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# Effects of *Opuntia humifusa* Seed Powder on Serum Lipid Profile in Ovariectomized Rats

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**Abstract**

***Opuntia humifusa* contains high levels of antioxidants including vitamin C, flavonoids and polyphenols, which may provide beneficial effects such as hypolipidemic activity and the reduction of atherosclerosis in postmenopausal women. This study was conducted to determine if the intake of *O. humifusa* seeds powder (OHS) regulates lipid concentrations, glutamate-oxaloacetate transaminase (GOT), and glutamate-pyruvate transaminase (GPT) in the serum of ovariectomized rats. Sprague-Dawley female rats were randomly assigned to either a sham-operated group (Sham) or one of the following four ovariectomy (OVX) subgroups: OVX with vehicle (OVX), OVX with 100, 200, and 500 mg/kg/day OHS (OHS100, OHS200, OHS500). Daily oral administration of OHS was initiated one week after ovariectomy and continued for seven weeks. Upon completion of treatments, organs were weighed and GOT, GPT, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were determined enzymatically. No significant differences in feed intake and organ index were observed among the groups. Significant decreases in GPT, TC and LDL-C ($p<0.05$) were observed in all of the OHS groups (OHS100, 200 and 500), while no significant changes in HDL-C were observed. In addition, the OHS200 and OHS500 treatment groups exhibited a lower level of serum GOT compared to the OVX group. These results indicate that supplementation with *O. humifusa* seeds could induce favorable changes in serum lipoprotein and lipid profiles, which frequently worsen with inadequate estrogen availability.**

**Key words:** *Opuntia humifusa* seeds, fatty acid, HDL-cholesterol, LDL-cholesterol, ovariectomized rats

## INTRODUCTION

Menopause is associated with a lack of estrogen and progesterone. In addition, it causes many physiological changes, including elevated levels of total cholesterol (TC) and LDL cholesterol (LDL-C) and increased risk of hypertension (1). Atherosclerotic lesions in humans and animals are correlated with elevated TC and LDL-C levels and decreased high-density lipoprotein cholesterol (HDL-C) levels (2). The incidence of atherosclerosis in women is lower than in men of the same age, but the incidence in women increases after menopause due to the decrease in estrogen level (3). Plant-based health products are frequently considered to be less toxic and have lower side effects than synthetic agents (4).

Plants in the genus *Opuntia* are members of the Cactaceae family that are widely distributed in semi-arid countries throughout the world, especially in the Mediterranean and Central America (5,6). *O. ficus-indica* var. *saboten* and other *Opuntia* spp. have been extensively characterized biochemically (7). Their biological effects, which include therapeutic properties against cancer (8), oxidative stress (9), and ulcers (10), have been well documented; however, less is known about the pharmacological properties of *Opuntia humifusa*, the cactus pear, which has long been cultivated in Korea. *O. humifusa* can be grown in Korean winters, even in areas where temperatures reach -20°C or below (11). In a recent study, it was shown that *O. humifusa* has high concentrations of total polyphenols and flavonoids compared to other cacti (12). Moreover, the *O. humifusa* displayed both antioxidant and free radical scavenging activity (13). The majority of plants with antioxidant compounds have a potential role in protecting humans from several illnesses (14,15). Additionally, cactus pear seeds are rich in minerals and sulfur-containing amino acids such as methionine and cysteine, providing about twice the recommended daily allowance for humans according to FAO/WHO (16).

Based on these characteristics, the *O. humifusa* seeds powder (OHS) could have blood cholesterol-lowering effects in post-menopausal women. Therefore, the effect of OHS on serum lipid levels in ovariectomized rats was evaluated.

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## MATERIALS AND METHODS

### *O. humifusa* seeds preparation

*O. humifusa* was collected in October in a field located in Goyang city, Korea. The seeds were separated from the fruit, freeze-dried (EYELA, Tokyo, Japan) and then ground through a 2 mm screen using a cyclone mill (3010-039, UDY Corp., Fort Collins, CO, USA). The samples were stored at -20°C until needed.

### Chemical analysis of diets and *O. humifusa* seeds

The moisture, crude fiber, and fat content were determined according to the AOAC methods (17). Nitrogen was determined using the Kjeldahl procedure and crude protein was calculated as N×6.25. Ash was determined by burning in a muffle furnace at 550°C.

### Analysis of the fatty acids in *O. humifusa* seeds

Fatty acids were evaluated by gas chromatography with flame ionization detection (GC-FID) using a HP-6890GC instrument (Hewlett Packard, Wilmington, DE, USA) with a FID and a HP-FFAP column (30 m ×0.32 mm×0.25 μm). Twenty-five mg of sample was weighed into a test tube and 2 mL of 0.5 N NaOH/methanol were added. The sample was then heated for 5 min in a heating block (100°C), after which 2 mL of 14% $BF_3$/methanol was added to the mixture, and the sample was boiled for 5 min. The oven temperature conditions were as follows: the initial temperature of the column, 100°C, was held for 2 min, after which the temperature was increased at 4°C/min to 240°C, where it was held for 20 min. The flow rate of the carrier gas (helium) was 1.5 mL/min. Fatty acids were identified by comparing the relative retention times of fatty acid methyl esters peaks with those of standards. The results were recorded and processed using the HP-Chemstation software and expressed as a relative percentage of each fatty acid.

### Test animals

Forty five 10-week old female Sprague-Dawley rats were purchased from Samtako Inc. (Gyeonggi, Korea). The animals were acclimated under controlled conditions (room temperature, 22±2°C; relative humidity, 50±5%; dark cycle, 12 hr/12 hr). The diet (NIH 31 M, Samtako Inc.), as shown in Table 1, and water were available *ad libitum* throughout the experimental period. All animal-based procedures were in accordance with the "Guidelines for the Care and Use of Experimental Animals of Korea University" (authority number KUIACUC-2010-96).

### Experimental animal design

After seven days of acclimation, the rats were ovariectomized or sham-operated. The rats were anesthetized by intramuscular injection with tiletamine plus zolazepam (Zoletil, Virbac, 5 mg/kg BW) and the ovaries were removed. The sham-operation was conducted in the same manner, but the ovaries were only exposed. One week later, ovariectomized rats (OVX) were randomly divided into four groups: OVX with vehicle (OVX, n=9) or OVX with 100, 200, or 500 mg/kg body weight/day of OHS (OHS100, n=9; OHS200, n=9; OHS500, n=9). The OHS was then suspended in distilled water and orally administered everyday using the Sonde once a day. Sham (n=9) and OVX rats received the vehicle (distilled water). According to the Human Rat Equivalent Dose Conversion Principle (18), the experimental dose for OHS in the present study was equivalent to a clinical dose for a 60 kg human subject. The treatment was continued for seven weeks and the body weight of each animal was measured weekly. At the end of the seven week trial, all the rats were deprived of feed overnight and urine samples were collected the following day. The animals were then anesthetized with diethyl ether and the blood was collected via abdominal aorta puncture. Afterward, the blood samples were centrifuged at 1800 ×*g* for 15 min to obtain the serum, which was kept at -70°C until analysis. The animals were then euthanized and the uterus, heart, liver, spleen, lungs and kidneys were carefully removed, cleaned and immediately weighed.

**Table 1.** Ingredient composition of the basal diet (NIH31M)

| Ingredient | Percentage by weight |
|---|---|
| Ground whole wheat | 35.17 |
| Ground whole yellow corn | 20 |
| Ground whole oats | 10 |
| Wheat middlings | 10 |
| Fish meal (60% protein) | 9 |
| Soybean meal (47.5% protein) | 5 |
| Soy bean oil (no additives) | 2.5 |
| Alfalfa meal (17% protein) | 2 |
| Corn gluten meal (60% protein) | 2 |
| Dicalcium phosphate | 1.5 |
| Brewer's dried yeast | 1 |
| Ground limestone | 0.5 |
| Salt | 0.5 |
| NIH #31 vitamin premix[1)] | 0.25 |
| NIH #31 mineral premix[1)] | 0.25 |
| Choline chloride | 0.13 |
| L-Lysine | 0.1 |
| DL-Methionine | 0.1 |

[1)]Vitamin and mineral mixtures.

### Biochemical analysis of serum

The serum total cholesterol was determined using a commercial kit (Wako, Osaka, Japan). HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), glutamate-pyruvate transaminase (GPT), and glutamate-oxaloacetate trans-

aminase (GOT) in the serum were measured using commercially available kits (Sekisui, Osaka, Japan).

**Statistical analysis**

All values were expressed as the mean±standard error (SE). One-way analysis of variance (ANOVA) was used to test for differences among groups. When ANOVA indicated significant differences among the means, Tukey's test was used to determine which means were significantly different. Data of proximate composition were only presented as the mean±standard deviations. All statistical analyses were conducted using the SAS software package (version 9.2, SAS Institute, Cary, NC, USA). Significant differences between groups and p values are indicated in the Figures and Tables.

## RESULTS AND DISCUSSION

**Proximate composition and fatty acids content of OHS**

The composition of OHS and the basal diet on a dry matter basis is shown in Table 2. The OHS contained 8.99% lipid and 36.91% crude fiber. The OHS contained about 8.9% fat, which was higher than that of *Opuntia ficus indica* seeds (6.77%) (19). However, the variations in seed composition of cactus pear fruits during its maturation period have been studied by Coskuner and Tekin (20). Dietary fiber intake has been recommended as a safe and effective approach for decreasing cholesterol and results in a lower risk of coronary heart events (21,22). The mechanisms of decreasing blood cholesterol induced by an increased intake of dietary fiber are controversial. Although soluble dietary fiber is known to be an effective hypercholesterolemia therapeutic agent, some recent findings have found that insoluble fibers isolated from some fruits, vegetables, and their pomace can also effectively decrease serum cholesterol and lower the risk of cardiovascular disease (23-27). The cholesterol-lowering actions of insoluble fibers might be related to some of their physicochemical properties, such as water-holding capacity, which alters cholesterol metabolism (28).

**Table 2.** Nutrient compositions of OHS and basal diets used in this study (DM basis, %)

| Assay | OHS | Basal diet |
|---|---|---|
| Dry matter | 98.04±0.04[1)] | 90.91±0.08 |
| Ash | 1.92±0.08 | 6.70±0.04 |
| Crude protein | 6.33±0.07 | 26.15±0.06 |
| Crude fat | 8.99±0.07 | 4.33±0.06 |
| Crude fiber | 36.91±0.06 | 6.61±0.05 |
| NFE[2)] | 43.89±0.05 | 47.12±0.06 |

[1)]Values are the mean±SD (n=3).
[2)]NFE: nitrogen-free extract.

**Table 3.** Fatty acid composition of *O. humifusa* seeds

| Fatty acid | OHS (% of total fat) |
|---|---|
| Myristic acid (C14:0) | 0.1 |
| Palmitic acid (C16:0) | 7.3 |
| Palmitoleic acid (C16:1) | 0.3 |
| Stearic acid (C18:0) | 3.1 |
| Oleic acid (C18:1) | 17.1 |
| Linoleic acid (C18:2) | 70.9 |
| Linolenic acid (C18:3) | 0.3 |
| Arachidic acid (C20:0) | 0.3 |
| Gadoleic acid (C20:1) | 0.2 |
| Behenic acid (C22:0) | 0.2 |
| Lignoceric acid (C24:0) | 0.1 |
| Unknown | 0.1 |
| Total | 100 |

The fatty acid composition of OHS is presented in Table 3. Oleic acid (C18:1) comprised 17.1% of the total fatty acid. The linoleic acids (C18:2) were present at the highest concentration (70.9%) in OHS. Fatty acids play a structural role in biological membranes and change the fatty acid composition of the lymphocyte plasma membrane, which may influence immune functions (29). The linoleic and oleic acid levels in OHS were similar to those of safflower oil (30). Linoleic and oleic acids have beneficial health effects including alleviation of cardiovascular dysfunction, inflammatory conditions, heart diseases, atherosclerosis, and autoimmune disorders (31). The effect of polyunsaturated fatty acids (PUFA) might be exerted via modulation and reduction of prostaglandin E2 (PGE2) synthesis, reducing inflammation and affecting alkaline phosphatase (ALP) activity (32). Furthermore, linoleic acid is an essential fatty acid and a precursor of arachidonic acid biosynthesis, which is a substrate for eicosanoid synthesis (33). The fatty acid composition (and high amounts of PUFA) makes the *O. humifusa* a special fruit for nutritional application.

**Effects of OHS on body weight, organ index and feed intake of rats**

The five groups of rats had a similar initial mean body weight. The body weights of the OVX groups were significantly higher than those of the Sham group ($p<0.01$) at one week after ovariectomy, and this difference was maintained throughout the entire experimental period ($p<0.01$ for all). The varying OHS dosages had no significant effect on preventing the body weight increase (Fig. 1A).

OVX caused significant atrophy of the uterine tissue when compared to the Sham group ($p<0.01$), indicating that the surgical procedure was successful. In addition,

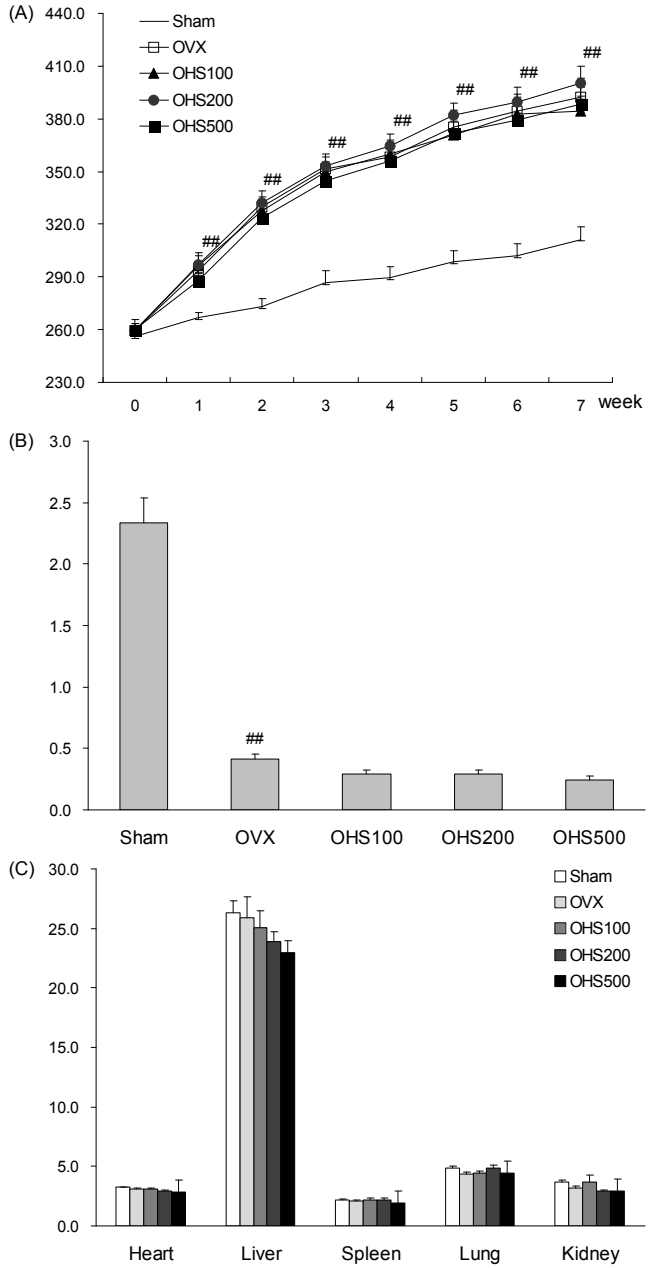


**Fig. 1.** Effect of a seven-week treatment with *O. humifusa* seeds on body and organ weight of ovariectomized (OVX) rats. Female SD rats (10 weeks old) were randomly assigned to sham-operated (Sham) and four ovariectomy (OVX) groups: OVX with vehicle (OVX), and OVX with 100, 200, or 500 mg/kg/day OHS. (A) The body weights of the animals were recorded weekly during the experimental period. (B) The uterus was isolated and weighed after the animals were euthanized. The uterus index is the uterus weight divided by the body weight. (C) The heart, liver, spleen, lungs and kidneys were also isolated and weighed after sacrificing the animals. The organ index is the organ weight divided by the body weight. Values are expressed as mean±SE (n=9). $^{\#\#}p<0.01$ vs. Sham, as evaluated by ANOVA.

OHS did not elicit any uterotrophic effect at any of the dosages (Fig. 1B). The tissue weights of the heart, liver, spleen, lungs and kidneys did not significantly differ among the groups (Fig. 1C). The feed intake of the OVX group was significantly higher than the Sham group ($p<0.01$) after ovariectomy (Table 4).

In general, ovarian hormone deficiency increased body weight, as seen in previous studies (34-36). Although the exact mechanisms by which OVX induces increases in body weight are not clear, a recent study has suggested that estrogen plays an important role in stimulating the differentiation of progenitor cells through the osteoblast lineage rather than the adipocyte lineage (37). Estrogen may be directly involved in energy metabolism by binding to estrogen receptors within abdominal, subcutaneous, and brown fat pads (38,39). None of the OHS doses tested prevented the increase in body weight induced by OVX in rats. In addition, OHS did not show any uterotrophic activity because uterine weight did not differ between the OVX and OHS groups. Zhang et al. (40) reported that Du-zhong cortex extract does not affect body or uterus weight in OVX rats, compared to 17α-ethinylestradiol effects. These results suggest that OHS may not function as 17α-ethinylestradiol in the regulation of body weight and uterus growth in OVX rats.

**Effects of OHS on the serum lipid profile**

The OHS groups had significantly ($p<0.05$) lower serum TC and LDL-C when compared to the OVX group (Table 5). In the OHS groups, the TC levels were 93.4, 85.0 and 77.0 mg/dL for the OHS100, OHS200, and OHS500 groups, respectively, suggesting a dose-dependent response. However, no significant differences in HDL-C were observed among similar groups. Interestingly, lower TC concentrations correlated directly with decreased liver weights in OHS fed rats.

One of the major problems when interpreting the differences among serum lipids and lipoproteins is establishing whether such differences are fully or partially dependent on the hormonal changes between pre- and post-menopausal women (41). A low estrogen concentration in both humans and ovariectomized rats is usually associated with elevated TC and LDL-C levels (42,43). These adverse changes in lipid metabolism following menopause may also contribute to an increased risk of coronary heart disease (44). The administration of OHS might protect the OVX rats from the negative changes associated with low estrogen availability. Our data clearly showed a significant decrease in TC and LDL-C levels among OHS groups, whereas HDL-C levels did not differ from those of the OVX group. In contrast to humans, both serum LDL-C and HDL-C levels decrease in rats treated with oral estrogen. This may occur because the predominant plasma cholesterol in rodents is HDL, which comprises approximately 60～70% of the total

**Table 4.** Effects of seven-week treatment with *O. humifusa* seeds on the feed intake of ovariectomized rats

| Feed intake, g/week | Sham | OVX | OHS100 | OHS200 | OHS500 |
|---|---|---|---|---|---|
| Day 1 to 7 | 151.7±6.3 | 190.3±10.1$^{\#\#}$ | 187.7±5.6 | 187.6±5.5 | 174.4±9.0 |
| Day 8 to 14 | 139.1±10.5 | 194.7±6.6$^{\#\#}$ | 196.8±5.5 | 193.4±5.3 | 190.4±6.6 |
| Day 15 to 21 | 152.3±6.9 | 189.9±6.8$^{\#\#}$ | 194.4±4.9 | 189.9±6.9 | 187.4±7.9 |
| Day 22 to 28 | 140.2±4.6 | 179.0±6.8$^{\#\#}$ | 176.7±4.8 | 181.2±4.5 | 175.4±5.8 |
| Day 29 to 35 | 141.9±6.7 | 175.7±6.5$^{\#\#}$ | 171.3±4.8 | 175.8±4.5 | 170.1±7.8 |
| Day 36 to 42 | 127.6±7.1 | 152.1±5.3$^{\#}$ | 149.1±4.3 | 151.2±4.3 | 143.1±6.8 |
| Day 43 to 49 | 123.0±14.2 | 152.3±5.3 | 148.8±5.9 | 154.3±3.8 | 146.9±6.2 |

Values are expressed as mean±SE (n=9). Sham-operated group (Sham) and four ovariectomy (OVX) subgroups: OVX with vehicle (OVX) and OVX with 100, 200, and 500 mg/kg/day OHS (OHS100, OHS200, OHS500). $^{\#}p<0.05$, $^{\#\#}p<0.01$ vs. Sham, as evaluated by ANOVA.

**Table 5.** Effect of *O. humifusa* seeds on the levels of total cholesterol, HDL-cholesterol and LDL-cholesterol in ovariectomized rat serum (mg/dL)

| Group | Total cholesterol | HDL-cholesterol | LDL-cholesterol |
|---|---|---|---|
| Sham | 79.80±2.65 | 31.24±1.96 | 8.33±0.62 |
| OVX | 129.00±1.85$^{\#}$ | 30.73±1.46 | 16.20±0.47$^{\#}$ |
| OHS100 | 93.40±1.63$^{*}$ | 36.66±0.77 | 8.80±0.66$^{*}$ |
| OHS200 | 85.00±1.84$^{*}$ | 27.66±1.29 | 9.20±0.25$^{*}$ |
| OHS500 | 77.00±2.05$^{*}$ | 27.38±0.94 | 9.20±0.24$^{*}$ |

Values are expressed as mean±SE (n=9). Sham-operated group (Sham) and four ovariectomy (OVX) subgroups: OVX with vehicle (OVX) and OVX with 100, 200, and 500 mg/kg/day OHS (OHS100, OHS200, OHS500). $^{*}p<0.05$ vs. OVX and $^{\#}p<0.05$ vs. Sham, as evaluated by ANOVA.

cholesterol pool (45). Direct correlations between the LDL-C level and atherosclerosis have previously been reported (46,47). The changes in serum TC level observed in the present study are similar to those reported by Gossell-Williams et al. (48), who found that supplementation with pumpkin seed oil decreased the TC levels in ovariectomized rats.

**Effects of OHS on hepatic enzymes in serum**

The effects of OHS on hepatic enzymes are shown in Fig. 2. The OVX group exhibited an elevation in serum GPT and GOT when compared to the Sham group ($p<0.05$). The oral administration of OHS significantly ($p<0.05$) suppressed serum GOT and GPT elevation when compared to the OVX group. The serum GPT and GOT levels often increase in response to liver injury, fatty liver and hepatitis (49). In the present study, decreases in the levels of GPT and GOT were observed in the OHS groups, suggesting that OHS confers protection on liver tissues against damage or disease. In addition, reductions in serum GPT and GOT levels were associated with lower liver weights in OHS-treated rats. The hepatoprotective effect of *O. humifusa* extracts in rats treated with carbon tetrachloride was reported by Park et al. (50).

Natural products with strong medicinal properties are gaining importance in light of the serious side effects posed by chemically produced medicinal derivatives (51). Thus, it may be of great potential benefit to evaluate the bioactivity of OHS as a whole mixture of phytochemicals rather than as single compounds. In conclusion, the results of this study suggest that OHS has favorable effects on the serum lipid profile and lipoprotein contents, translating to a potential role for treating atherosclerosis and hyperlipidemia in menopausal women.

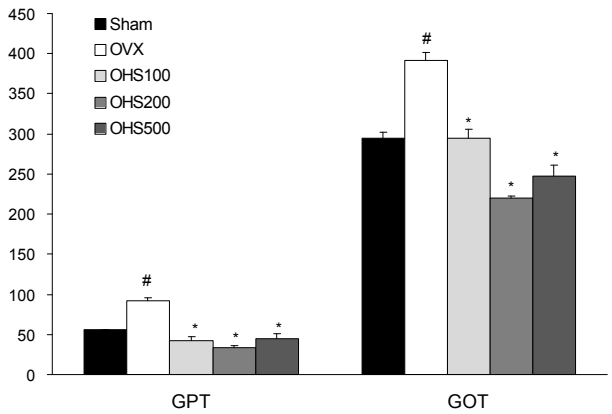


**Fig. 2.** Effect of *O. humifusa* seeds on hepatic enzymes in ovariectomized rats. Values are expressed as mean±SE (n=9). GPT, glutamate-pyruvate transaminase; GOT, glutamate-oxaloacetate transaminase. Sham-operated group (Sham) and four ovariectomy (OVX) subgroups: OVX with vehicle (OVX) and OVX with 100, 200, and 500 mg/kg/day OHS (OHS100, OHS200, OHS500). $^{*}p<0.05$ vs. OVX group and $^{\#}p<0.05$ vs. Sham group, as evaluated by ANOVA.

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