

A traditional Chinese Medicine Proves the Treatment of Endometrial Hyperplasia Via Regulating the HPO Axis in Rats

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Research

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Abstract

Background: Dysfunctional uterine bleeding, accompanied by endometrial hyperplasia, is a common gynecological disease regulated by multiple hormones, which seriously affects female physical and mental health. Some drugs have been prompted to cure the disease, but most drugs have certain side effects and limitations. Nonetheless, traditional Chinese medicine (TCM) could provide a novel perspective for the treatment of endometrial hyperplasia and related complications.

Methods: The active components from *Saururus chinensis*, *Celosia cristata* and *Spatholobus suberectus* (Referred to as SCS) were determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) methods. An acute high-dose SCS test was performed to evaluate the toxicity of SCS. The serum samples were used to identify the alterations involved in the biochemical index and concentration of four key hormones related to the hypothalamus-pituitary-ovary (HPO) axis. In addition, the expression of two essential genes regulated by the HPO axis was determined by the RT-qPCR method.

Results: In the present study, we identified the active components (e.g., sauchinone and formononetin) from the Chinese herbs via TLC and HPLC methods. In addition, the results from the serum biochemical index and histologic section found that acute high-dose SCS exerted no adverse impacts on the rats. We then illustrated that SCS could shorten hemostatic, coagulation time and degree of swelling on rats. Furthermore, SCS decreased the value of erythrocyte, hemoglobin, and hematocrit in serum. Further studies were conducted to provide direct evidence that the recovery of endometrial hyperplasia was associated with the modulation of four hormones (follicle-stimulating hormone, luteinizing hormone, estradiol and progesterone). Besides, SCS altered mRNA expression levels of matrix metalloproteinase-1 and tissue inhibitor of matrix metalloproteinase-1 on the uterine endometrium, thereby promoting the repair of proliferating endometrium in the rats.

Conclusion: Collectively, we demonstrated that an unexploited Chinese traditional medicine, a combination of *S. chinensis*, *C. cristata* and *S. suberectus*, could be used for the treatment of endometrial hyperplasia and associated complications in the rats, and uncovers the mechanism at molecular and gene expression levels.

1. Introduction

As one of the oldest healing methods, traditional Chinese medicine (TCM), including herbal medicine, acupuncture, moxibustion (Tang et al., 2008), has been profoundly promoted and used in treating congestion, diabetes and malaria for a long time (Gan et al., 2010; Li et al., 2018; Xiao and Luo, 2018). Furthermore, accumulating evidence indicates that TCM-related therapy is able to improve spasticity (Yang et al., 2018), neurological function deficit (He, 2015) and mental status (Bai et al., 2015) effectively. Acupuncture can induce the release of corticotrophin-releasing factor and adrenocorticotropic hormone thus decrease sympathetic tonus (Eisenhardt and Fleckenstein, 2016). Artemisinin, honored in the context of the Nobel Prize winners 2015, was first isolated and tested to fight against malaria by Chinese

researchers (Tu, 2016). Nonetheless, TCM was challenged by conventional medicine because most assessed therapies and effective ingredients remain uncertain. Furthermore, most verification trials were published in Chinese, inaccessible to traditional researchers, and not served in systematic reviews (Tang et al., 2008).

Dysfunctional uterine bleeding (DUB), accompanied by proliferating endometrium, which is characterizing as excessively heavy, prolonged and frequent bleeding of uterine origin, is a common disease to the Emergency and Gynecology Department (Deligeoroglou et al., 2013). The primary cause of proliferating endometrium and DUB is the dysfunction of the HPO (Hypothalamus-pituitary-ovarian) axis (Coll Capdevila, 1997), which is accounted for the expression of female hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), and estrogen (E) (Afrin et al., 2019; Kho and Mathur, 2015). The sudden decrease of E level in the proliferating endometrium patients induces apoptosis of epithelial cells of endometrial glands, resulting in irregular shedding of the endometrium (Deligeoroglou et al., 2013). TCM treatment studies have shown promising results for delayed menstrual cycles to cure the DUB and proliferating endometrium (Penn, 2018). Furthermore, most standard treatments are given priority to oral sex hormone drugs and injections, such as nonsteroidal anti-inflammatory drugs, tranexamic acid, prostaglandins, estrogen (Benetti-Pinto et al., 2017; Borzutzky and Jaffray, 2019). However, most drugs have certain side effects, which are accompanied by nausea, vomiting, and headaches (Bianchi Porro and Parente, 1989; FitzGerald, 2002; Grosser et al., 2017). Hence, it is essential to promote the development of new effective drugs for the treatment of endometrial hyperplasia and DUB patients.

Several drugs and extractions have been demonstrated to play a key role in strengthening coagulation, reducing inflammation and lowering uterine bleeding in the patients. Steroidal glycosides, extracted from the berries of *Solanum nigrum L.*, have been confirmed to enhance the ability of anti-inflammatory in injured cells (Xiang et al., 2018). Besides, Gongxuening capsule (GXN), a certified Chinese patent medicine with curative effects on DUB, can strengthen platelet aggregation, hemostasis and coagulation function, thereby increasing female estrogen and progesterone levels, regulating HPO axis and remitting endometrial hyperplasia (Liu et al., 2009). Here, we identified that Chinese medicine, a combination of *Saururus chinensis*, *Celosia cristata* and *Spatholobus suberectus* (SCS), possesses positive effects on the treatment of endometrial hyperplasia and relative complications. The key elements of the drug have been identified by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) methods. According to the animal test results, we found that SCS could shorten hemostasis and coagulation time and alleviate proliferative endometrium via the regulation of four hormones involved in the HPO axis. Collectively, this work provides multi-dimensional insights on the evaluation of the SCS and presents a novel perspective for the treatment of complications.

2. Materials And Methods

2.1 Drugs preparation

The *Saururus chinensis*, *Celosia cristata* and *Spatholobus suberectus* decoction pieces were purchased from Hunan Gaoqiao Market (Changsha, China), and the standard control decoction pieces were obtained from China Institute for the Inspection of Medicines and Biological Products to identify the components in the SCS decoction pieces. Each medicinal material was made into coarse powder by an ordinary pulverizer, and then pulverized by an ultrafine pulverizer after oven-dried (60°C, 12 h). The obtained medicinal ultrafine pieces were stored in a ventilated and dry place for later use.

2.2 Active components identification by TLC

The *S. chinensis* test solution (10 µL) including roots, stems, leaves, and standard control medicinal solution (10 µL) were respectively added into a silica gel plate, and then placed in the chromatographic cylinder containing 10 mL petroleum ether (60–90°C) and acetone. Finally, the dried samples were sprayed with 10% sulfuric acid ethanol coloring solution and heated at 105°C until showing clear spots.

The test *C. cristata* test solution (10 µL) and standard control medicinal solution (10 µL) were added into a silica gel plate and then placed in the chromatographic cylinder containing 10 mL cyclohexane-acetone (5:1) separately. The two samples were colored by 5% vanillin sulfuric acid and heated at 105°C until showing clear spots.

The *S. suberectus* test solution (10 µL) and standard control medicinal solution (10 µL) were respectively added into the silica gel plate, and then placed in the chromatographic cylinder containing 10 mL chloroform-methanol (20:1). The dried samples were observed at 254nm in the UV gel imaging system and photographed.

2.3 Quantitative determination of active components by HPLC

To evaluate the concentration of obtained decoction pieces, we then used the HPLC method to test the quality of *S. chinensis* and *S. suberectus* according to the *Chinese Pharmacopoeia 2010*. A total of 0.5 g *S. chinensis* was added into 25 mL methanol for 30 min and then treated with ultrasound (500 W, 25 kHz) for 40 min. The standard sample sauchinone was taken and made into a 40 ug/mL solution with methanol. The two types of samples were then detected by an HPLC (YOUNDAO2000). The previous treatment of *S. suberectus* was consistent with *S. chinensis*. The standard control sample formononetin was added into a 40 ug/mL solution with methanol. Again, the two types of samples were then detected by the HPLC.

2.4 Animals and treatment

According to the conversion reaction (Table S1), three herbs were added 1 L of 100% ethanol and boiled for 30 min. After cooling, we then filtered the residue and added 1 L of distilled water boiling for 30 min, and removed the residue again. The obtained alcohol extracts were placed in a vacuum freeze dryer and stored for later use.

Female SD rats purchased from the Hunan SJA Laboratory Animal Co., Ltd (Changsha, China) were used in all of the experiments. The SD rats were kept in specific pathogen-free conditions in the animal experiment center of Hunan Normal University. The diets of the rats were provided with Grade B rat breeding stocks daily, and the rats had ad libitum access to food and water at all times during the study.

2.5 Toxicity evaluation of acute high-dose SCS

Healthy SD female rats, weighing 130 ± 20 g, were habitually reared for one week and randomly divided into the following two groups: blank control group (CK, intragastrically administered equal volume of normal saline), normal dose administration group (SCS) and 20-dose SCS administration group (20 \times). Four SD female rats per cage were administered intragastrically 25 g/d solutions at a fixed time for 7 consecutive days, eventually collected plasma to calculate visceral coefficients and blood biochemical parameters. Rats were then sacrificed under light ether anesthesia.

In addition, to assess the safety of SCS on the rats, we collected two organs (liver and kidney) from the rats and depicted histologic sections based on the samples. The clean organ samples were immersed in neutral formalin for one week, and then dehydrated with a gradient of ethanol. The xylene solution was used for transparent treatment, and then the samples were embedded with paraffin. Paraffin sections were sectioned at 5 μm thickness and mounted on superfrost plus slides. Every tenth section was stained with hematoxylin and eosin and examined by light microscopy.

2.6 Determination of hemostasis and coagulation effects

Healthy SD female rats, weighing 200 ± 20 g, were adaptively reared for one week and randomly divided into the following three groups (each group has four rats): CK, Gongxuening positive control group (GXN), SCS group. Four female rats per cage were administered intragastrically at a fixed time every day for 30 d. Thirty minutes after the end of gavage on the 30th d, the tail was cut off 0.5 cm from the tip of the rat, and the time was counted from the blood flowed out spontaneously to the tail tip to stop bleeding. Meanwhile, we used a clean pin every 15 s to pick up the bottom of the blood drop until the fibrin filaments showing up and then recorded the clotting time. Furthermore, each rat's right foot was injected with 0.1 mL of a 10% egg white saline (EWS). The foot volume of the rats was recorded at 10 min, 20 min, and 30 min after the injection, and the foot swelling volume data were obtained to calculate the detumescent degree of the rats.

2.7 Endometrial hyperplasia rat establishment

Healthy SD female rats, weighing 200 ± 20 g, were reared adaptively for one week and randomly divided into blank control (CK, $n = 8$) and model group ($n = 24$). The rats in the model group were intramuscularly injected with estradiol benzoate 1 mg/kg every other day to yield endometrial hyperplasia (EH) model rats. After 60 d injections, the EH model rats were randomly divided into 3 groups: EH model group (EH, gavage of equal volume of normal saline), GXN group and SCS group. The two different drugs were uniformly prepared with saline for suspension, and the CK group was intramuscularly injected with 0.9%

sodium chloride injection. On the 0 d, the 3 d and the 7 d of administration, four rats were randomly selected from each group to test the thickness of the endometrium and serous hormone activity.

2.8 Scanning electron microscopy

The histological alterations in the endometrium were detected by scanning electron microscopy (SEM). Endometrial samples were fixed with 2% pentanediol and 1% osmic acid, followed by dehydration with a gradient of ethanol. The samples were then embedded, and semi-thin sections and ultra-thin sections were yielded by the microtome. The images were photographed under SEM (model Carl Zeiss-EVO-40) with the accelerated voltage of 10 kV at a magnification of $\times 2000$.

2.9 Determination of four key enzymes and gene expression of MMP-1 and TIMP-1

As previously mentioned (Coll Capdevila, 1997), four crucial hormones regulated by the H-P-O axis are related to endometrial hyperplasia. Hence, the expression activity of progesterone (P), estradiol (E_2), follicle-stimulating hormone (FSH) and luteotropic hormone (LH) in the blood of SD rats was detected by the enzyme-linked immunoassay kit following the manufacturing requirements.

The ovaries and uterus were taken immediately after dissection of rats, and the mRNA expression levels of matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) were detected by RT-qPCR technology. The primer design was listed in Table S2. The mRNA was then used as a template to produce cDNA by reverse transcription. Three biological replicates were run in the RT-qPCR system. The $2^{-\Delta\Delta CT}$ method was adopted to analyze the relative expression levels of genes.

2.10 Data analysis

Statistical analysis related to the concentration of organ coefficient, serum biochemical index, enzymes and gene expression levels was performed using Student's *t*-test and two-way ANOVA analysis (GraphPad Prism, V.5.02, GraphPad Software, Inc). Adobe Photoshop CS6 and Adobe Illustrator CC were utilized for figure processing. The values were presented as the mean \pm standard error.

3. Results

3.1 Detection of primary functional components of medicinal materials

In the present study, we first identified the active components from three decoction pieces (*S. chinensis*, *C. cristata* and *S. suberectus*) with relative standard drugs. As shown in Fig. 1, TLC chromatography of *S. chinensis* leaves, roots and stems suggested that the *S. chinensis* drug displayed the same color spots as the standard medicine of the control. Meanwhile, both *C. cristata* and *S. suberectus* presented similar spots on the corresponding positions of the standard control chromatography. In addition, the results of HPLC showed that *S. chinensis* and *S. suberectus* depicted the same expression pattern on the main

functional peaks as the corresponding control drugs of sauchinone ($y = 2.3904x + 0.435$, $R^2 = 0.9999$) and formononetin ($y = 1.6514x + 0.411$, $R^2 = 0.9997$) (Fig. 2), demonstrating that sauchinone and formononetin were two active substances in the SCS drug.

3.2 High-dose SCS exerted no significant toxic effect on the rats

The rats were closely observed after daily administration, and the results represented that no distinctive body weight alterations were found in the three comparisons (Fig. 3A). Notably, the organ coefficient value of the spleen, kidney, and ovary displayed no changes ($p > 0.05$), but the index of the liver increased significantly ($p < 0.05$) in the high-dose SCS treated rats (Fig. 3B). According to the results of blood biochemical indexes, acute high-dose SCS drug treatment did not trigger adverse effects on rats. To be more specific, the concentration of total protein (TP), albumin (ALB), urea, total cholesterol (TC), lactic dehydrogenase (LDH), alkaline phosphatase (ALP) and ratio of alanine aminotransferase/aspartate aminotransferase (ALT/AST) displayed no significant biological difference between acute high-dose SCS and control rats ($p > 0.05$, Fig. 3C ~ I). Notably, the normal dose SCS drug showed no alteration in the liver coefficient comparing with CK group, and other detected biomarkers remained unchanged. Besides, although the value was within the normal range, the content of plasma TP content was depleted in normal SCS-treated rats, suggesting that SCS might exert adverse effects on liver and kidney function. Hence, we subsequently demonstrated the safety of SCS by biopsy of the liver and kidney. The pathological section of the rat kidney showed that the number of glomeruli and tubules in the SCS group was higher than that of other rats (Fig. 3J). Liver pathological section results showed that gap junctions between the hepatocytes in the SCS group increased after 3 d of treatment. Nonetheless, the cell junction gap and the number of eosinophils in control and SCS-treated rats did not alter significantly on the 7 d (Fig. 3K), indicating that the SCS exerted no significant side effects in the kidney or liver.

3.3 SCS drug proved hemostasis, anti-inflammation and detumescence effects

Common drugs for the treatment of endometrial hyperplasia have the functions of hemostasis, anti-inflammatory and detumescence. Therefore, this study further compared the effects of SCS and the drug GXN on hemostasis, anti-inflammatory and detumescence in the rats. Compared with CK and GXN groups, SCS harbored an accelerated blood coagulation effect on tail-broken rats (Fig. 4A), and similar alterations in the hemostatic time were also observed in the rats (Fig. 4B). However, although both XBR and GXN had certain hemostatic functions, there was no significant difference between the two drugs. Compared with normal rats, the hemostatic time of both SCS ($p = 0.075256$) and GXN ($p = 0.056449$) was slightly decreased. Moreover, after injection with EWS, the relative toe edema in SCS rats reduced dramatically at 20 and 30 min comparing with 10 min, and the drug represented distinctively comparing with other experiment groups (Fig. 4C). Notably, the hematic phase results indicated that the content of erythrocyte, hemoglobin and hematokrit showed significant down-regulated differences between CK and

SCS rats, indicating that SCS drug could alleviate inflammation and swelling related to blood stasis (Table 1).

Table 1
Effects of SCS on the hematic phase in rats

Test items	CK	SCS
Leukocyte ($10^9/L$)	24.70 ± 1.68	14.27 ± 3.84
Lymphocyte (%)	57.30 ± 0.17	63.60 ± 6.51
Monocyte (%)	5.43 ± 0.58	5.40 ± 0.85
Neutrophil (%)	37.27 ± 0.44	40.00 ± 7.01
Erythrocyte ($10^{12}/L$)	8.16 ± 0.67	$3.67 \pm 0.99^*$
Hemoglobin (g/L)	174.67 ± 16.70	$77.67 \pm 20.34^*$
Hematokrit (%)	45.43 ± 4.17	$20.23 \pm 5.32^*$
MCV (fL)	55.63 ± 0.47	56.20 ± 1.01
MCH (pg)	21.30 ± 0.35	21.23 ± 0.96
MCHC (g/L)	384.00 ± 4.36	383.33 ± 9.60
RDW (%)	10.27 ± 0.18	12.57 ± 2.47
Platelet ($10^9/L$)	153.00 ± 23.30	200.00 ± 68.60
MPV (fL)	6.13 ± 0.18	6.80 ± 0.38
PDW (%)	17.23 ± 0.24	$18.50 \pm 0.21^*$
PCT (%)	0.26 ± 0.17	0.14 ± 0.05

MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; MPV: Mean platelet volume; PDW: Platelet distribution width; PCT: Thrombocytocrit. The data in the table are mean \pm SE, the sample size is ≥ 3 , and * indicate that there is a significant difference ($p < 0.05$) between CK and SCS group.

3.4 SCS drug restored proliferating endometrium

In the present work, we obtained endometrium hyperplasia rats for the study of SCS in the treatment of proliferating endometrium by injecting estradiol benzoate into rats for 60 consecutive days (Fig. 5B). According to the scanning electron microscopy results, we found that most of the pinopodes (a morphological biomarker of endometrial epithelial cells) in the EH (Fig. 5C, 5F) and GXN (Fig. 5D, 5G) groups were atrophic, hollow and sparsely arranged after 3 and 7 d of administration, while the pinopodes in the SCS cells gradually recovered to rounded after 7 d of administration (Fig. 5H). After 3 d administration, the thickness of the endometrium in the SCS rats was dramatically reduced, and it

showed extremely different from the rats of other experimental groups on the 7 d (Fig. 6). Importantly, the endometrial thickness of the SCS gradually recovered to the state of CK rats, indicating that SCS could be used for the treatment of proliferating endometrium.

3.5 SCS altered HPO axis related hormone and gene expression

To explore the molecular mechanism of SCS in the treatment of proliferating endometrium, we then detected four elemental hormones involved in the HPO axis. The results showed that the FSH content in the SCS rats increased gradually with gavage time, and was significantly higher than that in CK, EH and GXN groups at day 7 (Fig. 7A). Interestingly, both LH concentrations in GXN and SCS rats returned to normal after a short-term elevation (Fig. 7B). Coupled with these are the downtrend of E₂ (Fig. 7C) and uptrend of P (Fig. 7D). In addition, the endometrial extracellular matrix is also regulated by MMPs and TIMPs, and therefore both are key biomarkers for determining the treatment of endometrial hyperplasia. SCS significantly increased the expression of the MMP-1 gene, and its significance was higher than that of other experimental groups (Fig. 8A). Similarly, the ascending expression of the TIMP-1 gene in the SCS rats was observed, and the expression level was dramatically higher than in other treatments (Fig. 8B). Two types of up-regulated genes, MMPs and TIMPs, suggested that SCS could promote the degradation of hyperplasia endometrium by regulating the changes of HPO axis-related hormones.

Discussion

Taking into account the characteristics of TCM with low toxicity and difficulty in measuring lethal dose (Liu et al., 2015), thus the safety concerns such as treatment duration, long-term toxicity and dose-dependent toxicity need to be meticulously examined in the use of TCM. Indeed, several herbs have been demonstrated to cause severe intrinsic renal, hepatic, neurologic and carcinogenic toxicities (Haller et al., 2002; Nyirimigabo et al., 2015; Shaw, 2010; Teschke et al., 2014). Though some studies indicate that many species of Aristolochia in TCM were employed to treat symptoms such as acute arthritis and edema, but aristolochic acid extracted from the herb was found to contribute to renal impairment (Chen et al., 2013; Vanherweghem, 1998). Hence, before the drug is officially used, acute high-dose experiments need to be performed to detect the toxicity of SCS on rats. The results from the acute high-dose treatment elucidate that 20-dose SCS exerted no toxic effect on physiological parameters such as body weight in the rats. Nonetheless, the organ index of the rat liver changed significantly, suggesting that high doses of the drug might have adverse effects on the rat liver. Furthermore, both ALT and AST are important biomarkers of liver function testing (Liu et al., 2018), but the significance of the disease course and prognosis can only be determined when the ALT / AST ratio is changed (Khattab et al., 2015). Interestingly, liver histologic sections showed increased gap junctions between hepatocytes in the SCS rats, indicating that the drug can elevate liver metabolic activity in the model rats. In addition, SCS exerted no significant impact on other organ indexes (kidney, spleen, and genitals), it is therefore can reason that SCS constitutes no obvious toxic effect on SD rats.

DUB and proliferating endometrium can cause a range of bleeding symptoms, particularly a frequent and/or uncontrolled bleeding of the uterine, it is therefore necessary to identify the hemostatic, procoagulant and antiphlogistic effects of the drug. Total protein (TP) in the serum is composed of two main components, albumin (ALB) and globulin (GLO) (Alkan et al., 2015; Guo et al., 2018). ALB is a natural part of human blood, which has the function of protecting blood cells, platelets, and regulating coagulation, while globulin also has anti-inflammatory and antibacterial effects (Eljaiek et al., 2017). The symptoms of hyperviscosity are increased hematocrit and red blood cell count, while the value of erythrocyte, hemoglobin, and hematocrit decreased remarkably in Table 1, indicating that SCS regulated blood substances and reduced blood viscosity in the rats, thus reducing the occurrence of blood stasis. In addition, SCS also shortened coagulation and hemostatic time, and eliminated the relative toe edema in the rats, indicating that SCS could be served as an effective drug for complications caused by endometrial hyperplasias, such as bleeding, congestion and inflammation.

Normal uterine bleeding is caused by periodic changes in the endometrium, which is regulated by the HPO axis. The morphological variations of the functional layer of the endometrium can be divided into three phases, namely the proliferative phase, secretory phase, and menstrual phase (Tanos et al., 2020). During the menstrual period, the levels of E and P dropped, which subsequently causes the endometrial blood flow to decrease, resulting in endometrial necrosis due to ischemia. The area of the damaged and ischemic necrotic tissue gradually expanded and the permeability of the blood vessel wall boosted, eventually leading to tissue exfoliation (Jarrell, 2018; Lichten, 2018). Anovulatory DUB results from the prolonged E and limited progestin expression, which can trigger the endometrium growth dramatically and cause breakthrough-estrogen-bleeding in females (Jones and Sung, 2020; Urbanska et al., 2019). Importantly, our findings demonstrate that the pinopodes of EH rats can gradually return to functional morphology after SCS administration, which proves that the drug has an inhibitory effect on endometrial hyperplasia and is much better than the contrastive drug GXN. Hence, we reason that SCS can not only enhance the coagulation and anti-inflammatory ability of rats, reduce the occurrence of blood stasis, but also control the endometrial hyperplasia thus lessen the bleeding caused by the endometrium shedding.

The gonadotropin-releasing hormone secreted by the hypothalamus can induce the synthesis of FSH and LH, thereby promoting follicular development and increasing E secretion (Das and Kumar, 2018; Filatov et al., 2017; Smitz et al., 2016). LH increases P secretion and leads to ovulation. With the increase of E and P levels, it will produce negative feedback inhibition on the hypothalamus and pituitary gland, thus reducing the levels of FSH and LH, leading to luteal degradation (Di Renzo et al., 2016; Utian, 1989; Ye et al., 2018). Menstruation occurs when the endometrium loses the support of these two hormones and exfoliates and bleeds. The imbalance of FSH and LH secretion will affect the production of E_2 and P, and then affect the two-way feedback regulation of sex hormones, which causes female functional uterine bleeding (Abdollahi et al., 2018; Chuah et al., 2017). Estrogen can regulate uterine development and stimulate endometrium hyperplasia and thickening, while P can stimulate endometrium proliferation under the stimulation of E. If the expression level of both increases simultaneously, it will cause endometrium thickening and may cause functional uterine bleeding. Hence, we speculate that the

inhibited hyperplasia of the endometrium is due to SCS regulating the related hormone expression levels in the rats. Test results echoed our opinion: After administration of SCS, the levels of FSH in female rats elevated, LH increased first and then decreased, E2 declined, and P gradually facilitated. The result reflects the negative feedback mechanism of HPO, and the content of E and P does not boost dramatically together, thereby lowing the emergence of endometrial hyperplasia.

MMPs are a group of proteases that degrade the extracellular matrix (ECM), and TIMPs can specifically bind to activated MMPs to inhibit its activity and prevent further degradation of ECM (Arpino et al., 2015; Cui et al., 2017). Studies have shown that the MMPs and TIMPs changed distinctively in ectopic endometrial patients (Uzan et al., 2004). The imbalance between MMPs and TIMPs promotes the degradation of ECM, and increases the invasiveness of ectopic endometrium (Collette et al., 2004). Endometrial hyperplasia results from prolonged stimulation of the endometrium with unopposed estrogen, while many studies have shown that MMPs have significant differential expression in uterine fibroids, including high-expression of MMP-1, MMP-2, MMP-9 and low-expression of MT1-MMP, TIMP-1, -2, and-3 (Iurlaro et al., 1999; Knox et al., 1996; Maatta et al., 2000; McDonnell et al., 1991). MMPs promote sustained cellular growth by activating growth factors, inducing growth factor binding proteins, and releasing mitogenic molecules from the ECM (Pilka et al., 2004). In addition, MMPs and TIMPs are not only enzymes that regulate ECM, but also play an important role in organogenesis, angiogenesis, and wound repair (Hu et al., 2019; Liu et al., 2016; Smane-Filipova et al., 2016; Xing et al., 2017). In the present study, it is reported that there was a significant difference in the expression of MMP-1 and TIMP-1 between differently treated rats after 7 d gavage. Though the expression tendency of MMP-1 in SCS rats was downtrend, the relative expression levels were significantly higher than other treatment groups, indicating that SCS can induce the expression of the MMP-1 gene thereby promoting the degradation of ECM and reduce the endometrial hyperplasia.

Conclusion

In conclusion, the present study proves the active components from three traditional Chinese herbs by TLC and HPLC methods. An acute high-dose test identifies the safety of SCS in the treatment of endometrial hyperplasia and DUB. This work provides evidence that alterations to the hormones and mRNA induced by the gavage of SCS could affect the endometrial hyperplasia of the rats. In addition, the SCS drug is able to deplete blood stasis, enhance coagulation and anti-inflammatory effects, which provides an effective treatment for complications of endometrial hyperplasia. Collectively, this integrated analysis generates a prospective strategy for the treatment of endometrial hyperplasia and DUB and plays a great role in the promotion of Chinese medicine.

Abbreviations

TCM

Traditional Chinese medicine

SCS

Saururus chinensis, Celosia cristata and Spatholobus suberectus

TLC

Thin-layer chromatography

HPLC

High-performance liquid chromatography

HPO

Hypothalamus-pituitary-ovary

DUB

Dysfunctional uterine bleeding

FSH

Follicle-stimulating hormone

LH

Luteinizing hormone

P

Progesterone

E

Estrogen

E2

Estradiol

GXN

Gongxuening capsule

CK

Blank control group

EWS

Egg white saline

EH

Endometrial hyperplasia

SEM

Scanning electron microscopy

MMP-1

Matrix metalloproteinase-1

TIMP-1

Tissue inhibitor of matrix metalloproteinase-1

TP

Total protein

ALB

Albumin

TC

Total cholesterol

LDH

Lactic dehydrogenase
ALP
Alkaline phosphatase
ALT
Alanine aminotransferase
AST
Aspartate aminotransferase

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the guidelines of the Hunan Normal University ethics committee.

Consent to publish

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

Li-jun Chen & Zhi Wang: Conceptualization, Methodology. Bo Lv: Data Curation, Writing Original Draft. Yuan-de Peng: Writing Original Draft, Visualization. Material preparation. Wen-hui Fu & Wen-jing Li: Investigation. Material preparation.

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Figures

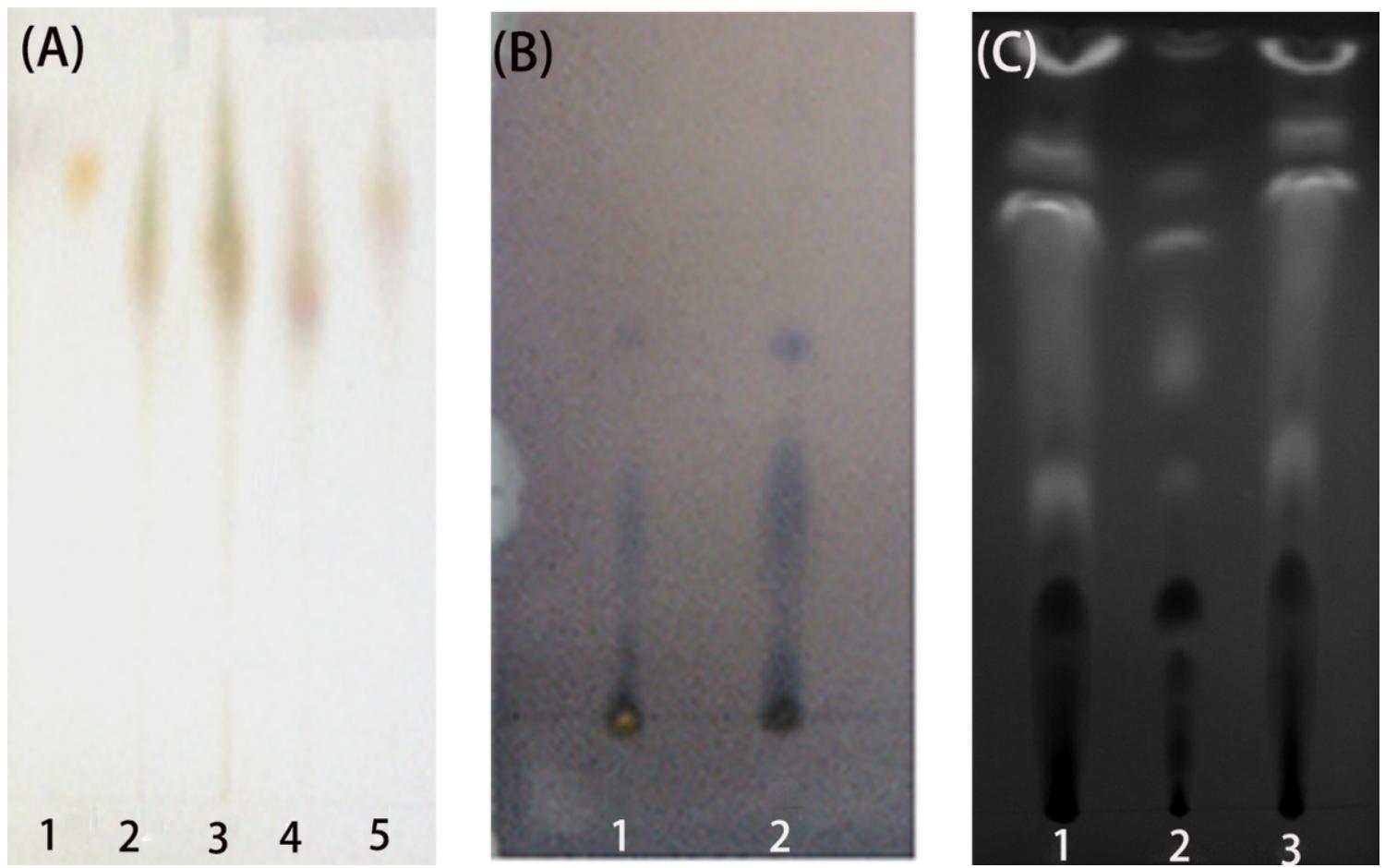


Figure 1

Thin-layer chromatography (TLC) images of three herbs. (A). TLC chromatogram of *S. chinensis*. (1~*S. chinensis*, 2~control herb, 3~leaf, 4~root, 5~stem). (B). TLC chromatogram of *C. cristata*. (1~control herb, 2~*C. cristata*). (C). TLC chromatogram of *S. suberectus*. (1,3~control herb, 2~*S. suberectus*).

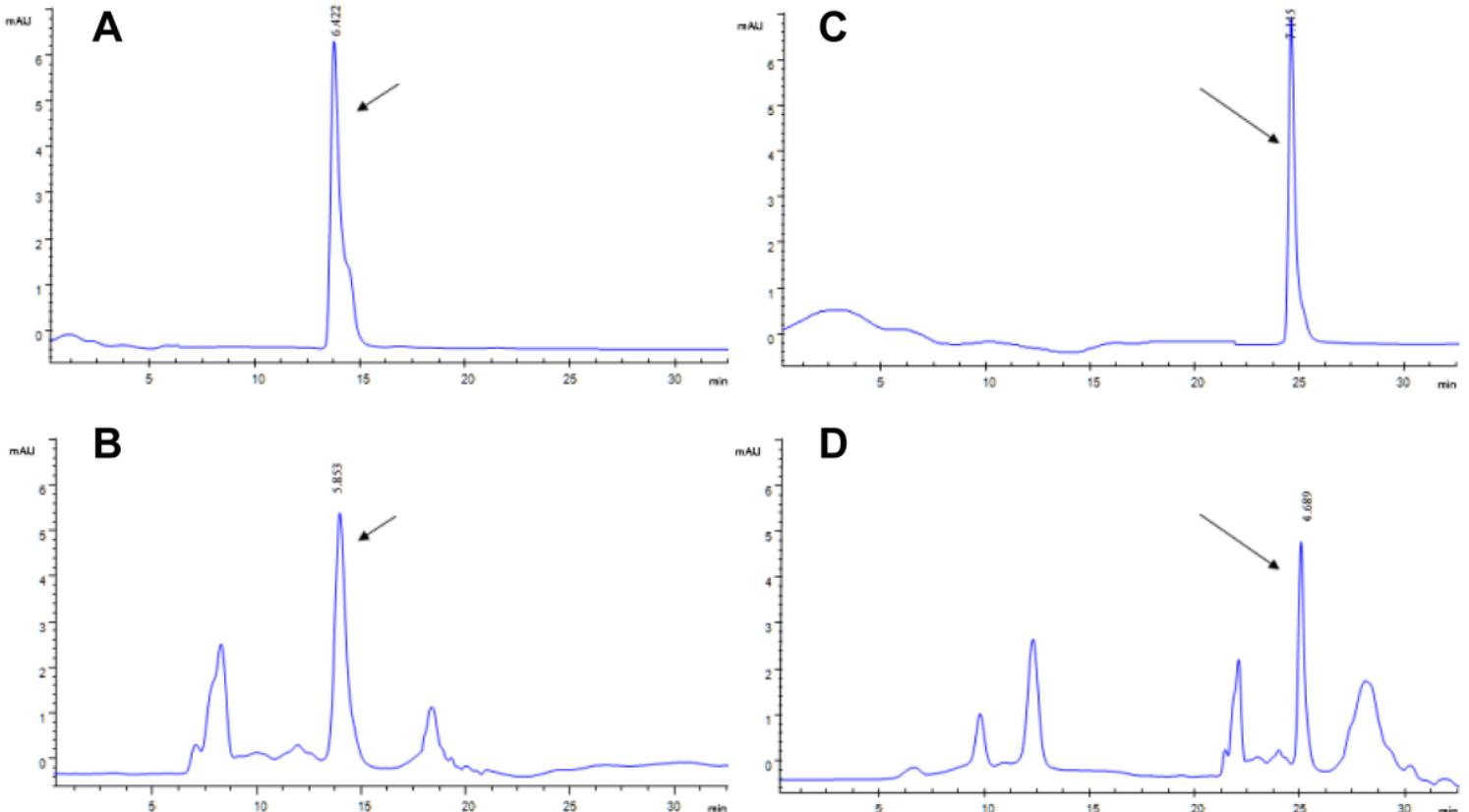


Figure 2

High-Performance Liquid Chromatography (HPLC) images of *S. chinensis* and *S. suberectus*. (A). HPLC chromatogram of control herb (sauchinone). (B). HPLC chromatogram of *S. chinensis*. Arrow refers to the sauchinone. (C). HPLC chromatogram of control herb (formononetin). (D). HPLC chromatogram of *S. suberectus*. Arrow refers to the formononetin.

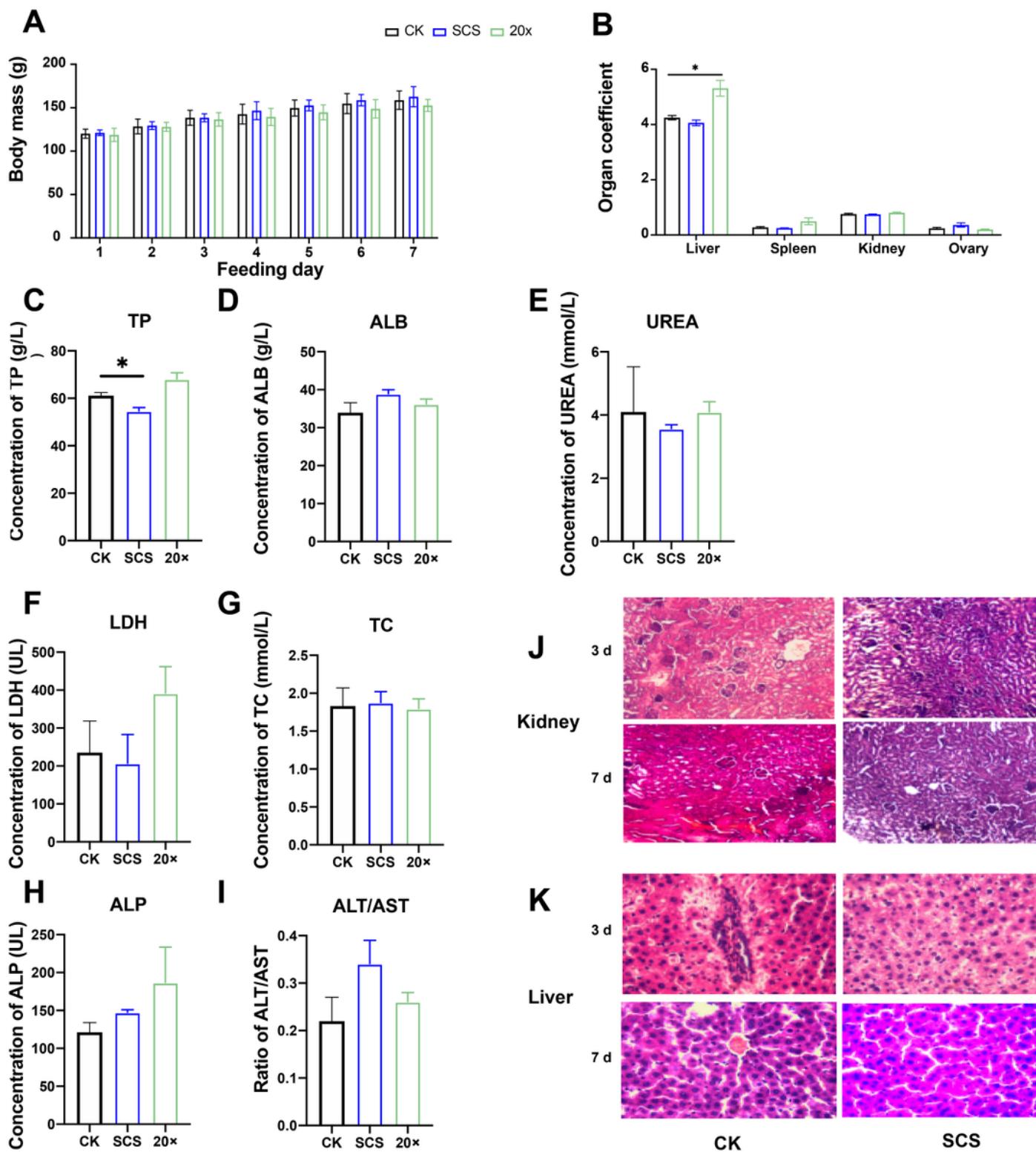


Figure 3

The effects of acute high-dose SCS drug on the SD rats. (A) Histogram of body mass alterations in high-dose SCS treated rats. (B). Effects of acute medication on organ coefficients in rats. Organ coefficient expressed by dividing the organ weight by body mass. (C~I) The alterations of blood biochemical parameters under the gavage of acute SCS. (J) Histologic section images of rat kidney after 3 and 7 d of gavage. (K) Histologic section images of rat liver after 3 and 7 d of gavage. Values are means \pm SEM. *

asterisk indicate the significant difference ($p < 0.05$, $n=4$). Abbreviations: TP, total protein; ALB, albumin; TC, total cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; ALP, alkaline phosphatase.

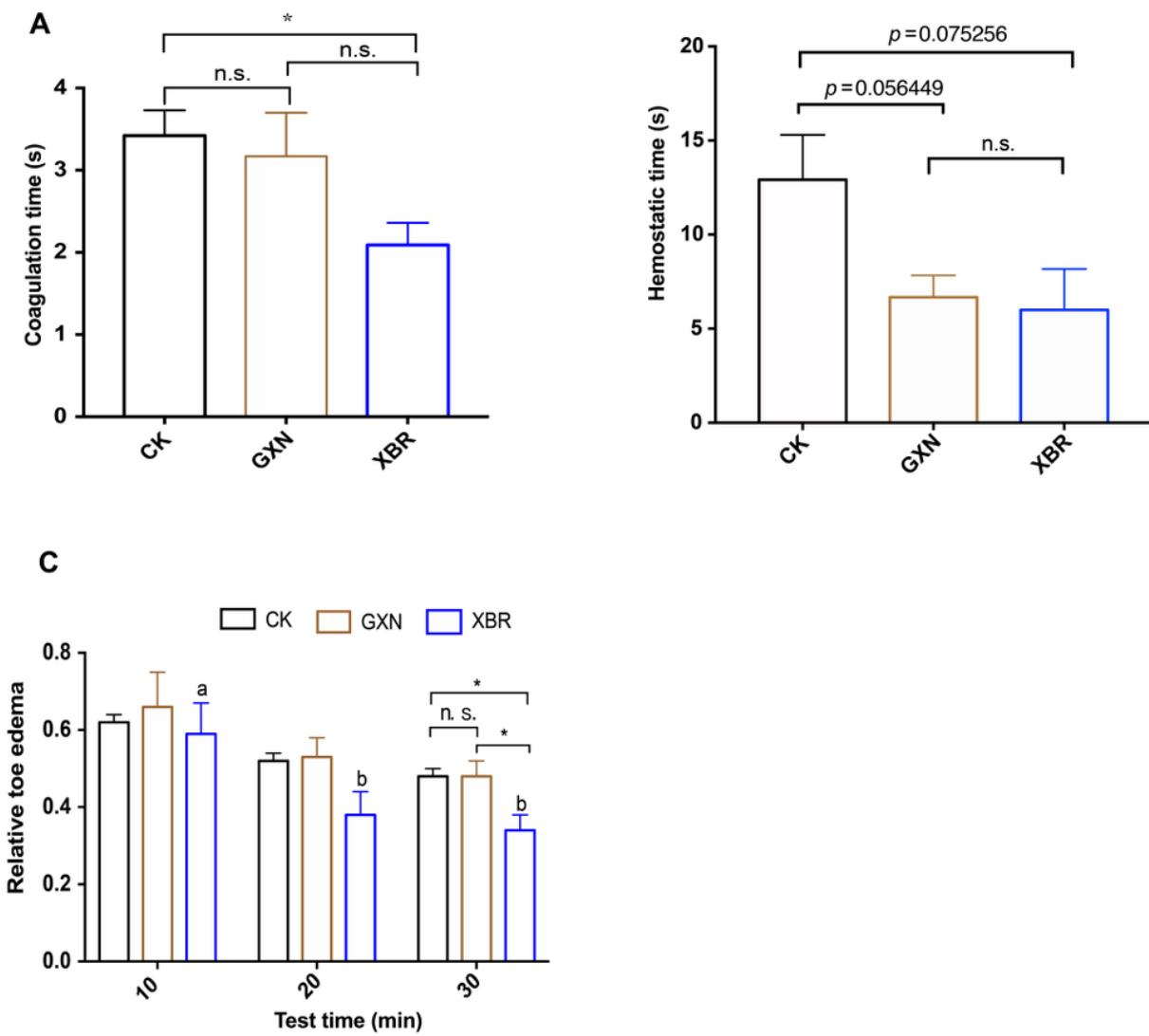


Figure 4

The effects of SCS drug on the hemostasis, coagulation and anti-inflammation of SD rats. (A) Effects of different medical treatments on coagulation time. (B) Effects of different medical treatments on hemostatic time. (C) The changes of paw detumescence. Relative toe edema expressed by (toe volume after swelling - toe volume before swelling) / toe volume before swelling. Values are means \pm SEM. ab Different superscripts or * asterisk indicate the significant difference in the different or same comparisons ($p < 0.05$, t-Student test, $n=4$).

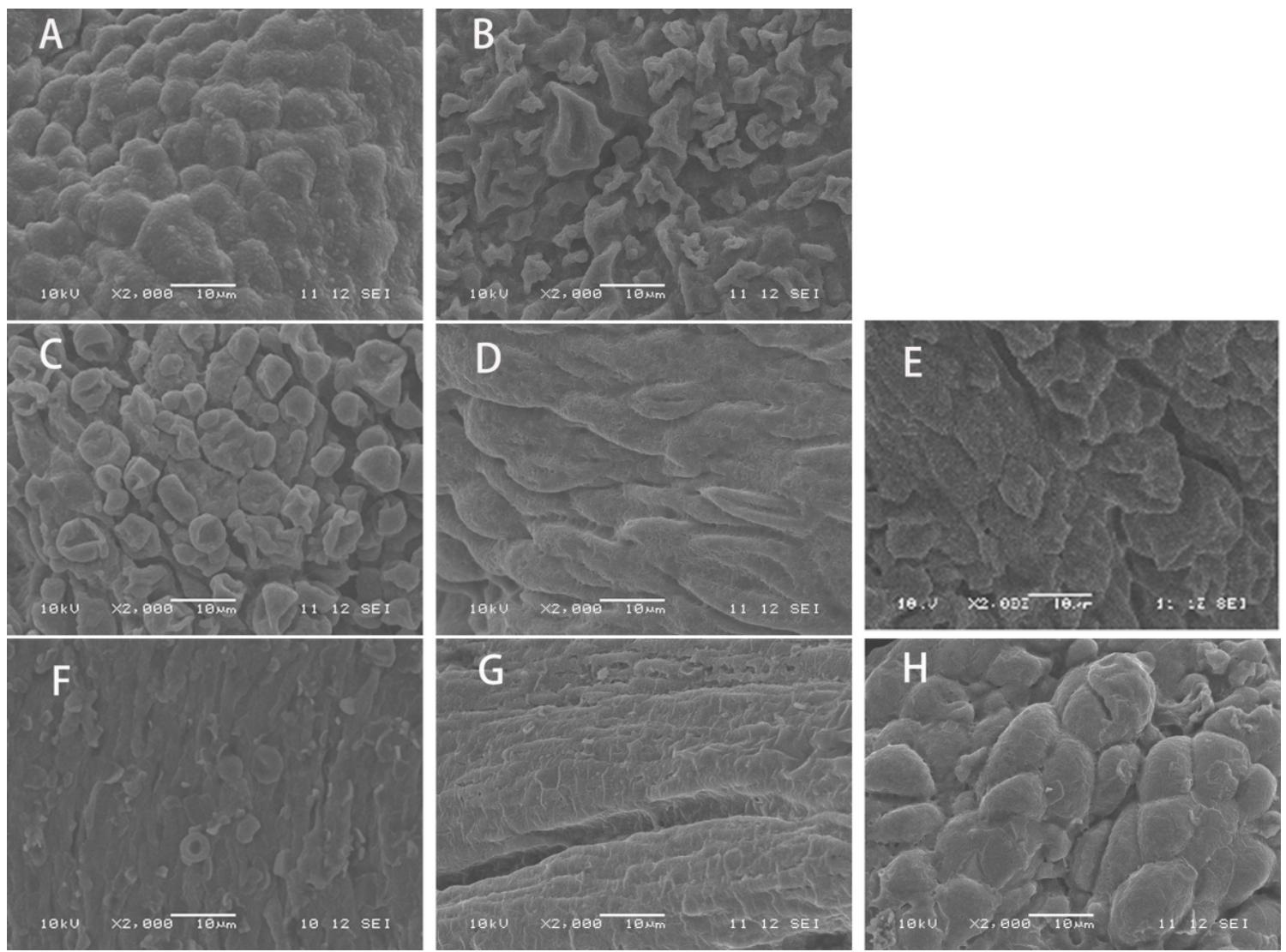


Figure 5

Scanning electron microscope (SEM) images of rat endometrium. (A) SEM image of CK rat endometrium. (B) SEM image of estradiol benzoate treated rat endometrium. (C, D, E) SEM images of rat endometrium after 3 d of gavage (C~EH, D~GXN, E~SCS). (F, G, H) SEM images of rat endometrium after 7 d of gavage (F~EH, G~GXN, H~SCS).

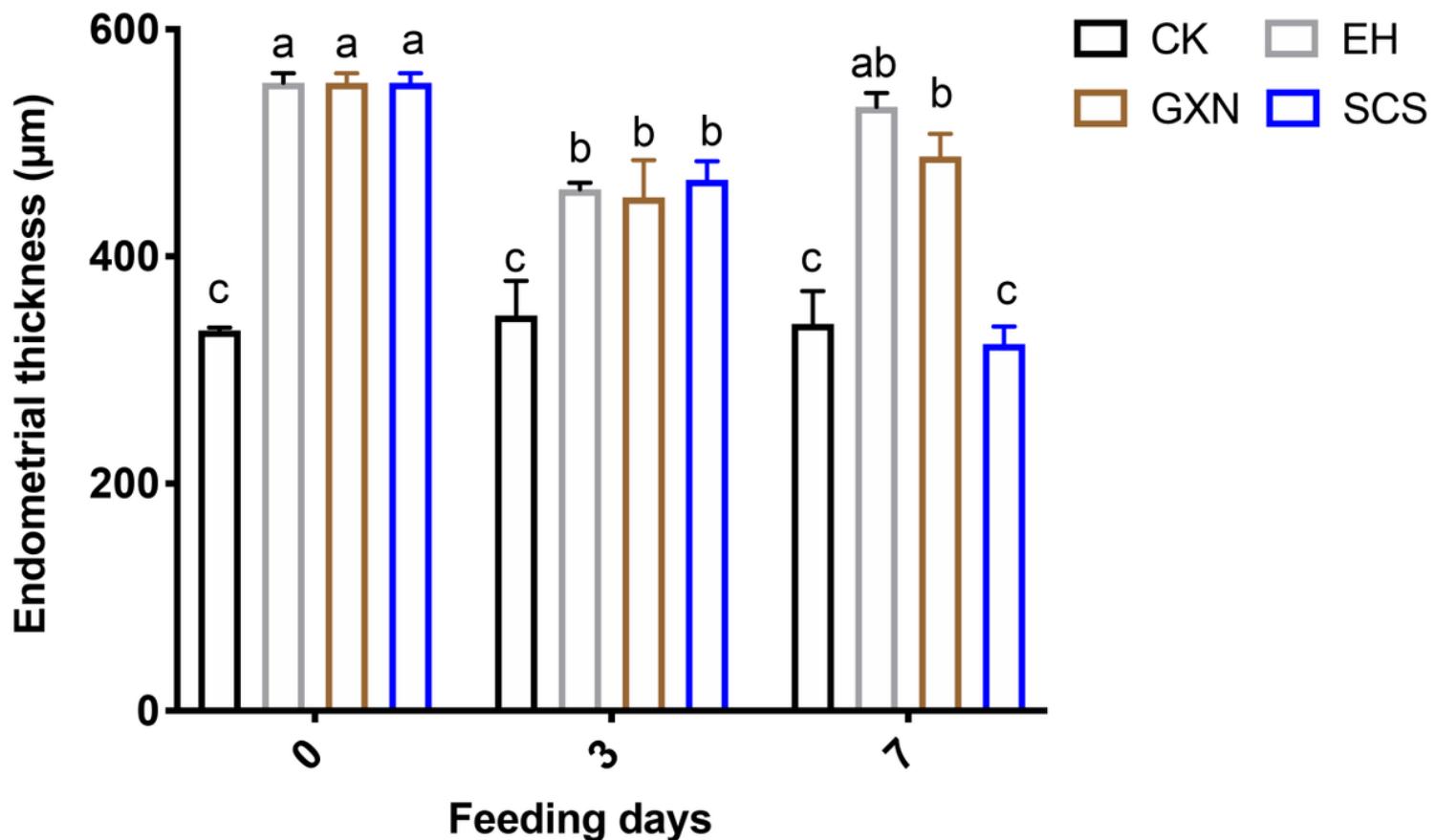


Figure 6

The effects of SCS drug on the thickness of endometrium in the endometrial hyperplasia rats. Values are means \pm SEM. abc Different superscripts indicate the significant difference ($p < 0.05$, t-Student test, $n \geq 4$).

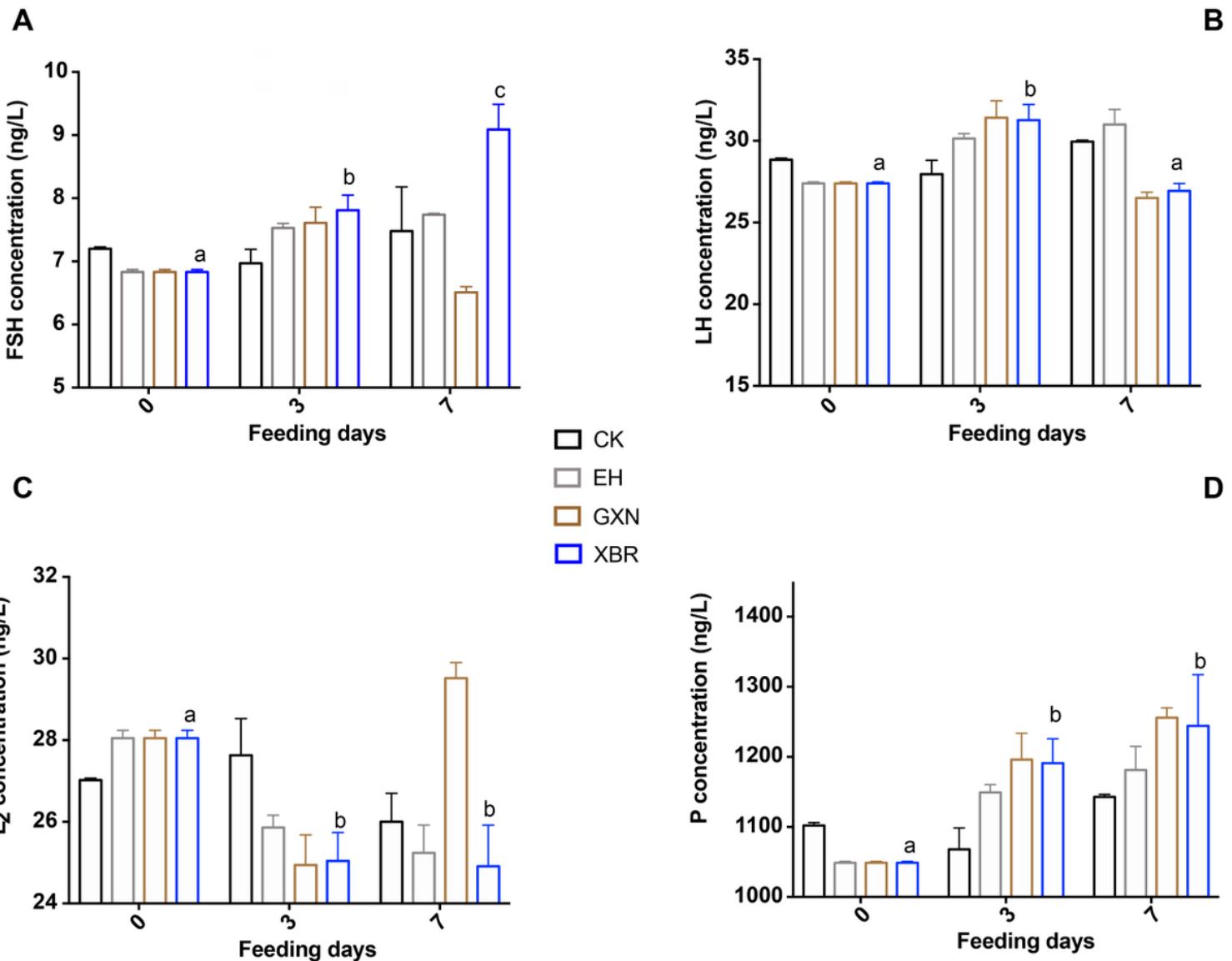
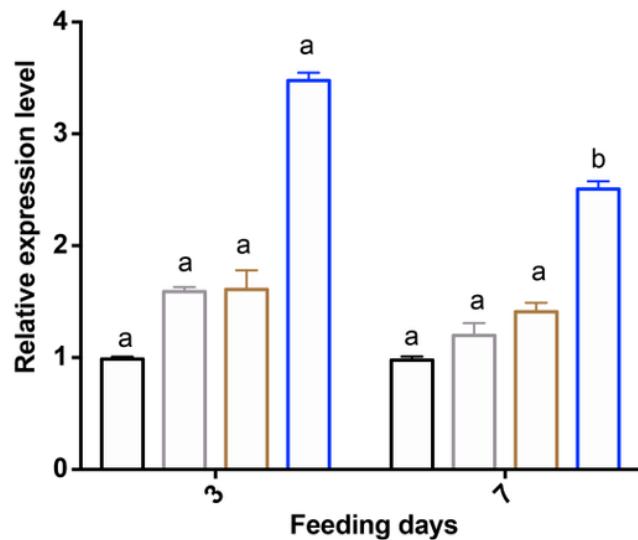
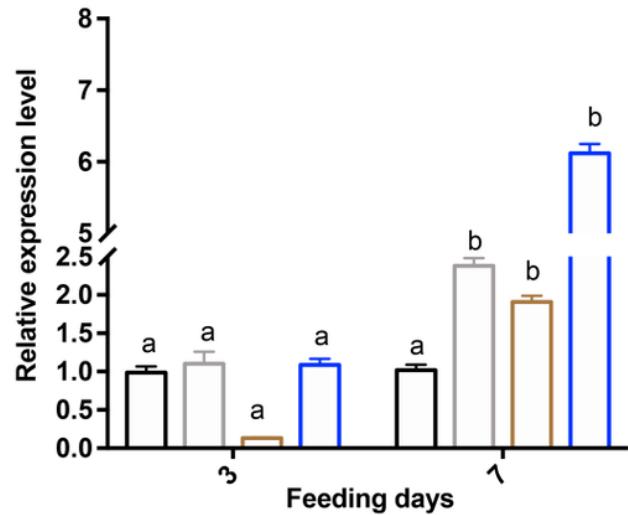


Figure 7

Effects of SCS drug on the hormone expression in the endometrial hyperplasia rats. The concentration of follicle-stimulating hormone (A), luteotropic hormone (B), estradiol (C) and progesterone (D) in the serum of rats. Values are means \pm SEM. abc Different superscripts indicate the significant difference ($p < 0.05$, t-Student test, $n=4$).

A**B****Figure 8**

Effects of SCS on the MMP-1 and TIMP-1 expression in the endometrial hyperplasia rats. (A) mRNA expression levels of MMP-1 in endometrium (B) mRNA expression levels of TIMP-1 in the endometrium. Values are means \pm SEM. ab Different superscripts indicate the significant difference ($p < 0.05$, t-Student test, $n=4$).

Supplementary Files

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