

## EFFECTS OF CHINESE MATERIA MEDICA-FUBAO DANGGUI JIAO ON EXPERIMENTAL ENDOMETRIOSIS

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### Abstract

The objective of this paper was to investigate the effects of a Chinese Materia Medica variant -Fubao Danggui Jiao (FDJ)-on experimental endometriosis. An endometriosis model was created by virtue of auto-transplantation of endometrial tissue onto rats' abdominal walls. The implants were allowed to grow for 30 days until the successful completion of the model. After that, forty endometriotic rats were randomly divided into four study groups and given different treatments: (1) negative control group (water, 2ml/kg, per os); (2) FDJ-A group (FDJ, 2ml/kg, per os); (3) FDJ-B group (FDJ, 4ml/kg, per os); (4) Danazol group (70mg/kg, per os). After 30 days with treatments, the volumes of endometriotic implants in each rat were measured. The implants and normal uterine horns were removed for routine histological examination. FDJ caused significant decreases in volumes of the surviving endometriotic implants, with two different doses having statistically equivalent effects. Upon histological examination, FDJ was observed to cause regression of epithelium and stroma of endometriotic implants. FDJ had revealed promising therapeutic effects on endometriosis.

**Keywords:** Fubao Danggui Jiao; Chinese Materia Medica; Endometriosis; Danazol.

**Non-standard Abbreviations:** Fubao Danggui Jiao - FDJ

### Introduction

Endometriosis is a common, chronic, estrogen-dependent gynecological disorder. In women of reproductive age, its incidence is as high as 10%, while that of infertile women is, over 35% (Vigan et al., 2004). Although the etiology of this disease remains unclear, the theory of retrograde menstruation is a widely accepted as an underlying mechanism, because the theory in reference explains how endometrial cells manage to present at ectopic sites. Nevertheless, the theory cannot account for the fact that these misplaced cells survive in women with endometriosis but not in healthy women (Gazvani et al., 2002; Nap et al., 2004; Tsaltas et al., 1998; Vinatier et al., 2001).

Although endometriosis represents a form of benign hyperplasia, it can infiltrate, metastasize and relapse in form of a cancer. It may cause pelvic pain, progressive dysmenorrhea, dyspareunia, and even infertility (Amer, 2008; Arya et al., 2006). The currently accepted medical treatments for this disease involve administration of Danazol, gonadotropin-releasing hormone agonists (GnRHa), progestogens, and oral contraceptives. In a word, these agents suppress the growth of ectopic endometrial tissues by inducing a hypoestrogenic state. However, hypoestrogenism suppresses the growth of normal

endometrium and prevents spontaneous ovulation. All of these medicines are therefore of limited value in patients who want to have children. Moreover, these therapies may cause various side-effects at high incidences, such as weight gain, abnormal lipid metabolism, liver dysfunction, thrombosis, and menopause-like symptoms (Olive et al., 2004; Rizner, 2009; Vercellini et al., 2008). Therefore, new medical strategies are needed to prevent the development of endometriosis.

In oriental countries, Chinese Materia Medica has a thousands-of-years long history of use. Many ancient prescriptions of Chinese Materia Medica have been handed down across generations, and are widely used because of their satisfactory therapeutic efficacy and low incidence of side-effects. Thus, researchers are searching for safe and effective Chinese Materia Medica variants to treat this intractable disease, endometriosis (Han et al., 2008; Qu et al., 2005; Wang et al., 2008; Zeng et al., 2006; Zhang et al., 2009; Zheng et al., 2005).

Fubao Danguai Jiao (FDJ) originates from Jiao Ai Tang, which is an ancient prescription of Chinese Materia Medica. Jiao Ai Tang was first mentioned in the *Synopsis of the Golden Chamber* written by Zhongjing Zhang of China in 219 A.D. It has remedial effects in terms of abortion prevention, and can regulate menstruation (Duan et al., 1984). Later generations chose four constituents of Jiao Ai Tang to develop a new extraction named the Decoction of Four Drugs. The Decoction of Four Drugs is a water extract of Chinese Angelica, Szechwan Lovage Rhizome, prepared Rehmannia root, and White Peony Alba. It enriches the blood, regulates menstruation, and absorbs clots (Xie, 1997). FDJ is a water extract based on the Decoction of Four Drugs with Colla Corri Asini, Indian Bread, Tangshen, Milkvetch root, and Radix Glycyrrhizae added. It was first introduced into the Chinese market in 1950s, and later into Japan. Its main function is to invigorate vital energy, enrich the blood, and regulate menstruation. This paper is the first to study the effects of FDJ on experimental endometriosis.

## Materials and Methods

### Test Drugs

FDJ was kindly provided by Iskra Industry Co., Ltd., Japan. FDJ is a water extract of Chinese Angelica, Colla Corri Asini, Szechwan Lovage Rhizome, prepared Rehmannia root, White Peony Alba, Indian Bread, Tangshen, Milkvetch Root, and Radix Glycyrrhizae. Danazol capsules were produced by Jiangsu Lianhuan Pharmaceutical Co., Ltd., China.

### Animals

Female Sprague-Dawley rats of specific pathogen-free (SPF) level, aged 8 weeks and weighing  $250\text{g}\pm 25\text{g}$ , were purchased from the Animal Department of Tongji Medical College, Huazhong University of Science and Technology, Permit Number: SYXK (Hubei) 2004-0028. All of the rats were kept in a SPF-level lab at the room temperature of  $23\pm 2$ , relative humidity of  $55\pm 15\%$ , with 12:12 light/ dark alteration. Approval of the protocol was obtained from the Institutional animal ethics committee.

## Methods

Forty female rats were injected with  $1\text{mg/kg}$  of diethylstilbestrol to stimulate estrus. After that, laparotomy was carried out under diethyl ether anesthesia. The left uterine horn was resected, and the endometrium was dissected from the myometrium. The endometrium was subsequently cut into  $5\times 5\text{ mm}^2$  - fragments. Four endometrial tissue fragments were implanted into the abdominal wall of the same rat, with epithelium facing the abdominal cavity. Next, streptomycin solution ( $52.5\text{ mg/kg}$ ) was dropped into the abdominal cavity so as to prevent infection. Finally, the wound was sewn up. The operation was conducted in a strict accordance with the aseptic technique.

Thirty days after the surgery, the rats were randomly divided into four study groups and given different treatments:

(1) negative control group (water, 2ml/kg, per os);(2) FDJ-A group (FDJ, 2ml/kg, per os); (3) FDJ-B group (FDJ, 4ml/kg, per os); (4) Danazol group (70mg/kg, per os) as the positive control. After 30 days of treatments, the volumes of surviving implants were evaluated by virtue of measuring their dimensions (length, width, height), volume thereby being equal to length\*width\*height. T-test was utilized for statistical analysis. In addition, the surviving implants and normal right uterine horns were removed and fixed in 10%-formalin for routine histological examination in form of hematoxylin – eosin (H-E) stained paraffin sections. The sections were examined under light microscope (200×) and photomicrographed.

**Table 1:** Volumes of ectopic endometrial tissues across study groups after 30 days of treatments

Study Group	Dose	Volume (mm <sup>3</sup> )
Negative Control	0	125.3±103.7
FDJ-A	2ml/kg	63.2±48.2*
FDJ-B	4ml/kg	58.5±52.4*
Danazol	70mg/kg	41.2±27.4*

All values are expressed as  $\bar{x} \pm s$ . \*P<0.05 vs negative controls.

## Results

### Volumes of Endometriotic Implants

After 30 days of treatments, the surviving endometriotic rat implants had grown well and had transformed into cystic structures protruding from the abdominal walls (Figure 1). The implants that failed to survive either perished or turned into small hard sites. On the surface of some of the surviving cysts, several newly-formed blood vessels had been observed, while some of the cysts contained a bright-yellow secrete inside their cavities. In addition, some of the cysts were closely adhered to the surrounding tissues.

FDJ caused a significant decrease in volumes of the surviving ectopic endometrial tissues, with two doses statistically equally effective (P>0.05). The volumes of the implants seen in the FDJ-A and FDJ-B arms were statistically equivalent to those seen in the Danazol arm (P>0.05). Table 1 shows the volumes of the ectopic endometrial tissues seen in each animal group.

### Histological Examination of Implants and Uterine Tissue

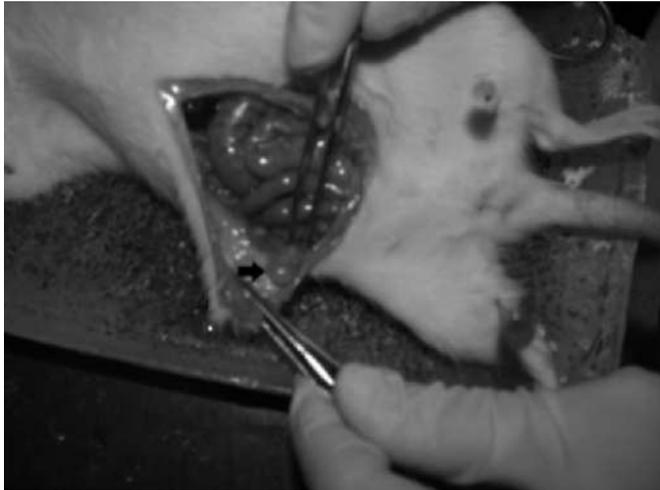
In the surviving implant sections across the negative control group, inflammatory hyperplasia in terms of stratified epithelia and dense inflammatory cell infiltration had been seen. In the FDJ and Danazol arms, some of the epithelial cells had atrophied into small cubic cells. In FDJ and Danazol -treated rats, stromal cells were arranged loosely (Figure 2).

In the right uterine horn sections, stromal inflammatory cell infiltration indicative of uterine inflammation had been seen with the negative controls, whereas the FDJ and Danazol groups had exhibited no pathology whatsoever (Figure 3).

## Discussion

Experimental results of the study presented herein, indicated that a 30 day-FDJ administration is capable of

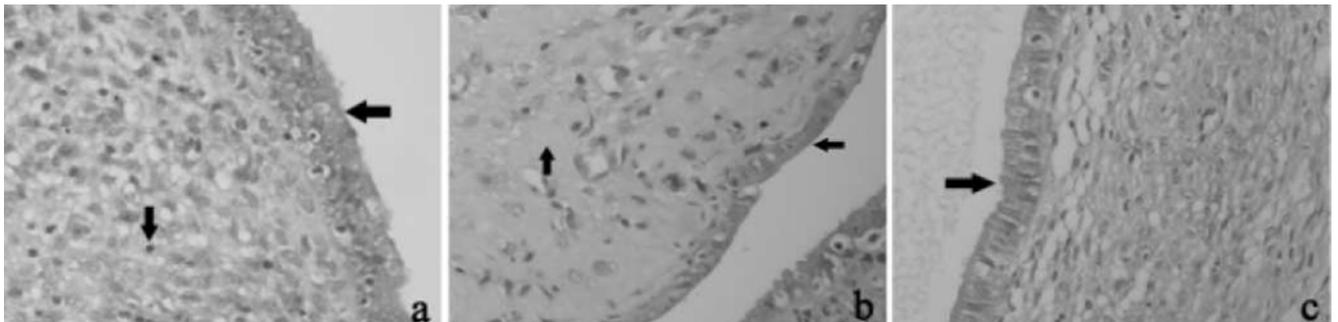
effectively inhibiting the growth of endometriotic implants. Upon histological examination, it was observed that FDJ caused



**Figure 1:** Surviving endometriotic implants

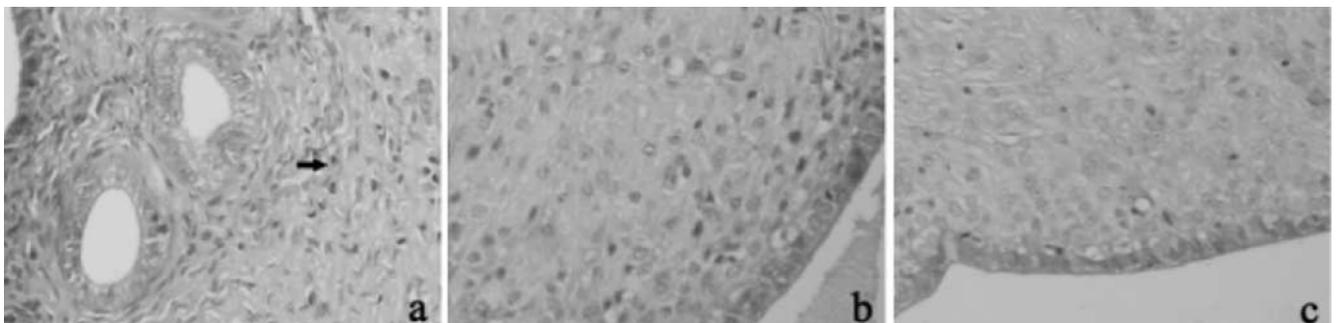
The surviving implants had grown to form cystic structures. Inside the cysts, transparent secrete had been found (arrow).

**Figure 2:** Sections of surviving implants (H-E staining; original magnification, 200×)



a. In the negative control group, epithelial cells of ectopic endometria had proliferated to form stratified epithelia (horizontal arrow). In these negative controls, chronic inflammatory cells were observed as well (vertical arrow). b. In FDJ-treated rats, epithelial cells of ectopic endometria had atrophied into a cubic shape (horizontal arrow). The cells in the stroma were arranged loosely (vertical arrow). c. In the ectopic endometria of Danazol-treated rats, normal, simple columnar epithelia had been seen (arrow).

**Figure 3:** Uterine tissue sections (H-E staining; original magnification, 200×)



a. In the negative controls, inflammatory cell infiltrations of the uterine stroma had been seen (arrow). b. In FDJ-treated rats, uterine tissue presented with no pathological changes at all. c. In Danazol-treated rats, the uteri presented with no pathological changes at all.

regression of epithelium and stroma in endometriotic implants. All of these results suggest that FDJ has promising therapeutic effects on endometriosis, statistically equivalent to those of Danazol. Moreover, the two doses exhibited no significant difference in efficacy of endometriosis management.

For decades now, FDJ has been used as a potent agent in drug treatment of irregular menstruation and deficiency of both vital energy and blood. However, its effects on endometriosis have never been examined before. The present study was the first to investigate the effects of FDJ on experimental endometriosis as its newly-discovered pharmacological potential.

FDJ is a water extract of several medicinal ingredients, including Chinese Angelica, Colla Corri Asini, Szechwan Lovage Rhizome, prepared Rehmannia root, White Peony Alba, Indian Bread, Tangshen, Milkvetch root, and Radix Glycyrrhizae. In traditional Chinese medicine Chinese Angelica is perceived as acrid-sweet in flavor and warm in nature; it enriches the blood, enhances blood circulation so as to alleviate pain, nourishes the liver, and regulates menstruation. Colla Corri Asini is sweet in flavor and neutral in nature; it stops bleeding, absorbs clots, and nourishes the blood and kidney. Szechwan Lovage Rhizome is pungent in flavor and fragrant in nature; it can increase vital energy and enhance blood circulation. Prepared Rehmannia root is sweet in flavor and warm in nature; it can nourish *yin*, supplement blood, and tonify the marrow. White Peony Alba is bitter-sour in flavor and cool in nature; it nourishes the blood and liver, puts down the middle warmer energy to alleviate pain, and decreases *yin* exhaustion by stopping excessive perspiration. Indian Bread is sweet in flavor and bland in nature; it can act as diuretic, supplement vital energy, and invigorate the spleen. Tangshen is sweet, slightly sour in flavor and mild in nature, while Milkvetch root is sweet in flavor and slightly warm in nature. Their actions are the same - invigoration of vital energy and spleen. Radix Glycyrrhizae is sweet in flavor and neutral in nature; it can invigorate vital energy and harmonize the nature of various medicinal ingredients (Li, 2002).

According to the traditional Chinese medical theory, endometriosis is an endometrial proliferation arising due to the stagnation of vital energy and blood outside the uterine cavity (Li et al., 2003). FDJ contains several ingredients capable of enhancing vital energy and blood circulation. Therefore, we theoretically deduced that FDJ has some promising properties possibly of use for endometriosis therapy.

In the present study, we investigated the therapeutic effects of FDJ. The results had demonstrated its effectiveness in suppressing endometriotic implants' growth. It was therefore deduced that FDJ has promising therapeutic effects on endometriosis; thus, further research is needed in order to further clarify its acting mechanisms and confirm its effectiveness in endometriosis. Additionally, the results of histological examinations of uterine tissue samples demonstrated that FDJ has an anti-inflammatory effect as well. Uterine inflammation may induce oviduct inflammation; should an oviduct inflammation become serious, it may result in oviduct obstruction and cause infertility. Our experimental results suggest the need for future research on therapeutic effects of FDJ on oviduct obstruction developed due to inflammation.

The present study showed that, by virtue of inhibiting the proliferation of ectopic endometrial tissues, FDJ may be of therapeutic value in endometriosis.

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