The effects of *Campsis grandiflora* on the model rats with endometriosis.

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**Abstract**

Endometriosis is associated with pelvic pain and infertility, which affects approximately 10% of women of reproductive age. In recent years, *Curcuma xanthorrhiza* (CG) has been administered by some clinical practitioners to treat endometriosis and get satisfactory curative effects. The present study was then designed to investigate the effects of CG on the model rats with endometriosis. Forty female Sprague-Dawley (SD) rats were randomly divided into CG group, Danazol group (DAN group), model control group (MOD group) and the sham-operation group (SHAM group). After all the treatment ended, the levels of Tumor necrosis factor-alpha (TNF-alpha), vascular endothelial growth factor (VEGF) and interleukin (IL)-18 in the peritoneal fluids and the levels of cancer antigen 125 (CA-125) and IL-6 in the serum were detected using enzyme-linked immune-sorbent assay (ELISA) as directed by the manufacturer. The levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA-125 and IL-6 in the serum of CG group, DAN group and SHAM group were significantly lower than those of the MOD group, respectively (P<0.05). CG significantly decreased the levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA-125 and IL-6 in the serum of the model rats with endometriosis (P<0.05). It may be through decreasing the levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA125 and IL-6 in the serum of model rats with endometriosis that CG relieves the disease significantly.

**Keywords:** Endometriosis, *Curcuma xanthorrhiza* (CG).

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**Introduction**

Endometriosis is characterized by the presence of endometrial-like tissue in locations outside the uterus. As a complex disease, endometriosis is associated with pelvic pain and infertility, which affects approximately 10% of women of reproductive age [1]. Surgical and hormonal treatment, as common interventions, often have unpleasant side effects and high rates of relapse [2, 3]. In recent years, medicinal botanicals with anti-inflammatory and pain-alleviating properties have been found to hold promise for treatment of endometriosis [4, 5]. More than 100 clinical trials exploring the effects of Chinese medicinal herbs on endometriosis have been conducted in China and most of them have demonstrated that Chinese medicinal herbs have eminent curative effects on the disease [2]. Post-surgical administration of Chinese medicinal herbs was also found to have comparable benefits to gestrinone but with fewer side effects[2]. A novel methodology for rigorously investigating the effects of individualized decoctions of Chinese medicine on endometriosis was also successfully established recently [6].

*Campsis grandiflora* (CG), as a creeping plant with large, deep orange to red flowers, have been used as a kind of Chinese medicinal herbs to promote Blood circulation and to remove Blood Stasis in diseases caused by Blood Stagnation clinically. The isolation of iridoids, phenyl propanoid glycosides and triterpenoids from the leaves and flowers of CG has been reported, and solvent extracts and active compounds isolated from the extracts have been found to possess several pharmacological actions. [7-12]. In recent years, CG has been administered by some clinical practitioners to treat endometriosis and get satisfactory curative effects. However, none has been conducted on the effects of CG on the endometriosis.
Although establishing a correct diagnosis of endometriosis is often problematic, some biomarkers for endometriosis have been found with clinical values[13, 14]. Advanced statistical analysis of a panel of interleukin (IL)-6, IL-8, tumor necrosis factor-alpha (TNF-alpha), high-sensitivity C-reactive protein (CRP), cancer antigen 125 (CA-125) and cancer antigen 199 (CA-199) on samples obtained during menstruation was reported to allow the diagnosis of both minimal-mild and moderate-severe endometriosis with high sensitivity and clinically acceptable specificity[15]. The present study was then designed to investigate the effects of CG on the levels of TNF-alpha, vascular endothelial growth factor (VEGF) and IL-18 in the peritoneal fluids and the levels of CA125 and IL-6 in the serum of model rats with endometriosis.

Materials and Methods

Animals

Forty female Sprague-Dawley (SD) rats (weighing 140 ±20 g) were provided by the Laboratory Animal Center of Zhejiang Chinese Medicine University (Hangzhou, China). The animals were kept in a room under a 12h light–12h dark cycle and environmentally controlled conditions of 22±2°C. All the animals were acclimatized one week before the experiment. The research was carried out according to the National Research Council’s protocol for the care and use of laboratory animals. Among the 40 rats, 10 rats were randomly taken as the sham-operation group (SHAM group). The other 30 rats were used to establish the model rats with endometriosis. The rats were randomized with the use of a randomization chart constructed in Microsoft Excel that randomized numbers into four groups.

The method of operational transplantation was used in the present research to establish the model rats with endometriosis. Each rat was injected with 0.2mg of diethylstilbestrol to stimulate estrus. Then 20% urethane (1.5g/kg) was injected intra-abdominally. The abdominal fur was shaved and the skin disinfected. The abdominal cavity was opened and the uterus was separated away from the right ovary by 0.5cm, and 2-cm long section of the uterus removed. Then the endometrium was separated and divided into three parts and the uterine branch, left ovary and peritoneal peritoneum were respectively sutured. Finally the abdominal cavity was closed and gentamycin sulfate (0.1ml) was injected into each rat for 3 days after finishing the above operation. Four weeks after the model rats were made, the 30 model rats were randomly divided into three groups (n=10 in each group). For each rat in the SHAM group, after the abdominal fur was shaved and the skin disinfected, the abdominal cavity was opened and then closed. The SHAM group was used to insure aseptic conditions.

The preparation of plant materials

CG was purchased from Huqing Yutang Pharmaceutical Co., Ltd (Hangzhou, China) and was identified by Zhejiang Chinese Medicine University (Hangzhou, China). 30g of CG was extracted by refluxing with 300ml of 70% ethanol for 20 min followed by filtration. The same extraction procedures were repeated once. The obtained solution was combined and condensed to a concentration of 1g/ml, which was then freeze dried and get extract of CG.

Group and administration:

The 40 rats were randomly divided into four groups: CG group (the rats were orally administrated the extracts of CG at 300 mg/kg once daily for 28 consecutive days), Danazol group (DAN group, the rats were orally administrated danazol at 36 mg/kg once daily for 28 consecutive days), model control group (MOD group, the rats were orally administrated saline at 8ml/kg once daily for 28 consecutive days), and SHAM group (the rats were orally administrated saline at 8ml/kg once daily for 28 consecutive days). After all the treatment ended, the rats were sacrificed and the samples of peritoneal fluids and serum were taken. The peritoneal fluid samples of the rats were centrifuged at 12,000 rpm for 10 min at 4 °C. Then, the supernatants were collected, aliquoted, and stored frozen at −80 °C until used for further evaluation. The levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA125 and IL-6 in the serum were detected using enzyme-linked immune-sorbent assay (ELISA) as directed by the manufacturer (RUIQI Bio Co. Ltd, Shanghai, China). Danazol was provided by Lianhua Medicine Company (Jiangsu, China).

Statistical analysis

Results were analyzed with Statistical Package for Social Sciences (SPSS 13.0 for Windows). Analysis of variance (ANOVA) was employed for analyzing all the data. A 5% significance level (P<0.05) and two-tailed tests were used for all hypothesis tests.

Results

The levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids

As shown in Figure 1A, the levels of TNF-alpha in the peritoneal fluids of CG group, DAN group and SHAM group were significantly lower than those of the MOD group respectively (P=0.0001, P=0.0002, P=0.0001). The levels of TNF-alpha in the peritoneal fluids of DAN group were significantly higher than those of SHAM group (P=0.0005), while there was no marked difference between CG group and SHAM group (P=0.1088). The levels of TNF-α in the peritoneal fluids of CG group were significantly lower than those of DAN group (P=0.0001).
Figure 1. The levels of tumor necrosis factor-alpha (TNF-alpha), vascular endothelial growth factor (VEGF) and interleukin (IL)-18 in the peritoneal fluids of *Curcuma xanthorrhiza* (CG) group, Danazol group (DAN group), model control group (MOD group) and the sham-operation group (SHAM group). (A) The levels of TNF-alpha in the peritoneal fluids of all the four groups. (B) The levels of VEGF in the peritoneal fluids of all the four groups. (C) The levels of IL-18 in the peritoneal fluids of all the four groups. Data were shown as mean ± SD. (N=10 in each group). The significant difference was set at \(^{a} p < 0.05\), compared with the MOD group; \(^{b} p <0.05\), compared with the SHAM group; \(^{c} p <0.05\), compared with the DAN group.

As shown in Figure 1B, the levels of VEGF in the peritoneal fluids of CG group, DAN group and SHAM group were significantly lower than those of the MOD group respectively (P=0.0001, P=0.0001, P=0.0001). The levels of VEGF in the peritoneal fluids of DAN group were significantly higher than those of SHAM group (P=0.0003), while there was no marked difference between CG group and SHAM group (P=0.1802). The levels of VEGF in the peritoneal fluids of CG group were significantly lower than those of DAN group (P=0.0004).

As shown in Figure 1C, the levels of IL-18 in the peritoneal fluids of CG group, DAN group and SHAM group were significantly lower than those of the MOD group respectively (P=0.0002, P=0.0002, P=0.0001). However, there were no marked difference among CG group, DAN group and SHAM group (P=0.7811, P=0.0619).

**The serum levels of CA125 and IL-6**

As shown in Figure 2A, the serum levels of CA-125 in CG group, DAN group and SHAM group were significantly lower than those of the MOD group respectively (P=0.0001, P=0.0001, P=0.0001). The levels of CA-125 in the peritoneal fluids of DAN group were significantly higher than those of MOD group (P=0.0003), while there was no marked difference between CG group and SHAM group (P=0.1802). The levels of CA-125 in the peritoneal fluids of CG group were significantly lower than those of DAN group (P=0.0004).

As shown in Figure 2B, the serum levels of IL-6 of all the four groups. Data were shown as mean ± SD. (N=10 in each group). The significant difference was set at \(^{a} p < 0.05\), compared with the MOD group; \(^{b} p <0.05\), compared with the SHAM group; \(^{c} p <0.05\), compared with the DAN group.

**Figure 2.**
cantly lower than those of the MOD group respectively (P=0.0003, P=0.0003, P=0.0001). The serum levels of CA-125 in DAN group and CG group were significantly higher than those of SHAM group respectively (P=0.0004, P=0.0004), while there was no marked difference between CG group and SHAM group (P=0.2322).

As shown in Figure 2B, the serum levels of IL-6 in CG group, DAN group and SHAM group were significantly lower than those of the MOD group respectively (P=0.0002, P=0.0002, P=0.0001). The serum levels of IL-6 in both of the CG group and DAN group were significantly higher than that of SHAM group respectively (P=0.0032, P=0.0021), and the serum levels of IL-6 in CG group were significantly lower than DAN group (P=0.0436).

Discussion

In the study, the levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA125 and IL-6 in the serum were selected as the biomarkers for endometriosis. TNF-alpha, a cytokine with pro-inflammatory and pro-angiogenic activities, was found elevated in serum of women with endometriosis compared with healthy controls and the serum TNF-alpha levels were reduced by treatment with danazol for 6 months [16]. A similar increase in the serum TNF-alpha levels in endometriosis women was found in a recent study [17]. The levels of TNF-alpha in peritoneal fluids and the levels of IL-6 in serum have been demonstrated as two biomarkers to discriminate between patients with endometriosis and those without[18]. VEGF may play an important role in the pathogenesis of endometriosis. In the patients with endometriosis, a positive correlation existed between the serum levels of CRP and VEGF [19]. In a Japanese population, a positive association was found between the VEGF +936 T allele and moderate-to-severe endometriosis [20]. The levels of IL-18 in peritoneal fluids were found markedly higher in women with peritoneal, minimal- to mild-stage endometriosis than in controls [21]. As early as in 1998, a meta-analysis showed that the performance of serum CA-125 measurement in the diagnosis of endometriosis grade I/IV is limited, however its performance in the diagnosis of endometriosis grade III/IV is better [22]. For the patients with advanced endometriosis and initially elevated CA-125 levels, CA-125 was found as a valuable adjutant in the follow-up of recurrence of the disease [23]. Serum CA-125 measurement is now a consolidated method for diagnosing this condition, and its interpretation has posed a number of problems, particularly regarding utility in diagnosing minimal-mild endometriosis [24]. In a study to test the ability of a group of serum cytokines, either individually or in combination, to serve as biomarkers for the nonsurgical diagnosis of endometriosis, serum IL-6 was finally selected as a promising serum marker for the nonsurgical prediction of endometriosis [25].

As early as in 2005, seven kinds of teri terpenoids were firstly isolated from CG [7]. An HPLC method was also successfully established in a recent study, which can determine the concentration of acteoside, oleanolic acid and ursolic acid simultaneously [9]. In another study, five pentacyclic triterpenoids isolated from CG were tested for insulin-mimetic and insulin-sensitizing activity [8]. Five pentacyclic triterpenoids, including oleanolic acid, hederagenin, ursolic acid, tormentic acid and myriantiacid, were isolated from the methanol extract of the leaves of CG [10]. In a following study by the same group, two new non-glycosidic iridoids, which were named cachinol and 1-O-methyl cachinol were isolated from the methanol extract of the leaves of CG together with a known iridoid cachinolides 1 [11]. The topical application of the extracts of CG dose-dependently inhibited arachidonic acid (AA)- and 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced ear edema in mice[12]. In the present study, CG was found to significantly decrease the levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA-125 and IL-6 in the serum of the model rats with endometriosis, which was consistent with the previous studies. Medicinal plants or herbs and their active components exhibit cytokine-suppressive, antioxidant, sedative and pain-alleviating properties, and each of these mechanisms of action would be predicted to have salutary effects in endometriosis[4], which is consistent to the findings of our study. In our studies, a series of main biomarkers for endometriosis respectively in the peritoneal fluids and the serum were detected, which may completely reflect the effects of GC on the disease. However, more studies on the expression of specific genes for endometriosis are needed to clarify other potential mechanisms involved in CG relieving endometriosis.

Conclusion

It may be through decreasing the levels of TNF-alpha, VEGF and IL-18 in the peritoneal fluids and the levels of CA125 and IL-6 in the serum of the model rats with endometriosis that CG relieves endometriosis significantly.

References

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