대한한방부인과학회지
J Korean Obstet Gynecol
VOL.26 NO.4 : 030-047 (2013)

만성 스트레스 모델에서 사물탕가향부자의 항우울 효과

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ABSTRACT

The Anti-depressive Effect of *Samul-tanggahyangbuja* on Chronic Mild Stress in Ovariectomized Rats

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Objectives: The purpose of the present study is to investigate anti-depressive effects of *Samul-tanggahyangbuja* (SGH) on ovariectomized and chronic mild stress (CMS) induced rats.

Methods: Ovariectomized rats were exposed to CMS for 4 weeks. Changes of depression behavior were tested by using sucrose intake test (SIT), elevated plus maze (EPM), forced swimming test (FST) and Morris water maze test (MWMT) in rats until being orally medicated with SGH (100 or 400 mg/kg/day). In addition, the serum levels of corticosterone (CORT), IL-4, IL-1β and changes of 5-HT in the brain were measured.

Results:

1. SGH 400 mg/kg treated group (SGH 400) significantly increased amount of sucrose intake compared with the control group ($p<0.05$).
2. SGH 100 mg/kg treated group (SGH 100) and SGH 400 significantly increased the time spent in the open arms of the EPM compared with the control group ($p<0.01$). SGH 400 also significantly increased the number of crossing of the open and closed arms compared with the control group ($p<0.05$).
3. SGH significantly shortened the immobility time in FST compared with the control group (SGH 100 $p<0.05$, SGH 400 $p<0.01$).
4. SGH significantly increased performance of acquisition trials compared with the control group ($p<0.05$, on day 4, 5 of SGH 100 and 400). SGH 400 also significantly increased performance of retention trials compared with the control group ($p<0.05$).
5. The serum levels of corticosterone and IL-4 were not significantly different among the groups. There were no changes on the serum levels of corticosterone, IL-1β and IL-4 after administration with SGH.
6. SGH 400 significantly increased the level of 5-HT in the hippocampus compared with the control group ($p<0.05$). SGH significantly increased the levels of 5-HT in the hypothalamus compared with the control group (SGH 100 $p<0.05$, SGH 400 $p<0.01$).

Conclusions: These results suggest that SGH has the anti-depressive effect on ovariectomized rat and affect 5-HT system rather than hypothalamic-pituitary-adrenal (HPA) axis and immune system.

Key Words: Menopausal Depression, *Samul-tanggahyangbuja*, CMS, 5-HT, Depression Behavior Test, Rhizome of Cyperus rotundus L

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Ⅰ. Introduction

Menopausal depression, common clinical problem[1], is reported that between 22 and 33% of menopausal women have mood deterioration and depression[2]. Its most major symptoms are anxiety and depression, which are so disabling both for the patient and her family[3].

Samul-tanggahyangbuja (SGH) is the prescription consisting of *Samul-tang* and Rhizome of Cyperus rotundus L.. *Samul-tang* has been long used for the treatment of gynecological diseases and various blood deficiency syndromes, and Rhizome of Cyperus rotundus L. is a major herb in treating gynecologic disease and neurological diseases[4]. In addition, *Samul-tang* and Rhizome of Cyperus rotundus L. has been frequently used together in malfunction of the Hypothalamic -pituitary-ovarian (HPO) axis and disorder of autonomic nervous system[5].

In laboratory studies, SGH has been shown to have anti-depressive effects on immobilization stress in ovariectomized rats[6,7].

However, no attempts have been to investigate the anti-depressive effect of SGH on chronic mild stress (CMS) which of procedure repeatedly exposes animals to various unpredictable and mild stressors over a period of weeks[8].

In particular, menopausal depression occurs through not acute and severe stress but chronic and mild stress. Therefore the CMS model is suitable for study of menopausal depression.

The aim of the present study was to explore the behavioral changes, immunoreactive effects, and 5-HT levels in the brain of SGH on ovariectomized rats and to investigate the clinical effect of SGH for menopausal depression. This study was designed to assess the anti-depressive effect of SGH on CMS in ovariectomized rats. It was tested via sucrose intake test (SIT), elevated plus maze (EPM), forced swimming test (FST), Morris water maze test (MWMT), and the serum levels of corticosterone (CORT), IL-1β, IL-4 and changes of 5-HT in the brain were measured.

Ⅱ. Materials and Methods

1. Subjects

Sprague Dawley female rats (200±20 g) at the age of 2-3 months (Orient, Inc. 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=9 per group): the nonoperated and nonstressed group (normal), the ovariectomized and CMS group (control), and the ovariectomized, CMS and SGH 100 mg/kg treated group (SGH 100), CMS and SGH 400 mg/kg treated group (SGH 400).

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.

2. CMS schedule procedure

The CMS group was placed under unpredictable stress for 4 weeks with same cage during CMS procedure. The normal group was not given any stress under the same conditions. The rats in the stress group were exposed to CMS in a separate room for 4 weeks. The various stressors included: depletion of water, depletion of water and food, soiled cages, a cage tilt of 45℃, white noise (100 dB), stroboscope light (300/min), wet bedding (100 ml of water per individual cage) and transfer to a small cage (3 per cage). From the day of the first stress, the SGH group was daily treated with the SGH extract (100 and 400 mg/kg, p.o.) for 4 weeks, and other groups were given sterile saline.

3. 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 Korean Medicine, Wonkwang University. The dried SGH samples (720 g) were immersed in a 10-fold volume of distilled water, boiled at 80℃ for 1 hour, 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

4. Sucrose intake test (SIT)

For the sucrose intake test, subjects were exposed to 1% sucrose solution for 22-28th days after the start of exposure with stress. Testing took place daily, between 14:00 and 17:00 on the same day of the week. Animals were subjected to sucrose preference test on the 28th day. Prior to each test, animals were food and water deprived for 24 hours. Sucrose solution consumption was recorded

by reweighing preweighed bottles of test solution.

5. Elevated Plus Maze (EPM)

On 29th day after CMS, EPM test was performed. The construction and the testing procedure of EPM were based on a method described by Pellow et al[9]. 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 (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 5-minutes period.

6. Forced swimming test (FST)

On 30th day after CMS and FST was performed, which was originally described by Porsolt et al[10], and is the most widely used pharmacological model for assessing antidepressant activity[11]. The apparatus consisted of a transparent Plexiglas cylinder (50 cm high×20 cm wide) filled to a 30 cm depth with water at room temperature. In the pre-test, rats were placed in the cylinder for 15 minutes, 24 hours prior to the 5-minutes swimming test. SGH extract (100, 400 mg/kg) or saline was administrated p.o. three times : immediately after the initial 15 minutes pre-test, 5-minutes test and 1 hour prior to the swimming test. During the 5-minutes swimming test, the following behavioral responses were recorded by a trained observer: Climbing behavior, Swimming behavior, Immobility.

7. Morris water maze test (MWMT)

The swimming pool of the Morris water maze was a circular water tank 200 cm in diameter and 35 cm deep. It was filled to a depth of 21 cm with water at 23±2℃. A platform 15 cm in diameter and 20 cm in height was placed inside the tank with its top surface being 1.5 cm below the surface of the water. The pool was surrounded by many cues that were external to the maze. A CCD camera was equipped with a personal computer for the behavioral analysis. Each rat was received four daily trials. For 6 consecutive days from day 31-36, the rats were tested with three acquisition tests. They also received retention tests on the 7th day. For the acquisition test, the rat was allowed to search for the hidden platform for 180 seconds and the latency to escape onto the platform was recorded. The animals were trained to find the platform that was in a fixed position during 6 days for the acquisition test, and then for the retention test, they received a 1 minute probe trial in which the platform was removed from the pool. The intertrial interval time was 1 minute. Performance of the test animals in each water maze trial was

assessed by a personal computer for the behavioral analysis (S-mart program, Spain).

8. Corticosterone measurements

After the behavior test, blood samples were collected from the rats. The total concentration of CORT 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 1000×g 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. Corticosterone (50 µl) was added into all of the wells. All of the wells were incubated for 2 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 corticosterone conjugate and 200 µl of p-nitrophenyl phosphate-substrate was 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.

9. Cytokine measurements

After the behavior test, plasma separated from the blood was used to estimate the cytokine levels. Enzyme-linked immunosorbent assay (ELISA) was performed using DuoSet ELISA development system according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Briefly, polystyrene microtiter plates (NUNC, U16 Maxisorp type, Roskilde, Denmark) were coated with monoclonal capture antibody (antirat IL-1β and 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 then tetramethylbenzidine substrate (Bangalore Genei, Bangalore, India) treatment followed this incubation. The reaction was stopped using 2 N 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.

10. Tissue analysis procedures

1) Sample preparation

The animals were sacrificed by decapitation immediately after behavioral testing. The brain was then rapidly removed and the hippocampus and hypothalamus was dissected out and placed onto an ice cold plate. All the tissue samples were quickly frozen and stored in a deep freezer at -80℃ until assayed. The samples weighed and then homogenized with a ultrasonic disruptor (Sonics Materials, INC, USA) in an ice cold 0.1 M perchloric acid (PCA) solution (600 μl) containing 0.1% sodium metabisulfate and 40 ng/ml of dihydroxybenzylamine (DHBA) was used as an internal standard. After homogenization, the solution was centrifuged at 15000 rpm in a micro 17R centrifuge (Micro 17R, Hanil Co. Korea) for 30 minutes at 4℃.

2) Determination of tissue level of monoamines

The levels of 5-HT were determined by performing HPLC coupled with ECD. A 20 μl sample of the supernatant was injected into a Bondapak C18 reverse -phase column (Waters Co, with a 300×3.9 mm internal diameter and a particle size of 5 μm) for the separation of 5-HT (flow rate of 1 ml/min). Determination of monoamines was done with an ECD (ESA, Coulochem II, Model 5200A), and a pump (ESA, Model 580). A guard cell(ESA, Model 5020) was set at +400 mV, the first and second electrodes of the analytical cell (ESA, Model 5011) were set at -40 and +200 mV, and the output of the second electrode was recorded as a chromatograph with using a HP 3395B printer (Hewlett Packard, Germany). The composition of the mobile phase was 150 mM sodium phosphate monobasic, 0.7 mM sodium octane sulfonate, 0.1 mM EDTA and 10% acetonitrile, and this was adjusted to pH 3.2 using 0.1 M phosphoric acid. The tissue level of the monoamines was determined by performing a linear regression analysis for the peak heights obtained from a range of standard curves, and expressed as ng of monoamine per g of fresh tissue weight.

11. Data analysis

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

Ⅲ. Results

1. Sucrose intake test

Amount of sucrose intake was significantly decreased in the control group compared to the normal group ($p<0.05$). SGH 400 significantly increased amount of sucrose

intake, compared with the control group ($p<0.05$). The amount of sucrose intake were respectively 8.2±1, 6.5±0.6, 6.3±1.2 and 8.33±1.2 (ml) in the Normal, Control, SGH 100 and SGH 400 (Fig. 1).

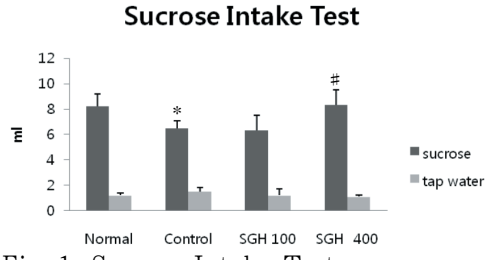

Fig. 1. Sucrose Intake Test.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
* : $p<0.05$ in comparison with Normal
# : $p<0.05$ in comparison with Control

2. Elevated plus maze

1) Latency in the open arms of EPM

The time of spent on the open arms shows the percentage (normal group =100%). Total time of in the open arms was significantly decreased in the control group, compared with the normal group ($p<0.01$). SGH 100 and 400 significantly increased the time spent in the open arms of the EPM ($p<0.01$). The latency in the open arms of EPM were respectively 100±5.9, 69.3±6.9, 98.2 ±4.5 and 98.3±3.6 (%) in the Normal, Control, SGH 100 and SGH 400 (Fig. 2).

2) Numbers of crossing of the open and closed arms in the EPM

Locomotor activity was significantly decreased in the control group compared to the normal group ($p<0.05$). SGH 400 significantly increased numbers of crossing of the EPM, compared with the control group ($p<0.05$). The numbers of crossing were respectively 43.0±6.2, 29.0±6.3, 36.0±8.2 and 41.0±7.5 in the Normal, Control, SGH 100 and SGH 400 (Fig. 3).

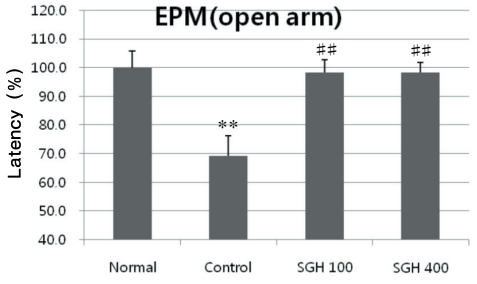

Fig. 2. Latency in the Open Arms of EPM.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
** : $p<0.01$ in comparison with Normal
: $p<0.01$ in comparison with Control

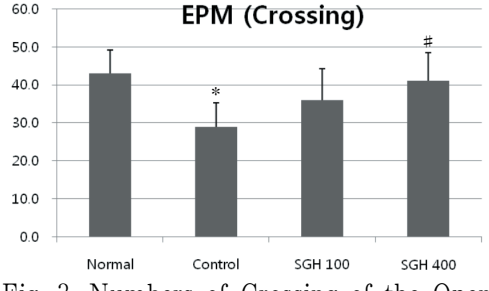

Fig. 3. Numbers of Crossing of the Open and Closed Arms in the EPM.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
* : $p<0.05$ in comparison with Normal
# : $p<0.05$ in comparison with Control

3. Forced swimming test

CMS significantly increased immobility time in the FST compared to the normal group ($p<0.01$) and SGH groups significantly shortened the immobility time in comparison to control values (SGH 100 $p<0.05$, SGH 400 $p<0.01$). The immobility time were respectively 9±3.6, 29±8.9, 11±6.5 and 10±3.6 (sec) in the Normal, Control, SGH 100 and SGH 400 (Fig. 4).

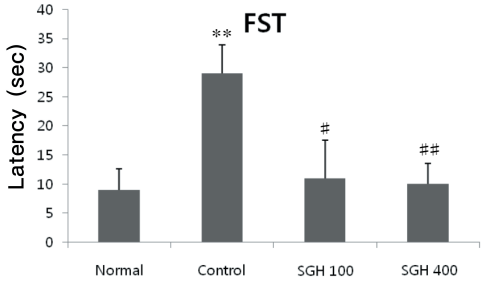

Fig. 4. Forced Swimming Test.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGHa 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
** : $p<0.01$ in comparison with Normal
# : $p<0.05$ in comparison with Control
: $p<0.01$ in comparison with Control

4. Water maze test

1) The acquisition trial of the water maze

The escape latency differed among the groups when the results were averaged over all the session. The control group significantly showed a worse performance than the normal group (at the Day 3, 4, 5 respectively). SGH groups significantly increased performance of acquisition trials ($p<0.05$, on day 4, 5 of SGH 100 and 400). The latency of acquisition trial were respectively 54±5.3 38±5.2 22±2.9, 110±5.6 99±3.5 84±5.7, 98±6.3 74±5.6 30±4.6 (sec) and 92±8.1 77±4.2 38±3.9 in the Normal, Control, SGH 100 and SGH 400 at the Day 3, 4, 5 (Fig. 5).

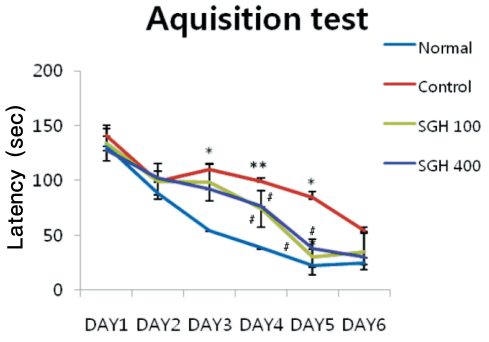

Fig. 5. The Acquisition Trial of the Water Maze.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group.
* : $p<0.05$ in comparison with Normal
** : $p<0.01$ in comparison with Normal
# : $p<0.05$ in comparison with Control

2) The retention trial of the water maze (target zone)

To examine the spatial memory of rats, the performance on the probe trial with comparing the time spent to the platform was analyzed. The times spent to the platform were significantly different among the groups, and the control group spent less time around the platform than the normal group ($p<0.01$). CMS severely impaired spatial cognition on MWMT. However, SGH 400 significantly increased performance of retention trials, compared with the control group ($p<0.05$).

The latency of the water maze were respectively 6.9±1.2, 2.6±1, 2.3±2.1 and 4.8±1.2(sec) in the Normal, Control, SGH 100 and SGH 400 (Fig. 6).

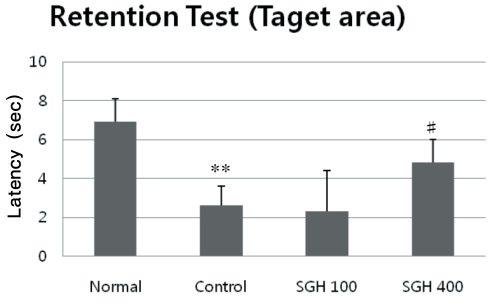

Fig. 6. The Retention Trial of the Water Maze(Target Zone).
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
** : $p<0.01$ in comparison with Normal
# : $p<0.05$ in comparison with Control

3) The retention trial of the water maze (quadrant zone)

The times spent to the platform were significantly different among the groups, and the control group spent less time around the platform than the normal group ($p<0.01$). CMS severely impaired spatial cognition on MWMT. However, SGH 400 significantly increased performance of retention trials, compared with the control group ($p<0.05$). The latency of the water maze (quadrant) were respectively 40.2±4.2, 29.3±2.6, 30.6±4.5 and 35.6±3.7 (%) in the Normal, Control, SGH 100 and SGH 400 (Fig. 7).

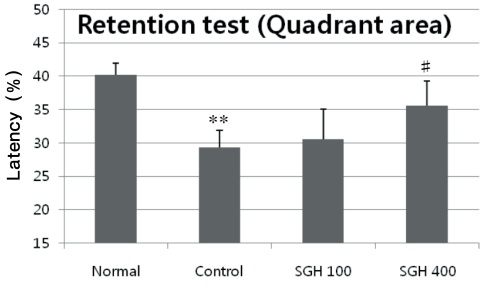

Fig. 7. The Retention Trial of the Water Maze (Quadrant Zone).
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
** : $p<0.01$ in comparison with Normal
# : $p<0.05$ in comparison with Control

5. ELISA

1) The serum levels of CORT

The serum levels of CORT were not significantly different in comparisons among the groups. There were no changes on the serum levels of CORT after administration with SGH. The serum levels of CORT were respectively 189±24, 212±27, 206±14 and 212±22 (μg/ml) in the Normal, Control, SGH 100 and SGH 400 (Fig. 8).

2) The serum levels of IL-1β

The serum levels of IL-1β were significantly different in comparisons among the groups. The LSD test results indicated a significantly increased the serum levels of IL-1β in the control group compared to the normal group ($p<0.05$). Administration with SGH (100, 400) did not produce any significant effect on the serum levels of IL-1β. The serum levels of IL-1β were respectively 19±3.9, 26±3.5, 25±4.5 and 24±3.6 (pg/ml) in the Normal, Control,

SGH 100 and SGH 400 (Fig. 9).

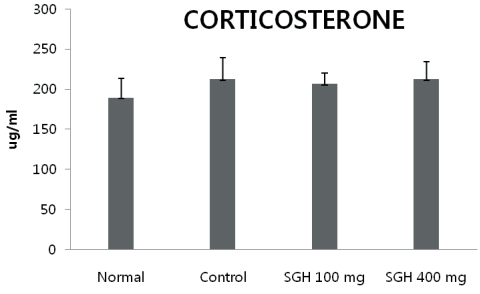

Fig. 8. The Serum Levels of CORT.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group

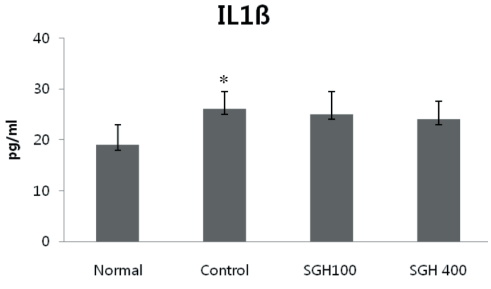

Fig. 9. The Serum Levels of IL-1β.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
* : $p<0.05$ in comparison with Normal

3) The serum levels of IL-4

The serum levels of IL-4 were not significantly different in comparisons among the groups. Administration with SGH (100 and 400) did not produce any significant effect on the serum levels of IL-4. The serum levels of IL-4 were respectively 18.9±2.3, 15.9±3.6, 16.5±4.5 and 16.3±3.6 (pg/ml) in the Normal, Control, SGH 100 and SGH 400 (Fig. 10).

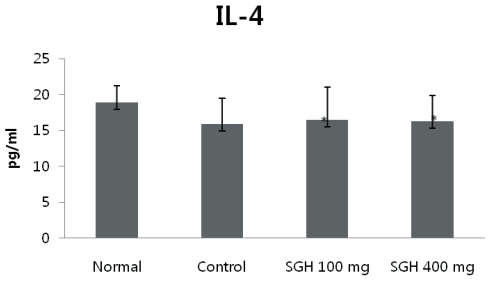

Fig. 10. The Serum Levels of IL-4.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group

6. 5-HT levels in the brain

1) 5-HT levels in the hippocampus

The levels of 5-HT in the hippocampus were significantly different in comparisons among the groups. The LSD test results indicated a significantly decreased levels of 5-HT in the control group compared to the normal group ($p<0.05$). SGH 400 significantly increased level of 5-HT, compared with the control group ($p<0.05$). The levels of 5-HT were respectively 958±66, 725±86, 824±78 and 954±85 (ng/mg) in the Normal, Control, SGH 100 and SGH 400 (Fig. 11).

2) 5-HT levels in the hypothalamus

The levels of 5-HT in the hypothalamus were significantly different in comparisons among the groups. The LSD test results indicated a significantly decreased levels of 5-HT in the control group compared to the normal group ($p<0.01$). SGH 100

and 400 significantly increased levels of 5-HT, compared with the control group (SGH 100 $p<0.05$, SGH 400 $p<0.01$). The levels of 5-HT were respectively 1325±98, 926±85, 1120±79 and 1230±89 (ng/mg) in the Normal, Control, SGH 100 and SGH 400 (Fig. 12).

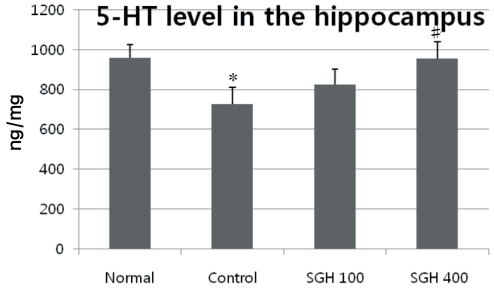

Fig. 11. 5-HT Levels in the Hippocampus.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
* : $p<0.05$ in comparison with Normal
# : $p<0.05$ in comparison with Control

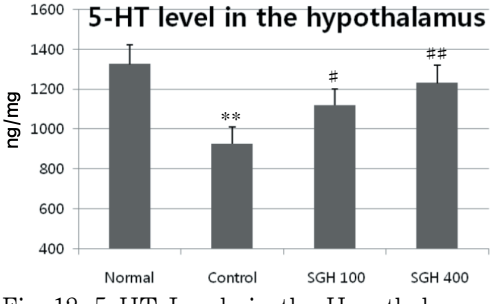

Fig. 12. 5-HT Levels in the Hypothalamus.
Normal : non-ovariectomized group
Control : after ovariectomized, exposed to CMS group
SGH 100 : after ovariectomized, exposed to CMS SGH 100 mg/kg/day treated group
SGH 400 : after ovariectomized, exposed to CMS SGH 400 mg/kg/day treated group
** : $p<0.01$ in comparison with Normal
# : $p<0.05$ in comparison with Control
: $p<0.01$ in comparison with Control

Ⅳ. Discussion

Women are at a two-fold greater risk for developing depression as compared to men[12,13]. Particularly, depression is prevalent during the menopausal transition[14-19]. Depression is one of the most prevalent mood disorders with high morbidity and mortality that is characterized by either depressed mood or anhedonia, according to DSM-IV[20]. The symptoms of depression include sadness, anxiety, apathy, sleeping problems, loss of appetite desire, feeling of worthlessness, and most importantly, suicidal thoughts. The World Health Organization estimates that depression is now the fourth most important worldwide cause of loss in human disability adjusted life years, and predicts it will have become the second most by the year 2020[21].

There have been many studies relating the causes of menopausal depression with a hormonal change. However, psychosocial factors may play more important role in menopausal depression. Physical discomfort such as fatigue, insomnia and a pain, concern about aging and death, a sense of emptiness felt by growth and independence of the children, and pessimism to a relative situation to the husband in social life are associated with an increased risk of the menopausal depression[3].

In the traditional oriental herbal medicine, *Samul-tang* is widely used in blood deficiency that is a lot of related to the aging and Rhizome of Cyperus

rotundus L. is known to be effective in releasing depression[4,22]. Other studies reported that *Samul-tang* improved brain function by reacting on biochemical of the senile brain[23], *Samul-tang* (with Puerariae Radix) improved uterus atrophy of ovariectomized rat[24]. Thus SGH which consists of *Samul-tang* and Rhizome of Cyperus rotundus L. is common prescription that can be used in a clinic for treatment of menopausal depression caused by aging and chronic stress. *Samul-tang* was first written on ≪Taepyeonghyeminhwajegukbang · Jibuinjugi≫[25], consists of Root of Angelica gigas N., Root of Paeonia lactiflora P., Rhizome of Cnidium officinale M. and Prepared root of Rehmannia glutinosa L.. In a number of studies, it has been reported that SGH and its constituent herbs has anxiolytic, anti-depressive effects[6,7,26-32]. Recent studies showed that SGH has anti-depressive effects on immobilization stress in ovariectomized rats[6,7].

CMS model, developed by Willner and colleagues, is widely used as an animal model of depression for development of antidepressant, and its primary focus is the induction of anhedonia which is a core symptom of human depression[33-34]. In rats, application of CMS procedures resulted in a variety of behavioral, neurochemical, neuroendocrine and neuroimmune alterations resembling some of the dysfunctions observed in human depression[35-41]. The repeated, weak stressors are effective methods for inducing depression-like symptoms in an animal model[42], as they reflect menopausal depression symptoms attributed to the weak and chronic stress during menopause transition. Thus, the CMS model was chosen in the present study to be suitable for study of menopausal depression.

The decrease in sucrose intake has been demonstrated as a core symptom of depression in the CMS[43]. In the present study, SGH significantly increased amount of sucrose intake, consistent with the previous study on immobilization stress[6]. Based on the result of these studies, SGH may reflect decreased response to anhedonia which is a core symptom of human depression.

The EPM test is one of the most popular tests in all currently available animal models of anxiety[44,45]. In the present study, SGH significantly increased the time spent in the open arms and the numbers of crossing of the open and closed arms, consistent with the previous study on immobilization stress[7]. The result of these studies suggest that SGH may be effective for treatment of anxiety disorder.

In the present study, SGH significantly shortened the immobility time in FST, consistent with the previous study on immobilization stress[6]. Based on the result of these studies, SGH may be effective on depression.

The MWMT is one of the most frequently used laboratory tools in behavioral neuroscience. This informs the affect that uncertainties influences on the cognition disorder which is one of

depression symptoms[46]. In the present study, SGH treatment significantly increased performance of acquisition trials and retention trials. In the previous study, SGH treatment significantly increased performance of acquisition trials[6]. These results suggest that SGH may reverse memory and learning deficits induced by depression.

These results of behavioral study suggest that SGH has the anxiolytic, anti-depressant, increase of memory and learning effects on ovariectomized rats regardless of animal models of depression.

CORT is a steroid hormone involved in regulation of immune reaction, stress response. It sensitively reacts to the stress and it's secretion is increased in depressive condition[47,48]. IL-1β is representative pro-inflammatory cytokine and IL-4 is an anti-inflammatory cytokine. Some studies have shown that pro-inflammatory cytokines were significantly higher, whereas anti-inflammatory cytokines were significantly lower in patients with major depression than those of healthy controls[49]. In the present study, the serum level of CORT, IL-4 were not significantly different in comparisons among the groups. There were no changes on the serum level of CORT, IL-1β, IL-4 after administration with SGH. In the previous study, the serum level of CORT were significantly increased in the control group compared to the normal group, there no changes on the serum level of CORT after administration with SGH[6]. There were significantly changes on the serum level of IL-1β, IL-4 after administration with SGH[6,7]. In contrast to earlier studies on immobilization stress[6,7], SGH did not have effects in HPA axis and immune system on the CMS. These results may be due to the different animal models of depression.

Serotonin or 5-Hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Serotonin in the CNS is synthesized in serotonergic neurons where it has various functions. These include the regulation of mood, appetite, sleep, as well as muscle contraction. Serotonin also has some cognitive functions including in memory and learning. Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants. Reduced activity of CNS serotonin is reported in unipolar depression and serotonin is the major target of recent antidepressant drugs. The most prescribed drugs in depression are drugs which alter serotonin levels. Some drugs inhibit the re-uptake of serotonin, making it stay in the synapse longer. Some drugs prevent the breakdown of monoamine neurotransmitters including serotonin, and therefore increase concentrations of the neurotransmitter in the brain[50]. In the present study, SGH significantly increased levels of 5-HT in the hippocampus and hypothalamus. These results support anti-depressive effects of SGH related to serotonin. In previous studies, it has been reported that Rhizome

of Cyperus rotundus L. has anti-depressive effects associated with serotonin[32] and *Samul-tang* significantly increased 5-HT levels in the brain of senile rat[23], consistent with the current result of the study.

In the present study, a significant changes of depression behavior was observed in CMS rats, consistent with earlier studies[6,7]. In contrast to earlier studies[6,7], immunoreactive effect was not observed in CMS rats. SGH significantly increased the level of 5-HT in the brain. These results suggest that SGH has the anti-depressive effect regardless of animal models of depression and SGH affect 5-HT system rather than HPA axis and immune system producing the anti-depression action.

In conclusion, the present study cleary demonstrated that SGH has anti-depressant effects on CMS in ovariectomized rats. Therefore, it is suggested that SGH may be useful for treatments of menopausal depression.

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 CMS in ovariectomized rats, the present study was performed using SIT, EPM, FST, MWMT, the serum levels of CORT, IL-1β, IL-4, and changes of 5-HT in the brain. The results of this study were as follows.

1. SGH 400 significantly increased amount of sucrose intake, compared with the control group ($p<0.05$).
2. SGH 100 and 400 significantly increased the time spent in the open arms of the EPM compared with the control group ($p<0.01$). SGH 400 significantly increased the number of crossing of the open and closed arms compared with the control group ($p<0.05$).
3. SGH significantly shortened the immobility time in FST compared with the control group (SGH 100 $p<0.05$, SGH 400 $p<0.01$).
4. SGH significantly increased performance of acquisition trials compared with the control group ($p<0.05$, on day 4, 5 of SGH 100 and 400). SGH 400 significantly increased performance of retention trials compared with the control group ($p<0.05$).
5. The serum levels of CORT, IL-4 were not significantly different among the groups. There were no changes on the serum levels of CORT, IL-1β, IL-4 after administration with SGH.
6. SGH 400 significantly increased level of 5-HT in the hippocampus compared with the control group ($p<0.05$). SGH significantly increased levels of 5-HT in the hypothalamus compared with the control group (SGH 100 $p<0.05$, SGH 400 $p<0.01$).

In conclusion, the present results demonstrated that SGH possesses the anti-depressive effect on ovariectomized rat. These results suggest that SGH may be useful for treatments of menopausal depression and SGH affect 5-HT system rather than HPA axis and immune system.

□ 투 고 일 : 2013년 10월 14일
□ 심 사 일 : 2013년 10월 29일
□ 게재확정일 : 2013년 11월 8일

국문초록

목 적: 본 연구는 만성 스트레스로 인한 갱년기 우울증 모델에서 사물탕가향부자(SGH)의 항우울 효과를 검증하여 실험적 유의성을 확인하고자 시행되었다.

방 법: 난소적출 흰쥐에 4주간 만성 스트레스를 가하고 SGH(100 and 400 mg/kg/day)를 투여한 후 행동검사인 sucrose intake test(SIT), elevated plus maze (EPM), forced swimming test(FST) 및 Morris water maze test(MWMT) 등을 통한 우울행동 변화와 혈청 corticosterone(CORT), IL-1β 및 IL-4 등의 변화를 측정하였고, 또한 뇌 내 hippocampus와 hypothalamus에서 5-HT의 변화를 측정하였다.

결 과:

1. SIT에서 SGH 400 mg/kg/day 투여군은 대조군에 비해서 자당 섭취가 유의하게 증가하였다($p<0.05$).
2. EPM에서 SGH 400 mg/kg/day 투여군은 대조군에 비해서 open arms에 머무는 시간이 유의하게 증가하였고($p<0.01$), open arms와 closed arms를 교차하는 횟수도 대조군에 비해서 유의하게 증가하였다($p<0.05$).
3. FST에서 SGH 100 mg/kg/day, 400 mg/kg/day 투여군 모두 대조군에 비해서 immobility시간이 유의하게 줄어들었다(SGH 100 $p<0.05$, SGH 400 $p<0.01$).
4. MWMT에서 SGH 100 mg/kg/day, 400 mg/kg/day 투여군은 모두 대조군에 비해서 실험 4일째에서 acquisition trials 수행 시간이 유의하게 단축되었고($p<0.05$), SGH 400 mg/kg/day 투여군은 대조군에 비해서 retention trials 수행 시간이 유의하게 증가하였다($p<0.05$).
5. CORT와 IL-4 측정에서 대조군과 SGH 투여군 모두 유의한 변화가 없었고, IL-1β 측정에서 대조군에서는 유의하게 증가하였지만 SGH 투여군에서는 대조군에 비해서 유의한 변화가 없었다.
6. Hippocampus에서의 5-HT 측정에서 SGH 400 mg/kg/day 투여군은 대조군에 비해서 유의하게 증가하였다($p<0.05$). Hypothalamus에서의 5-HT수준은 SGH 투여군 모두 대조군에 비해서 유의하게 증가하였다(SGH 100 $p<0.05$, SGH 400 $p<0.01$).

결 론: 이상의 결과로 SGH는 만성 스트레스를 가한 난소적출 흰쥐에서 항우울 효과가 있음을 알 수 있었고, HPA axis, immune system 보다 5-HT system에 작용하는 것으로 사료된다.

중심단어: 갱년기 우울증, 사물탕가향부자, CMS, 5-HT, 우울행동검사, 향부자

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