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# 사물탕가향부자가 항우울행동 및 면역기능에 미치는 영향

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## ABSTRACT

### The Effect of *Samul-tanggahyangbuja* on Anti-Depressive Behavior and Immunity

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**Objectives:** *Samul-tang* and Rhizome of Cyperus rotundus L. have been frequently used in gynecologic disease. The purpose of the present study is to explore the behavioral and neurobiological effects of *Samul-tanggahyangbuja* (SGH) on ovariectomized rats and to form a basis for clinical treatment.

**Methods:** Ovariectomized rats were repeatedly stressed for over 2 weeks. After orally medicated with SGH (200 or 400 mg/kg/day), the anxiety response was tested using the Elevated Plus Maze (EPM) in rats. The serum levels of estradiol and IL-4, and immunohistochemical changes of IL-4 in the Locus coeruleus (LC) and Paraventricular Nucleus (PVN) were measured.

**Results:**

1. In the EPM, SGH 400 mg significantly increased time spent on the open arms and decreased time spent on the closed arms, compared with the control group ($p<0.05$).
2. SGH tended to increase numbers of crossings in the open and closed arms in the EPM. However, it did not reach statistical significance.
3. SGH significantly increased the serum levels of estradiol compared with the control group ($p<0.05$).
4. SGH 400 mg significantly increased the serum levels of IL-4 compared with the control group ($p<0.05$).
5. IL-4 immunoreactivity was reduced in the control group compared with the normal group ($p<0.05$). However, SGH groups (200 and 400 mg) did not produce any significant effects on levels of IL-4 in the LC and PVN.

**Conclusions:** These results suggest that SGH possesses the anti-depressant and immuno-modulatory effects on ovariectomized rats.

**Key Words:** Menopausal Depression, *Samul-tanggahyangbuja*, Elevated Plus Maze (EPM), Estradiol, IL-4, Immunohistochemistry, Rhizome of Cyperus Rotundus L., Cyperi Rhizoma

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# Ⅰ. Introduction

Menopause is based on the cessation of estradiol and progesterone production by the ovaries, which are a part of the body's endocrine system of hormone production[1]. The menopause transition is a period of marked hormone instability and the later phases of the transition are characterized by a steep increase in Follicle Stimulating Hormone (FSH) levels, followed by a dramatic decline in estradiol levels[2].

The average age of natural menopause is known to be in late 40s. In the perimenopause years, many women undergo noticeable and clinically observable physical changes resulting from hormonal fluctuations. In addtion, the menopause represents a time of vulnerability for onset of depressive disorders[3-5]. It is reported that between 22 and 33% of menopausal women have mood deterioration and depression[6].

Depressive disorder is one of the most frequently occurring psychiatric diseases and is prevalent in at least 15% of the population. However, the prevalence of depressive disorders is twice as high in women as in men[7]. The higher incidence and severity of depression in women are associated with the presence or absence of ovarian hormones[8]. The ovarian hormones related to the menopause-induced depression influence the hippocampal anatomy and physiology; thus, they affect behavior in adult female rats[9-11].

Estrogen has been implicated in depressive disorders due to the increase in risk of depressive episodes after puberty, the antenatal period, and reduced rates after menopause. Conversely, the premenstrual and postpartum periods of low estrogen levels are also associated with an increased risk of depressive disorder[12]. In other words, changes in estradiol are associated with an increased risk of depressive symptoms in postmenopausal women. Women with a decline in total serum estradiol over the 2 year period had a more than 3 fold increased risk of depressive symptoms[13].

Accumulating evidence suggests that Major Depressive Disorder (MDD) in adults is associated with immune system dysregulation and activation of the inflammatory response system[14]. Cytokines, signaling molecules that mediate key steps in cellular and humoral immunity, have been increasingly implicated in adult MDD[15]. Some study found that Interleukin-4 (IL-4) is positively correlated with estrogen[16].

*Samul-tanggahyangbuja* (SGH) is the prescription consisting of *Samul-tang* and rhizome of cyperus rotundus L.. A composite traditional oriental medicine, *Samul-tang* is a basic prescription consisting of four herbs: prepared root of rehmannia glutinosa L., root of angelica gigas N., rhizome of cnidium officinale M., root of paeonia lactiflora P.. For hundreds of years in oriental medicine, *Samul-tang* has been used for the treatment of gynecological diseases, chronic inflammation and pain relief[17]. It has also been widely applied

to malfunction of the hypothalamus or ovary and to disorder of autonomic nervous system including menstrual irregularity and dysmenorrhea[18]. The main constituents of *Samul-tang* are phenolic compounds, phthalides, alkaloids, terpene glycosides and iridoid glycosides[19]. Some of these are known to have anti-oxidant or anti-ischemic effects[20-22].

The aim of the present study was to explore the behavioral changes, the serum levels of estradiol and IL-4 immunoreactive effects of SGH on ovariectomized rats and to form a basis for clinical treatment. This study was designed to assess the anti-depressant effects of SGH in the experimental animal models. It was tested via an Elevated Plus Maze, and the serum levels of estradiol and IL-4, and immunohistochemical changes of IL-4 in the brain were measured.

# Ⅱ. Materials and Methods

### 1. Subjects and stress procedure

Sprague Dawley female rats (about 200 g) at the age of 3 months (Orient, Inc., Gyeonggi-do, Korea) were used for the study. The rats were housed under a controlled temperature (22-24℃) with a 12 hours light/dark cycle. The lights were on from 8:00 to 20:00. Food and water were made available ad libitum. They were allowed at least 1 week to adapt to their environment before the experiments. The animal experiments were carried out in accordance with the Prevention of Cruelty to Animals Act 1986 and NIH guidance for the care and use of laboratory animals for experimental procedures, and were approved by local committee review.

The female rats were randomly divided into four groups (n=8 per group): the nonoperated and nonstressed group (Normal), the ovariectomized and stressed group (Control), the ovariectomized, stressed and SGH 200 mg/kg/day treated group (SGH 200 mg), the ovariectomized, stressed and SGH 400 mg/kg/day treated group (SGH 400 mg).

Using aseptic conditions, bilateral ovariectomy was performed under general anesthesia with pentobarbital sodium (50 mg/kg, i.p.). After postoperative recovery for 7 days, the ovariectomized rats were stressed daily. Stress was produced by forcing the animals into an immobilizer device (a disposable rodent restraint cone, Harvard Instrument, U.S.A.) for 2 hours (10:00-12:00 a.m.) for 14 days. From the day of the first immobilization, the SGH group was daily treated with the SGH extract (200 and 400 mg/kg, p.o.) for 2 weeks, and other groups were given sterile saline. Immobilization was kept for 30 minutes after the treatments.

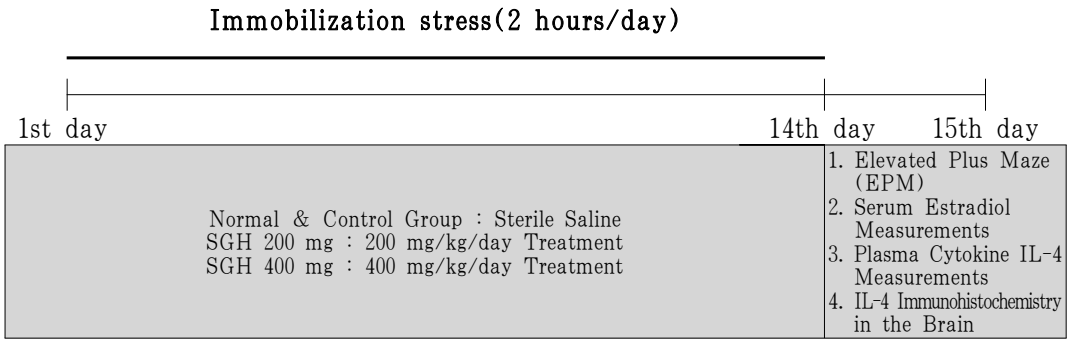


Fig. 1. Experimental Procedure.

## 2. Preparation of herbal extracts

SGH was purchased from an oriental drug store (Omniherb, Inc., Gyeongsangbuk-do, Korea), as prescribed in Table 1. The voucher specimens are deposited at the herbarium located in the College of Oriental Medicine, Wonkwang University. The dried SGH samples (720 g) were immersed in a 10 fold volume of distilled water, boiled at 80℃ for 1hour, and then the water extract was collected. The process was repeated once, and the extracts were combined and concentrated with a rotary evaporator and vacuum-dried to yield about 8.0% (w/w) of the extract.

Table 1. Prescription of *Samul-tanggahyangbuja*

| Pharmaceutical name | Dose (g) |
|---|---|
| *Rhizome of Cyperus rotundus L.* | 8 |
| *Prepared root of Rehmannia glutinosa L.* | 4 |
| *Root of Angelica gigas N.* | 4 |
| *Rhizome of Cnidium officinale M.* | 4 |
| *Root of Paeonia lactiflora P.* | 4 |
| Total amount | 24 |

## 3. Elevated Plus Maze (EPM)

The construction and the testing procedure of an Elevated Plus Maze (EPM) were based on a method described by Pellow et al. (1985)[23]. The EPM is a rodent model of anxiety that is used as a screening test for putative anxiolytic compounds and as a general research tool in neurobiological anxiety research. It consisted of two open arms (the arms extended from a central 50×10 cm space) and two enclosed arms (50×10×40 cm). The apparatus was elevated 50 cm above the floor.

Two behavioral measures were recorded for each rat; (1) the duration of time spent on the open arms and closed arms and (2) the number of entry points to the two compartments of the maze.

The frequency of entries into the open arms and the closed arms and the time spent on the respective arms were recorded for a period of five minutes.

## 4. Serum estradiol measurements

After the behavior test, blood samples were collected from the rats. The total concentration of estradiol was measured by

an ELISA kit (DuoSet ELISA development system, R&D Systems, Inc., Minneapolis, MN., USA). Cardiac blood was collected just prior to sacrificing the rats. The blood was centrifuged for 15 minutes at 1000g within 30 minutes of collection. The samples were immediately assayed or stored at ≤-60℃. All reagents, working standards and samples were prepared. The excess microplate strips were removed from the plate frame, returned to the foil pouch containing the desiccant pack and sealed. All of the samples or standards (100 µl) were added into the appropriately labeled wells and 50 µl of conjugated serum was placed into all of the wells except for the nonspecific binding wells and total count wells. Estradiol (50 µl) was added into all of the wells. All of the wells were incubated for two hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500±50 rpm. Each well was washed three times with wash buffer. After the last washing, any remaining wash buffer was removed by aspirating or decanting. 5 µl of estradiol conjugate and 200 µl of p-nitrophenyl phosphate-substrate were added to all of the wells. The well was incubated for 1 hour at room temperature (without shaking). Next, 50 µl of Stop Solution was added to each well. Using a microplate reader, the optical density of each well was immediately determined. The absorbance was read at 450 nm and 550 nm, and the sample values were calculated from a standard curve.

### 5. Cytokine measurements

After the behavior test, plasma separated from the blood was used to estimate the cytokine IL-4 levels. Enzyme-linked immunosorbent assay (ELISA) was performed using DuoSet ELISA development system according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA). Briefly, polystyrene microtiter plates (NUNC, U16 Maxisorp type, Roskilde, Denmark) were coated with monoclonal capture antibody (antirat IL-4) obtained from mouse (R&D Systems) and incubated at 4℃ overnight. The following day, the plates were blocked and then incubated for 2 hours with plasma. This was followed by the addition of corresponding biotinylated detection antibody obtained from goat (R&D Systems) and incubated for 2 hours. Streptavidin horseradish peroxidase (R&D Systems) and tetramethylbenzidine substrate (Bangalore Genei, Bangalore, India) treatment followed this incubation. The reaction was stopped using 2N sulfuric acid and optical density reading was taken at 450 nm. All the experiments were conducted in duplicate. A standard curve was obtained based on the standards provided by the manufacturer.

### 6. IL-4 immunohistochemistry

All of the animals were deeply anesthetized with sodium pentobarbital (80 mg/kg, ip) and they were perfused through the ascending aorta with normal

saline (0.9%), followed by 800 ml of 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS). The brains were removed, postfixed overnight and cryoprotected in 20% sucrose in 0.1 M PBS at 4℃. The brains were cut by cryostat sectioning into 30 μm coronal sections, and these slices were processed histochemically as free-floating sections.

The brain sections were washed in PBS containing 0.3% Triton X-100. The primary goat polyclonal antibodies against the following specific antigen were used : IL-4 (concentration 1:100 ; Santacruz biotechnology, Delaware Avenue Santa Cruz, CA, USA). The primary antibodies were diluted with blocking solution (10% fetal bovine serum in PBS, pH=7.4) and the tissues were incubated for 72 hours at 4℃ with constant agitation. Following rinsing in PBS, the sections were incubated for 2 hours at room temperature in biotinylated goat anti-serum (Vector Laboratories, Burlingame, CA, USA) that was diluted 100:1 in PBS with tween-20 containing 2% normal rabbit serum. The sections were placed in Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA, USA) for 2 hours at room temperature. Following a further rinsing in PBS, the tissue was developed using diaminobenzidine chromogen with nickel intensification. The sections were mounted on gelatine-coated slides, air-dried and coverslipped for microscopic observation. The number of stained nuclei of Paraventricular Nucleus (PVN) and Locus coeruleus (LC) cells were counted at 100× magnification using a microscope rectangle grid that measured 100×100 μm.

### 7. Data analysis

Statistical comparisons were done for the behavioral and immunological studies using the one-way ANOVA, and LSD post hoc was done. All of the results were presented as means±S.E.M., and SPSS 15.0 for Windows was used for analysis of the statistics. The significance level was set at $p<0.05$.

## Ⅲ. Results

### 1. Latency in the open arms of EPM

Latency in the open arms of EPM for 5 minutes was recorded. The time spent on the open and closed arms was expressed as a percentage (100× time spent on the open arms/total time in EPM, normal group=100%). The percentage of time spent on the open arms was significantly decreased in the control group, compared with the normal group ($p<0.01$). However, SGH 400 mg significantly increased time of open arms, compared with the control group ($p<0.05$), as shown in Fig. 2.

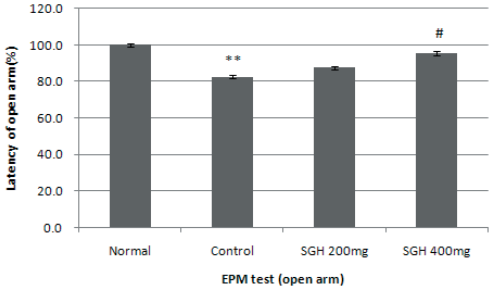


Fig. 2. The Time Spent on the Open Arms in the EPM.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
** : $p<0.01$ in comparison with the Normal group
# : $p<0.05$ in comparison with the Control group

## 2. Latency in the closed arms of EPM

The percentage of time spent on the closed arms was significantly increased in the control group, compared with the normal group ($p<0.01$). However, SGH 400 mg significantly decreased the percentage of time spent on the closed arms, compared with the control group ($p<0.05$), as shown in Fig. 3.

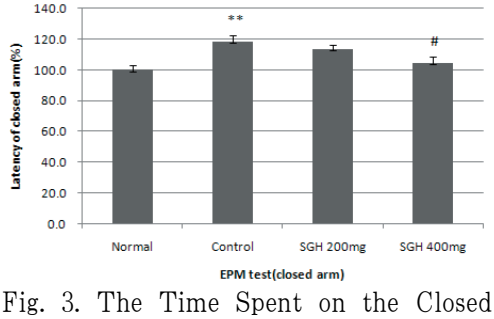


Fig. 3. The Time Spent on the Closed Arms in the EPM.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
** : $p<0.01$ in comparison with the Normal group
# : $p<0.05$ in comparison with the Control group

## 3. The number of crossings the open and closed arms in the EPM

Locomotor activity was decreased in the control group compared with the normal group ($p<0.05$). SGH groups tended to increase the number of crossings the open and closed arms in the elevated plus maze, as shown in Fig. 4. However, it did not reach statistical significance.

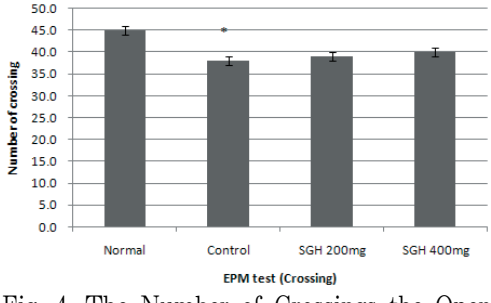


Fig. 4. The Number of Crossings the Open and Closed Arms in the EPM.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
* : $p<0.05$ in comparison with the Normal group

## 4. ELISA

1) Effects of SGH on the serum levels of estradiol

The serum levels of estradiol were significantly different among the groups. The serum levels of estradiol were significantly reduced in the control group compared with the normal group ($p<0.05$). However, SGH 400 mg significantly increased the serum levels of estradiol compared with the control group ($p<0.05$), as shown in Fig. 5.

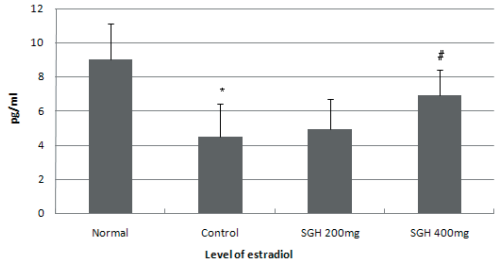


Fig. 5. The Serum Levels of Estradiol.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
* : $p<0.05$ in comparison with the Normal group
# : $p<0.05$ in comparison with the Control group

2) Effects of SGH on the serum levels of IL-4

The serum levels of IL-4 were significantly different among the groups. The serum levels of IL-4 were significantly reduced in the control group compared with the normal group ($p<0.01$). SGH 400 mg significantly increased the serum levels of IL-4 compared with the control group ($p<0.05$), as shown in Fig. 6.

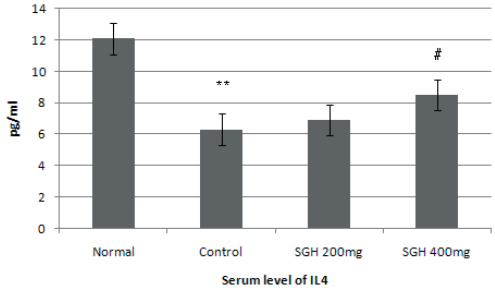


Fig. 6. The Serum Levels of IL-4.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
** : $p<0.01$ in comparison with the Normal group
# : $p<0.05$ in comparison with the Control group

3) IL-4 immunohistochemistry in the brain

The number of IL-4 immunoreactive neurons were significantly different among the groups. IL-4 immunoreactivity was reduced in the control group compared with the normal group ($p<0.05$). SGH groups (200 and 400 mg) did not produce any significant effect on levels of IL-4 in the LC and PVN, as shown in Fig. 7, 8.

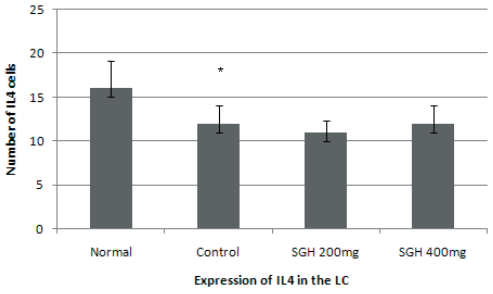


Fig. 7. IL-4 Immunohistochemistry in the LC.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
* : $p<0.05$ in comparison with the Normal group

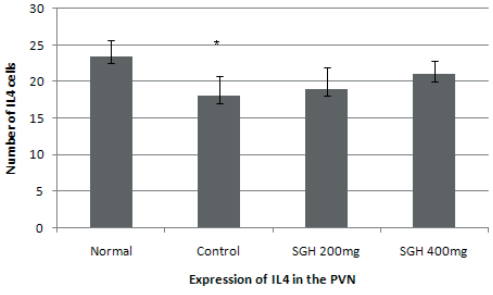


Fig. 8. IL-4 Immunohistochemistry in the PVN.
Normal : the nonoperated and nonstressed group
Control : after ovariectomized, exposed to immobilization stress group
SGH 200 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 200 mg/kg treated group
SGH 400 mg : after ovariectomized, exposed to immobilization stress, *Samul-tanggahyangbuja* 400 mg/kg treated group
* : $p<0.05$ in comparison with the Normal group

# Ⅳ. Discussion

The menopause transition is a period of marked hormone instability[24], with intense and irregular fluctuations in the levels of estrogen, which tend to decline after the postmenopause[25]. Sudden drops or fluctuations, or long periods of sustained low levels of estrogen are associated with an increased risk of depressive symptoms in postmenopausal women[13]. External stresses during the menopause transition also appear to have more negative impacts on women's mood[26]. In addition, studies have shown that women who have undergone a surgical menopause and thus experienced an abrupt drop in estrogen concentrations are also at a greater risk of depression[27-29].

Medical treatment for these issues has improved greatly, as well as the pharmaceutical treatments available for mental disorders like depression and anxiety. Recent research showed that melatonin supplementation in perimenopausal women can produce a significant improvement in thyroid function and gonadotropin levels, as well as restoring fertility and menstruation and preventing the depression associated with the menopause[30]. Antidepressants have been used with some success in the treatment of hot flush, improving sleep, mood and quality of life. But, some of them may cause nausea, insomnia, dry mouth, headache, constipation and decreased appetite. Therefore, it is necessary to examine the claim that herbal remedies help relieve menopausal symptoms[31].

*Samul-tang* was first written on 〈Taepyeonghyeminhwajegukbang · Jibuinjugi〉[32], it has been widely used in various blood deficiency syndromes and gynecologic

disease. *Samul-tang* has effects on hormone secretion and adjustment, related to menstruation and ovulation of the female[18]. Also, it has been believed to prevent and remedy all kinds of blood diseases such as menstrual irregularity and metrorrhagia. In a number of studies, it has been reproted that *Samul-tang* is effective in treating leucopenia, thrombocytopenia, anemia, and has anti-stress, anti-inflammatory effects and reduces side-effects of some anti-cancer agents[33-35].

*Samul-tang* is a decoction consisting of 4 kinds of medical herbs: Prepared root of Rehmannia glutinosa L., Root of Angelica gigas N., Rhizome of Cnidium officinale M., Root of Paeonia lactiflora P..

Through the experimental study in mice, Prepared root of Rehmannia glutinosa L. treated group increased in cell-mediated and humoral immune response[36]. Essential oil of Root of Angelica gigas N., a component of *Samul-tang*, exhibited an anxiolytic-like effect in the EPM[37]. Matsumoto et al. reported that ligustilide, an ingredient of Root of Angelica gigas N. had a beneficial effect on psychological stress-induced pathophysiological changes in the central nervous system. For example, it could reverse the decrease in the duration of pentobarbital sleep in mice that was caused by social isolation stress and activation of the central noradrenergic system[38]. Methanol extract of Rhizome of Cnidium officinale M. showed anxiolytic action in the EPM[39]. Root of Paeonia lactiflora P. is reported to influence the processes related to the mechanism of antidepressant action[40].

Also, Rhizome of Cyperus rotundus L. is a major herb in treating gynecologic disease and neurological diseases. In a prevoius study, Rhizome of Cyperus rotundus L. had antidepressant activity in the Forced Swimming Test (FST)[41].

*Samul-tang* has been often used to accompany with Rhizome of Cyperus rotundus L.. In the traditional oriental herbal medicine, *Samul-tang* and Rhizome of Cyperus rotundus L. were frequently used together in gynecologic disease, such as 'menstrual irregularities', 'flooding and spotting' and 'infertility'[18].

In this paper, *Samul-tanggahyangbuja* was used to demonstrate its antidepressant-like effects in menopausal depression. Ovariectomized rats were repeatedly stressed for over 2 weeks. After being orally medicated with SGH (200 or 400 mg/kg/day), the anxiety response was tested using the Elevated Plus Maze (EPM) in rats. Moreover, measurements of blood serum estradiol and the change of IL-4 in blood samples were made.

The EPM test is one of the most popular tests in all currently available animal models of anxiety[23,42]. This test for anxiety-like behavior has been used for screening and phenotyping transgenic and knockout mice[42] and for drug discovery[42,43]. The EPM test has a strong predictive validity for screening anxiolytic drugs[23,42,44]; anxiolytic drugs

specifically increase, and anxiogenic drugs specifically decrease the number of entries into the open arms and the time spent there. Total entry score is also an index of anxiety, and the percentages of entries and time spent in each arm constitute the index of primary anxiety[23,44]. Avoidance of the open arms is considered to be a result of the induction of higher levels of fear[23] and it is thought that the aversion of mice to explore the open arms of the maze is caused by fear of open and elevated spaces.

In the present study, SGH 400 mg prolonged the time spent in the open arms and the number of entries into the open arms compared with the control group in the EPM. These results of behavioral study suggest that SGH has the anxiolytic effects on the ovariectomized rats. In the previous study, SGH decreased the immobility time in Forced swimming test (FST), also demonstrated the antidepressant -like effects.

Significant changes in hormonal concentrations occur at the time of menopause. In this period, estrogen concentrations decline precipitously and the ensuing hypoestrogenic state has been linked with substantial alterations in physical and psychological function. Estrogen deficiency has been proposed to increase the susceptibility to depression[45]. Estrogen receptors are present in the brain and estrogen has been shown to modulate neurotransmitter turn-over and stimulates serotonergic activity through the regulation of receptor number and function[46], as well, estrogen can influence mood and well-being. These findings are further supported by in vivo animal studies, where a sudden decrease in the brain's exposure to estrogen has been shown to disrupt neurosteroid signalling, leading to an increase in anxiety and depression[47].

The results of this study showed that SGH significantly increased level of the serum levels of estradiol compared with the control group ($p<0.05$).

Other research has explored potential roles of molecules necessary for overall cellular functioning: cytokines. The symptoms of MDD are nearly identical to those of sickness behavior, the response of the body when the immune system is fighting an infection. This raises the possibility that depression can result from a maladaptive manifestation of sickness behavior as a result of abnormalities in circulating cytokines[48].

IL-4 is an anti-inflammatory cytokine. Some studies have shown that the pro-inflammatory cytokines (IL-2, IL-12, and TNF-α) and Monocyte Chemotactic Protein-1 (MCP-1) were significantly higher, whereas anti-inflammatory cytokines IL-4 and Transforming Growth Factor-beta 1 (TGF-β1) were significantly lower in patients with major depression than those of healthy controls. It seems likely that the anti-depressant therapy (sertraline) might have exerted immuno-modulatory effects through a decrease in the pro-

inflammatory cytokine IL-12 and an increase in the anti-inflammatory cytokines IL-4 and TGF-β1[49]. Similarly, SGH 400 mg significantly increased the serum levels of IL-4 compared with the control group ($p<0.05$).

Locus coeruleus (LC) is a nucleus in the brain stem involved with physiological responses to stress and panic. It is studied in relation to clinical depression, panic disorder and anxiety. LC provides an endogenous anti-inflammatory agent in the microenvironment around the neurons, glial cells and blood vessels in the neocortex and hippocampus[50].

Paraventricular Nucleus (PVN) is a neuronal nucleus in the hypothalamus. It contains multiple subpopulations of neurons that are activated by a variety of stressful and physiological changes. The PVN receives afferent inputs from many brain regions. Among these, input from neurons in structures adjacent to the anterior wall of the third ventricle carries information about the electrolyte composition of the blood, and about circulating concentrations of such hormones as angiotensin and relaxin, to regulate the magnocellular neurons. Input from the hippocampus to the CRH (Corticotropin -releasing hormone) neurons is important regulators of stress responses[51].

IL-4 immunoreactivity was reduced in the control group compared with the normal group ($p<0.05$). But, SGH groups (200 and 400 mg) did not produce any significant effect on levels of IL-4 in the LC and PVN. It is considered that there is a limit to detect IL-4 by ELISA due to the often low numbers of specifically responding cells, the relatively low amounts of IL-4 produced per cell. So, further measurement by more sensitive methods would be needed.

In conclusion, these results support anti-depressant and immuno-modulatory effects of SGH and it may be effective for treatment of depression by stress. Therefore, it is suggested that SGH can be an attractive candidate for depression treatment in menopausal women.

Further studies to fully elucidate the mechanism of underlying SGH antidepressant -like effects and define its clinical efficacy would be desired.

# V. Conclusion

In order to investigate the effects of *Samul-tanggahyangbuja* on repetitively stressed ovariectomized rats, the present study was performed using the Elevated Plus Maze, the levels of estradiol and IL-4 of serum. The results of this study were as follows.

1. In the EPM, SGH 400 mg significantly increased time spent on open arms and decreased time spent on closed arms, compared with the control group.
2. SGH groups (200 and 400 mg) tended to increase numbers of crossings in the open and closed arms in the EPM.

However, it did not reach statistical significance.

3. SGH significantly increased the serum levels of estradiol compared with the control group.
4. SGH 400 mg significantly increased the serum levels of IL-4 compared with the control group.
5. IL-4 immunoreactivity was reduced in the control group compared with the normal group. But, SGH groups (200 and 400 mg) did not produce any significant effect on levels of IL-4 in the LC and PVN.

In conclusion, the present results demonstrated that SGH effectively reduced patho-physiological depression-like responses and exerted immuno-modulatory effects. These results suggest that SGH may be useful for treatments of menopausal depression.

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## 국문초록

**목 적:** 본 연구는 갱년기 우울증 모델에서 사물탕가향부자(SGH)의 항우울 효능 및 면역반응에 대한 실험적 유의성을 확인함으로써 임상적인 응용에 기초자료로 사용하기 위하여 시행되었다. SGH가 반복적인 스트레스를 가한 난소적출 흰쥐에서 항우울행동 효과와 estradiol level 및 anti-inflammatory cytokine인 IL-4에 미치는 영향을 연구하고자 하였다.

**방 법:** 난소적출 흰쥐에 2주간 반복적인 스트레스를 주고 동시에 SGH(200 and 400 mg/kg/day)를 경구 투여한 후 행동검사인 Elevated Plus Maze(EPM)를 통한 우울행동과 혈청 estradiol, IL-4의 변화를 측정하였고, 또한 뇌 내 Locus coeruleus (LC)와 Paraventricular Nucleus(PVN)에서 IL-4의 변화를 측정하였다.

**결 과:**
1. EPM에서 SGH 400 mg 투여군은 대조군에 비해서 open arms에 머무는 시간이 현저히 증가하였고, closed arms에 머무는 시간이 현저히 감소하였다.
2. EPM에서 SGH 투여군은 대조군에 비해서 open arms와 closed arms를 교차하는 횟수가 증가하였으나, 통계적인 유의성은 없었다.
3. Estradiol 측정에서 SGH를 투여한 후 estradiol 수준이 현저하게 증가하였다.
4. IL-4 측정에서 SGH를 투여한 후 혈청 IL-4 수준이 현저하게 증가하였다.
5. 뇌에서의 IL-4 면역반응은 대조군에서 감소하였으나, SGH를 투여한 후 LC와 PVN에서 IL-4 수준의 유의성 있는 변화는 나타나지 않았다.

**결 론:** 이상의 결과로 사물탕가향부자는 난소적출 흰쥐의 항우울행동과 면역조절에 유효함을 알 수 있었다.

**중심단어:** 갱년기 우울증, 사물탕가향부자, Elevated Plus Maze, Estradiol, IL-4, 면역조직화학법, 향부자

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