A Botanical Extract from Channel Flow Inhibits Cell Proliferation, Induces Apoptosis, and Suppresses CCL5 in Human Endometriotic Stromal Cells

Fritz Wieser,2,3 Jie Yu,3 John Park,3 Andrew Gaeddert,4 Misha Cohen,5 Jean-Louis Vigne,6 and Robert N. Taylor3

Department of Gynecology and Obstetrics,3 Emory University School of Medicine, Atlanta, Georgia Health Concerns,4 Oakland, California Institute for Health & Aging5 and Center for Reproductive Sciences,6 University of California, San Francisco, California

ABSTRACT

Growing evidence suggests that medicinal herbs have direct actions on endometrial cells. By screening multiple herbs using an in vitro model of endometriosis, we found that a commonly used herbal formula exerted considerable antiproliferative effects. Our purpose was to investigate the effects of this antendometriosis herbal mixture on cell proliferation, apoptosis, and CCL5 expression and secretion in endometriotic stromal cells in vitro. Isolated normal endometrial, eutopic, and ectopic endometriotic stromal cells were cultured under established conditions. Cell proliferation, apoptosis, and CCL5 gene expression protein secretion was evaluated after incubation with different concentrations of an antiendometriosis herbal mixture extract. Cell proliferation was assessed by cell counting, 3H-thymidine incorporation, and MTS assays. Apoptosis was determined by blotting using anti-cleaved caspase 3 antibodies and by a TUNEL assay. CCL5 gene expression and protein secretion were determined by transient transfection of gene promoter reporters and ELISAs in cell supernatants. Extracts of a traditional herbal mixture dose-dependently decreased cell proliferation in normal, eutopic, and ectopic endometriotic stromal cells. 3H-Thymidine uptake and MTS confirmed these findings. The herbal extracts induced apoptosis, as evidenced by activation of caspase 3 and the presence of TUNEL-positive cells after treatment. The herbal extracts also suppressed CCL5 gene transcription and protein secretion in endometriotic stromal cells, even when corrected for cell number. Extracts from a medicinal herbal mixture have direct effects on cell proliferation, apoptosis, and CCL5 production in endometriotic stromal cells. Our findings support the further investigation of novel, potentially safe and well-tolerated botanical products as future endometriosis treatments.

INTRODUCTION

Endometriosis is a chronic inflammatory disease, affecting from 10% of all reproductive-aged women to as many as 70% of women with infertility or chronic pelvic pain and accounting for up to $22 billion in U.S. health care costs per year [1–3]. Local inflammation is augmented in endometriosis, as shown by significantly elevated levels of peritoneal inflammatory and angiogenic cytokines [4]. In endometriotic tissues, key anti-inflammatory transcription factors (e.g., progestrone receptor B [PR-B, also known as PR-B]) are downregulated, whereas proinflammatory transcription factors (e.g., nuclear factor-kappa B [NFkB]) are upregulated [5–9]; as a consequence, expression of proinflammatory cytokines and chemokines is increased [4, 5, 10]. Endometriotic cells and peritoneal macrophages are the primary sources of cytokine (e.g., tumor necrosis factor alpha [TNF], interleukin 1 beta [IL1B, IL6]), chemokine (e.g., chemokine [C-C motif] ligand 2 [CCL2], also known as monocyte chemoattractant protein 1 [MCP-1], CCL5, also known as regulated on activation, normal T-cell expression and secreted [RANTES]), inflammatory enzyme (e.g., prostaglandin-endoperoxide synthase 2 [PTGS2], also known as cyclooxygenase [COX-2]), and vascular endothelial growth factor (VEGF) expression [4, 5, 11–14]. In addition, increased immune responses observed in endometriosis and models of endometriosis promote growth of endometriotic cells [15–18]. Genetic factors only partly explain the increased proliferative and inflammatory responses in endometriotic cells of women with endometriosis [19–24].

Hormonal drugs, including progestins, danazol, and gonadotropin-releasing hormone (GnRH) agonists, have been used for decades to treat endometriosis-associated symptoms. In addition to their endocrine actions, these drugs exhibit anti-inflammatory, antiproliferative, and proapoptotic effects on in vitro and in vivo models of endometriosis [25, 26]. Unfortunately, these classical endocrine ablative treatments carry adverse side effects, including vasomotor symptoms, suppression of reproductive function, and in some cases a negative impact on bone health [27]. Hence, current drugs are prescribed reluctantly by physicians for long-term management of women with endometriosis, particularly in adolescents. Moreover, because current medical endometriosis treatment is only partially effective, many women report persistent symptoms during a period of more than 20 years [2].

Growing experimental evidence indicates that botanicals have antiproliferative and anti-inflammatory activity on endometrial cells and can suppress endometriotic implants in...
animal models via their antiproliferative and anti-inflammatory effects [28–31]. Botanicals have been used frequently in various combinations in Chinese herbal mixtures to treat endometriosis-associated symptoms [29]. The principal approach of herbal medicine is based on synergistic interaction of components that lead to higher efficacy and lower toxicity. In our study, we sought to analyze the cellular effects of a mixture of medicinal herbs (Channel Flow), commonly used to ameliorate endometriosis symptoms. We generated extracts to assess the effects of this herbal remedy on cell proliferation, apoptosis, and CCL5 production in endometriotic cells in vitro.

MATERIALS AND METHODS
Reagents

We selected a commonly used antendometriosis herb preparation (Channel Flow; Health Concerns, Oakland, CA) consisting of nine individual herbs. The herbs of the formula, with their English names, Chinese, and botanical names, are: frankincense (RuXian, Boswellia carterii Birdw.), cordyceps (YanHsSuo, Cordydis tunschotianis), salvia (DanShen, Salvia miltiorrhiza Bge.), cinnamon (GuiZhi, Cinnamomum cassia Presl.), Chinese angelica (Baizhi, Angelica sinesis), dahurian angelica (BaiZhi, Angelica dahurica), licorice (GanCao, Radix Glycyrrhizae), myrrh (MoYao, Commiphora myrrha Engl.), and white peony (BaiShao, Paeonia lactiflora Pall.) [30]. We originally chose this formula because of its long traditional use in health-related conditions, commercial over-the-counter availability, and numerous recent anecdotal reports of its beneficial effects in endometriosis [32]. The formula was developed by M.C. and A.G. and is based on the traditional Chinese formula Huo Luo Xiao Ling Dan (HLXL), which has been used in patients with a variety of inflammatory conditions [33]. The identities of the raw materials of the herbal mixture were authenticated based on the Chinese Herbal Materia Medica [34], shipped from China to the United States in whole for further testing and evaluation. The herbal formula was manufactured and processed in the United States using a water and alcohol extraction process by Health Concerns. We investigated the herbal mixture from two different lots of Channel Flow (lot numbers G7237 and F5947). After the raw materials were tested, they were powdered and processed together. For the in vitro experiments, aqueous extraction of the pulverized herbs was performed with ethanol (50%) for 1 day at room temperature and was subsequently diluted in minimum essential medium-a (MEM-a) media. Control extracts using the same solvents but omitting the herb combination also were prepared. Dose-response experiments were carried out to assess solubility and toxicity of the herbal mixture extract (HME). The 17β-estradiol, medroxyprogesterone acetate (MPA), sulindac, and actinomycin D were obtained from Sigma-Aldrich (St. Louis, MO).

Patient Recruitment and Characterization

Women with surgically documented endometriosis and women without endometriosis with regular menstrual cycles, who had not received hormones or GnRH agonist therapy for at least 3 mo before surgery, were recruited after they had provided written informed consent under a study protocol approved by the institutional review boards at Emory University (Atlanta, GA) and University of California, San Francisco (San Francisco, CA). Endometrial biopsies were collected and cultured under established conditions [37]. In addition, we used normal endometrial stromal cells. Briefly, the endometrial tissue was retrieved from the Pipelle device, minced into small pieces, digested with collagenase (2 mg/ml) for 1 h at 37°C, and separated using serial filtration. Debris was removed by 100-μm aperture sieves, and epithelial glands were retained on 40-μm aperture sieves. Stromal cells in the filtrate were subcultured twice to eliminate contamination by macrophages and other leukocytes. Cells were grown to confluence in the presence of MEM-α with 10% charcoal-stripped, heat-inactivated fetal calf serum (FCS) containing nonessential amino acids, penicillin/streptomycin, and 10 mM E2.

Extensive characterization of the stromal cells confirmed that they retained phenotypic proteins (e.g., IGFBP1, vimentin [VIM] and functional markers (e.g., estrogen, progesterone, and EGFR receptors) of their origin in vivo [37]. High proliferation (95%) of the EE stromal cell population was confirmed by negative staining with the following antibodies: CD3 (T cells), CD11B (granulocytes), CD45 (monocytes and other leukocytes), and cytokeratin (epithelial cells).

3H-Thymidine Incorporation Assay

Proliferative activity was determined by measuring the incorporation of 3H-thymidine into nascent DNA, as described previously by DiCorleto and Bowen-Pope [38]. Briefly, aliquots of the HME or vehicle were added to triplicate wells (in 24-well plates) containing confluent quiescent, stromal cells. After 48 h of incubation, tritiated thymidine (1 μCi/well, 15.7 Ci/mmol; New England Nuclear, Boston, MA) was added to each well, and pulse labeling was carried out for 4–6 h. Cells in representative wells were counted via hemocytometer. Thymidine incorporation into 10% trichloroacetic acid-insoluble nuclear acid was determined by scintillation counting. Proliferative activity was expressed as total counts per minute of 3H-thymidine incorporated per 10,000 cells. Medroxyprogesterone acetate and sulindac were used as controls in the proliferation assays. All values were expressed as the mean ± SEM of triplicate assays.

MTS Assay

Cell proliferation was also assessed by a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (MTS; Promega Corp., Madison, WI). In brief, the cells were seeded in 96-well microplates and incubated overnight. Then, cells were treated with different concentrations of extracts of the herbal mixture or vehicle for 24–72 h. At the end of the incubation periods, 20 μl of MTS stock solution (12.5 μg/ml; Promega Corp., Madison, WI) was added to each well, and the plates were further incubated for 4 h at 37°C. The absorbency at a wavelength of 570 nm was measured with Multiscan MCC 340 microplate reader (TiterTek). All of the measurements were performed in triplicate. Results were calculated as the percentages of proliferation with respect to vehicle-treated cells.

TUNEL Assay

Eutopic endometriotic stromal cells were grown on chamber slides. After treatment with or without the herbal mixture extract, the slides were gently washed three times in 0.1 mol/L PBS (pH 7.4), fixed with 4% paraformaldehyde-PBS solution (Boston Bioproducts, Worcester, MA), and immediately transferred to a freezer until use. To study apoptosis of cultured cells, TUNEL assay was performed using In Situ Cell Death Detection KiT POD (number 11684 817910) according to the manufacturer’s instructions (Roche, Indianapolis, IN). The terminal deoxyribonucleotidyl transferase (TDT)-mediated TUNEL assay was used to detect DNA fragmentation in situ. Fewer than 3% of the cells detached from culture dishes and were not counted. Actinomycin D and sulindac were used as positive controls for apoptosis [30, 39].

Western Blotting

An alternative assessment of apoptosis was afforded by the detection of EE stromal cell caspase 3 activation. Samples of whole-cell lysates of EE stromal cells were prepared for Western blot analysis. Cell culture media were removed by aspiration, and the cells were washed with 10 ml of cold PBS and scraped into 200 μl of cell extraction buffer (20 mM Tris [pH 8.0], 137 mM NaCl, 10% glycerol, 1% Triton X-100, 2 mM EDTA, 1 mM Pefabloc [Pentapharm AG, Basel, Switzerland], 2 μM leupeptin, 0.14 U/ml aprotinin, and 1 mM vanadate). Cells were burst by repeated freeze-thaw cycles and centrifuged at 12,000 × g for 15 min at 4°C. The lysates were denatured in Laemmli buffer, and 80 μg of total protein was electrophoresed on 4%–12% polyacrylamide SDS gels at 500 mA for 2 h. Proteins were transferred to polyvinylidene difluoride membranes (Millipore Corp., Bedford, MA) for 2 h in a semidry transfer system (Hoefer Scientific, San Francisco, CA). Membranes were blocked for 1 h in 50 mM Tris, 150 mM NaCl, and 0.1% Tween-20 containing 5% instant nonfat dry milk and then probed with 1:1000 of the anti-caspase 3 antibody at 4°C overnight. The polyvinylidene difluoride membranes were washed three times.
FIG. 1. Effects of increasing concentrations of the HME on cell count. Eutopic endometriotic stromal cells were plated at concentrations of 5000 cells/well, cultured in the Lab-Tek II Chamber Slide System (NUNC, Roskilde, Denmark), and fixed with 4% paraformaldehyde. Before staining, cells were permeabilized with 1:5 diluted Super Block buffer (Sey Tel Laboratories Inc., Logan, UT), and rabbit anti-caspase 3 antibody (catalog number 9664; Cell Signaling Technology, Danvers, MA) and a goat anti-rabbit antibody (1:30 000; catalog number 31460; Pierce Biotechnology Inc., Rockford, IL) for use with primary rabbit antibodies. Hematoxylin was used as a nuclear counterstain.

CCL5 Promoter Activation: Transfection and Cytokine Treatment of EE Cells

Transfections were performed in EE stromal cells grown in MEM-α with 10% FBS in 12-well plates at about 50% confluence. The pGL2-477-CCL5 promoter or pGL2-basic control vector was added to each well using Effectene reagents (Qiagen, Chatsworth, CA). The firefly luciferase reporter construct transfection efficiencies were normalized to an independent control plasmid (0.1 µg of Renilla luciferase reporter) with constructs that had been cotransfected simultaneously. In each case, a total of 0.45 µg of plasmid DNA per well was added. The cells then were incubated for 24 h in MEM-α with 10% FBS and antibiotics in the absence or presence of TNF before luciferase activity was quantified. The concentration of TNF (2.9 nM) used previously in these assays was shown to stimulate CCL5 expression between 50% and 85% of maximal [5]. The results are presented as the ratio of firefly luciferase activity (transfection efficiency) in treated and untreated cells after correcting for basal activity (pGL2-basic), as reported previously [5]. Each reporter vector was assayed in three independent cultures.

CCL5 ELISA

Cell culture supernatants were collected and ELISA performed using a commercial human CCL5 ELISA kit (Quantikine; R&D Systems). The samples and known CCL5 standard protein preparations were incubated in 96-well plates precoated with monoclonal CCL5 antibodies. All CCL5 analyses were performed contemporaneously and in duplicate according to the manufacturer’s instructions.

Statistics

All experiments were performed in triplicate. The effects of the HME and control compounds on endometrial cells were tested by Mann-Whitney tests or ANOVA with posthoc analysis (Fisher least significant difference [LSD]). Statistical analyses were performed by using SPSS (SPSS Inc., Chicago, IL) for Windows 10.0.1 (1999). The significance level was set at α = 0.05.

RESULTS

Effects of the HME on Cell Proliferation

Incubation in the presence of the herbal extract inhibited proliferation of EE stromal cells by 80% (half-maximal inhibitory concentration, 200 ng/ml). Significant decreases in cell counts were noted after 4 days in culture with the HME, even in the presence of 10% FCS (Fig. 1). To further characterize the effect on cell number, the activity of the herbal extract on stromal mitogenesis was tested by ³H-thymidine incorporation. The HME (300 ng/ml) inhibited cell count by 64% (P < 0.001, ANOVA with Fisher LSD) after 4 days (Fig. 1) and inhibited ³H-thymidine incorporation in EE stromal cells by 80% (P < 0.05, ANOVA with Fisher LSD; Fig. 2). Treatment with MPA (1 µM) and sulindac (300 µM), used as positive controls, resulted in decreases of ³H-thymidine incorporation of 17% and 30%, respectively, in EE stromal cells (P < 0.01, ANOVA with Fisher LSD; Fig. 2). It should be noted that the HME also suppressed ³H-thymidine incorporation in normal endometrial and ectopic (ovarian) endometriotic cells by 53% and 69%, respectively (P < 0.05, ANOVA with Fisher LSD; data not shown). In our hands, GnRH agonist had no direct effects on endometriotic cell mitogenesis (data not shown), in contrast to prior reports [40]. Interestingly, TNF increased mitogenesis of EE cells by 56% (P < 0.05, ANOVA with Fisher LSD). The MTS assay results confirmed the ³H-thymidine incorporation experiments and showed a 50% decrease in cell proliferation after treatment with the HME at 300 ng/ml (data not shown). Having documented dose-responsive effects of the herbal extract on cell cycle kinetics, we postulated that the botanical compound might activate apoptosis in these cells.
Effects of the HME on TUNEL Assay

First, the HME induced apoptosis, as assessed by TUNEL (Fig. 3). Phase contrast revealed no discernible differences in cell shape or number in the absence (Fig. 3A) or presence (Fig. 3B) of the herbal extract (200 ng/ml for 48 h). However, although TUNEL was undetectable under control conditions (Fig. 3C), punctuate staining indicated that essentially all cell nuclei exposed to the herbal extract had evidence of DNA nicking (Fig. 3D).

Effects of the HME on Caspase 3 Expression in EE Stromal Cells

To further characterize whether the herbal mixture induced apoptosis in EE stromal cells, lysates from treated EE cells were analyzed by Western blotting to detect the effects of the herbal extract on caspase 3 expression. The herbal extract increased caspase 3 activation, as assessed by conversion of pro-caspase 3 to its cleaved forms within 12 h of treatment in EE stromal cells (Fig. 4A). Sulindac and actinomycin D were used as positive controls [5, 39]. In addition, the herbal extracts (1000 ng/ml) induced apoptosis, as evidenced by the perinuclear accumulation of caspase 3 shown by immunocytochemistry (Fig. 4B). In previous findings, we could show that the HME induces apoptosis not only in endometrial stromal cells but also in epithelial cells from women with endometriosis [30].

Effects of the Herbal Mixture on CCL5 Gene Expression

We previously have used CCL5 gene expression as a sensitive and specific indicator of endometriotic cell activation [5, 10]. To evaluate the effects of the botanical mixture on CCL5 gene expression, we transfected EE stromal cells with a well-characterized human CCL5 gene promoter-reporter construct. Treatment with the HME decreased basal as well as TNF-induced CCL5 promoter activation in EE cells by 39% and 53%, respectively. The effects on the cytokine-stimulated CCL5 promoter activity were statistically significant by the conservative Mann-Whitney test (P < 0.05; Fig. 5).

FIG. 3. Incubation with HME (1000 ng/ml) induced fluorescent TUNEL-positive changes in EE stromal cell nuclei after 48 h of treatment. A) Control (phase contrast). B) HME (phase contrast). C) Control (TUNEL). D) HME (TUNEL). Original magnification ×200.

Effects of the Herbal Mixture on CCL5 Secretion

To verify that the gene suppression effects were also manifested at the level of protein secretion, EE cells were incubated in the presence and absence of TNF and the presence and absence of the extract for 48 h, after which cell supernatants were analyzed using a specific ELISA for CCL5. As we have reported previously [5], under basal conditions, EE cells fail to secrete CCL5. However, the HME resulted in a significant suppression of TNF-induced CCL5 protein secretion by 91% (P < 0.05, Mann-Whitney test; Fig. 6). Similar effects were shown when IL1B was used as the CCL5-inducing cytokine (data not shown).

DISCUSSION

Studies from our own laboratory and others’ laboratories have demonstrated an important role for inflammatory mediators underlying the pathophysiology of endometriosis [4, 12, 41–43]. Endometriotic cell-derived cytokines (e.g., TNF, IL8, CCL5) have been shown to play an important role in regulating increased inflammatory and proliferative responses in women with endometriosis, leading to endometriosis-associated symptoms, such as infertility and chronic pelvic pain [44]. The current experiments focus on the effects of proinflammatory cytokines on proliferation of endometriotic stromal cells and CCL5 production in vitro. In this model, an herbal mixture was shown to inhibit proliferation, induce apoptosis, and blunt CCL5 gene and protein expression.

Simple and complex herbal mixtures, sometimes employing up to 10 different medicinal herbs, have been used traditionally to treat endometriosis-associated symptoms [29]. Active constituents of these botanicals have been shown to exhibit potent pharmacological effects, including cytokine suppression, PTGS2 inhibition, antioxidant effects, and pain relief in women with endometriosis and in models of endometriosis [29,
FIG. 5. Eutopic endometriotic stromal cells were transfected with a 477-bp human CCL5 promoter-luciferase construct. Treatment with TNF (0.6 nM) induced a >2-fold increase in normalized promoter activation (LUC:REN ratio, labeled as LUC/REN) measured after 24 h. Pretreatment (4 h) of endometrial stromal cells with the HME (500 ng/ml) decreased CCL5 promoter activation in control and TNF-treated EE stromal cells by 38% and 53%, respectively. The latter reached statistical significance (*P < 0.05, Mann-Whitney test). Error bars represent mean ± SD of triplicate measures in a representative experiment.

FIG. 6. Effects of the HME (500 ng/ml) on CCL5 protein secretion. Eutopic endometriotic stromal cells were plated in basal medium containing 2% FCS and were treated in the absence (control) or presence (HME) of the herbal mixture for 2 days. As we have observed previously, EE cells do not secrete CCL5 into the media under basal conditions (control and HME histograms). However, whereas TNF induced a robust release of CCL5, pretreatment with HME reduced CCL5 secretion by >90% (*P < 0.05, Mann-Whitney test). *Significant inhibition by HME (P < 0.05, Mann-Whitney test). Error bars represent mean ± SD of triplicate measures in a representative experiment.

45]. Although there is promising clinical evidence of the potential efficacy of medicinal herbal mixtures, interpretation of the extent studies is difficult because most were not conducted according to Western evidence-based guidelines. Data from our current studies help to explain, at least in part, the observed efficacy of herbal treatment in vivo.

We investigated the antiproliferative effects of a medicinal herbal mixture consisting of nine different anti-inflammatory botanicals (Chinese angelica, corydalis, cinnamon, dahurian angelica, frankincense, licorice, myrrh, salvia, and white peony). This herbal mixture was adapted from a commonly known formula Huo Luo Xiao Ling Dan (HXLX) [34]. HXLX and its adaptations have been used to treat chronic inflammatory diseases, such as rheumatoid arthritis and endometriosis [34, 45, 47].

Evidence of anti-inflammatory effects of HXLX and its components was shown previously in in vitro and murine models of inflammation [29, 46]. In our study, the HME from Channel Flow dose-dependently decreased endometriotic stromal cell proliferation in vitro. In preliminary studies, we have observed that single components of the herbal mixture (licorice, corydalis, and salvia) [30] also suppress cell proliferation significantly. By contrast, endometriotic cells showed an increased mitogenic responsiveness to TNF. Our data correspond to the findings of Ohama et al. [18], who reported a 38% increase in proliferation of endometriotic stromal cells in response to TNF, which appeared to be mediated by an increase in IL8.

The herbal mixture used in our study induced apoptosis, as shown by TUNEL staining and an increase of caspase 3 activation in the EE stromal cells (Figs. 3 and 4). It has been reported previously that endometrial cells from women with endometriosis exhibit resistance to apoptosis [48–52]. Correspondingly, several cell proliferation/apoptosis markers and transcription factors regulating proliferation are expressed aberrantly, such as increased B-cell lymphoma/leukemia-2 (BCL2), NFKB1, MYC, and TGFBI, and decreased expression of PGR B and BAX in the endometrium of women with endometriosis [5, 9, 53–56]. Our data show that the botanical combination in HME has antiproliferative and proapoptotic effects (Figs. 1–4) comparable to MPA and sulindac in EE stromal cells [5]. As observed for endometriosis drugs, including MPA, dienogest, and danazol, in our studies and others [26, 57, 58], similarly, the herbal mixture also exhibited direct antiproliferative effects in normal endometrial stromal cells. The direct antiproliferative and proapoptotic effects of the HME on endometriotic stromal cells confirm the therapeutic potential of botanicals in the treatment of endometriosis.

Medicinal herbs and their components also exert anti-inflammatory effects in animal models and in women with endometriosis [29, 59]. Botanicals suppress eicosanoid (i.e., PTGS2), chemokine (i.e., CCL2), and cytokine (i.e., TNF, IL8) production, as observed in animal models of endometriosis and other models of inflammation [29, 60]. Cao et al. [61] reported that curcumin, an active component of turmeric, decreased CCL2 expression in an in vitro model of endometriosis [61]. In the current study, we demonstrated that an herbal mixture decreased CCL5 secretion by endometriotic stromal cells. The potential efficacy of botanicals in the treatment of endometriosis-associated symptoms shown in preliminary clinical trials can be explained partly by the anti-inflammatory effects of single or combinations of herbs, as shown in this study (Figs. 5 and 6). Suppression of key mediators, such as CCL5, is one theoretical therapeutic approach in the treatment of endometriosis-associated infertility.

Standard hormonal endometriosis drugs (e.g., progestins, GnRH agonists, danazol), experimental endocrine therapies (e.g., selective estrogen and progesterone receptor modulators [PRMs]), and thiazolidinediones and novel nonhormonal treatments (e.g., TNF inhibitors) exhibit untoward side effects [29, 62–64]. Surprisingly, raloxifene, prescribed after endometriosis surgery, actually shortened the time to recurrence of pain [65], and PRMs were observed to induce cystically dilated glands in endometrium of treated women [66]. Although thiazolidinediones showed beneficial effects in models of fertility [67, 68], clinical studies on the effect of nonhormonal treatments using thiazolidinediones (rosiglitazone) on endometriosis were suspended because of the cardiovascular side effects of this drug class. In contrast, the promising advantage of nonhormonal phytotherapy is the expectation of reduced side effects and the potential fertility-preserving and fertility-enhancing effects. The completed NCT 00043047 trial (treatment, randomized, open label, active control, parallel assignment, efficacy study) on the effects of botanicals in
REFERENCES

EFFECTS OF BOTANICALS IN ENDOMETRIOSIS


