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난소적출로 유발된 랫트 갱년기 장애에 대한 가감귀비온담탕의 생리활성 효과 평가

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ABSTRACT

Anti-climacterium Effects of *Gagamguibiondam-tang* in Ovariectomized Rats

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Purpose: The object of this study was to observe the anti-climacterium activity of *Gagamguibiondam-tang* (GGOT) on ovariectomized (OVX) rats, a well-documented rodent models resembles with women postmenopausal climacterium symptoms, as including cardiovascular diseases, obesity, hyperlipidemia, osteoporosis, organ steatosis and mental disorders.

Methods: In this study, anti-climacteric effects were evaluated separated into three categories; 1) anti-obese, 2) anti-uterine atrophy and 3) anti-osteoporotic effects. Five groups were used (8 rats in each group); sham control, OVX control, GGOT 500, 250 and 125 mg/kg administered groups. Twenty-eight days after bilateral OVX surgery, GGOT were orally administered, once a day for 84 days, and then the changes on the body weight and gain during experimental periods, serum estradiol levels, abdominal fat pad and uterus weights with histopathology of abdominal fat pads (total thickness and mean adipocyte diameters) and uterus (total, epithelial and mucosal thickness, percentages of uterine gland regions) for anti-obese and estrogenic effects. In addition, femur, tibia and fourth or fifth lumbar vertebrae (L4 or L5) wet, dry and ash weights, mineral density (BMD), bone strength (failure load), serum osteocalcin and bone specific alkaline phosphatase (bALP) contents, histological and histomorphometrical analyses - bone mass and structure with bone resorption, were monitored for anti-osteoporosis activity.

Results: As a result of OVX, noticeable increases of body weight and gains, food and water consumption, weights of abdominal fat pad deposited in dorsal abdominal cavity, serum osteocalcin levels were demonstrated in this experiment with decrease of uterus, femur, tibia and L5 weights, serum bALP and estradiol levels. In addition, marked hypertrophic changes of adipocytes located in deposited abdominal fat pads, uterine disused atrophic changes, decreases of bone mass and structures of femur, tibia and L4 were also observed in OVX control rats with dramatic increases of bone resorption markers, the Ocn and OS/BS at histopathological and histomorphometrical analysis in this study as compared with sham-operated control rats, suggesting the estrogen-deficient climacterium symptoms - obese and osteoporosis were induced by OVX, respectively. However, these estrogen-deficient climacterium symptoms induced by bilateral OVX in rats were significantly inhibited by 84 days of continuous oral treatment of GGOT 500, 250 and 125 mg/kg, respectively. Especially, GGOT 500, 250 and 125 mg/kg showed clear dose-dependent inhibitory activities on the OVX-induced climacterium signs.

Conclusion: The results suggest that oral administration of GGOT 500, 250 and 125 mg/kg has clear dose-dependent favorable anti-climacterium effects - estrogenic, anti-obese and anti-osteoporotic activities in OVX rats in this experiment.

Key Words: *Gagamguibiondam-tang*, Ovariectomize, Climacteric, Obesity, Osteoporosis

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Ⅰ. Introduction

Climacterium involve perimenopausal phases inculding premenopausal, menopausal and postmenopausal periods[1]. The climacteric corresponds to the period which women gradually loose their reproductive ability in consequence of aging[2]. During the climacteric period, nearly 70% of women complain some type of symptom. Normally, these symptoms are attributed to lack of estrogen. Estrogen deficiency may cause directly these metabolic diseases such as obesity, diabetes, heart disease and hypertension. And these metabolic diseases may also happen partly as secondary effects of obesity owing to the orexigenic effects of estrogen deficiency[3,4]. Also during this period, social and psychological stress increases for relationship trouble in family or work, loss of healthy confidence, recognition of aging. These factor can increase physical symptoms indirectly as well as mental symptoms such as depression, emotional instability[1].

Hormone treatment is often recommended to reduce the effect of ovarian failure on women's health. However, it has been raised serious fears regarding the safety and use of hormone replacement therapy in connection to breast cancer and cardiovascular events in long treatment[5]. Because of this reason, many researchers have searched alternative therapies, such as phytoestrogens to soften menopausal symptoms[2].

In Korean Medicine, climacterium is classified as several parts such as deficiency of the Kidney (腎虛), stagnation of Liver (肝鬱), disharmony between Heart and Kidney (心腎不交), insufficiency of both of the Heart and the Spleen (心脾兩虛)[6]. ≪Dongebogam≫ said that if worry and thinking do hurt Heart, so can't produce blood, Spleen is son of Heart, Spleen can't be reared, so do not eat much, cut the root of birth, cause menopause and menstrual irregularity[7]. This means that insufficiency of both of the Heart and the Spleen is one of the main causes related climacteric.

Gagamguibiondam-tang (GGOT, 加減歸脾溫膽湯) used in this study is combination of *Guibi-tang* and *Gami-ondam-tang*. GGOT can be used in case of forgetfulness, palpitation, easily scare when worry and thinking do hurt the Heart and the Spleen[7]. And GGOT has shown good effect in the clinic. For this reason, GGOT is chosen for this study.

Many studies reported about effect of GGOT. There are studies of the effect of GGOT in an animal model of depression by chronic mild stress[8], the effect of GGOT on immune reponse and in concentration of catecholamine in immobilization stressed rats[9]. However, study about effect of GGOT for climacteric syndrome and experimental study about anti-climacterium effects of GGOT was not found until now.

So this study was conducted to observe anti-climacterium effects of GGOT. In

this study, anti-climacteric effects were estimated at three categories as follows; 1) anti-obese, 2) anti-uterine atrophy and 3) anti-osteoporotic effects. Whereupon this experiment, I got good effectiveness, report this study.

Ⅱ. Materials and methods

1. Animals and husbandry

Whole one hundred healthy female SPF/VAF Crl: CD1 [Sprague-Dawley (SD)] rats (6-week old ; OrientBio, Seungnam, Korea; Body weight ranged in 120~150 g), were used after acclimatization for 7 days. Animals were distributed five rats per polycarbonate cage. Room was controlled as a humidity (50~55%) and temperature (20~25℃). Water were provided free to access. Light and dark cycle was one-on-one each 12 hr, and standard rodent chow (Samyang, Seoul, Korea). After 27 days after OVX operation, eight rats were chosen in each group based on the body weight deviations (Average 324.40±7.20 g of OVX rats, ranged in 315-338 g; Average 256.00±10.10 g of sham-operated rats, ranged in 239-273 g, respectively) as follows. According to the national regulations of the welfare and usage of laboratory animals, all laboratory animals were treated, and approved by the Institutional Animal Care and Use Committee in Daegu Haany University (Kyeongsan, Kyeongbuk, Korea) prior to animal experiment [Approval No DHU2015-028, March 27, 2015].

2. Preparation and administration of test materials

Aqueous extracts of GGOT as brown powders, were prepared by routine methods using programmable freeze dryer (FDB-5503, Operon, Kimpo, Korea) and rotary vacuum evaporator (N-1110, Eyela, Tokyo, Japan) from appropriated mixtures of 18 types of herbs(80 g) (Table 1), which were purchase from local voucher after confirm the morphology under microscopy (Jecheon Hanbang Yakcho, Jecheon, Korea). Total 800 g of GGOT were boiled in 2,000 ml of distilled water for 4 hrs and 3 times at 60℃, and evaporized using automated round flasked evaporator (Eyela N-1110, Tokyo, Japan), and then totally lyophilized. Total 176.24 g (yield=22.03%) of lyophilized aqueous GGOT extracts were acquired. GGOT extracts were kept at -20℃ in a refrigerator to defend from humidity and light until used. They were also stored at -20℃ in a refrigerator to preserve from degeneration and light until use. GGOT extracts were well suspended or disolved upto 100 mg/ml in distilled water, at least. From 28 days after OVX, GGOT 500, 250 and 125 mg/kg were orally performed, for 84 days once a day, respectively. Appropriated amounts of GGOT (100, 50 and 25 mg/ml concentrations) were directly suspended or dissolved into aqua pura, and performed in a volume of 5 ml/kg. In sham control and OVX rats, only distilled water as

vehicle, were orally provided as same volumes and periods, in place of herbal formulas, in this test (Table 2).

Table 1. Composition of GGOT

Korean name	Herbs	Scientific name	Amounts (g)
白茯苓	*Poria* (*Hoelen*)	*Poria cocos* (Schw.) Wolf	12
龍眼肉	*Longanae Arillus*	*Dimocarpus longan* Loureiro	8
當　歸	*Angelicae Gigantis Radix*	*Angelica gigas* Nakai	8
香附子	*Cyperi Rhizoma*	*Cyperus rotundus* Linne	6
陳　皮	*Citri Unshii Pericarpium*	*Citrus unshiu* Markovich	4
半　夏	*Pinelliae Tuber*	*Pinellia ternata* Breitenbach	4
枳　實	*Ponciri Fructus*	*Poncirus trifoliata* Rafinesque	4
酸棗仁 (炒)	*Zizyphi Spinosae Semen*	*Zizyphus jujuba* Mill	4
白　朮	*Atractylodis Rhizoma Alba*	*Atractylodes macrocephala* Koidzumi	4
柴　胡	*Bupleuri Radix*	*Bupleurum falcatum* Linne	4
升　麻	*Cimicifugae Rhizoma*	*Cimicifuga heracleifolia* Komarov	4
遠　志	*Polygalae Radix*	*Polygala tenuifolia* Willdenow	3
石菖蒲	*Acori Gramineri Rhizoma*	*Acorus gramineus* Solander	3
木　香	*Aucklandiae Radix*	*Aucklandia lappa* Decne	2
竹　茹	*Bambusae Caulis In Taeniam*	*Phyllostachys nigra* Munro var. *henonsis* Stapf	2
甘　草	*Glycyrrhizae Radix*	*Glycyrrhiza uralensis* Fischer	2
生　薑	*Zingiberis Rhizoma Crudus*	*Zingiber officinale* Roscoe	3
大　棗	*Zizyphi Fructus*	*Zizyphus jujuba* Miller var. *inermis* Rehder	3
Total	18 types		80

Table 2. Experimental Design

Groups	Operation	Group identification	Treatment
Anti-climacterium effects of GGOT on the OVX rat model			
Control	Sham surgery	Sham	Distilled water 5 ml/kg/day, *oral gavage*
Control	OVX	OVX	Distilled water 5 ml/kg/day, *oral gavage*
Active	OVX	GGOT500	GGOT 500 mg/kg/day, *oral gavage*
Active	OVX	GGOT250	GGOT 250 mg/kg/day, *oral gavage*
Active	OVX	GGOT125	GGOT 125 mg/kg/day, *oral gavage*

3. Climacterium inducement

Rats were narcotized with 2 to 3% isoflurane (Hana Pharm. Co., Hwasung, Korea) in the blend of 70% N_2O and 28.5% O_2, and were retained with 1 to 1.5% isoflurane in the blend of 70% N_2O

and 28.5% O_2. The surgical procedure was conducted according to our established methods[10] as follows. The OVX treatment group was performed open surgery accompanying bilateral OVX via a linea alba midline incision. Next, the incision was closed in two layers. The muscular layers were stitched up using dissolvable 3-0 vicryl sutures separately from peripheral tissues, and the skin sutured using silk 3-0 by continuous sutures. The second group of rats was performed a sham operation, in which a equal incision in the linea alba, but bilateral OVX were not operated.

4. Body weight measurements

Changes of body weight were taken using an automatic electronic balance (Precisa Instrument, Zuerich, Switzland) once a week, at least from at OVX, 1 day before administration, initiation of administration to sacrifice, respectively. At OVX, beginning of administration and at a ending, all animals were overnight fasted (no water about 18 hrs) to decrease the differences from feeding. In addition, body weight gains were computed as follow Equation [1].

EQUATION [1]. Body Weight Gains (g)
OVX recovery/induced periods (28 days)
= Body weight at 1 day before start of administration (27 days after OVX)-body weight at OVX
After administration (84 days) = Body weight at sacrifice-body weight at start of administration

5. Organ weight measurements

The left sides of abdominal fat pad located into dorsal abdominal wall, uterus including vagina located in abdominal cavity were gathered at sacrifice after separations of the surrounding muscles, connective tissues and any debris. The weights of organs were taken at g levels concerning absolute wet-weights. To decrease the individual body weight differences, the relative weights (% of body weight) were also measured using body weight at sacrifice and absolute wet-weight as follow Equation [2].

EQUATION [2]. Relative Organ Weights (% of body weight)
=[(Absolute abdominal fat pad, uterus weights/Body weight at sacrifice)×100]

6. Bone weight measurements

At end of 84 days continuous oral administration from 28 days after bilateral OVX surgery, the right sides of femur and tibia with L5 were gathered after removals of the surrounding muscles, connective tissues and any debris. The weight of bones was checked at g levels concerning absolute wet-weights, and they were dried for 8 hrs at 120℃ in high temperature dry oven (LDO-080N, Daihan Labtech Co., Seoul, Korea) for measurements dry bone weights. After that dried bones were carbonized at 800℃ for 6 hrs in furnace (LEF-1055-1, Daihan Labtech Co., Seoul, Korea) regarded as ash absolute weights. To decrease the several body weight differences, the relative

weight (%) was computed using body weight at sacrifice and absolute wet/dry/ash weight as follow Equation [3].

EQUATION 3. Relative Bone Weights (% of body weight)

=[(Absolute bone weight/Body weight at sacrifice)×100]

7. Serum biochemistry

For serum biochemistry, 10 ml of whole blood was gathered from *vena cava* at sacrifice, and extracted the serum by centrifugation at 15,000 rpm under 4℃ for 10 mins, using clotting activated serum tube. All serum samples were frozen at -150℃ using ultradeepfreezer (Model MDF1156, Sanyo, Tokyo, Japan) until they were assayed.

Serum osteocalcin levels were detected using Rat Osteocalcin ELISA Kit (Immutopics, San Clemente, CA, USA) at ng/ml levels, serum bALP levels were detected by Rat bALP ELISA kit (Quidel Corp., San Diego, CA, USA) at U/L levels a pg/ml with ELISA Reader (Tecan, Männedorf, Switzerland), respectively.

8. Measurement of BMD and FL

Total, epiphyseal and mid-shaft BMD of right femur and tibia were detected by dual-energy x-ray absorptionmetry (Norland pDEXA; Fort Atkinson, WI, USA) with total L5 regions. In addition, bone strength was observed as FL. FL of mid-shaft regions of right tibia and femur was observed using computerized testing machine (SV-H1000, Japan Instrumentation System Co., Japan) by a three-point bending test to failure as N (Newton).

9. Histological procedures for abdominal fat pads, uterus

After measuring of organ weights, left dorsal abdominal fat pads connected on the *muscularis quadratus lumborum*, left uterus horn was sampled and crossly trimmed. In 10% neutral buffered formalin (NBF), sampled tissues were fixed. 3-4 μm serial sections were prepared after paraffin embedding. With hematoxylin and eosin, delegate sections were stained for light microscopically examination. To observe more detail changes, total thickness of dorsal abdominal fat pads were measured using a computer-based automated image analysis process (iSolution FL ver 9.1, IMT i-solution Inc., Vancouver, Quebec, Canada) under microscopy (Model Eclipse 80i, Nikon, Tokyo, Japan) as mm/rat, and mean diameters of abdominal dorsal white adipocytes were computed in limited view fields on a computer monitor using an automated image analysis process as μm; at least 10 white adipocytes in each fat pad were considered as histomorphometrical analysis according to our previous established methods[10]. In addition, total (mm/uterus), mucosa (μm/uterus) and epithelial (μm/uterus) thicknesses of the uterus were also detected using an automated image analyzer with percentages of uterine glands set in the mucosa (%/mucosa of uterus), respectively[10]. When this analysis was made, the

histopathologist was blinds about group allocation.

10. Histological procedures for bones

The left side of femur and tibia with L4 of each rats were gathered and fixed in 10% NBF, and decalcified in decalcifying solution for 5 days. Mixed decalcifying solution (0.5 N sodium hydroxide and 24.4% formic acid) was exchanges once a day for 5 days. After that, embedded in paraffin, sectioned (3~4 μm) and stained with Safranin O stain. When this analysis was made, the histopathologist was blinds to group allocation. In addition, bone histomorphometry was performed using an automated image analysis process (iSolution FL ver 9.1, IMT i-solution Inc., Vancouver, Quebec, Canada) under microscopy (Model Eclipse 80i, Nikon, Tokyo, Japan) as for bone structure and mass with bone resorption in a uniform area of cortical or epiphyseal bone regions of femur, tibia or L4 (growth plate regions were excluded). Cortical bone thickness was measured in the mid-shaft regions of femur, tibia and L4. Length of trabecular bone (Tbl; mm/trabecular bone), thickness (Tbt; μm/trabecular bone), number (Tbn; mean numbers of trabecular bone/epiphyseal regions), trabecular bone volume (TV/BV, TBV; %) and cortical bone thickness (Cbt; μm/mid-shaft cortical bone) were checked for bone structure and mass, and ratio (OS/BS; %) and osteoclast cell number (Ocn; mean osteoclast cell numbers/epiphyseal regions) were taken for bone resorption as previous methods[10], respectively.

11. Statistical analyses

All Data was stated as mean± standard deviations (SD) of eight rats. And for different dose groups, multiple comparison tests were performed. Variance homogeneity was examined using the Levene test[11]. If the Levene test showed no meaningful deviations from variance homogeneity, the gained data were analyzed by one way ANOVA test followed by least-significant differences multi-comparison (LSD) test. If significant deviations from variance homogeneity were detected at Levene test, Kruskal-Wallis H test, a non-parametric comparison test was carried out. When a meaningful difference is detected in the Kruskal-Wallis H test, the Mann-Whitney U (MW) test was performed to discover the specific pairs of group comparison, which are meaningfully different. SPSS for Windows (Release 14.0K, IBM SPSS Inc., Armonk, NY, USA) were used for statistical analyses[12]. In addition, the percent changes between sham and OVX control rats were calculated and the percent changes as compared with OVX control and test material treated rats were also calculated to help the comprehension of the anti-climacterium effects of test substances as follow Equation [4] and [5], according to previous method described[13], respectively.

EQUATION [4]. Percent Changes in Comparison with Sham Control (%)

=[((Data of OVX control-Data of sham control rats)/Data of sham control rats) ×100]

EQUATION [5]. Percent Changes in Comparison with OVX Control (%)

=[((Data of test material treated rats -Data of OVX control)/Data of OVX control)×100]

Ⅲ. Results

1. Effects on body weight and gain

We selected eight rats in each group showing more increases of body weights as compared with sham-operated rats, and regarded as good OVX animals at 27 days after OVX surgery, meaningful ($p<0.01$) increases of body weights were observed in all OVX rats as compared with sham control rats from 27 days after OVX with significant ($p<0.01$) increases of body weight gains during 4 weeks of OVX recovery/induce periods, in this experiment. However, meaningful ($p<0.01$ or $p<0.05$) decreases of body weights were indicated in GGOT 500 mg/kg treated rats from 35 days after initial administration, and from 49 and 56 days after initial treatment in GGOT 250 and 125 mg/kg treated rats as contrasted with OVX control rats, respectively. In addition, during 84 days of administration periods all test substance treated rats showed meaningful ($p<0.01$) decreases of body weight gains as compared with OVX control, in this experiment (Fig. 1).

The body weight gains during 84 days of continuous oral administration periods in OVX control were changed as 69.77% as compared with sham control, but they were changed in GGOT 500, 250 and 125 mg/kg treated rats as -23.09, -17.67 and -15.73% as compared with OVX control, respectively.

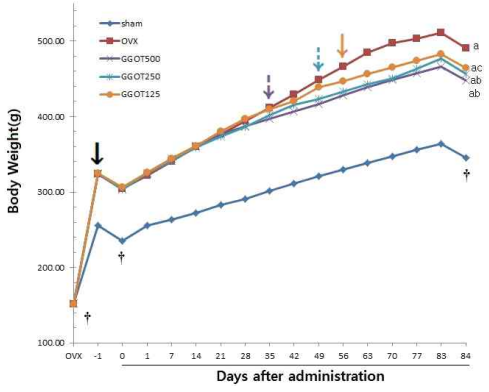

Fig. 1. Changes on the body weight after GGOD treatment.
All rats were overnight fasted (†).
27(↓) days after OVX
35(↓), 49(↓) and 56(↓) days after initial administration in GGOT500, GGOT250 and GGOT125
a : $p<0.01$ as compared with sham control by LSD test
b : $p<0.01$ and c : $p<0.05$ as compared with OVX control by LSD test
Treated rats as compared with OVX control rats

2. Effects on the abdominal fat pad weights

Meaningful ($p<0.01$) increases of abdominal fat pad located into left dorsal abdominal muscles, relative and absolute weights were detected in OVX control rats as contrasted with sham control rats, respectively. However, dose-dependent and meaningful ($p<0.01$) decreases of

abdominal fat pad weights were detected in GGOT 500, 250 and 125 mg/kg treated rats in this experiment (Table 3, Fig. 2).

The abdominal fat pad deposited into left dorsal abdominal muscle absolute and relative weights in OVX control were changed as 389.01 and 240.29% as compared with sham control, and they were altered as -63.49, -46.52 and -30.83% of absolute weights, and -59.91, -42.34 and -26.83% of relative weights in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

Table 3. Changes on the Abdominal Fat Pad Weights

Items / Groups	Absolute wet-weight (g)	Relative wet-weight (% of body weight)
	Abdominal fats	
Sham	3.115±0.787	0.912±0.265
OVX	15.234±1.231[a]	3.104±0.229[a]
GGOT500	5.563±0.524[ab]	1.244±0.128[b]
GGOT250	8.148±1.027[ab]	1.790±0.272[ab]
GGOT125	10.538±1.256[ab]	2.271±0.246[ab]

a : $p<0.01$ as compared with sham control by MW test
b : $p<0.01$ as compared with OVX control by MW test

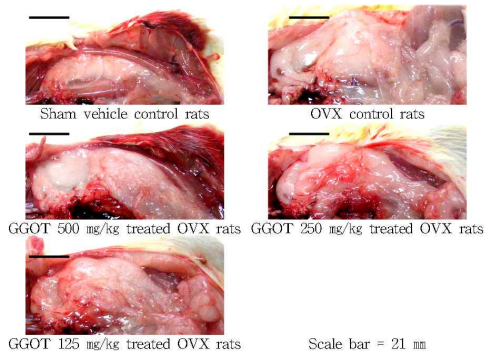

Fig. 2. Representative gross images of the abdominal fat pads, taken from intact or OVX rats located into left abdominal muscles.

3. Effects on the uterus weights

Meaningful ($p<0.01$) decreases of the uterus relative and absolute wet-weights were noticed in OVX control rats as contrasted with sham control rats, respectively. However, meaningful ($p<0.01$) increases of the uterus weights were observed in all test substance treated rats as compared with OVX control rats, respectively (Table 4, Fig. 3).

The uterus absolute and relative wet-weights in OVX control were changed as -88.84 and -92.11% as compared with sham control, and they were altered as 94.83, 47.33 and 24.17% of absolute weights, and 113.64, 58.62 and 31.42% points of relative weights in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

Table 4. Changes on the Uterus Weights

Items / Groups	Absolute wet-weight (g)	Relative wet-weight (% of body weight)
	Uterus	
Sham	0.672±0.208	0.193±0.056[a]
OVX	0.075±0.012[a]	0.015±0.002[a]
GGOT500	0.146±0.028[ab]	0.033±0.006[ab]
GGOT250	0.111±0.010[ab]	0.024±0.002[ab]
GGOT125	0.093±0.011[ac]	0.020±0.002[ab]

a : $p<0.01$ as compared with sham control by MW test
b : $p<0.01$ and c: $p<0.05$ as compared with OVX control by MW test

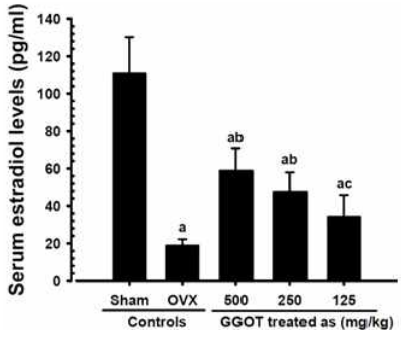

Fig. 3. Representative gross images of the uterus, taken from intact or OVX rats.

4. Effects on the serum Estradiol

Meaningful ($p<0.01$) decreases of the serum estradiol levels in OVX control rats as contrasted with sham control rats, respectively. However, significant ($p<0.01$ or $p<0.05$) increases of the serum estradiol levels were demonstrated in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control rats, in the present study (Fig. 4).

The serum estradiol levels in OVX control were changed as -82.98% as compared with sham control, and they were altered in GGOT 500, 250 and 125 mg/kg treated rats as 211.26, 151.66 and 80.79% as compared with OVX control, respectively.

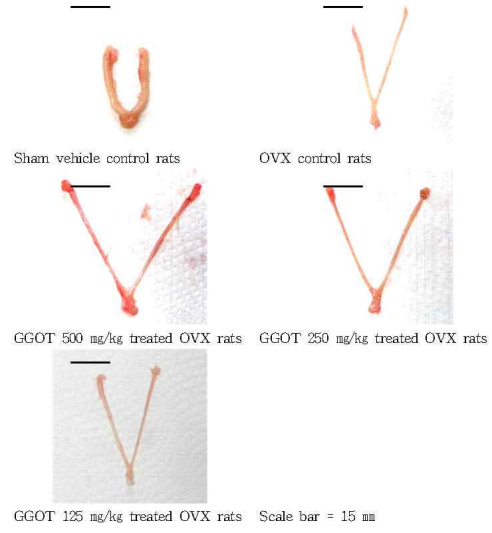

Fig. 4. Changes on the serum estradiol levels.
a : $p<0.01$ as compared with sham control by LSD test
b : $p<0.01$ and c : $p<0.05$ as compared with OVX control by LSD test

5. Effects on the serum Osteocalcin and bALP

Significant ($p<0.01$) increases of the serum osteocalcin levels, and decreases of serum bALP levels in OVX control rats were observed as compared with sham control rats, respectively. However, significant ($p<0.01$) decreases of the serum osteocalcin and increases of bALP levels were demonstrated in all test herbal formula treated rats including GGOT 125 mg/kg as compared with OVX control rats, in this experiment (Fig. 5, 6).

The serum osteocalcin and bALP levels in OVX control were changed as 75.35 and -55.88% as compared with sham control, and they were altered as -21.39, -31.59, -22.76 and -16.85% of osteocalcin levels, and 43.33, 81.18, 45.01 and 29.14%

of bALP levels in RC 40 mg/kg, GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

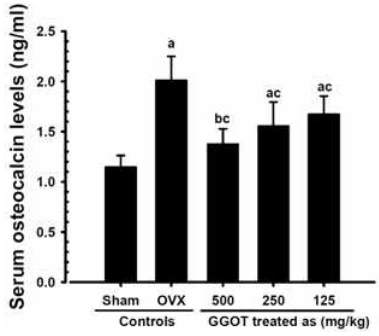

Fig. 5. Changes on the serum osteocalcin levels.
a : $p<0.01$ and b : $p<0.05$ as compared with sham control by LSD test
c : $p<0.01$ as compared with OVX control by LSD test

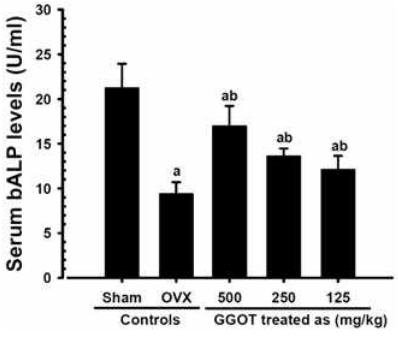

Fig. 6. Changes on the serum bALP levels.
a : $p<0.01$ as compared with sham control by MW test
b : $p<0.01$ as compared with OVX control by MW test

6. Effects on BMD

The BMD in all seven detecting points, the total, epiphyseal and mid-shaft of femur and tibia, total L5 regions of OVX control rats were significantly ($p<0.01$) decreased as compared with sham control. However, significant ($p<0.01$) increases of BMD in all measured regions were detected in all test substance administrated rats as compared with OVX control rats, in the current experiment (Table 5).

The femur total, neck and mid-shaft BMD in OVX control were changed as -24.94, -24.31 and -22.15% as compared with sham control, and they were altered as 16.88, 9.52 and 7.47% of BMD in total regions, 22.59, 14.30 and 9.53% in neck regions, and 18.84, 11.38 and 9.48% in mid-shaft regions in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The tibia total, neck and mid-shaft BMD in OVX control were changed as -25.15, -23.69 and -33.58% as compared with sham control, and they were altered as 23.59, 13.23 and 9.55% of BMD in total regions, 20.51, 12.97 and 10.42% in neck regions, and 38.62, 25.90 and 17.27% in mid-shaft regions in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The total BMD of L5 in OVX control were changed as -25.17% as compared with sham control, and they were altered in GGOT 500, 250 and 125 mg/kg treated rats as 21.47, 12.06 and 9.07% as compared with OVX control, respectively.

Table 5. Changes on the Bone Mineral Density of Right Femur and Tibia with L5

Items / Groups	Controls		GGOT		
	Sham	OVX	500 mg/kg	250 mg/kg	125 mg/kg
Femur					
Total	0.154±0.012	0.116±0.005[a]	0.135±0.011[ac]	0.127±0.007[ad]	0.124±0.006[ad]
Neck	0.159±0.011	0.121±0.005[a]	0.148±0.008[ac]	0.138±0.007[ac]	0.132±0.008[ac]
Mid-shaft	0.127±0.006	0.099±0.007[a]	0.118±0.004[ac]	0.110±0.005[ac]	0.108±0.005[ac]
Tibia					
Total	0.145±0.007	0.109±0.008[a]	0.134±0.007[ac]	0.123±0.008[ac]	0.119±0.005[ac]
Neck	0.148±0.009	0.113±0.005[a]	0.136±0.006[ac]	0.127±0.007[ac]	0.125±0.007[ac]
Mid-shaft	0.120±0.008	0.080±0.008[a]	0.110±0.008[bc]	0.100±0.012[ac]	0.093±0.008[ac]
L5-Total	0.146±0.010	0.109±0.008[a]	0.132±0.007[ac]	0.122±0.006[ac]	0.119±0.003[ac]

a : p<0.01 and b : p<0.05 as compared with sham control by LSD test
c : p<0.01 and d : p<0.05 as compared with OVX control by LSD test

7. Effects on FL

The strengths (FL) of femur and tibia mid-shaft regions in OVX control rats were significantly (p<0.01) decreased as compared with sham control rats, respectively. However, significant (p<0.01 or p<0.05) increases of FL on the both femur and tibia were detected in all test substance administrated rats including three different dosages of GGOT as compared with OVX control rats, in the current study (Fig. 7, 8).

The mid-shaft FL of femur and tibia in OVX control were changed as -66.63 and -59.85% as compared with sham control, and they were altered as 130.18, 86.53 and 57.65% of femur FL, and 88.66, 52.94 and 37.01% of tibia FL in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

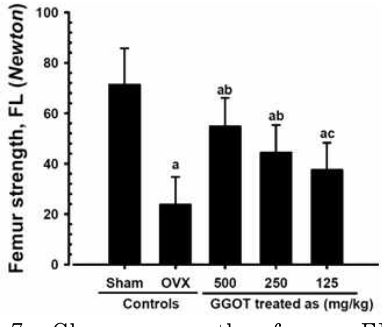

Fig. 7. Changes on the femur FL in OVX rats.
a : p<0.01 as compared with sham control by LSD test
b : p<0.01 and c : p<0.05 as compared with OVX control by LSD test

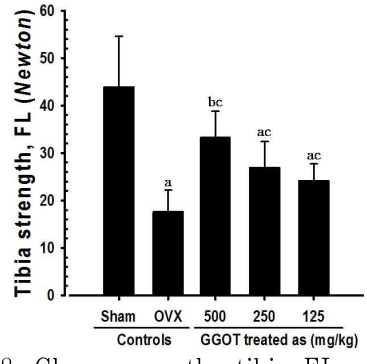

Fig. 8. Changes on the tibia FL.
a : p<0.01 and b : p<0.05 as compared with sham control by MW test
c : p<0.01 as compare d with OVX control by MW test

8. Histopathology: Abdominal fat pad, uterus

We performed the general histopathological profiles and histomorphometrical analysis of abdominal fat pads for anti-obese effects of test substances, of uterus for estrogenic effects, respectively.

1) Abdominal fat pads

Significant ($p<0.01$) increases of the thickness of abdominal fat pads deposited into left abdominal muscles, and also increases of mean adipocyte diameters were noticed in OVX control rats, due to remarkable deposition of adipose tissues on the abdominal cavity and their hypertrophy of adipocytes, respectively. However, meaningful ($p<0.01$) decreases of mean diameters of adipocytes and the thickness of abdominal fat pads were observed in all test substance administrated rats as contrasted with OVX control rats, in the present result (Table 6, Fig. 9).

The thickness of abdominal fat pads and mean adipocyte diameters in OVX control were changed as 126.33 and 81.12% as compared with sham control, and they were altered as -45.41, -30.79 and -15.06% of thickness of abdominal fat pads, and -39.16, -29.12 and -22.65% of mean adipocyte diameters in GGOT 500, 250 and 125 mg/kg treated rats treated rats as compared with OVX control, respectively.

Table 6. Changes on the Histopathology-Histomorphometry for Abdomianl Fat Pads, Uterus

Items / Groups	Controls		GGOT		
	Sham	OVX	500 mg/kg	250 mg/kg	125 mg/kg
Fat pads					
Total Th (mm)	4.41±0.88	9.97±1.08[a]	5.44±0.88[bc]	6.90±0.72[ac]	8.47±0.66[ac]
Adipocyte DM (μm)	86.09±15.84	155.93±13.21[a]	94.87±18.80[c]	110.52±18.12[ac]	120.61±13.20[ac]
Uterus					
Total Th (mm)	2.49±0.76	0.56±0.09[d]	0.89±0.35[df]	0.85±0.08[de]	0.71±0.11[df]
Epi Th (μm)	37.68±4.36	9.38±1.89[d]	25.42±4.54[de]	16.87±2.18[de]	14.67±3.23[de]
Mucosa Th (μm)	969.03±164.33	156.52±39.84[d]	357.17±49.51[de]	301.90±33.37[de]	269.93±35.02[de]
UG percentage (%)	31.71±10.04	5.20±1.40[d]	15.52±2.83[de]	13.74±3.29[de]	9.86±1.41[de]

a : $p<0.01$ and b : $p<0.05$ as compared with sham control by LSD test
c : $p<0.01$ as compared with OVX control by LSD test
d : $p<0.01$ as compared with sham control by MW test
e : $p<0.01$ and f : $p<0.05$ as compared with OVX control by MW test

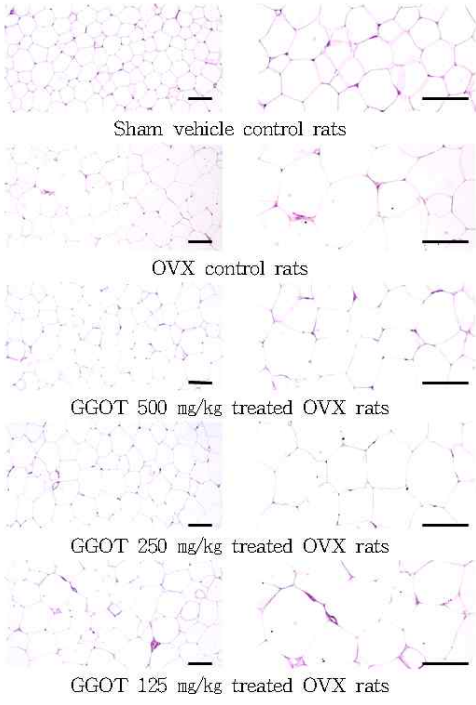

Fig. 9. Representative histological images of the adipocytes, taken from sham-operated or OVX rat abdominal fat pads located into left dorsal abdominal muscles.
All hematoxylin-eosin stain
Scale bars = 120 μm

2) Uterus

Significant ($p<0.01$) decreases of the total thickness, mucosa and epithelial thicknesses of the uterus, and of the percentages of uterine glands in the mucosa were demonstrated in OVX control rats, due to estrogen-depletion related disused atrophic changes, respectively. However, meaningful ($p<0.01$ or $p<0.05$) increases of the total thickness, mucosa and epithelial thicknesses of the uterus, and of the percentages of uterine glands in the mucosa were detected in all treated rats as compared with OVX control rats, in the current result (Table 6, Fig. 10).

The total and epithelial thickness of the uterus in OVX control were changed as -77.56 and -75.10% as compared with sham control, and they were altered as 58.84, 52.13 and 27.07% of total thickness, and 170.95, 79.79 and 56.36% of epithelial thickness in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The mucosa thickness and uterine gland occupied regions of the uterus in OVX control were changed as -83.85 and -83.60% as compared with sham control, and they were altered as 128.19, 92.88 and 72.45% of mucosa thickness, and 198.44, 164.22 and 89.62% of uterine gland occupied regions in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

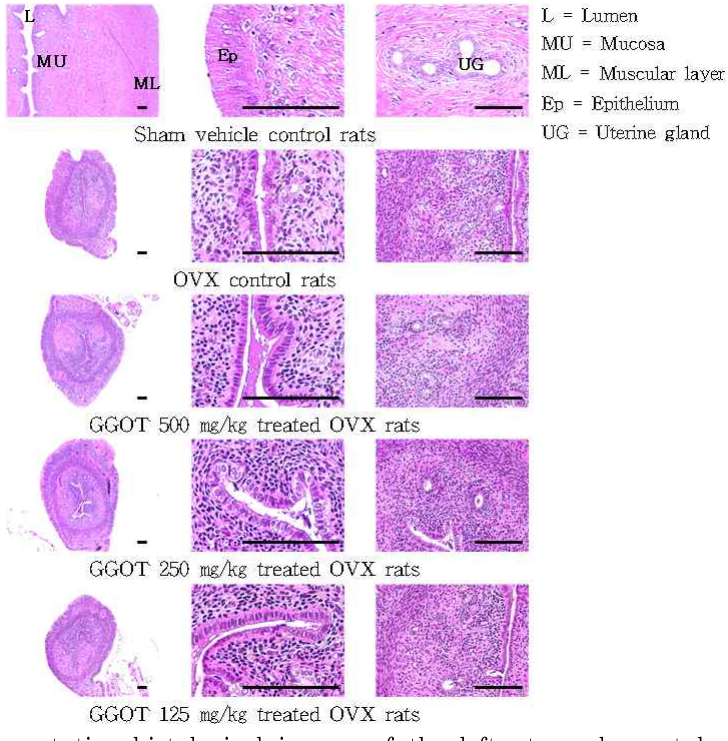

Fig. 10. Representative histological images of the left uterus horn, taken from sham-operated or OVX rats.
All hematoxylin-eosin stain
Scale bars = 120 μm

9. Histopathology

1) Femur, tibia and L4

Although comparatively well-developed cortical and trabecular bone were detected in L4, femur and tibia of sham control rats, classical osteoporotic histological profiles were indicated in OVX control rats as impressive decreases of cortical and trabecular bone masses, increase of connective tissues in periosteum of cortical bone results from resorption of osteoid tissues associated to osteocalst activations, in this experiment. However, impressive increases of the bone structures and mass, the both cortical and trabecular bones were observed in all test substance administered rats including GGOT 500 mg/kg as compared with OVX control rats, associated to their inhibitory activities on osteoclast cell activities, in this experiment (Table 7-9, Fig. 11-13).

2) Bone mass and structures

Significant ($p<0.01$) decrease of Tbn, Tbt, Tbl, TV/BV and Cbt were detected in OVX control rats as compared with sham-operated control rats in the femur, tibia and L4, respectively. However, these decreases of bone mass and structures were meaningfully ($p<0.01$ or $p<0.05$) inhibited by treatment of all test herbal

formulas as compared with OVX control rats, in this study (Table 7-9, Fig. 11-13).

The TV/BV and Cbt of the femur in OVX control were changed as -68.99 and -23.81% as compared with sham control, and they were altered as 142.36, 59.68 and 28.08% of TV/BV, and 16.53, 9.23 and 7.33% of Cbt in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The TV/BV and Cbt of the tibia in OVX control were changed as -70.85 and -50.72% as compared with sham control, and they were altered as 147.18, 108.70 and 82.92% of TV/BV, and 38.92, 25.91 and 21.36% of Cbt in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The TV/BV and Cbt of the L4 in OVX control were changed as -42.06 and -43.25% as compared with sham control, and they were altered as 52.55, 38.42 and 28.21% of TV/BV, and 68.70, 55.36 and 36.58% of Cbt in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

3) Bone resorption

Significant ($p<0.01$) increases of Ocn and OS/BS were detected in OVX control rats as compared with sham control rats in the femur, tibia and L4. However, these activation and increase of osteoclast cells were dramatically inhibited by treatment of all test substances as compared with OVX control rats, in the present study (Table 7-9, Fig. 11-13).

The Ocn and OS/BS of the femur in OVX control were changed as 210.14 and 256.48% as compared with sham control, and they were altered as -52.80, -42.99 and -24.77% of Ocn, and -63.12, -43.27 and -26.08% of OS/BS in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The Ocn and OS/BS of the tibia in OVX control were changed as 113.91 and 362.29% as compared with sham control, and they were altered as -39.84, -23.17 and -19.51% of Ocn, and -67.55, -47.75 and -28.11% of OS/BS in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

The Ocn and OS/BS of the L4 in OVX control were changed as 147.06 and 314.69% as compared with sham control, and they were changed as -47.02, -30.36 and -18.45% of Ocn, and -67.50, -32.73 and -14.41% of OS/BS in GGOT 500, 250 and 125 mg/kg treated rats as compared with OVX control, respectively.

Table 7. Changes on the Histopathology-Histomorphometry for Bone Mass and Resorption of Left Femur

Items / Groups	Controls		GGOT		
	Sham	OVX	500 mg/kg	250 mg/kg	125 mg/kg
TBV, BV/TV	49.26±10.16	15.28±2.62^{c}	37.03±11.01^{e}	24.40±3.80^{ce}	19.57±1.63^{ce}
Tbn	26.88±10.27	11.00±2.78^{c}	20.13±3.56^{e}	15.88±3.44^{cf}	14.50±1.60^{ce}
Tbl	6.56±1.82	1.82±0.33^{c}	4.60±0.57^{de}	3.78±0.69^{ce}	3.22±0.39^{ce}
Tbt	155.21±16.34	93.01±10.22^{a}	136.91±9.13^{ab}	133.55±12.31^{ab}	111.02±10.26^{ab}
Cbt-shaft	883.75±121.32	673.36±42.40^{c}	784.69±22.28^{de}	735.54±18.10^{ce}	722.73±17.38^{ce}
Ocn	8.63±2.13	26.75±4.46^{a}	12.63±2.67^{ab}	15.25±2.12^{ab}	20.13±2.30^{ab}
OS/BS	4.56±1.12	16.27±3.40^{c}	6.00±1.19^{de}	9.23±1.29^{ce}	12.03±1.22^{ce}

a : $p<0.01$ as compared with sham control by LSD test
b : $p<0.01$ as compared with OVX control by LSD test
c : $p<0.01$ and d : $p<0.05$ as compared with sham control by MW test
e : $p<0.01$ and f : $p<0.05$ as compared with OVX control by MW test

Table 8. Changes on the Histopathology-Histomorphometry for Bone Mass and Resorption of Left Tibia

Items / Groups	Controls		GGOT		
	Sham	OVX	500 mg/kg	250 mg/kg	125 mg/kg
TBV, BV/TV	48.15±10.32	14.04±3.46^{a}	34.70±6.95^{ac}	29.29±4.57^{ac}	25.68±6.02^{ac}
Tbn	35.88±6.03	10.63±1.92^{d}	21.75±4.53^{de}	16.63±1.69^{de}	13.75±2.25^{df}
Tbl	7.27±1.01	2.47±0.68^{a}	4.18±0.82^{ac}	3.96±0.59^{ac}	3.53±0.38^{ac}
Tbt	123.33±11.83	75.85±4.99^{a}	116.21±11.34^{c}	106.24±7.81^{ac}	101.84±13.23^{ac}
Cbt-shaft	838.44±53.00	413.19±74.20^{a}	574.00±46.61^{ac}	520.26±39.44^{ac}	501.46±46.32^{ac}
Ocn	14.38±2.92	30.75±3.65^{a}	18.50±1.77^{bc}	23.63±4.00^{ac}	24.75±4.37^{ac}
OS/BS	6.15±0.86	28.41±4.77^{d}	9.22±0.94^{de}	14.84±3.34^{de}	20.42±1.64^{de}

a : $p<0.01$ and b : $p<0.05$ as compared with sham control by LSD test
c : $p<0.01$ as compared with OVX control by LSD test
d : $p<0.01$ as compared with sham control by MW test
e : $p<0.01$ and f : $p<0.05$ as compared with OVX control by MW test

Table 9. Changes on the Histopathology-Histomorphometry for Bone Mass and Resorption of L4

Items / Groups	Controls		GGOT		
	Sham	OVX	500 mg/kg	250 mg/kg	125 mg/kg
TBV, BV/TV	53.00±10.18	30.70±5.46^{d}	46.84±10.02^{e}	42.50±8.00^{f}	39.36±5.14^{df}
Tbn	25.38±4.75	10.75±1.67^{d}	22.50±3.25^{e}	14.88±2.03^{de}	14.13±2.23^{de}
Tbl	4.33±0.39	2.27±0.41^{a}	3.97±0.60^{c}	3.18±0.60^{ac}	2.99±0.21^{ac}
Tbt	148.77±7.93	99.03±13.45^{a}	144.77±15.73^{c}	136.31±7.92^{c}	121.78±15.85^{ac}
Cbt-shaft	244.35±46.82	138.68±18.31^{a}	233.94±33.90^{c}	215.45±21.00^{c}	189.40±25.65^{ac}
Ocn	8.50±2.07	21.00±2.78^{a}	11.13±2.36^{bc}	14.63±1.85^{ac}	17.13±2.03^{ac}
OS/BS	5.06±1.22	20.99±2.84^{a}	6.82±1.29^{c}	14.12±2.48^{ac}	17.97±1.84^{ac}

a : $p<0.01$ and b : $p<0.05$ as compared with sham control by LSD test
c : $p<0.01$ as compared with OVX control by LSD test
d : $p<0.01$ as compared with sham control by MW test
e : $p<0.01$ and f : $p<0.05$ as compared with OVX control by MW test

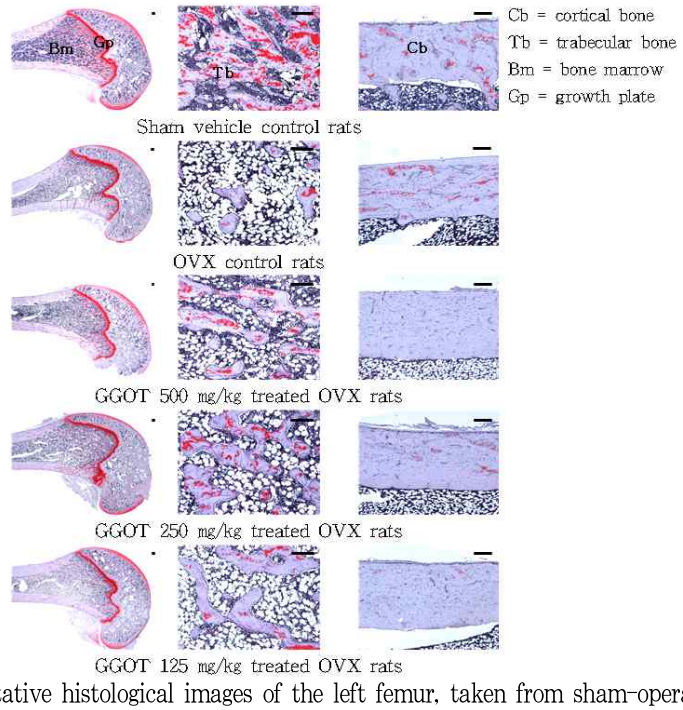

Fig. 11. Representative histological images of the left femur, taken from sham-operated or OVX rats. All Safranin O stain
Scale bars = 240 μm

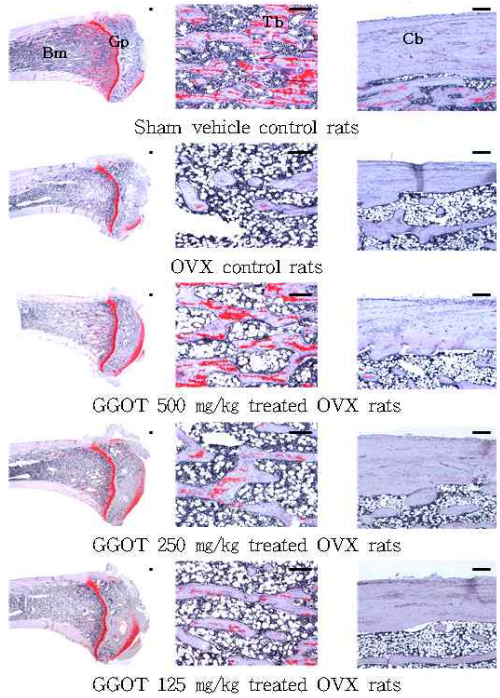

Fig. 12. Representative histological images of the left tibia, taken from sham-operated or OVX rats. All Safranin O stain
Scale bars = 240 μm

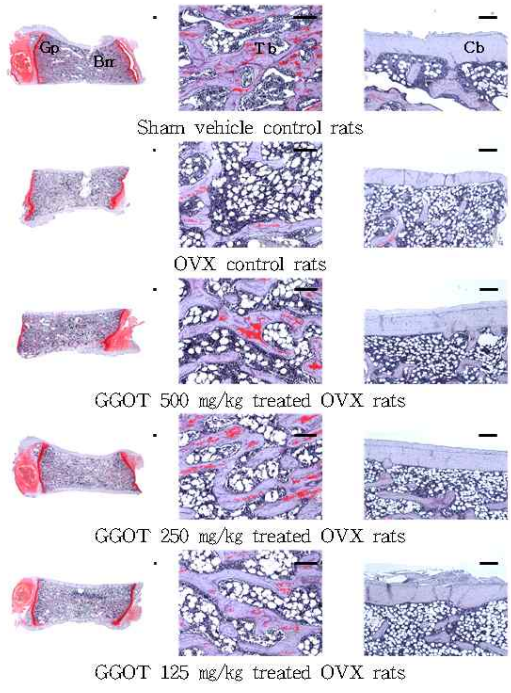

Fig. 13. Representative histological images of the L4, taken from sham-operated or OVX rats. All Safranin O stain
Scale bars = 240 μm

Ⅳ. Discussion

Climacteric symptoms referred to multi-abnormal conditions related to estrogen deprivation during postmenopausal periods, including cognitive impairment, insomnia, depression, irritability, fatigue, psychological symptoms, and increased risk for osteoporosis and cardiovascular disease[14], and the climacteric state is related with an increased risk of metabolic diseases such as heart disease, diabetes, obesity and hypertension[15].

Hormone therapy is often recommended to treat several climacteric symptoms. However, hormone therapy cause many side effects, when used in short-term as well as long treatment. Short-term hormone therapy is able to cause these side effects such as mammalgia, weight gain, similar premenstrual-sydrome symptoms, migraine, nausea, abdomianl distension, ophthalmoxerosis, mouth dryness. Also long-term hormone therapy can cause breast cancer, endometrial cancer and cardiovascular disorders[5].

GGOT is a novel aqueous polyherbal formula as consisted of 18 herbs, combined famous traditional aqueous polyherbal formulas - *Guibi-tang* and *Gami-ondam -tang*, which have been used for several hundred years, mosttly to treat various symptoms on obstetrics and gynecological fields[16,17]. In addition, the composition of herbs were modified to maximization

the anti-climacterium effects, as ginseng radix, astragali radix was excepted and cimicifugae rhizoma, acori gramineri rhizoma was added. *Guibi-tang* has been used for centuried years, mainly to treat amnesia, insomnia, anxiety, palpitations, fatigue, poor appetite, and depression[16]. Up to date studies have reported the precise bioactivities of *Guibi-tang*, which include immune regulation[18], antioxidant effects[19], anticancer activities[20] and protective effect of the gastric mucosa[21]. *Gami-ondam-tang* has been traditionally used for the alleviation of various neuropsychiatric disorders including neurosis, insomnia and climacterium related neural disorders in traditional medicine[17]. It has been reported that oral administration of *Gami-ondam-tang* improves cognitive function in aged rats through the increase of cholineacetyltransferase expression in the basal forebrain[22]. Others also observed that *Gami-ondam-tang* prevents depressive behavior in thiamine-deficient mice and this may be closely related to the activation of cholinergic functions in the hippocampus[23]. Moreover, it also recently reported that the subchronic administration of *Gami-ondam-tang* for 14 days improved cognitive performance in normal naive mice through the enhancement of neurogenesis and proteinkinase B - cAMP response element-binding protein-brain-derived neurotrophic factor signaling in the hippocampus[24,25].

Estradiol has been shown to control eating and body weight mainly via modulating the potency of feedback signals that control meal size[26]. It is a well-established phenomenon that the absence of estradiol leads to a temporary increase in eating and a sustained increase in body weight[26,27]. This phenomenon is of clinical relevance because estradiol levels decrease in postmenopausal women; importantly, postmenopausal women make up a high percentage of the obese population[28]. In this study, OVX induced significant increases of body weight and gains, increases of abdominal fat depositions with adipocyte hypertrophy. However, these estrogen-deficiencies related obese was dramatically inhibited by treatment of all three different dosages of GGOT dose-dependently, suggesting possible anti-obese effects of GGOT, may be mediated the enhancement activity on the digestive tract motility or diuretic effects.

Estrogens play a vital role in grow the function and regulation in numerous female target organs such as vagian, uterus and skeletal and cardiovascular systems[29]. Estrogen depletion is accompanied with a marked atrophy of organs such as vagina and uterus[30]. In addition, OVX-induced estrogen deficiency induced severe atrophic changes on the uterus[30]. Our results showed that OVX induced a significant decrease in uterine weights with marked decreases of serum estradiol levels and related uterine atrophic changes, the decreases of total thickness, mucosa and epithelial thicknesses, and uterine glands in the mucosa. However, these estrogen-deficient uterine atrophy in rats induced

by bilateral OVX were significantly inhibited by 84 days of continuous oral treatment of GGOT 500, 250 and 125 mg/kg with obvious dose-dependent, in the current result. These results are considered as direct evidences that GGOT has favorable estrogenic effects in OVX rats, at least in a condition of this experiment. The increase of uterine mass is believed to involve the mechanism of the protective effects of GGOT against OVX-induced uterine atrophy. However, more intimate mechanism should be researched in future.

Osteoporosis is a metabolic bone disease, which arises from a disorder in the normal bone remodeling, tilting the balance to bone resorption over formation. Osteoporosis is due to an imbalance between bone formation and bone resorption, which results in bone fractures and loss after mineral flux[31]. Until now, osteoporosis has been believed to be related with estrogen-deficiency, and estrogen-deficient OVX osteoporosis animal models have been treated as useful animal model for evaluation of osteoporotic drugs, because several parameters are clearly decreased by ovariectomy within 4 to 6 weeks after operation, as summarized by other investigators[32]. The increases trends of bone weights have been considered as a valuable markers of anti-osteoporotic activities[33]. Serum osteocalcin levels were generally accepted as a marker of bone turnover, and bALP level was generally accepted as serum markers of bone formation[34]. As progression of OVX related osteoporosis, serum osteocalicin levels were generally increased along the increases of bone turn over, but serum bALP contents were dramatically decreased along inhibition of bone formations[34]. BMD of bone provided good predictable information about efficacy of anti-osteoporotic agents[35]. BMD and bone strengths were markedly decreased in osteoporosis regardless of causes[35]. A microscopic observation of bone informed good information about bone morphology[36]. In osteoporotic animals, the histological profiles were clearly changed as compared with sham control regardless of the cause, especially on the cortical and trabecular bone, and the efficacy of various anti-osteoporosis agents have been evaluated on the histology of bones[37]. In other words, some histomorphometrical indices for bone mass and bone formations are clearly decreased but histomorphometrical indices for bone resorption are increased, and they informed trustworthy information to predict the efficacy of anti-osteoporotic agents[37]. In the present study, noticeable increases of osteocalcin level was demonstrated in OVX control rats with decrease of femur, tibia and L5 weights and serum bALP levels. In addition, marked decreases of bone mass and structures of femur, tibia and L4 were also observed in OVX control rats with dramatic increases of bone resorption markers, the Ocn and OS/BS at histopathological and histomorphometrical analysis in this study as compared with sham-operated control rats, suggesting

the estrogen-deficient osteoporosis was also induced by OVX. However, these estrogen-deficient osteoporosis induced by bilateral OVX in rats were significantly inhibited by 84 days of continuous oral treatment of all three different dosages of GGOT, respectively. Especially, GGOT showed clear dose-dependent anti-osteoporotic activities.

As a result of OVX, noticeable increases of body weight and gains, weights of abdominal fat pad deposited in dorsal abdominal cavity, osteocalcin levels were demonstrated in this experiment with decrease of uterus, femur, tibia and L5 weights, serum bALP and estradiol levels. In addition, marked hypertrophic changes of adipocytes located in deposited abdominal fat pads, uterine disused atrophic changes, decreases of bone mass and structures of femur, tibia and L4 were also observed in OVX control rats with dramatic increases of bone resorption markers, the Ocn and OS/BS at histopathological and histomorphometrical analysis in this study as compared with sham-operated control rats, suggesting the estrogen-deficient climacterium symptoms - obese, osteoporosis were induced by OVX, respectively. However, these estrogen-deficient climacterium symptoms induced by bilateral OVX in rats were significantly inhibited by 84 days of continuous oral treatment of GGOT 500, 250 and 125 mg/kg, respectively.

As a result of this study, GGOT showed clear dose-dependent activities for three categories as follows: anti-obese, anti-uterine atrophy and anti-osteoporotic effects. Therefore, it is expected that GGOT will be promising as a novel alternative agents for relieving the climacterium symptoms, especially on obese, uterine atrophy and osteoporosis in menopausal women. And GGOT consisted of 18 herbs and each herb has various active ingredients, the screening of the biological active compounds should be conducted in future with more detail mechanism studies.

V. Conclusion

After experiment this study to evaluate anti-climacterium effects of GGOT in OVX rats, obtained the following conclusions.

1. Anti-obese: OVX induced significant increases of body weight and gains, abdominal fat depositions with adipocyte hypertrophy. However, these estrogen-deficiencies related obese was significantly inhibited by treatment of all three different dosages of GGOT dose-dependently.
2. Anti-uterine atrophy: OVX induced a significant decrease in uterine weights with marked decreases of serum estradiol levels and related uterine atrophic changes, the decreases of total thickness, mucosa and epithelial thicknesses, and uterine glands in the mucosa. However, these estrogen-deficient uterine atrophy were significantly inhibited

by treatment of GGOT with obvious dose-dependence.

3. Anti-osteoporotic effects: OVX induced significant increases of serum osteocalcin level, bone resorption markers with decreases of serum bALP level, bone weights, BMD, bone strengths, bone mass and bone structures markers. However, these OVX-induced estrogen-deficient osteoporosis in rats were significantly inhibited by treatment of all three different dosages of GGOT with clear dose-dependent anti-osteoporotic activities.

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국문초록

목 적: 이 연구에서는 한의학에서 다양한 부인과 질환에 사용되어온 전통 복합처방인 귀비탕과 가미온담탕을 합방, 가감한 가감귀비온담탕 열수 추출물의 갱년기 장애 개선 효과를 심혈관 장애, 비만, 고지혈증, 골다공증, 장기 지방축적 및 신경정신 장애를 포함한 다양한 사람의 갱년기 장애를 나타내는 것으로 알려진 난소적출(Ovariectomized, OVX) 랫트 모델을 이용하여 평가하였다.

방 법: 난소적출수술 28일 후부터 가감귀비온담탕 추출물(수율 : 22.03%)을 각각 100, 50 및 25 mg/ml의 농도로 멸균 증류수에 용해시키고, 체중 kg 당 5 ml의 용량(500, 250 및 125 mg/kg)으로 매일 1회씩 84일(12주 : 3개월)간 경구 투여한 다음, 항비만효과, 항자궁위축효과 및 골다공증 억제 효과의 3가지 생리활성 효과로 구분하여 평가하였다. 항비만 효과 및 항자궁위축효과를 평가하기 위해, 체중 및 증체량, 혈청중 에스트라디올 함량, 복부 축적 지방 및 자궁의 중량의 변화와 복부 축적 지방의 두께 및 평균 지방세포 직경, 자궁 전체, 상피 및 점막의 두께와 점막내 자궁샘이 차지하는 비율의 변화를 각각 평가하였다. 또한 골다공증 개선 효과, 즉 골 보호효과를 평가하기 위해, 대퇴골, 경골 및 요추골의 습, 건조 및 탄화 중량, 골밀도, 골강도, 혈중 osteocalcin 및 bone specific alkaline phosphatase(bALP) 함량, 골량 및 구조와 골흡수에 대한 조직병리학적 변화를 각각 측정하였다.

실험군(5개군; 군당 8마리의 랫트 사용)
거짓수술 대조군(거짓수술 후, 증류수 투여 대조군)
난소적출 대조군(난소적출 수술 후, 증류수 투여 대조군)
GGOT500(난소적출 수술 후, 가감귀비온담탕 추출물 500 mg/kg 투여 고용량 실험군)
GGOT250(난소적출 수술 후, 가감귀비온담탕 추출물 250 mg/kg 투여 중간용량 실험군)
GGOT125(난소적출 수술 후, 가감귀비온담탕 추출물 125 mg/kg 투여 저용량 실험군)

결 과: 난소적출 대조군에서는 거짓수술 대조군에 비해 현저한 체중 및 증체량, 사료 및 물 섭취량, 축적 복부 지방 중량, 혈청 중 osteocalcin 함량의 증가가 자궁, 대퇴골, 경골 및 L5 중량과 혈중 bALP 및 에스트라디올 함량의 감소와 함께 인정되었으며, 현저한 복벽 축적 지방 두께의 증가 및 자궁의 위축, 대퇴골, 경골 및 L4의 골량 및 구조의 감소 소견이 골 흡수 지표(Ocn 및 OS/BS)의 현저한 증가와 함께 조직병리학적 및 조직형태계측학적으로 인정되었다. 즉, 전형적인 에스트로겐 결핍성 갱년기 장애가 난소적출에 의해 유발되었다. 한편 이러한 난소적출에 의한 에스트로겐 결핍성 폐경기 관련 갱년기 장애 소견이, 가감귀비온담탕 추출물 500, 250 및 125 mg/kg의 84일에 걸친 연속 경구 투여에 의해 투여 용량 의존적으로 억제되었다.

결 론: 이상의 결과에서, 가감귀비온담탕 500, 250 및 125 mg/kg의 경구투여는 난소적출 랫트에서 에스트로겐 결핍성 폐경기 관련 갱년기 장애 개선 효과를 투여 용량 의존적으로 나타내었다. 따라서 가감귀비온담탕은 효과적인 갱년기 장애 개선제로서 개발 가능성이 높을 것으로 기대되며, 특히 에스트로겐 결핍성 비만 및 골다공증의 개선에 유효할 것으로 판단된다. 한편 가감귀비온담탕은 총 18종의 약제로 구성되어 있고, 각각 수많은 생리활성 물질을 함유하고 있어, 이후 생리활성을 나타내는 화학성분의 검색과 더불어 다양한 방면으로 기전적인 연구가 체계적으로 수행해야 할 것으로 판단된다.

중심단어: 가감귀비온담탕, 난소적출, 갱년기, 비만, 골다공증

References

1. The Society of Korean Medicine Obstetrics and Gynecology. Korean Obstetrics & Gynecology Ⅰ. Seoul:Jungdam publisher. 2007:237-8.
2. del Giorno C, et al. Effects of *Trifolium pratense* on the climacteric and sexual symptoms in postmenopause women. Rev Assoc Med Bras. 2010;56(5):558-62.
3. Liang YQ, et al. Estrogen receptor β is involved in the anorectic action of estrogen. Int J Obes Relat Metab Disord. 2002;26(8):1103-9.
4. Saengsirisuwan V, et al. Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement. Metabolism. 2009;58(1):38-47.
5. Lew YO. Side effects and management of postmenopausal hormone replacement therapy. J Menopausal Med. 2004;10(1):14-20.
6. Kim KS, Yoo DY. Literary Study on the Climacteric Syndrome. J Oriental medical laboratory of Daejeon univ. 2004;13(1):107-28.
7. Heo J. Dongeuibogam (東醫寶鑑). Seoul: Hanmibook. 2008:47, 51, 172.
8. Cha YJ, et al. The Effect of *Guibiondamtang* in an Animal Model of Depression using Chronic Mild Stress. J Oriental Neuropsychiatry. 2001;12(2):53-68.
9. Whang WW, Jo JY. The Effect of *Guibiondamtang* on Immune Reponse and in Concentration of Catecholamine in Immobilization Stressed Rats. J Oriental Neuropsychiatry. 1995;6(1):1-17.
10. Kang SJ, et al. Dried pomegranate potentiates anti-osteoporotic and anti-obesity activities of red clover dry extracts in ovariectomized rats. Nutrients. 2015;7(4):2622-47.
11. Levene A. Pathological factors influencing excision of tumours in the head and neck. Part I. Clin Otolaryngol Allied Sci. 1981;6(2):145-51.
12. Ludbrook J. Update: microcomputer statistics packages. A personal view. Clin Exp Pharmacol Physiol. 1997;24(3-4):294-6.
13. Kang SJ, et al. Fermentation with Aquilariae Lignum enhances the anti-diabetic activity of green tea in type II diabetic db/db mouse. Nutrients. 2014;6(9):3536-71.
14. Wolff LPG, et al. Avaliação do endométrio em menopausadas após uso de isoflavonas. Rev Assoc Med Bras. 2006;52(6):419-23.
15. Choi JS, Koh IU, Song J. Genistein reduced insulin resistance index through modulating lipid metabolism in ovariectomized rats. Nutr Res. 2012;32(11):844-55.
16. Tohda C, et al. Inhibitory effects of *Eleutherococcus senticosus* extracts on amyloid beta(25-35)-induced neuritic atrophy and synaptic loss. J Pharmacol Sci. 2008;107(3):329-39.
17. Nakagawasai O, et al. Effect of kami-untan-to on the impairment of learning and memory induced by thiamine-

deficient feeding in mice. Neuroscience. 2004;125(1):233-41.
18. Busta I, Xei HS, Kim MS. The use of Gui-Pi-Tang in small animals with immune-mediated blood disorders. J Vet Clin. 2009;26(2):181-4.
19. Kang IH, et al. Effects of Gwibitang on glutamateinduced apoptosis in C6 glial cells. J Korean Orient Med. 2001; 22(4):45-57.
20. Yim NH, et al. Guibitang, a traditional herbal medicine, induces apoptotic death in A431 cells by regulating the activities of mitogen-activated protein kinases. BMC Complement Altern Med. 2014;14:344.
21. Kim HJ, Choi JH, Lim SW. The defensive effect of Keuibi-tang on the gastric mucous membrane of mouse injured by stress and ethanol. J Orient Med. 2003;24(1):155-68.
22. Wang Q, et al. Potentiation of brain acetylcholine neurons by Kami-Untan-To (KUT) in aged mice: implications for a possible antidementia drug. Phytomedicine. 2000;7(4):253-8.
23. Nakagawasai O, et al. Preventive effect of kami-untan-to on performance in the forced swimming test in thiamine-deficient mice: relationship to functions of catecholaminergic neurons. Behav Brain Res. 2007;177(2):315-21.
24. Oh HK, et al. Kami-ondam-tang, a traditional herbal prescription, attenuates the prepulse inhibition deficits and cognitive impairments induced by MK-801 in mice. J Ethnopharmacol. 2013;146(2):600-7.
25. Hong JG et al. The memory-enhancing effects of Kami-ondam-tang in mice. J Ethnopharmacol. 2011;137(1):251-6.
26. Geary N. The estrogenic inhibition of eating. Handbook of behavioral neurobiology (Stricker EM, Woods SC, Eds.). Vol. 14. New York:Kluwer Academic. 2004: 307-45.
27. Eckel LA. Estradiol: a rhythmic, inhibitory, indirect control of meal size. Physiol Behav. 2004;82(1):35-41.
28. Lutz TA. Amylin may offer (more) help to treat postmenopausal obesity. Endocrinology. 2011;152(1):1-3.
29. Korach KS, Migliaccio S, Davis VL. Estrogens. Principles of Pharmacology: Basic Concepts and Clinical Applications (Munson PL, Mueller RA, Breese GR, Eds.). New Yotk:Chapman and Hall. 1995:809-25.
30. Ateba SB et al. *Eriosema laurentii* De Wild (Leguminosae) methanol extract has estrogenic properties and prevents menopausal symptoms in ovariectomized Wistar rats. J Ethnopharmacol. 2013; 150(1):298-307.
31. Sakai A, et al. Bone marrow cell development and trabecular bone dynamics after ovariectomy in ddy mice. Bone. 1998;23(5):443-51.
32. Wronski TJ, Yen CF, Scott KS. Estrogen and diphosphonate treatment provide long-term protection against osteopenia in ovariectomized rats. J Bone Miner Res. 1991;6(4):387-94.
33. Puel C, et al. Preventive effect of

Abelmoschus manihot (L.) Medik. on bone loss in the ovariectomised rats. J Ethnopharmacol. 2005;99(1):55-60.
34. Kuo YJ, et al. Calcitonin inhibits SDCP-induced osteoclast apoptosis and increases its efficacy in a rat model of osteoporosis. PLoS One. 2012;7(7):e40272.
35. Syed Z, Khan A. Bone densitometry: applications and limitations. J Obstet Gynaecol Can. 2002;24(6):476-84.
36. Yamaguchi K, et al. Suppressive effect of norzoanthamine hydrochloride on experimental osteoporosis in ovariectomized mice. Biol Pharm Bull. 1999;22(9):920-4.
37. Jakubas-Przewlocka J, Przewlocki P, Sawicki A. Assessment of changes due to the long-term effect of estrogen and calcium deficiency in the trabecular bone structure in rats. Clin Exp Rheumatol. 2005;23(3):385-8.