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Original Article

Literature Review on Biological Effects of *Gyejibokryeong-hwan* against Gynaecological Diseases

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Objectives: To investigate therapeutic mechanisms of *Gyejibokryeong-hwan* (GJBRH) against gynaecological diseases, articles on biological assay were gathered and analyzed.
Methods: The articles were classified as being from domestic or international journals, and by their year of publication. The mechanisms of the biological effects against gynaecological diseases were noted.
Results: Of the 14 articles analyzed, 13 were published in China and 1 was from Japan. GJBRH showed therapeutic effect against uterine and mammary gland diseases. Uterine-related diseases such as endometriosis, hysteromyoma, adenomyosis, cancer, and inflammation can be improved by the administration of GJBRH through anti-angiogenesis, anti-inflammation, the modulation of immune cell and immunoglobulin, and the regulation of hormone secretion. GJBRH also reduced mammary hyperplasia by regulating hormone and cytokine release.
Conclusions: We speculate that the inhibitory effect against uterine and mammary gland diseases could be related to the therapeutic efficacy of GJBRH in improving gynaecological diseases.

Key Words : *Gyejibokryeong-hwan*, gynaecological disease, therapeutic mechanism, uterine, mammary gland.

Introduction

Gyejibokryeong-hwan (GJBRH) is a traditional herbal formula consisting of 5 herbal medicines i.e. Cinnamomi ramulus, Poria sclerotium, Moutan cortex, Paeoniae radix, and Persicae semen. GJBRH has been used to treat symptoms caused by stagnant blood which leads to abnormal mass in lower abdomen, amenorrhea, dysmenorrhea, menstrual pain, difficult delivery, retention of placenta, and abnormally prolonged discharge of lochia, which were mainly involved in uterine disorders[1,2].

These same symptoms in Korean medicine can be explained as gynaecological diseases of modern western medicine, especially uterus-related diseases. The uterus is the organ in which offspring are carried and nourished before birth, and menstruation occurs. Another name for the uterus is 'blood chamber' which means the uterus is easily influenced by the state of blood flow and its pathogenic symptoms are observed as blood-related disorders in most cases[2]. GJBRH can be applied to stagnant blood-induced uterus-related disorders through promoting blood flow and dispelling blood stasis. Clinical studies support that GJBRH can improve clinical symptoms of hypermenorrhea, dysmenorrhea

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and uterine myomas[3,4], and decrease the severity of menorrhalgia[5].

In vivo experiment is a method that uses an animal model to investigate the efficacy of a treatment or medicine of interest by diverse administration routes including gastrointestinal tract, subcutaneous, intravenous, and intraperitoneal injection. Numerous researches testing Korean medicine have been performed using in vivo experiments to evaluate the therapeutic effect of herbal medicines or herbal formulas, although controversy remains regarding whether experimental methods could properly explain therapeutic mechanism of traditional Korean medicine. Nonetheless, the trials to find the point of connection between Korean medicine and biological experiments would be beneficial to construct scientific and objective establishment of Korean medicine.

In the present study, we searched articles dealing with the biological effects of GJBRH. Articles on treating gynaecological diseases classified by Korean standard classification of diseases (KCD) were further investigated to figure out the relationship between the biological and therapeutic effects of GJBRH and its mechanisms of action.

Materials and Methods

1. Search strategy and terms

We searched a variety of published papers in Korean and foreign electronic bibliographic databases between 1990 and the present through the Korea Education and Research Information Service (KERIS), National Discovery for Science Leaders (NDSL), Korean Studies Information Service System (KISS), Korean Traditional Knowledge Portal, Oriental Medicine Advanced Searching Integrated System (OASIS), PubMed, ScienceDirect, Google Scholar, China National Knowledge Infrastructure (CNKI), and Citation Information from the National Institute of Informatics (CiNii) using search terms such as “Gyejibokryeonghwan”, “Gyejibokryonghwan”, “Gyejibokryunghwan”, “Keishi-bukuryo-gan”, “Guizhi-fuling-wan”, “Guizhi-fuling-capsule”, “계지복령환”, and “桂枝茯苓丸” (Table 1).

2. Selection criteria and data extraction

We selected 14 full text-papers regarding in vivo biological experiments dealing with gynaecological diseases referring to KCD index (Code No. N60 - N99). Papers were categorized by the distribution of their publication year and country of origin. From the selected papers, data

Table 1. Electronic Bibliographic Databases and Search Terms for *Gyejibokryeong-hwan*

Electronic bibliographic databases	Search terms
Korea Education and Research Information Service http//www.riss4u.net	Gyejibokryeonghwan Gyejibokryonghwan Gyejibokryunghwan Keishi-bukuryo-gan Guizhi-fuling capsule 계지복령환 桂枝茯苓丸
Korean Studies Information Service System http://kiss.kstudy.com	
National Discovery for Science Leaders http://www.ndsl.kr	
Oriental Medicine Advanced Searching Integrated System http://oasis.kiom.re.kr	
Korea Institute of Science and Technology Information http://society.kisti.re.kr/main.html	
Korean Traditional Knowledge Portal http://www.koreantk.com	
PubMed http://www.ncbi.nlm.nih.gov/pubmed	
Google Scholar http://scholar.google.co.kr	
ScienceDirect http://www.sciencedirect.com/	
National Institute of Informatics http://ci.nii.ac.jp	
China National Knowledge Infrastructure http://www.cnki.net	

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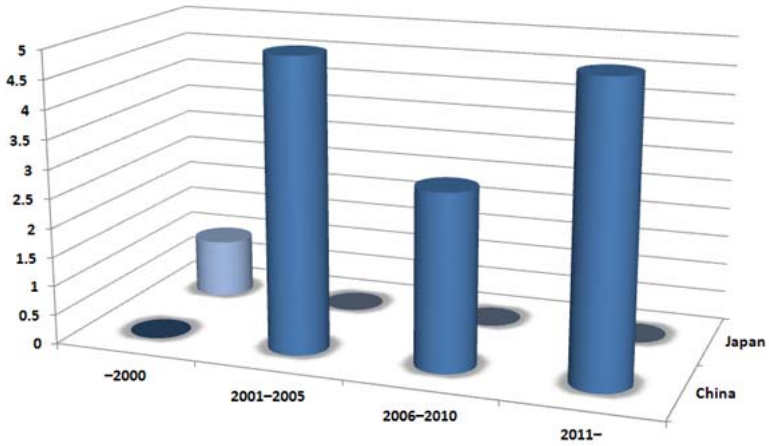

Fig. 1. Distribution of papers classified by the publication year and country

extraction was conducted as follows: target disease, animal species, induction of symptoms, and factors of treatment for parts of the body such as serum, organ, or tissue. The outcome measures were further investigated to determine the mechanism of the therapeutic effect of GJBRH.

Results

1. Distribution of papers by the publication year and country

As shown in figure 1, most papers dealing with gynaecological diseases were published in China (92.86%), followed by one paper from Japan. In China, the numbers of published papers sharply increased in the period from 2001 to 2005 and showed slight decrease in the period from 2006 to 2010. Thereafter, there has been increasing frequency of papers published since 2011 to the present year. The single Japanese paper was published in 1995; no other researches were found throughout the period of publication years searched.

2. Biological effect of GJBRH on gynaecological diseases

Gynaecological diseases were divided by their lesions which were uterine & pelvic lesions and mammary lesions. Uterine and pelvic diseases included endometriosis, hysteromyoma, uterine adenomyosis, cervical cancer, and pelvic inflammation. Mammary lesions included mammary hyperplasia.

1) Biological effects on the diseases of uterine & pelvic lesions

As shown in Table 2, most of the reported papers have dealt with biological effects of GJBRH on endometriosis. Rats, especially Sprague-Dawley rats, were used as an animal model and endometriosis symptoms were induced by the endometrial autografts in the abdomen. After the oral and intragastric administration of GJBRH, histological changes were observed in the endometriotic area such as reduced endometrial volume and gland or a decrease in microvessel density[6,8,10,11]. Additionally, the levels of vascular endothelial growth factor (VEGF), MCP-1

Table 2. Therapeutic Effects of *Gyejibokryeong-hwan* on Uterine Diseases

Target disease	Animal	Induction	Outcome (cytokines or molecules)	
			Organ & tissue	Blood & fluid
Endometriosis	SD rats[6-10] Wistar rats[11]	Autograft of endometrium in abdomen[6-11]	Ectopic endometrium VEGF ↓[6] MCP-1 & ICAM-1 ↓[8,10] MMP-2 & MMP-9 ↓[11] Spleen CD4+Tcell ↑[9,10] Cytotoxic NK cell activity ↑[9,10]	Peripheral fluid & blood Macrophage ↓[6] IL-8 ↓[6] TNF-α ↓[6] IgG, IgM, IgA ↓[7] CD3+,CD4+ ↑[7] CD8+ ↓[7] Serum & plasma 6-keto-PGF1α ↑[11] β-EP ↑[11] TXB2 ↓[11]
Hysteromyoma	Wistar rats[12] Kunming mice[13]	Estradiol[12] Estradiol benzoate[13]	-	Serum & blood estradiol ↓[12,13] progesterone ↓[12,13]
Uterine adenomyosis	SHN mice[14]	Ectopic pituitary isografting[14]	Uterine TS activity ↓[14]	-
Cervical cancer	BALB/c nu mice[15]	HeLa cell[15]	Tumor MMP-2, MMP-9 expression ↓[15] angiogenesis ↓[15]	-
Pelvic inflammation	Wistar rats[16]	Bacteria & mechanical damage[16]	Uterine tissue TNF-α & TNF-β1expression ↓[16] VEGF expression ↓[16]	-

VEGF, vascular endothelial growth factor; MCP-1, monocyte chemoattractant protein-1; ICAM-1, inter-cellular adhesion molecule-1; MMP, matrix metalloproteinases; IL, interleukin; TNF, tumor necrosis factor; Ig, immunoglobulin; TS, thymidylate synthetase; 6-keto-$PGF1_{\alpha}$, 6-ketone-prostaglandin $F1_{\alpha}$;EP, β-endorphin;TXB_2,thromboxaneb_2.

(monocyte chemoattractant protein-1), ICAM-1 (inter-cellular adhesion molecule-1), and matrix metalloproteinases (MMP) were reduced in the endometrium while there were increases of CD4+ T cell and NK cell activity in the spleen from treatment with GJBRH[6,8-11]. In the peripheral fluid and blood, GJBRH reduced the production of macrophages, interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and immunoglobulin A, G, and M. GJBRH also inhibited the production of CD8+ cells while stimulated those of CD3+ and CD4+. Serum expressions of 6-keto-prostaglandin F1α (PGF1α) and β-endorphin (EP) were enhanced while that of thromboxane B2 (TXB2) was inhibited by the administration of GJBRH.

GJBRH showed therapeutic effect against hysteromyoma induced by estradiol and its derivative. Excessive uterine weight and smooth muscle proliferation were reduced by the administration of GJBRH. It also decreased the levels of estradiol and progesterone in serum and platelet aggregation, and viscosity of blood whereas enhanced blood coagulation time, kaolin partial thromboplastin time, and prothrombin time[12,13]. Thymidylate synthetase (TS) activity in rats with adenomyosis induced by pituitary isografting was inhibited by the treatment of GJBRH, which showed decreased adenomyosis development[14]. GJBRH suppressed the growth of cervical cancer and angiogenesis, and the expressions of MMP-2 and MMP-9 were also inhibited[15]. Pelvic inflammation was improved by GJBRH through the inhibition of TNF and VEGF expression in uterine tissues[16].

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Table 3. Therapeutic Effects of *Gyejibokryeong-hwan* on Mammary Gland

Target disease	Animal	Induction	Outcome	
			Organ & tissue	Blood & fluid
Mammary hyperplasia	SD rats[17-19)]	Estradiol & progesterone[17-19)]	Mammary gland ER ↓[17-19)] PR ↓[17-19)]	Blood & plasma & serum Estradiol ↓[17-19)] Progesterone ↑[17-19)] IL-2 ↑[18,19)] TNF-α ↓[18,19)]

ER, estrogen-receptor; PR, progesterone- receptor; IL, interleukin; TNF, tumor necrosis factor.

2) Biological effects on the disease of mammary gland

Table 3 shows that GJBRH inhibited mammary hyperplasia of rats which was induced by the stimulation of estradiol and progesterone through reducing duct epithelia, acinus and nipple height, and suppressing papilledema, lobular proliferation and hyperemia[17-19)]. It also decreased hematocrit and viscosity in blood, and the levels of estradiol and TNF-α while enhance the secretions of progesterone and interleukin-2 (IL-2) in serum and plasma[17-19)].

Discussion

In the present study, we gathered articles regarding biological effects of GJBRH against gynaecological diseases and investigated the outcomes to show whether the therapeutic effects of the herbal formula could be related to experimental results.

Endometriosis is characterized by endometrial-like tissue outside the uterus in adjacent organs or body parts such as pelvic peritoneum, ovaries, and abdomen[20)]. Endometrium was surgically auto-grafted in the abdomen, which is conducted by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall as depicted in the literature[21)]. The development of endometriosis is known to relate to the recruitment of blood vessels to the endometriotic lesions which induce angiogenesis[22)]. Vascular endothelial growth factor (VEGF), an important mediator of angiogenesis, is expressed at high levels in the peritoneal fluid and endometrial tissues[22-24)]. Matrix metalloproteinases-2 and -9 (MMP-2 and -9), a family of zinc-dependent endopeptidases, can degrade the collagen IV and play a key in the pathogenesis of endometriosis by degrading extracellular cellular matrix (ECM) and promoting the release of key factors[25)]. The serum level of MMP-2 is elevated in infertile women with advanced stages of endometriosis and the expressions of MMP-2 and 9 are also increased in the patients of ectopic endometrium [23,26)]. In addition, it is reported that MMPs is highly correlated with tumor aggressiveness of various human cancers[27)]. The oral administration of GJBRH can reduce the expressions of VEGF and MMPs by preventing the angiogenesis and the degradation of ECM.

Endometriosis is associated with an immune-inflammatory process that occurs in the peritoneal cavity of patients[28)]. GJBRH can inhibit monocytes migrated from the peripheral blood to the peritoneal cavity by monocyte chemotactic protein-1 (MCP)-1 which makes monocytes transform into macrophages and bring about peritoneal inflammation characterizing endometriosis[29)]. The increased accumulation of activated macrophages and their products found in patients with endometriosis are reported to influence the development of endometriotic tissues, and it is also known that

the cytokines such as IL-6 and TNF-α, released by activated macrophages, can promote aromatase activity in endometriotic stromal cells and increase the production of estrogen charging the growth of endometriotic lesions[30].

Intercellular adhesion molecule-1 (ICAM-1) found in the human endometrium is known to be related to the defective functions of natural killer (NK) cells and mediate interactions between endometrial cells and lymphocytes during the initial and sustained formation of endometriosis[31-33]. NK cells recruited to eutopic endometrium in the onset of menstruation participate in endometrial remodelling and repair by clearing the endometrial products following menstrual shedding, so decreased NK cell activity and the resulting impaired clearance of endometrium can contribute the development of endometriosis[34-36]. Interleukin-8 (IL-8), a pro-inflammatory chemokine, initiates many different signalling pathways and results in angiogenesis, mitogenesis and motogenesis by binding to the chemokine receptors CXCR1 and CXCR2, which is observed at higher concentration in patients with endometrioma[37,38]. TNF-α, a primary effector of inflammatory responses, proceeds one of the major mechanisms of endometriosis by increasing expression of cytokines such as MCP-1 and IL-8 and its production is increased in endometriotic epithelial cells[39]. TNF-α also stimulates the expression of matrix metalloproteinases (MMPs) in endometrial tissue[40]. Transforming growth factor-β1 (TGF-β 1), a molecular mediators of pathological tissue fibrosis, can stimulate the fibroblasts to produce collagen, fibronectin, and integrins, and also inhibit the production of collagenase and heparinase to degrade the extracellular matrix in various cell types, including platelets, macrophages, ovarian cells, uterine tube cells, and uterine endometrial cells[41]. Inflammatory responses characterized in endometriosis can be ameliorated by decreasing macrophage accumulation, the expressions of MCP-1, ICAM-1, IL-8, TNF-α and TGF-β1, and activating NK cells by the administration of GJBRH.

GJBRH also improves the immune responses through regulating immunoglobulin (Ig) secretion and T lymphocyte activation. Among the three major classes (IgG, IgA, and IgM), IgG and IgA which are detected in sera, cervical, and vaginal secretions in patients with endometriosis are considered as candidates for the autoantigens responsible for the immune response[42]. IgM is also immunodominant in the sera of endometrial patient and the serum levels of IgG, IgA as well as IgM are increased in endometriosis[43,44]. Impaired Th immune response has been reported as a main factor causing the development and progression of endometriosis[45]. T-cells in CD4+ (helpers) suppress the proliferation and function of T cells in CD8+ suppressor (cytotoxic) phenotype[46]. The decreased level and ratio of CD3, CD4/CD8 observed in peripheral blood of patients with endometriosis represented the autocrine and regulatory function of T cells in endometriotic tissues[47].

6-ketone-prostaglandin F1α (6-keto-PGF1α) has been used as a substitute of prostacyclin which is a major metabolite of arachidonic acid (AA) produced by vascular endothelial cells, and its level is decreased in rats with endometriosis[48-50]. β-Endorphin, a pain-reducer released following exposure to a painful stimuli, is found at low level in the endometriosis patients with moderate or severe pain[51,52]. Thromboxane b2 (TXB2) is a hydrolyzed metabolite of TXA2, which is an oxidation product derived from AA in cyclooxygenase (COX) and thromboxane synthase dependent reactions[53]. The production of serum TXB2 is a specific and most common index for evaluation of COX-1 activity in humans and

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others, and the plasma level of TXB2 is increased in rats with endometriosis[50,53]. GJBRH can improve the expressions of 6-keto-PGF1α and EP while reduce that of TXB2, which leads to regulating the inflammatory and immune response.

Adenomyosis is described as a diffuse invasion of endometrial elements into the uterine myometrium without apparent border between the normal uterine tissue and the lesion[54]. SHN mice are known to develop uterine adenomyosis spontaneously and the development is can be easily induced by ectopic pituitary isografts (EPI) which are found in a high incidence of uterine adenomyosis[55,56]. Thymidylate synthase (TS) are recognized as an indicator of cell proliferation and promotes DNA precursor synthesis, especially de novo pyrimidine synthesis[57,58]. Hysteromyoma, a benign tumor growing from the muscle or connective uterine tissue, causes heavy and prolonged menstrual bleeding, painful menstruation, pain below the stomach, and increased demand of urination associated with pressure on the bladder and constipation, and is known to be related with the growth of uterine myomas and activity of estrogens[59]. The rat hysteromyoma model can be established by the injection of estradiol benzoate and progesterone[60]. The amount of estradiol and progesterone secreted by the cells and endometrium of hysteromyoma was significantly larger than those of normal control groups[61,62]. GJBRH inhibits the development of pathogenic invasion of endometrium and benign uterine tumor growth by decreasing TS activity and hormones such as estradiol and progesterone.

Mammary hyperplasia is characterized by an enlargement of multiple mammary glands and increases breast cancer risk when hyperplasia is aggravated[63]. Estrogens, especially 17β-estradiol (estradiol), and progesterone have critical functions in mammary gland development and carcinogenesis. The estrogen/estrogen receptor (ER)-α signaling pathway stimulates proliferation of mammary epithelium, and estrogens can have epithelial cells and stromal cells secrete growth factors and pituitary prolactin that induce mitogenesis in the epithelium[64,65]. Progesterone receptor (PR) is expressed by the great number of epithelial cells within the estradiol-induced atypical hyperplastic foci and the mammary carcinomas[66]. Tumor necrosis factor-α (TNF- α) involved in the pathogenesis of inflammatory, autoimmune and malignant diseases can be also produced in the mammary glands changed by tumor infiltrating lymphocytes or by cells of tumor stroma, and promote angiogenesis by stimulating endothelial cell proliferation and modulating expression of pro-angiogenetic factors[67,68]. Interleukin 2 (IL-2), a lymphocytotrophic cytokine, is involved in the growth and differentiation of T and B cells and improves NK cells to enhance the cytolytic[69]. The serum level of IL-2 is decreased in rats with mammary gland hyperplasia[70]. Mammary hyperplasia which can worsen to breast tumors is suppressed by the administration of GJBRH through regulating hormone levels (estradiol and progesterone) and cytokines (IL-2, TNF-α).

Conclusions

We researched articles regarding the curative effect against gynaecological diseases of GJBRH to evaluate the relationship between the biological effect and therapeutic efficacy as defined in Korean medicine. Most papers were published in China, followed by Japan, and studies of GJBRH have been reported constantly up to the present. GJBRH inhibited uterine-related diseases including endometriosis, hysteromyoma, adenomyosis, cancer, and inflammation by suppressing the anti-angiogenesis and anti-inflammation, modulating

the immune cells and immunoglobulin, and regulating hormone secretion. GJBRH also decreased = hyperplasia of the mammary gland through the down-regulation of hormones and cytokine release. These biological effects against gynaecological diseases could be associated with the therapeutic efficacy of GJBRH as defined by Korean medicine, namely curing uterine and mammary gland-related disorders.

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