VEGF and MMP-2/9 and secondary antibodies were purchased from Abcam (UK). Rabbit antibodies against CD31 and TGF-β were obtained from Boster Biological Technology Co. (Wuhan, China). Anti-beta actin monoclonal antibody was purchased from Zhongshan Goldenbridge Biotechnology Co. (Peking, China). Chemiluminescent HRP substrate was purchased from Thermo Scientific (Waltham, MA, USA). All other chemicals were commercial products of analytical grade.

2.2. Preparation of drug and UPLC-Q-TOF-MS analysis

2.2.1. Preparation of drug

The total extract of *C. chinense* (TEC) was obtained by water extraction and alcohol precipitation according to the Chinese Pharmacopoeia (2015). The procedure for the extraction of *C. chinense* involved: The *C. chinense* herb was dried in the sun (30~35 °C) until the water content was less than 10% to meet the requirement of the Chinese Pharmacopoeia (2015). The plant material was crushed into a powder using an electric grinder. The obtained plant powder was extracted twice with a 10-fold volume of purified water and each extraction time was 1.5 h. The water solutions were combined and concentrated until the relative density of concentration was 1.15 g/cm^3 (80 °C), and the same volume of absolute ethanol was added to the concentration and stirred fully, the mixture was filtered after stewing for 24 h, and the filtrate was collected. The solvents in the filtration were evaporated under 50 °C to obtain the TEC. The TEC was stored at 4 °C prior to use. Three samples of TEC were precisely weighed and placed into 3 conical flasks (500 mL). Distilled water was added to the 3 flasks, and TEC was dissolved using ultrasound, the concentration of L-TEC, N-TEC and H-TEC were 22.5 mg/mL, 45 mg/mL, 90 mg/mL, respectively.

2.2.2. UPLC-Q-TOF-MS analysis

UPLC separation was performed using a Waters Acquity UPLC system (Waters, USA). The separation was performed on a C18 column (2.1 × 100 mm, 1.7 μm). The mobile phase consisted of solvent A (0.1% formic acid in water, v/v) and solvent B (ACN). The optimized UHPLC elution program was: 0–5 min, 10–15%B; 5–8 min, 15–20%B; 8–14 min, 20–30%B; 14–17 min, 30–33%B; 17–30 min, 33–43%B; 30–35 min, 43–80%B; 35–40 min, 80–10% B; 40–45 min, 10–10%B. The column temperature was maintained at 30 °C. The flow rate was 0.2 mL/min, and the injection volume was 2 μL. MS detection was performed using a Waters Xevo G2QTOF (Waters, USA) mass spectrometer equipped with an ESI source. The MS was operated in positive and negative ion modes. The following operating parameters were used for the MS analysis: ion spray voltages were set at −2.5 KV and 3 KV; turbo spray temperature was 350 °C; and TOF MS and TOF MS/MS were performed over *m/z* ranges of 50–1200. All of the operations and acquisitions were controlled.

2.3. Experimental animals and treatments

Female Sprague Dawley (SD) rats (Certificate No. SCXK2015-001), weighing 270~300 g; and male SD rats (Certificate No. SCXK2015-001), weighing 300~330 g were purchased from the Experimental Animal Center of Anhui Medical University (Anhui, China). All animals were kept in an animal house at 25 ± 1 °C under a 12/12-h dark and light cycles (lights on 7:00 a.m.) with free access to standard laboratory food and water. The Anhui University of Chinese Medicine on Ethics in the Care and Use of Laboratory Animals approved all animal experiments (LLSC20160336).

Female rats in oestrus were screened and mated with male rats. Female and male rats were mated in a separate cage at a ratio of 2:1 overnight. Vaginal smears were performed the next morning to check for the presence of a vaginal plug or spermatozoid. The presence of either was considered evidence of breeding, and that day considered the first day of pregnancy. The AUB model was induced by oral administration of mifepristone (12.4 mg/kg) and misoprostol (130 μg/kg) at 8 a.m. and 6 p.m. respectively on the 7th day of pregnancy.[23,24] The rats were randomly divided into 5 groups (8 rats in each group): the model, L-TEC (225 mg/kg), N-TEC (450 mg/kg), H-TEC (900 mg/kg) and LG (4 g/kg). Non-pregnant females were selected as the control group. A weighted cotton ball (85~100 mg) was placed in the vagina. The cotton balls were removed at 8 a.m. and 6 p.m. on the next day, placed in an EP tube and refrigerated. A new cotton ball was placed in the vagina, and this procedure was continued until the 7th day. Rats in the drug groups were intragastrically administered corresponding drugs on the 8th day. The volume of administration was 1 mL/100 g, and the actual volume was calculated according the weight of the rats. The control group and the model group were given an equal volume of distilled water intragastrically once daily for 7 days.

2.4. The measurement of metrorrhagia volume

As previously described by Hallberg and Zhang[25,26] venous blood (20 μL) from the tail was taken from each individual rat, and 4 mL

(V_1) of a 5% NaOH solution was mixed with the blood. The absorbance (A) of the mixture was measured using ultraviolet–visible (UV) spectrophotometer at the wavelength of 546 nm.

The cotton ball containing uterine bleeding from each rat was collected and placed in a beaker. An appropriate amount of 5% NaOH was used to soak and wash the blood absorbed on the cotton ball. The actual amount of NaOH solution was based on the actual quantity of bleeding. The process was repeated 2 or 3 times until the washed solution was colourless. Finally, the consumed volume of NaOH was recorded as V_2. Meanwhile, the washed solution was transferred to a metering tank and the absorbance value was measured. The volume of metrorrhagia was calculated according to the following formula.

$$\text{Volume(mL)} = \frac{\text{venous blood}(0.02\ \text{mL}) \times \text{the A of washed solution} \times \text{V2}}{\text{the A of venous blood} \times \text{V1}}$$

2.5. ELISA measured the levels of plasma metabolic markers

Eight rats in each group were anaesthetized, and blood from the abdominal aorta was collected and centrifuged to obtain plasma. The plasma levels of TXB2, 6-keto-PGF1α, IL-6 and TNF-α were assessed using the respective ELISA kits, according to the manufacturer's instructions.

2.6. Pathological observation of the endometrium in rats

Uterine tissues from each rat were collected following blood collection from the abdominal aorta. The middle of left uterus tissues were removed and fixed in a 4% paraformaldehyde solution. Endometrium sections (5 μm) were collected from the uterus, stained with haematoxylin and eosin (HE) and observed under a microscope, and the results were scored based on the criteria of uterine pathological grading.[27]

2.7. Detection of the micro vessel density (MVD) of the endometrium in rats by immunofluorescence staining detected

Uterine tissues were harvested from 3 rats in each group, and the endometrial tissue (5 μm thick) was sealed for 30 min in serum. Rabbit anti-CD31 (1:100) antibody was added and incubated at 37 °C for 2 h. The secondary antibody (IgG, 1:100) was added and incubated at 37 °C for 1 h, DAPI was added to stain the nucleus. The image was observed under a fluorescence microscope (Nikon, Japan).

2.8. Western blot for relative proteins

Soluble proteins were harvested from uterine tissues using lysis buffer. Lysed protein samples were loaded and separated on 8~15% sodium dodecyl sulfate (SDS)-polyacrylamide gels. Separated proteins were transferred to PVDF membranes, and the membranes were incubated overnight at 4 °C with the following primary rabbit monoclonal antibodies: MMP-2 (1:1000); MMP-9 (1:1000); VEGF (1:100); TGF-β (1:500), and β-actin (1:800). The targeted antigens were detected by enhanced chemiluminescence analysis. The proteins bands were scanned using Amersham Imager 600 software, and the IntDen were calculated using a computerized image analysis system.

2.9. Statistical analysis

IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The data are presented as the mean ± standard deviation. Statistical analyses were performed using one-way ANOVA, and the significance of the difference between groups was determined using LSD tests. Differences were considered statistically significant at $P < 0.05$ or 0.01.

3. Results

3.1. Chemical constituents of TEC

Qualitative analyses of the chemical constituents in TEC were performed using UPLC-Q-TOF-MS. Positive and negative ion modes were used to scan the components of TEC. The results revealed that the compounds in the negative ion mode were greater than the compounds in the positive ion mode. Therefore the total ion chromatograms (TIC) of the reference substances and TEC were obtained under the negative ion mode (shown in Fig. 1A, B). There were 11 chemical components, including caffeic acid, hesperidin, linarin, luteolin, quercetin, naringenin, apigenin, kaempferol, buddlejasaponin IVb, sakosaponin b1 and acacia, identified by comparison of the retention time and ion fragments of the reference substances. The chemical structures are shown in Fig. 1C and Table 1. Other compounds were also detected but the molecular formulae could only be tentatively determined using UNIFI software. The structures of the chemicals could not be confirmed due to the lack of reference substances, and these compounds are not described in this article.

3.2. TEC reduced metrorrhagia volume

According to the results shown in Fig. 2A, the metrorrhagia volume in the model group increased significantly ($p < 0.01$) compared with control group. Meanwhile, the metrorrhagia volume in the TEC groups decreased significantly compared with the model group ($p < 0.05$ for L-TEC group; $p < 0.01$ for N-, H-TEC groups) and the H-TEC group showed the best haemostatic effect among all the groups.

3.3. Effects of TEC on coagulation and anti-coagulation factors

ELISA detection revealed that the level of TXB2 decreased in the model group ($p < 0.01$). N-TEC, H-TEC and LG increased levels of TXB2 compared with the model group ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively); meanwhile, the level of 6-keto-PGFlα increased in the model group ($p < 0.05$), H-TEC decreased the level of 6-keto-PGFlα compared with the model group ($p < 0.05$) (Fig. 2B).

3.4. TEC improved the pathological morphology of the endometrium

The results of HE showed that the gastrulas were not completely discharged, the decidua remained in the uterus and endometrial cells were damaged in the model group (Fig. 3B). The

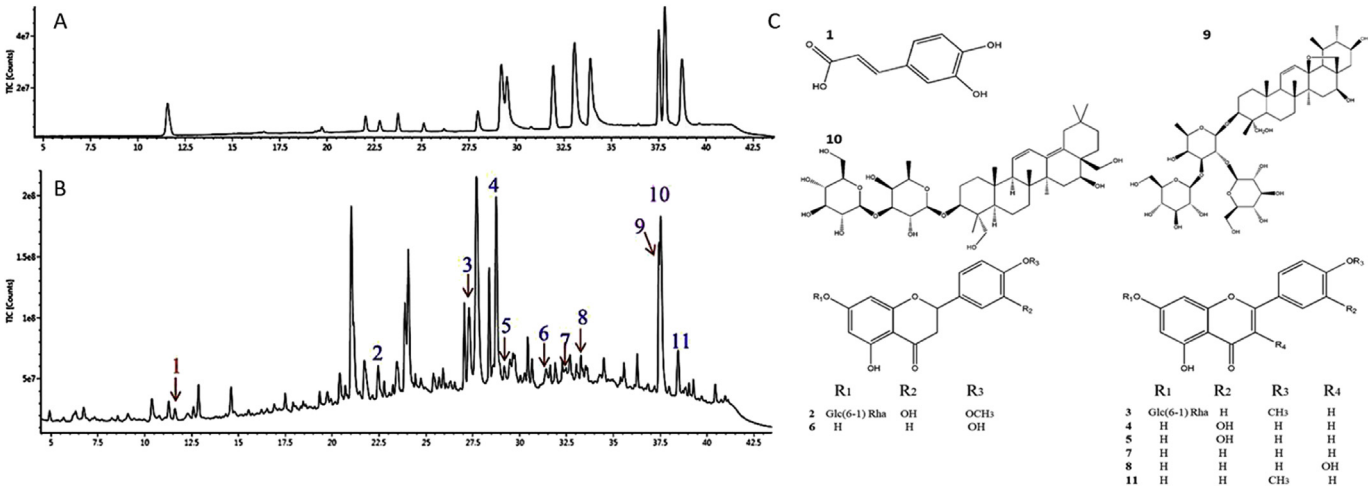

Fig. 1. The total ion chromatograms of samples by UPLC-Q/TOF-MS in negative ion mode. (A) TIC of standard substances monitored in negative ion mode; (B) TIC of the total extract of *C. chinense* in negative ion mode; (C) Chemical structures of the 11 standard reference compounds in *C. chinense*.

Table 1
The information of compounds identified in TEC by UPLC-Q/TOF-MS in negative ion mode.

Peak	RT (min)	Identification	Formula	Neutral mass (Da)	Observed neutral mass (Da)	Observed *m/z*	Fragments
1	11.49	Caffeic acid	$C_9H_8O_4$	180.04226	180.0422	179.0342	135.04512, 133.02921
2	22.43	Hesperidin	$C_{28}H_{34}O_{15}$	610.18977	610.1901	609.1870	577.15063, 431.09723, 323.05339, 301.07179, 268.03737, 164.01125
3	27.37	Linarin	$C_{28}H_{32}O_{14}$	592.17921	592.178	637.1762	283.06136, 268.03737, 89.02435
4	29.32	Luteolin	$C_{15}H_{10}O_6$	286.04774	286.0477	285.0453	133.02921
5	29.82	Quercetin	$C_{15}H_{10}O_7$	302.04265	302.0424	301.0419	151.00345, 107.01352
6	32.05	Naringenin	$C_{15}H_{12}O_5$	272.06847	272.0685	271.0634	177.01917, 151.00367, 119.0499
7	33.21	Apigenin	$C_{15}H_{10}O_5$	270.05282	270.0528	269.0492	151.00368, 117.03463, 107.01352
8	33.97	Kaempferol	$C_{15}H_{10}O_6$	286.04774	286.0477	285.0431	
9	37.48	Buddlejasaponin IVb	$C_{48}H_{78}O_{18}$	942.51882	942.5218	987.5176	779.46112, 617.40707, 518.38513, 289.09225, 161.04521, 391.30235
10	37.55	Saikosaponin b1	$C_{42}H_{68}O_{13}$	780.46599	780.4689	779.4608	617.40687, 541.35236, 471.34716, 145.05035
11	38.64	Acacia	$C_{16}H_{12}O_5$	284.06847	284.0684	283.0637	268.03777

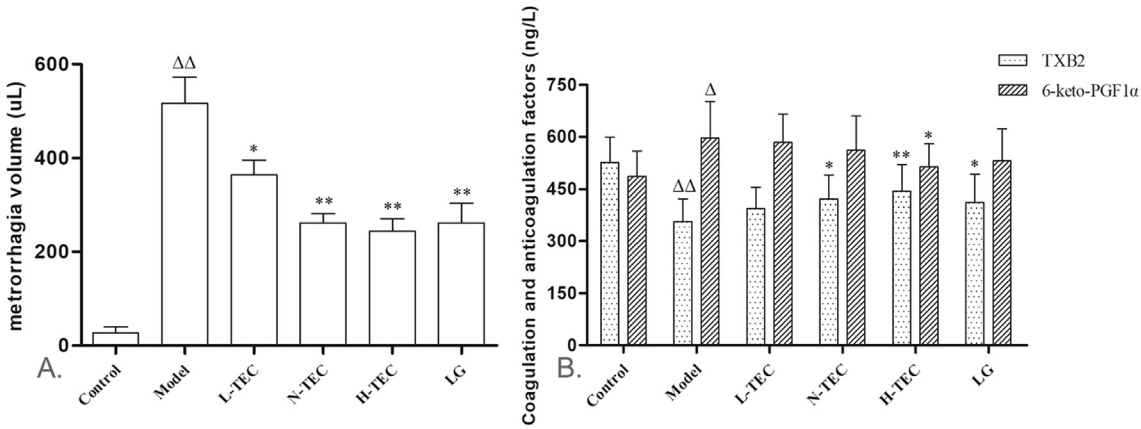

Fig. 2. Effects of TEC on metrorrhagia volume and the levels of TXB2 and 6-keto-PGF1α in rats induced by AUB (n = 8). (A) The results of metrorrhagia volume. $^{\triangle\triangle}P < 0.01$ in comparison between the model group and the control group; $^{*}P < 0.05$ in comparison the L-TEC group and the model group; $^{**}P < 0.01$ in comparison other treatment groups with model group, respectively. (B) The results of the levels of TXB2, $^{\triangle\triangle}P < 0.01$ in comparison between the model group and the control group; $^{*}P < 0.05$ in comparison the N-TEC group, LG group with the model group, respectively; $^{**}P < 0.01$ in comparison between the H-TEC group and the model group. The results of the levels of 6-keto-PGF1α, $^{\triangle}P < 0.05$ in comparison between the model group and the control group; $^{*}P < 0.05$ in comparison between the H-TEC group and the model group. Data were shown as mean ± SD.

N-TEC, H-TEC and LG groups showed a reduction in pathological phenomena compared to the model group (Fig. 3C–E). Histopathological scores in the model group increased significantly compared with the control group ($p < 0.01$), and uterine histopathological scores decreased significantly in the N-TEC and H-TEC groups ($p < 0.05$) compared with the model group (Table 2). LG reduced the histopathological scores, but there was no significant difference.

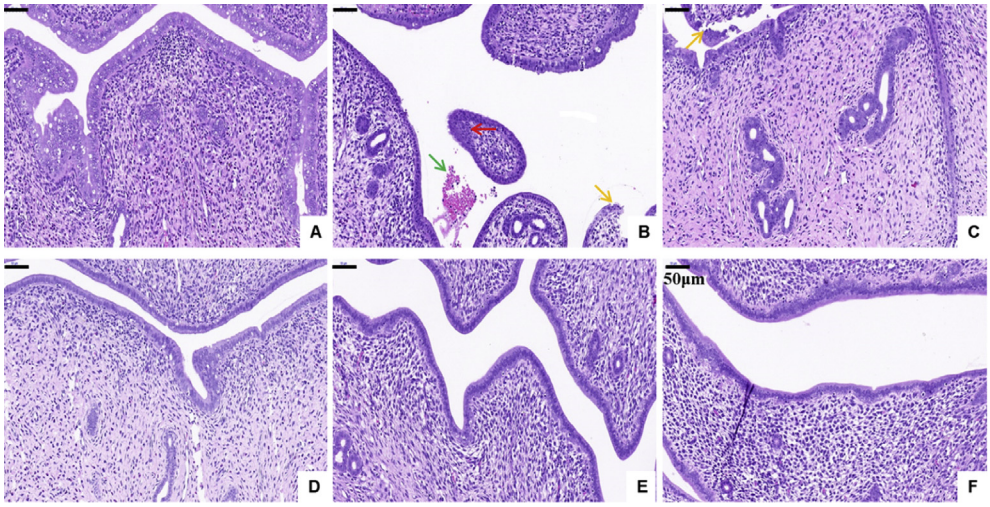

Fig. 3. Effects of TEC on pathological changes of endometrium in rats by HE Staining. A: control; B: model; C: L-TEC (225 mg/kg); D: N-TEC (450 mg/kg); E: H-TEC (900 mg/kg) and F: LG (4 g/kg) group. Green arrow showed haemorrhage (B), red arrow showed remained of decidua (B), and yellow arrow showed endometrial cell damage (B,C). The pictures are from 200× microscopic fields. Each group contained eight rats. Scale bars, 50 μm.

Table 2
Effects of TEC on uterine histopathological score in rats (n = 8).

Group	Dosage (mg/kg)	Number of rats					Score
		I (score 0)	II (score 1)	III (score 2)	IV (score 3)	V (score 4)	
Control	–	6	2	0	0	0	0.25 ± 0.46
Model	–	1	0	4	3	0	2.13 ± 0.99$^{\triangle\triangle}$
TEC	225	0	4	4	0	0	1.50 ± 0.53
	450	1	5	2	0	0	1.13 ± 0.64*
	900	2	4	2	0	0	1.00 ± 0.76*
LG	4000	1	5	1	1	0	1.25 ± 0.88

The criteria of each uterine histopathological grade was as follows: grade I (score 0): endometrial villi or a small residual villi, but no remains of decidua; grade II (score 1): endometrial villi or a small residual villi, and a small decidua tissue; grade III (score 2): a large number of residue of endometrial villi and decidua; grade IV(score 3): the embryo sac was not completely discarded, and a large number of residue of endometrial villi and decidua; grade V (score 4): the embryo sac is intact in the uterus, and a large number of residue of endometrial villi and decidua. Data were shown as mean ± SD. $^{\triangle\triangle}P < 0.01$ in comparison between the model group and the control group. $^{*}P < 0.05$ in comparison between the N-TEC group and the model group, and $^{*}P < 0.05$ in comparison between the H-TEC group and the model group.

3.5. TEC increased MVD in the endometrium

As shown in Fig. 4, CD31 labelled endothelial cells in uterine tissue exhibited red fluorescence, and DAPI re-stained nuclei exhibited blue fluorescence. The bright colours in the merged images are micro-vessels. The results demonstrated that MVD decreased in the model group. MVD increased in the treatment groups compared with the model group. These results suggest that TEC promotes angiogenesis and repair of the endometrium.

3.6. TEC decreased plasma IL-6 and TNF-α levels in rats

As shown in Fig. 5A, B, the levels of IL-6 and TNF-α were elevated significantly in the model group ($p < 0.01$). Besides, the levels of IL-6 and TNF-α decreased significantly in the N-TEC, H-TEC and LG groups compared with the model group ($p < 0.05$, $p < 0.01$). Furthermore, the levels of IL-6 and TNF-α in H-TEC group were lower than those in LG group, but there were no significant differences.

3.7. Effects of TEC on relative protein levels in rats

The expressions of MMP-2, MMP-9, VEGF and TGF-β in uterine tissues were detected to investigate the possible therapeutic mechanism of TEC. The results demonstrated that the protein levels of MMP-2 and MMP-9 in the model group were significantly higher than those in the control group ($p < 0.01$, Fig. 6b); TEC could decrease the expression of MMP-2 ($p < 0.05$ at 225 mg/kg, $p < 0.01$ at 450, 900 mg/kg) and MMP-9 ($p < 0.05$ at 450 mg/kg, $p < 0.01$ at 900 mg/kg). Additionally, protein levels of VEGF and TGF-β in the model group were significantly lower ($p < 0.01$, Fig. 6c); TEC could increase the expression of TGF-β ($p < 0.01$ at 225, 450, 900 mg/kg) and VEGF ($p < 0.01$ at 225, 450, 900 mg/kg); LG could also down-regulate the expression of MMP-2 and MMP-9 and up-regulate the expression of TGF-β and VEGF ($p < 0.01$, $p < 0.05$).

4. Discussion

There was a heavy menstrual bleeding and residual necrotic decidua was present in rats after incomplete abortion, which was consistent with the symptoms of AUB.[3] The results indicate that incomplete abortion serves as an appropriate model of AUB. The main features of AUB are large amounts of bleeding and unexplained menorrhagia associated with inherited coagulation defects.[28] Thromboxane and 6-keto-PGF1α are the most common factors that affect coagulation function. TXB2 is transformed from TXA2, which affects platelet aggregation and vasoconstriction; and 6-keto-PGF1α plays a role in anti-coagulation via inhibition of platelet aggregation.

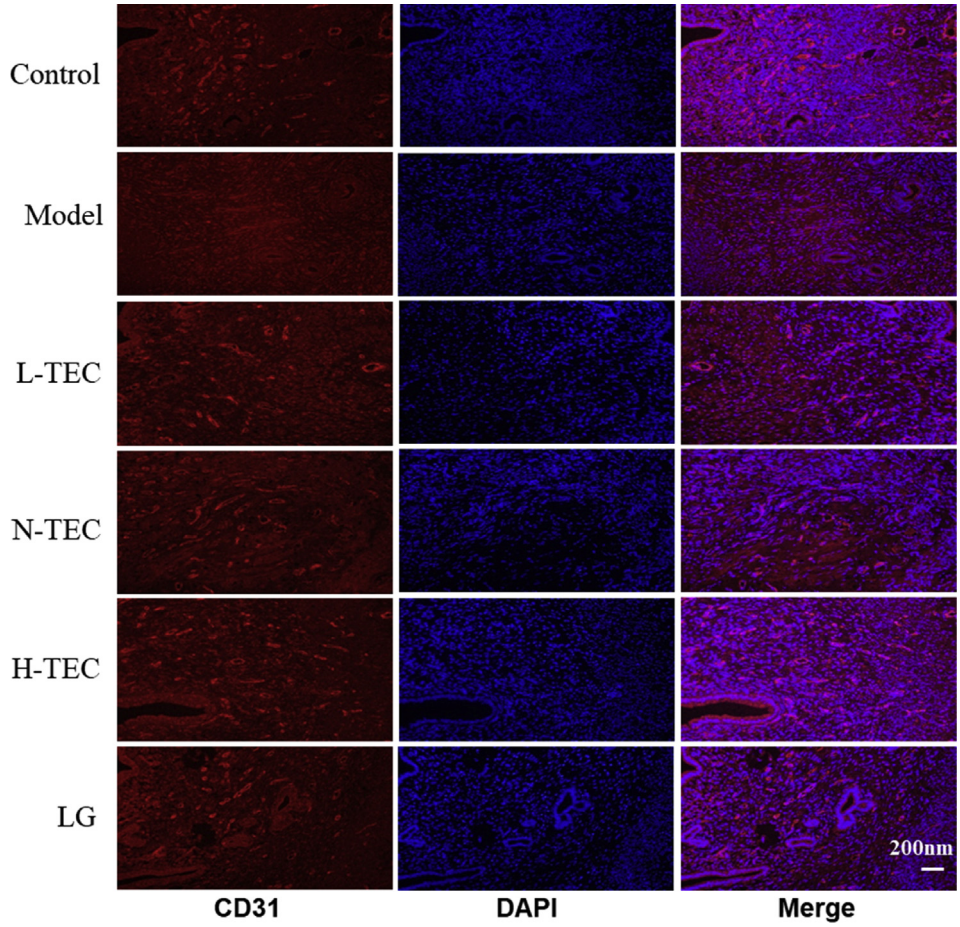

Fig. 4. Effects of TEC on MVD of endometrium in rats by immunofluorescence staining. Red fluorescence represented endothelial cells in the uterine tissue labelled with CD31, blue fluorescence represented nucleus of endothelial cells labelled with DAPI, and bright colours in the merge were micro vessels. The pictures are from 400× microscopic fields. Each group contained three rats. Scale bars, 200 nm.

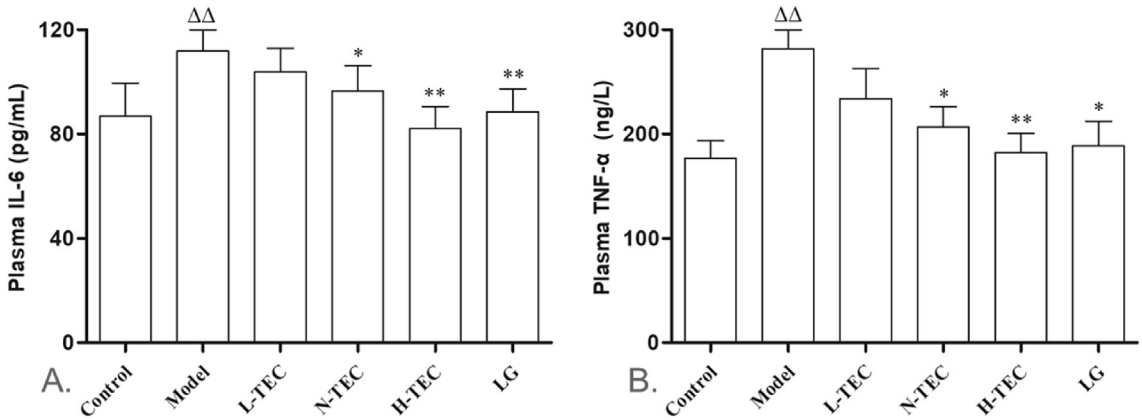

Fig. 5. Effects of TEC on IL-6 and TNF-α levels in rats induced by AUB (n = 8). (A) The results of the level of IL-6, $^{\triangle\triangle}P < 0.01$ in comparison between the model group and the control group; $^{*}P < 0.05$ in comparison between the N-TEC group and the model group; $^{**}P < 0.01$ in comparison the H-TEC group, LG group with the model group, respectively. (B) The results of the level of TNF-α, $^{\triangle\triangle}P < 0.01$ in comparison between the model group and the control group; $^{**}P < 0.01$ in comparison between the H-TEC group and the model group; $^{*}P < 0.05$ in comparison the N-TEC group, LG group with the model group, respectively. Data were shown as mean ± SD.

Hormone withdrawal after abortive drug-induced incomplete abortion triggers the influx of inflammatory cells into the endometrium, which leads to the degradation and necrosis of the decidua and villi.[29,30] TNF-α, IL-6 and TGF-β are common cytokines related to inflammation. Over expression of TNF-α has adverse effects on pregnancy and induces abortion.[31] Excessive IL-6 also has a negative effect on pregnancy and low expression of TGF-β increases miscarriage.[32,33]

Many factors are involved in the regulation of the menstrual cycle, such as microangiogenesis in the endometrium, the

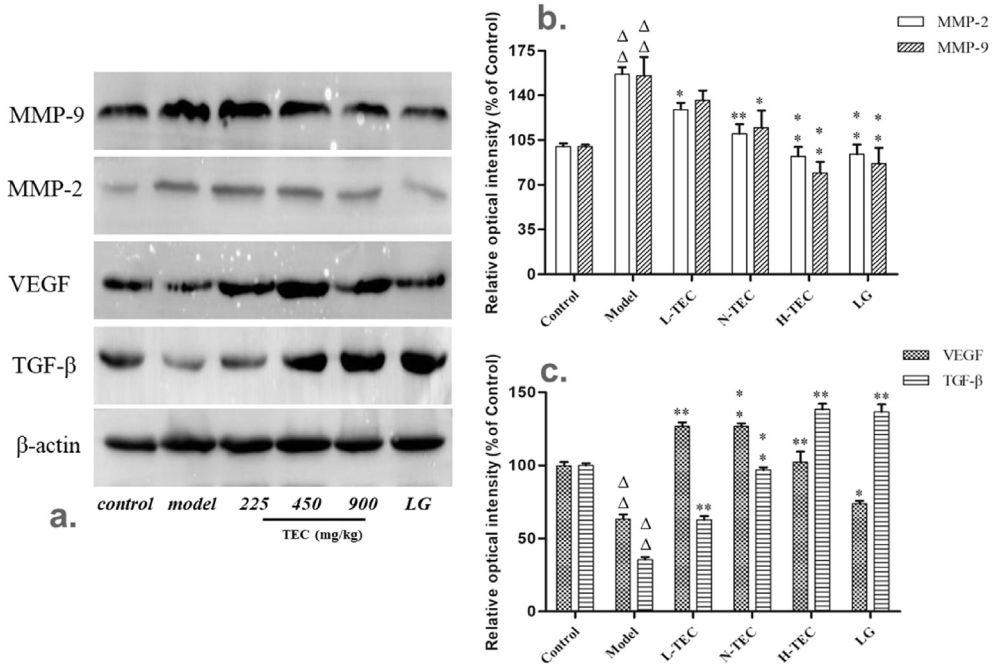

Fig. 6. Effects of TEC on relative protein levels in uterine tissue (n = 3). (a) Left panel: Representative Western blotting for the immunodetection of MMP-9, MMP-2, VEGF, TGF-β and β-actin in uterine tissue. (b, c) Right panel: Integrated data for MMP-9, MMP-2, VEGF and TGF-β expression normalised against β-actin. The results of the expression of MMP-2, $^{*}P < 0.05$ in comparison between the L-TEC group and the model group; $^{**}P < 0.01$ in comparison other treatment groups with the model group, respectively. The results of the expression of MMP-9, $^{*}P < 0.05$ in comparison between the N-TEC group and the model group; $^{**}P < 0.01$ in comparison the H-TEC group, LG group with the model group, respectively. The results of the expression of VEGF, $^{*}P < 0.05$ in comparison between the LG group and the model group; $^{**}P < 0.01$ in comparison other treatment groups with the model group, respectively. The results of the expression of TGF-β, $^{**}P < 0.01$ in comparison treatment groups with the model group, respectively. Data were shown as mean ± SD. $^{\triangle\triangle}P < 0.01$ in comparison between the model group and the control group.

concentration of vasoactive substances of the endometrium, and the repair and reconstruction of endometrial vascular damage.[34] MVD is a quantitative indicator to measure the angiogenesis of micro-vessels, that is, the higher the value of MVD in endometrial tissue, the richer it is in new capillaries.[35] It was recently reported that VEGF is also related to the development and repair of the endometrium.[36] Furthermore, MMPs may participate in the regulation of the menstrual cycle via the reconstruction and repair of the endometrium.[37]

C. chinense is an effective haemostatic agent, that is widely used in Chinese folk medicine, and it contains many kinds of active ingredients, such as triterpenoid saponins (buddlejasaponin IVb and saikosaponin b1), flavonoids (hesperidin, quercetin, and luteolin), and phenolic acid (caffeic acid), etc. The curative effect of TEC on AUB may be attributed to the synergistic effect of the active ingredients that cause the total effect of the drugs. Buddlejasaponin IVb was selected as the indicator of TLC identification of *C. chinense* in the Chinese Pharmacopoeia (2015), and it produces anti-inflammatory effects by the inhibition of NO and TNF-α production.[38] Quercetin showed a good protective effect on subarachnoid haemorrhage and intracerebral haemorrhage by inhibition of IL-6 and MDA expression.[39,40] Luteolin and caffeic acid exhibited the haemostatic activity by inhibition of prothrombin time (PT) and activated partial thromboplastin time (APTT),[41] which may be related to the intrinsic coagulation pathway of TEC. The metrorrhagia volume in H-TEC group was less than that in LG group, and the levels of IL-6 and TNF-α were lower than that in the LG group in the present study. Although there was no significance ($p > 0.05$), it could be concluded that the therapeutic effect of TEC on AUB was not worse than that of LG. Hesperidin suppressed the expression of MMP-2 and MMP-9 in rats with nitric oxide deficiency-induced cardiovascular remodelling.[42] Notably, the protein levels MMP-2 and MMP-9 were significantly decreased in the model group after treatment with TEC. These results indicated that TEC has therapeutic effects on AUB by promotion of coagulation and the suppression of inflammation.

5. Conclusion

Qualitative analysis results based on UPLC-Q-TOF-MS showed that the components in TEC were partially composed of saponins and flavonoids, which provides a basis for the comprehension of the chemical constituents of TEC.

We believe that these results are the first to report the potential haemostatic effects of TEC in the treatment of rats with AUB. The preliminary mechanism of TEC in the treatment of AUB include the reduction the metrorrhagia volume, the inhibition of the inflammatory response of the uterus tissue to promote endometrial repair, the down-regulation of the expression of MMP-2/9 and the up-regulation the expression of VEGF and TGF-β. These results provide a scientific basis for the further clinical use of *C. chinense*.

Declaration of Competing Interest

The authors declare no conflict of interest.

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