

Research Paper

Amelioration of estrogen-deficiency-induced obesity by *Ocimum gratissimum*

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Abstract

Objectives: Menopausal transition in women initiates with declining estrogen levels and is followed by significant changes in their physiological characteristics. These changes often lead to medical conditions, such as obesity, which is correlated with chronic low-grade/subclinical inflammation. *Ocimum gratissimum* L. is a food spice or traditional herb in many countries; the plant is rich in antioxidants, which possess anti-inflammation activities and multitude of other therapeutic functions.

Methods: In this study, we evaluated effects of *O. gratissimum* extract (OGE) in preventing obesity by using ovariectomized (OVX) animal models to mimic menopausal women.

Methods: OVX rats showed increase in body weight and in adipocyte size in perigonadal adipose tissue ($p < 0.05$) and decrease in uterus weight. By contrast, OGE (0.2 mg/ml) significantly reduced body weight gain and adipocyte in OVX rats and showed insignificant changes in uterus weight. Further investigation indicated that OGE exerted no influence on levels of dorsal fat, serum total cholesterol, and serum triacylglycerol and on serum biochemical factors, calcium, phosphorus, and glucose.

Conclusion: These findings suggested that OGE dietary supplements may be useful in controlling body weight of menopausal women.

Key words: *Ocimum gratissimum*, obesity, ovariectomized animal models

Introduction

Onset of menopause in females is often associated with increased visceral fat accumulation, a condition known as central obesity [1], and

subsequent increase in incidence of hypertension, atherosclerosis, metabolic syndrome, and cardiovascular diseases. Although body composition

of postmenopausal women results from long-term interactions among between intake, energy expenditure, and sex hormonal status or combination thereof [2], research remains ongoing regarding methods of alleviating menopausal symptoms. Krumm et al. [3] reported that physical activity is an important component of long-term weight maintenance in postmenopausal women. However, in a recent large prospective study among women consuming normal diet, although physical activity was inversely related to weight gain among normal-weight women, no relation was observed among heavy-weight women [4]. Result of such studies invoked interest on other factors that may be involved in preventing physical activities from affecting weight reduction among obese menopausal women.

As inflammation contributes to progression of obesity and its comorbidities [5], numerous studies reported that women undergoing menopausal transition exhibit deleterious changes in circulating inflammatory markers and adipokines correlated with increased visceral adiposity [6], which plays significant pathophysiological role in development of metabolic syndrome and its sequelae, possibly because it serves as source of a number of substances, including free fatty acids and adipokines, such as adiponectin, tumor necrosis factor- α , and plasminogen activator inhibitor type 1 [7–9]. These observations highlighted the importance of inflammation to menopause-associated obesity.

Ocimum gratissimum is a commonly used herbal ingredient in Traditional Chinese medicine and widely distributed in tropical and warm-temperate geolocations. *O. gratissimum* aqueous extract (OGE) consists of abundant antioxidants and possesses many therapeutic functions, including anti-inflammation [10], analgesic and spasmolytic [11], antidiarrheal [12], antitumor [13–17], antiviral [18], and antihyperglycemic activities [19,20]. This extract also improves phagocytic functions without affecting humoral or cell-mediated immune system [21]. Our report revealed that expressions of cell cycle regulators, apoptosis-related factors, and cell proliferation markers modified by OGE treatment cause cell apoptosis in human osteosarcoma U2-OS and HOS cells [17]. Antitumor activities of OGE were also observed in breast, prostate, and lung cancers [13–16]. With its many antioxidant components, OGE also exhibits potential to protect body organs from free radical damage and oxidative stress [22–28].

In obesity models, ovariectomy can induce weight gain and fat deposition in rodents, whereas estradiol or antioxidant treatment can restore these changes [29]. Therefore, we investigated effects of

OGE in alleviating obesity-related inflammation by using ovariectomized (OVX) animal models.

Materials and Methods

Preparation of OGE and composition analysis

OGE was prepared as described in [22]. *O. gratissimum* leaves were harvested and washed in clear running water and air-dried for 1 week to obtain coarse powders. Powdered plant materials (20 g) were homogenized with distilled water (1000 ml) using a polytron. Homogenate was boiled for 1 h and then filtered through two-layer gauze. Filtrate was centrifuged at $20,000 \times g$ and 4°C for 30 min to remove insoluble pellets. Supernatant was collected, lyophilized, and stored at -20°C until usage. Prior to assays, OGE powder was dissolved at indicated concentration. Total polyphenol and flavonoid contents were determined by Folin-Ciocalteu [30] and Lamaison methods [31], respectively. Polyphenol and flavonoid contents were quantified by comparison with pure standards.

OVX Sprague-Dawley rat model

All female Sprague-Dawley rats (200 g) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All procedures involving laboratory animal use were in accordance with guidelines of the Institutional Animal Care and Use Committee of China Medical University (IACUC-CMU) for the care and use of laboratory animals. All experimental procedures were also approved by the IACUC-CMU.

Twenty-four rats were used. Six rats were given sham operation under Nembutal (pentobarbital sodium; 50 mg/kg body weight) anesthesia. The remaining rats were OVX and were equally divided into second, third, and fourth groups. For drinking, the first (sham) and second (OVX) groups received sterile water only. The third (OVX + OGE (0.2 mg/ml)) and fourth (OVX + OGE (0.1 mg/ml)) groups received OGE solution diluted with sterile water.

All animals were maintained under regulated 12 h/12 h light-dark illumination cycles at constant temperature ($22\pm 0.5^\circ\text{C}$) and humidity (45%–50%). Food and drinking water were supplied ad libitum. Body weight was measured at indicated time of the day during experiments. After 12 weeks, animals were sacrificed, and blood and perigonadal adipose tissues were immediately obtained. Dorsal fat and uterus of each rat were dissected and weighed.

Histology of adipose tissues

Perigonadal adipose tissues were fixed in zinc formaldehyde at 4°C overnight prior to their transfer

and storage in phosphate-buffered saline at 4 °C. Tissues were subsequently embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Digital images were captured with an Olympus DX51 light microscope (Tokyo, Japan).

Measurement of serum biochemical markers

Blood was coagulated by maintenance at room temperature for 15–20 min and subsequent centrifugation for 10 min. Serum biochemistry analyzer (AU400, Olympus, Tokyo, Japan) was used to measure calcium ion, glucose, inorganic phosphorus, total cholesterol, and triglyceride.

Statistical analysis

Data were obtained from six measurements and expressed as means \pm SEM. Statistical comparisons were performed using ANOVA. Student's *t*-test was used in two-group comparisons. $p < 0.05$ was considered statistically significant.

Results

OGE supplementation reduced ovariectomy-associated increase in body weight but not uterine weight

Initial investigations determined effects of OGE uptake on estrogen-deficiency-induced obesity. Increased body weight in OVX rats was significantly higher ($p < 0.01$) than that in sham control rats (Figure 1A). After 12 weeks, body weights of sham and OVX rats measured 313 ± 8 and 353 ± 11 g, respectively. Increased body weight of OVX + 0.2 mg/ml OGE group was significantly lower than that of OVX group ($p < 0.01$) but not that of OVX + 0.1 mg/ml OGE group.

Observations also determined changes in uterus weight as response to OGE supplementation. Uterus/body weight ratio in OVX group significantly decreased compared with that of sham group ($p < 0.05$) (Figure 1B). Uterus/body weight ratio in OVX groups with OGE supplement (OVX + 0.1 mg/ml OGE and OVX + 0.2 mg/ml OGE) significantly decreased compared with that of sham group ($p < 0.01$), but observed change was insignificant compared with that of OVX group.

OGE supplementation decreased OVX-associated increase in adipocyte size

OGE supplementation attenuated weight gain in OVX rats. Investigation on effects of supplementation on adipocyte properties revealed that adipocytes in perigonadal adipose tissues of OVX rats were clearly

enlarged compared with those from sham-operated control rats (Figure 2A). High OGE supplementation (0.2 mg/ml) reduced enlarged adipocyte size in response to ovariectomy. Changes in dorsal fat mass were also analyzed. Ratio of dorsal fat mass/body weight was insignificantly changed among four experimental groups, though dorsal fat mass in OVX group was higher than that in sham group (Figure 2B).

Effects of OGE on blood biochemical factors

Blood lipid, total cholesterol, and triacylglycerol levels were determined to investigate whether OGE supplementation can maintain lipid metabolism. As shown in Figure 3 (upper panel), serum total cholesterol level was slightly changed in OVX and OVX groups supplemented with OGE ($p > 0.05$). A similar phenomenon was observed in serum triacylglycerol levels (Figure 3, lower panel). Other blood biochemical factors were also determined. Serum calcium, phosphorus, and glucose showed no change in response to ovariectomy or ovariectomy with OGE supplementation (Figure 4).

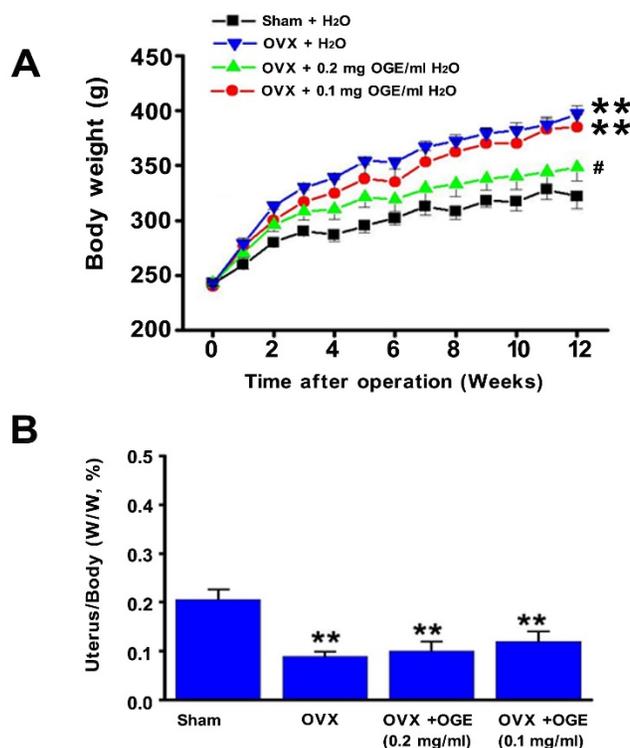


Figure 1. Effects of OGE supplement on body weights. Rats were sham-operated or OVX. OVX rats were divided in three groups; two groups were supplied with water containing OGE (0.1 and 0.2 mg/ml), and the other one received sterile water. (A) Body weight was measured each week for 12 weeks. (B) After 12 weeks, rats were sacrificed, and uteruses were obtained and weighed. #, $p < 0.05$ compared with OVX. **, $p < 0.01$ compared with sham.

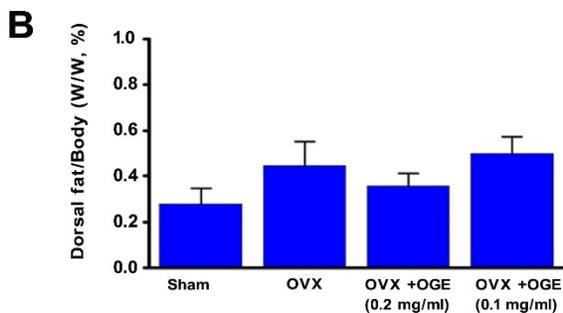
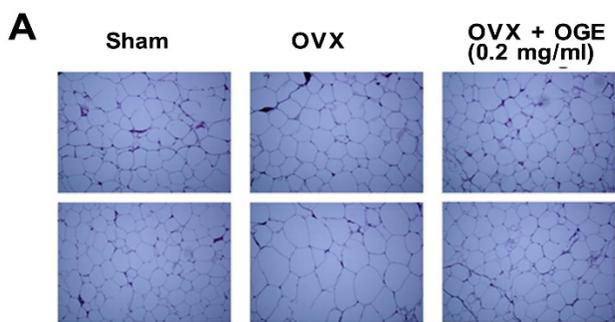


Figure 2. Effects of OGE supplement on adipocyte size and dorsal fat content. Rats were sham-operated or OVX. OVX rats were divided in three groups; two groups were supplied with water containing OGE (0.1 and 0.2 mg/ml), and the other one received sterile water. After 12 weeks, rats were weighed and subsequently sacrificed to obtain periuterine adipose tissues and dorsal fat. (A) After hematoxylin and eosin staining, adipocytes were observed by using light microscope (200×). (B) Dorsal fat samples were collected and weighed. n.s.: no statistical significance compared with sham or OVX.

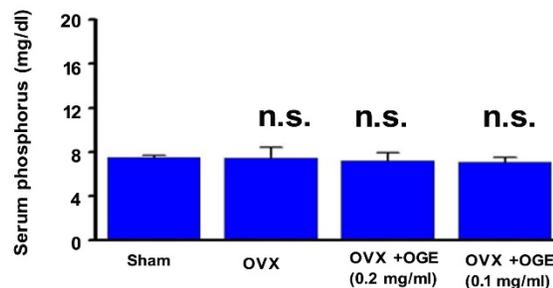
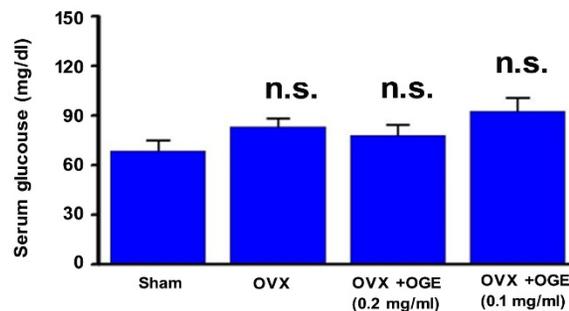
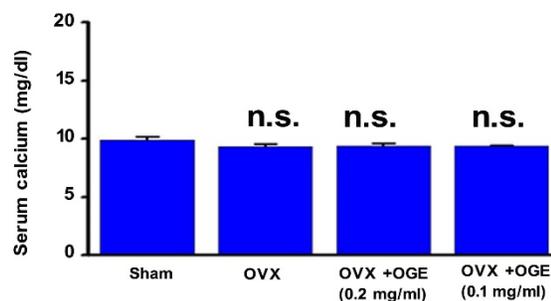


Figure 4. OGE supplement insignificantly affected blood lipids. Rats were sham-operated or OVX. OVX rats were divided in three groups; two groups were supplied with water containing OGE (0.1 and 0.2 mg/ml), and the other one received sterile water. After 12 weeks, sera were obtained for determination of calcium, phosphorus, and glucose levels. n.s.: no statistical significance compared with sham or OVX.

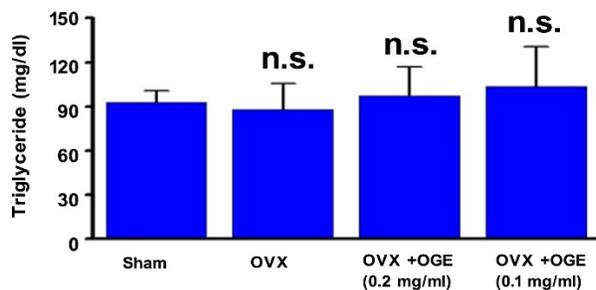
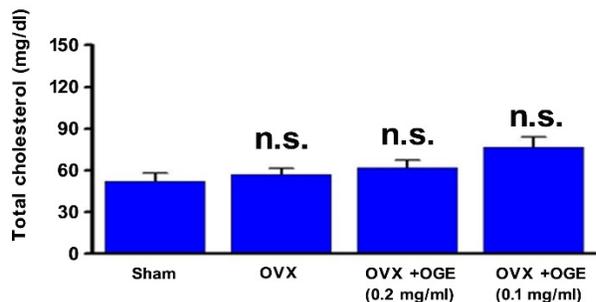


Figure 3. OGE supplement insignificantly affected blood lipids. Rats were sham-operated or OVX. OVX rats were divided in three groups; two groups were supplied with water containing OGE (0.1 and 0.2 mg/ml), and the other one received sterile water. After 12 weeks, blood samples were obtained for determination of serum total cholesterol and triacylglycerol. n.s.: no statistical significance compared with sham or OVX.

Discussion

Both human (menopause) and rodent (reproductive senescence) studies indicated that age-related ovarian hormone loss contributes to changes in distribution of adipose tissues [32]. Significant decrease in estrogen and progesterone results from follicular depletion, yielding many androgenic patterns of fat distribution, that is, an increase in central or abdominal adiposity. Similarly, when consuming high-fat diet, male mice and OVX mice can obtain significant increase in body weight [33]. However, treating mice with estrogen results in reduced body weight.

Recently, considerable amount of evidence indicated that flavonoid intake can reduce body fat deposition in OVX animals [34]. Wu et al. [35] demonstrated that combined intervention of flavonoid and exercise prevents body fat accumulat-

ion in the whole body, increases lean body mass and restoration of bone mass, and reduces high serum cholesterol in OVX mice. These findings suggested that flavonoid intervention can be beneficial by improving both body composition and bone mass in estrogen-deficient condition. As OGE also contains many flavonoids [27], we evaluated effects of OGE in preventing obesity by using the same OVX animal models. The present results show effects of OGE on restoring normal body composition and lipid metabolism under estrogen-deficient conditions, suggesting that OGE supplementation may offer an effective regimen to prevent lifestyle-related health problems.

Reports showed that isoflavone supplementation effectively lowered serum cholesterol in OVX rats [34, 35] and in postmenopausal women [36]. However, other reports showed different results [37]. In the present study, no change was noted in total cholesterol levels in OVX rats. Thus, whether OGE can reverse total cholesterol of OVX rats remains unknown. Possibly, in our previous laboratory, OGE can reduce total cholesterol level in high-fat diet and high-cholesterol diet rats [28].

With significant amount of simple phenols and flavonoids, including catechin and epicatechin [27], discovered in OGE and scientific evidence proving their beneficial anti-obesity effects [38], these compounds may potentially decrease weight gain in OVX rats. Future research should explore molecular mechanisms of such effects. Ovarian adipose tissue macrophages (adipose tissue macrophage clusters) and state of nuclear factor- κ B activation should also be further explored to understand inflammation in estrogen-deficiency-related obesity and natural compounds for clinical use after menopause to achieve weight loss and to improve body figures of menopausal women.

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Competing Interests

The authors have declared that no competing interests exist.

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