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 64 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 analyses 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
I. Introduction

Climacterium involve perimenopausal phases including premenopausal, menopausal and postmenopausal periods\(^1\). The climacteric corresponds to the period during which women gradually lose 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 factors can increase physical symptoms indirectly as well as mental symptoms such as depression, emotional instability\(^5\).

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 term\(^6\). Because of this reason, many researchers have searched alternative therapies, such as phytoestrogens to soften menopausal symptoms\(^7\).

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)\(^8\). «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\(^9\). 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 response 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 flaked evaporator (Eyela N-1110, Tokyo, Japan), and then totally lyophilized. Total 176.24 g (yield=22.03%) of lyophilized aaqueous 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 dissolved 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

<table>
<thead>
<tr>
<th>Korean name</th>
<th>Herbs</th>
<th>Scientific name</th>
<th>Amounts (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>白茯苓</td>
<td><em>Poria (Hoelen)</em></td>
<td><em>Poria cocos</em> (Schw.) Wolf</td>
<td>12</td>
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<tr>
<td>龙眼肉</td>
<td><em>Longanae Arillus</em></td>
<td><em>Dimocarpus longan</em> Loureiro</td>
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</tr>
<tr>
<td>痛归</td>
<td><em>Angelicae Gigantis Radix</em></td>
<td><em>Angelica gigas</em> Nakai</td>
<td>8</td>
</tr>
<tr>
<td>香附子</td>
<td><em>Cyperi Rhizoma</em></td>
<td><em>Cyperus rotundus</em> Linne</td>
<td>6</td>
</tr>
<tr>
<td>陈皮</td>
<td><em>Citri Unshii Pericarpium</em></td>
<td><em>Citrus unshiu</em> Markovich</td>
<td>4</td>
</tr>
<tr>
<td>半夏</td>
<td><em>Pinelliae Tuber</em></td>
<td><em>Pinellia ternata</em> Breitenbach</td>
<td>4</td>
</tr>
<tr>
<td>桔梗</td>
<td><em>Ponciri Fructus</em></td>
<td><em>Poncirus trifoliata</em> Rafinesque</td>
<td>4</td>
</tr>
<tr>
<td>酸枣仁（炒）</td>
<td><em>Zizyphi Spinosae Semen</em></td>
<td><em>Zizyphus jujuba</em> Mill</td>
<td>4</td>
</tr>
<tr>
<td>白术</td>
<td><em>Atractylodis Rhizoma Alba</em></td>
<td><em>Atractylodes macrocephala</em></td>
<td>Koidzumi</td>
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<td>柴胡</td>
<td><em>Bupleuri Radix</em></td>
<td><em>Bupleurum falcatum</em> Linne</td>
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<td><em>Cimicifuga heracleifolia</em></td>
<td>Komarov</td>
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<td>远志</td>
<td><em>Polygalae Radix</em></td>
<td><em>Polygala tenuifolia</em> Willdenow</td>
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<tr>
<td>石菖蒲</td>
<td><em>Acori Gramineri Rhizoma</em></td>
<td><em>Acorus gramineus</em> Solander</td>
<td>3</td>
</tr>
<tr>
<td>木香</td>
<td><em>Aucklandiae Radix</em></td>
<td><em>Aucklandia lappa</em> Decne</td>
<td>2</td>
</tr>
<tr>
<td>竹茹</td>
<td><em>Bambusae Caulis In Taeniam</em></td>
<td><em>Phyllostachys nigra</em> Munro var.</td>
<td>2</td>
</tr>
<tr>
<td>苦草</td>
<td><em>Glycyrrhizae Radix</em></td>
<td><em>Glycyrrhiza uralensis</em> Fischer</td>
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<tr>
<td>生薑</td>
<td><em>Zingiberis Rhizoma Crudus</em></td>
<td><em>Zingiber officinale</em> Roscoe</td>
<td>3</td>
</tr>
<tr>
<td>大棗</td>
<td><em>Zizyphi Fructus</em></td>
<td><em>Zizyphus jujuba</em> Miller var.</td>
<td>inermis Rehder</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>18 types</td>
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Table 2. Experimental Design

<table>
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<tr>
<th>Groups</th>
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<th>Group identification</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Control</td>
<td>Sham</td>
<td>Sham</td>
<td>Distilled water 5 ml/kg/day. oral gavage</td>
</tr>
<tr>
<td>Control</td>
<td>OVX</td>
<td>OVX</td>
<td>Distilled water 5 ml/kg/day. oral gavage</td>
</tr>
<tr>
<td>Active</td>
<td>OVX</td>
<td>GGOT500</td>
<td>GGOT 500 mg/kg/day. oral gavage</td>
</tr>
<tr>
<td>Active</td>
<td>OVX</td>
<td>GGOT250</td>
<td>GGOT 250 mg/kg/day. oral gavage</td>
</tr>
<tr>
<td>Active</td>
<td>OVX</td>
<td>GGOT125</td>
<td>GGOT 125 mg/kg/day. oral gavage</td>
</tr>
</tbody>
</table>

3. Climacterium inducement

Korea) in the blend of 70% N₂O and 28.5% O₂, and were retained with 1 to 1.5% isoflurane in the blend of 70% N₂O
and 28.5% O₂. 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, Switzerland) 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].

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

\[ \text{After administration (84 days)} = \text{Body weight at sacrifice - body weight at start of administration} \]

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].

\[ \text{EQUATION } [2]. \text{ Relative Organ Weights (% of body weight)} = \left[ \frac{\text{Absolute abdominal fat pad, uterus weights}}{\text{Body weight at sacrifice}} \right] \times 100 \]

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°C 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°C 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].

\[
\text{EQUATION 3. Relative Bone Weights (\% of body weight)} = \left(\frac{\text{Absolute bone weight}}{\text{Body weight at sacrifice}}\right) \times 100
\]

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°C for 10 mins, using clotting activated serum tube. All serum samples were frozen at -150°C using ultradepfreezer (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.\(^\text{10}\) 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.\(^\text{10}\) 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\(^\text{10}\), 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\(^\text{11}\). 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\(^\text{12}\). 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\(^\text{13}\), respectively. EQUATION \([4]\). Percent Changes in Comparison with Sham Control (\%)

\[\text{Percent Changes} = \frac{\text{Test Value} - \text{Sham Control}}{\text{Sham Control}} \times 100\]

\[\text{Percent Changes} = \frac{\text{Test Value} - \text{OVX Control}}{\text{OVX Control}} \times 100\]


= [((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.

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

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

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 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>
<thead>
<tr>
<th>Items</th>
<th>Absolute wet-weight (g)</th>
<th>Relative wet-weight (% of body weight)</th>
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<tr>
<td>Groups</td>
<td>Uterus</td>
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<tr>
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<tr>
<td>OVX</td>
<td>0.075±0.012a</td>
<td>0.015±0.002a</td>
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<tr>
<td>GGOT500</td>
<td>0.146±0.028ab</td>
<td>0.033±0.006ab</td>
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<tr>
<td>GGOT250</td>
<td>0.111±0.010ab</td>
<td>0.024±0.002ab</td>
</tr>
<tr>
<td>GGOT125</td>
<td>0.093±0.011ac</td>
<td>0.020±0.002ab</td>
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</tbody>
</table>

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

Table 3. Changes on the Abdominal Fat Pad Weights

Table 4. Changes on the Uterus Weights
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.

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.

![Serum osteocalcin levels](image)

**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

![Serum bALP levels](image)

**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.
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 GGT 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 GGT 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 compared 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 Abdominal Fat Pads, Uterus

<table>
<thead>
<tr>
<th>Groups</th>
<th>Items</th>
<th>Controls</th>
<th>GGOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat pads</td>
<td></td>
<td>Sham</td>
<td>OVX</td>
</tr>
<tr>
<td>Total Th (mm)</td>
<td>4.41±0.88</td>
<td>9.97±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.44±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adipocyte DM (μm)</td>
<td>86.09±15.84</td>
<td>155.93±13.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.87±18.80&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td>Sham</td>
<td>OVX</td>
</tr>
<tr>
<td>Total Th (mm)</td>
<td>2.49±0.76</td>
<td>0.56±0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.89±0.35&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epi Th (μm)</td>
<td>37.68±4.36</td>
<td>9.38±1.89&lt;sup&gt;de&lt;/sup&gt;</td>
<td>25.42±4.54&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucosa Th (μm)</td>
<td>969.03±164.33</td>
<td>156.52±39.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>357.17±49.51&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>UG percentage (%)</td>
<td>31.71±10.04</td>
<td>5.20±1.40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15.52±2.83&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: p<0.01 and <sup>b</sup>: p<0.05 as compared with sham control by LSD test
<sup>c</sup>: p<0.01 as compared with OVX control by LSD test
<sup>d</sup>: p<0.01 as compared with sham control by MW test
<sup>e</sup>: p<0.01 and <sup>f</sup>: p<0.05 as compared with OVX control by MW test
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.
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 osteoclast 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 -62.73, -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**

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

- 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**

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

- 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**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Items</th>
<th>Sham</th>
<th>Controls</th>
<th>500 mg/kg</th>
<th>250 mg/kg</th>
<th>125 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBV, BV/TV</td>
<td>53.00±10.18</td>
<td>30.70±5.46&lt;sup&gt;d&lt;/sup&gt;</td>
<td>46.84±10.02&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>42.50±8.02&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>39.36±5.14&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tbn</td>
<td>25.38±4.75</td>
<td>10.75±1.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.50±3.25&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>14.88±2.03&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>14.13±2.23&lt;sup&gt;de&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Tbl</td>
<td>4.33±0.39</td>
<td>2.27±0.41&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.97±0.60&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.18±0.60&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.99±0.21&lt;sup&gt;ef&lt;/sup&gt;</td>
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<td></td>
<td>Tbt</td>
<td>148.77±7.93</td>
<td>99.03±13.45&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>144.77±15.73&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>136.31±7.92&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>121.78±15.85&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cbt-shaft</td>
<td>244.35±46.82</td>
<td>138.68±18.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>233.94±33.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>215.45±21.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>189.40±25.65&lt;sup&gt;c&lt;/sup&gt;</td>
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<td></td>
<td>Ocn</td>
<td>8.50±2.07</td>
<td>21.00±2.78&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>11.13±2.36&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>14.63±1.85&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>17.13±2.03&lt;sup&gt;ac&lt;/sup&gt;</td>
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<td></td>
<td>OS/BS</td>
<td>5.06±1.22</td>
<td>20.99±2.84&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.82±1.29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14.12±2.48&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>17.97±1.84&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- 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
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\(^5\), and the climacteric state is related with an increased risk of metabolic diseases such as heart disease, diabetes, obesity and hypertension\(^5\).

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-syndrome symptoms, migraine, nausea, abdominal 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, mostly to treat various symptoms on obstetrics and gynecological fields\(^1\&^7\). 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 graminei 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\(^{(27)}\). 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\(^{(20)}\). Moreover, it also recently reported that the subchronic administration of *Gami-ondam-tang* for 14 days improved cognitive performance in normal naïve mice through the enhancement of neurogenesis and protein kinase 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\(^{(23)}\). 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 body 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 vagina, 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\textsuperscript{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 anti-osteoporotic drugs, because several parameters are clearly decreased by ovariectomy within 4 to 6 weeks after operation, as summarized by other investigators\textsuperscript{32}. The increases trends of bone weights have been considered as a valuable markers of anti-osteoporotic activities\textsuperscript{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\textsuperscript{34}. As progression of OVX related osteoporosis, serum osteocalcin levels were generally increased along the increases of bone turn over, but serum bALP contents were dramatically decreased along inhibition of bone formations\textsuperscript{34}. BMD of bone provided good predictable information about efficacy of anti-osteoporotic agents\textsuperscript{35}. BMD and bone strengths were markedly decreased in osteoporosis regardless of causes\textsuperscript{35}. A microscopic observation of bone informed good information about bone morphology\textsuperscript{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\textsuperscript{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\textsuperscript{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

목적: 이 연구에서는 한의학에서 다양한 부인과 질환에 사용되어온 전통 복합 처방인 귀비탕과 가미은단탕을 함방, 개량한 가감귀비은단탕 열수 추출물의 갱년기 장애 개선 효과를 실험관 장애, 비만, 고지혈증, 골다공증, 장기 지방적출 및 신경성장 기능에 포함된 다양한 사람의 갱년기 장애를 나타내는 것으로 알려진 난소적출(Ovariectomized, OVX) 헬드 모델을 이용하여 평가하였다.

방법: 난소적출수술 28일 후부터 가감귀비은단탕 추출물(수용 : 22.03%)을 각각 100, 50 및 25 mg/ml의 농도로 일정 주위수에 용해시키고, 체중 kg 당 5 ml의 용량(500, 250 및 125 mg/kg)으로 매일 1회씩 84일(12주 : 3개월)간 경구 투여한 다음, 항병안호흡, 방사성위측효과 및 골다공증 억제 효과의 3가지 생리활성 효과로 구분하여 평가하였다. 항병안 효과 및 방사성위측효과를 평가하기 위해, 체중 및 주장, 월령 점검 알라미드를 함방, 복부 측위 지방 및 자궁의 증가량으로 복부 측위 지방의 두께 및 평균 지방세포의 경과. 자궁 전체, 색전 및 적립의 두께와 전막막 자궁심이 차지하는 비율의 변화를 각각 평가하였다. 또한 골다공증 개선 효과, 즉 골 보호효과를 평가하기 위해, 대퇴골, 경골 및 요추골의 습, 건조 및 단합 증강, 골밀도, 골질도, 혈중 osteocalcin 및 bone specific alkaline phosphatase(ALP) 함량, 평면 및 구조의 곰혹수에 대한 조직병리적 변화를 각각 측정하였다.

실험군(5개군: 군단 8마리의 헬드 사용)


결과: 난소적출 대조군에서는 거짓수술 대조군에 비해 현저한 체중 및 주장량, 사료 및 물 심취량, 측위 복부 지방 증량, 월령 중 osteocalcin 함량의 증가가 자궁. 대퇴골, 경골 및 L5 측위와 혈중 ALP 및 에스트로皐담 함량의 감소와 함께 인질되었으며, 현저한 복부 측위 지방 두께의 증가 및 자궁의 위축, 대퇴골, 경골 및 L4의 골량 및 구조의 감소 소견이 골 곰혹 지표(Ocn 및 OS/BS)의 현저한 증가와 함께 조직병리학적 및 조직형태계측학적으로 인정되었다. 즉, 전형적인 에스트로皐텐 결절성 갱년기 장애가 난소적출에 의해 유발되었다. 한편 이러한 난소적출에 의한 에스트로皐텐 결절성 폐경기 관련 갱년기 장애 소견이, 가감귀비은단탕 추출물 500, 250 및 125 mg/kg의 84일에 걸친 연속 경구 투여에 의해 유발된 용량 양의 결로 억제되었다.

결론: 이상의 결과에서, 가감귀비은단탕 500, 250 및 125 mg/kg의 경구투여는 난소적출 실험에서 에스트로皐텐 결절성 폐경기 관련 갱년기 장애 개선 효과를 투여 용량 의존적으로 나타내었다. 따라서 가감귀비은단탕은 효과적인 갱년기 장애 개선체로서 개발 가능성이 높을 것으로 기대되며, 특히 에스트로皐텐 결절성 비만 및 골다공증의 개선에 유용함으로 판단된다. 한편 가감귀비은단탕은 총 18종의 약재로 구성되어 있고, 각각 수많은 생리활성 물질을 함유하고 있어, 이로 생리활성 물질을 나타내는 화학적분의 검색과 더불어 다양한 방면으로 기전적인 연구가 개계적으로 수행해야 할 것으로 판단된다.

중심단어: 가감귀비은단탕, 난소적출, 갱년기, 비만, 골다공증
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