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Comparison of the Effects of Deer Antler, Old Antler, and Antler Glue on Osteoporosis in Ovariectomized Rats

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ABSTRACT

Background: Examination of the effects of deer antler, old antler, and antler glue on postmenopausal osteoporosis in an ovariectomized Sprague-Dawley rat model.
Methods: The study involved 7 experimental groups; SHAM (sham-operated rats), OVX (ovariectomized rats), E2 (ovariectomized rats with estradiol 10 μg/kg daily, orally), DA (ovariectomized rats with deer antler extract 5.83 mg/kg), OA (ovariectomized rats with old antler extract 3.8 mg/kg), low-AG (ovariectomized rats with low dose of antler glue powder 12.5 mg/kg), high-AG (ovariectomized rats with high dose of antler glue powder 37.5 mg/kg). After 6 weeks of treatment, body weight, blood calcium, phosphorus, estradiol, liver [alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT)] and kidney [blood urea nitrogen (BUN)/creatinine ratio] function, and femoral bone mineral density (BMD) were measured.
Results: The body weights of DA, OA, low-AG, and high-AG groups did not significantly differ from OVX group. Blood estradiol levels were significantly increased in the DA, low-AG, and high-AG groups compared to the OVX group. Blood calcium, phosphorus, ALP, AST, and ALT levels and BUN/creatinine ratio did not show significant changes in the DA, OA, low-AG, and high-AG groups.
BMDs of the femur, and femoral head and neck were significantly increased in the low-AG group. In the OA group, the BMD of the femoral head and neck was significantly increased.
Conclusion: Treatment with deer antler, or antler glue for 6 weeks was effective for increasing estradiol and femoral BMD in ovariectomized rats, suggesting that this may be of therapeutic benefit for osteoporosis.

Introduction

Osteoporosis is a skeletal disorder resulting in weakening of bone mineral density (BMD) leading to an increased risk of bone fracture. According to the World Health Organization (WHO), osteoporosis is a "systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk." In general, peak bone mass is achieved in the mid-20s to early 30s and maintained until middle age (50s), after which bone mass declines as bone resorption outpaces bone formation [1-3].

Osteoporosis may be classified into primary and secondary osteoporosis, and primary osteoporosis can be further divided into Type 1 (postmenopausal osteoporosis) and Type 2 (senile osteoporosis) osteoporosis. Type 1 osteoporosis occurs as a result of estrogen deficiency and bone resorption outpacing bone formation, though both increase. Type 2 osteoporosis occurs as a result of a reduced rate of bone formation caused by a gradual reduction of osteoblast production. In general, bone loss is expedited in postmenopausal women with osteoporosis due to the effect of aging to a state of female hormone deficiency [3,4].

Several herbal medicinal ingredients have been reported to tonify the liver and kidney and strengthen the sinew and bone, including deer antler, antler glue [5], old antler [6], semen cuscutae [7], lycii fructus [8], dried root of rehmannia glutinosa [9], corni fructus [10], and semen ziziphi spinosae [11]. Some of these prescriptions include yukmijiwhang-tang with radix puerariae [12], and daeyoungjeonganokyong [13], which tonifies kidney essence and invigorates the sinew and bone, and samul-tang with

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radix puerariae [14], which tonifies yin and blood. Studies on pharmacopuncture identified drynaria rhizome [15], rubi fructus [16], epimedii herba [17], and deer antler [18], which tonify kidney essence, and artemisia vulgaris folium [19], which warms the meridian and dissipates cold.

With regard to the efficacy of deer antler, old antler, and antler glue on osteoporosis, Kwak et al [20] reported that deer antler extract dose-dependently inhibited the differentiation of osteoclasts without cytotoxicity, while Zhang et al [21] stated that deer antler polypeptides prevented bone mass loss by inhibiting IL-1 and IL-6 in white rats with induced osteoporosis. Tseng et al [22] reported the therapeutic efficacy of a mixture containing deer antler and blood from deer antler on osteoporosis. Hwang et al [6] suggested that old antler water extracts were effective in treating osteoporosis in white rats with induced osteoporosis, as shown by the significant elevation of peroneotibial BMD. Further, Meng et al [5] reported that high-dose deer antler was the most effective in increasing BMD, although all 3 types of regimen (high and low doses of deer antler, and antler glue) improved BMD.

Osteoporosis is usually treated with herbal medicine ingredients that tonify the kidney essence. Based on the determination that deer antler, old antler, and antler glue are all appropriate for osteoporosis, which occurs as a result of a weakened kidney essence, we orally administered deer antler, old antler, low-dose antler glue, and high-dose antler glue extracts to white rats whose ovaries had been removed for 6 weeks.

Materials and Methods

Animal

Female (7 weeks old, 240 ± 12 g), white, Sprague-Dawley rats (Daehan, BioLink, Korea). underwent sham-operated (SHAM) surgery or bilateral ovariectomy. During the sham surgery, the skin on the back was incised but sutured without removing the ovaries. The experimental groups comprised of 5 rats per group and were housed in a polycarbonate cage, with 2 or 3 rats per cage at a temperature of 20–25℃, humidity of 45–55%, and 12-hour light and 12-hour dark cycles. Food (RodFeed, DBL Co., Korea) and water were given ad libitum. This animal study was approved by the Institutional Animal Care and Use Committee per the animal ethics regulations of Sangji University (IRB No.: 2016-19).

Purchase of medicinal herbs and drugs

Deer antler, old antler, and antler glue powder were purchased from Omniherb, Korea, and estradiol was purchased from SIGMA-ALDRICH Co. Korea.

Herbal medicine preparation

Old antler and deer antler (20 g) powder was heated in an electric kettle with 1,000 mL of distilled water for 4 hours. The heated solution was filtered through Whatman filter paper, and the filtrate was concentrated under low pressure in a vacuum rotary evaporator. The resulting concentrate was freeze-dried for 5 days. The yield was 1.866 g (9.33%) for the deer antler and 1.215 g (6.075%) for the old antler.

Medication

According to the method suggested by Lim et al [9] and Tseng et al [22], we set the standard dosage of estradiol at 10 μg/kg and administered 3 μg per white rat (300 g). For deer antler, old antler, and low-dose and high-dose antler glue, adult doses were converted according to the weight of the rats based on the method described by Jeon [11]. For deer antler and old antler, the daily dose for a 60-kg adult was assumed to be 3.75 g and so 18.75 mg was the calculated dose for a rat. After converting this to the weight of freeze-dried deer antler and old antler (mg), 1.75 mg of deer antler and 1.14 mg of old antler was administered to each rat. For the antler glue that we used, the instruction specifies that 0.75 g of antler glue is equivalent to 3.75 g of old antler. Thus, we calculated the weight-dependent dose based on the assumption that the daily dose for a 60-kg adult is 0.75 g (12.5 mg/kg). Low- and high-dose of antler glue were calculated to be 3.75 mg and 11.25 mg (threefold of the low dose), respectively. One white rat drinks about 25 mL of water daily, so the above amount was dissolved in 25 mL of normal saline in a water bottle to allow the rats to drink ad libitum (Table 1).

Classification of the study groups and study design

Five rats were assigned to each of 7 study groups: SHAM group that underwent sham surgery, ovariectomized rats (OVX) that underwent ovariectomy, E2 (estradiol) group that received estradiol after undergoing ovariectomy, deer antler extract (DA) group that received deer antler, old antler extract (OA) group, the low dose antler glue (low-AG) group, and the high dose antler glue (high-AG) group.

After 1 week following surgery, the rats were administered their corresponding drugs and the therapeutic efficacy of the drugs on osteoporosis were assessed 6 weeks later.

Body weight measurement

Body weight was measured once during the first week (Week 0), and once every week following drug administration, using electronic scales (CAS Co., Korea).

Blood sampling and blood test

After 6 weeks of drug administration, the rats were fasted for 1 day prior to blood sampling. First, anesthesia was induced with Zoletil 50 (Virbac SA, France) then, a blood sample was drawn through cardiac puncture. The blood sample was centrifuged at 2,000 rpm at 4℃ for 10 minutes (HA-1000-3, Hanil, Korea), and the resulting sample was submitted to the GC Labs (Korea) to analyze calcium, phosphorus, estradiol, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), and creatinine levels.

Table 1. Daily Dose in Ovariectomized Rats.

	Dose standard	Daily dose
Estradiol (E2)	10.00 μg/kg	3.00 μg
Deer antler (DA)	5.83 mg/kg	1.75 mg
Old antler (OA)	3.80 mg/kg	1.14 mg
Low antler glue (low-AG)	12.50 mg/kg	3.75 mg
High antler glue (high-AG)	37.5 mg/kg	11.25 mg

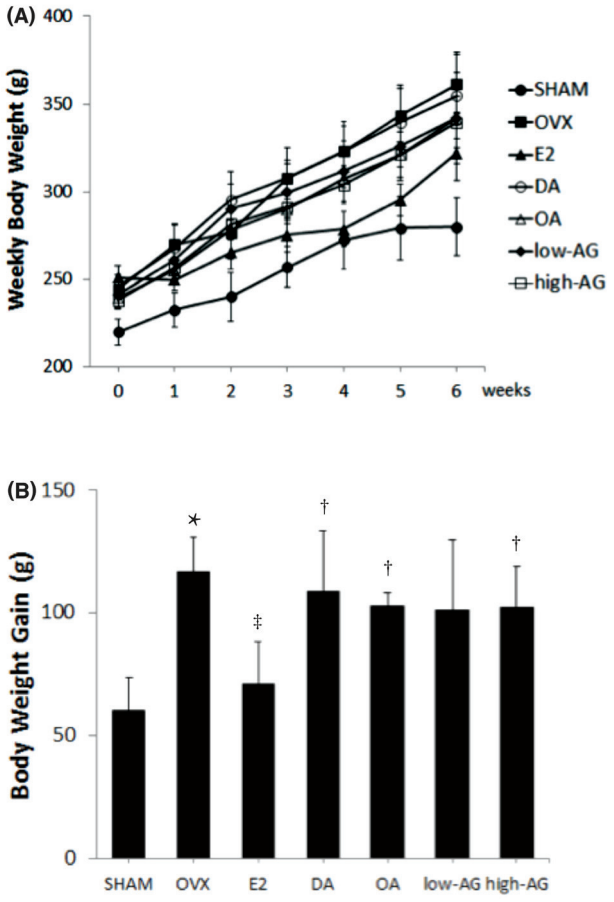

Fig. 1. Effects of deer antler, old antler, and antler glue on weekly body weight (A), and body weight gain (B) in ovariectomized rats. The body weight gain was calculated by the equation: final body weight – initial body weight [17].

Values are presented as mean ± SD.
*$p< 0.01$ and †$p< 0.05$ compared with SHAM group. ‡$p< 0.05$ compared with OVX group.
DA, ovariectomized rats with deer antler extract (5.83 mg/kg, daily, orally) for 6 weeks; E2, ovariectomized rats with estradiol (10 μg/kg, daily, orally) for 6 weeks; high-AG, ovariectomized rats with high dose of antler glue powder (37.5 mg/kg, daily, orally) for 6 weeks; low-AG, ovariectomized rats with low dose of antler glue powder (12.5 mg/kg, daily, orally) for 6 weeks; OA, ovariectomized rats with old antler extract (3.8 mg/kg, daily, orally) for 6 weeks; OVX, ovariectomized rats; SHAM, SHAM operated rats.

BMD measurement

After drawing blood samples the rats were sacrificed by cervical dislocation, and their right femurs were taken to measure BMD using dual-energy X-ray absorptiometry (DXA) (PIXImus, Lunar Co., USA)

Statistical analysis

Statistical analyses were performed using the SPSS Statistics 22.0 (SPSS Inc., Chicago, USA) software, and measurements were presented as mean ± standard deviation. Univariate Analysis of Variance (ANOVA) was performed, and Tukey Honest Significant Difference (HSD) was used as a post-hoc test. Statistical significance was set at a $p< 0.05$.

Results

Changes of body weight

All groups gained weight, but the highest mean final weight was in the OVX group (361.2 ± 16.94 g). Compared to the SHAM group, the OVX group ($p< 0.01$) and DA, OA, and high AG groups ($p< 0.05$) gained significantly more weight, while the E2 group gained significantly less weight ($p< 0.05$). The DA, OA, low AG and high AG groups gained less weight than did the OVX group, but the differences were not statistically significant (Fig. 1).

Blood analysis

There were no significant differences in the calcium and phosphorus levels across the groups. Compared to the SHAM group, the OVX group had a significantly lower estradiol level ($p< 0.01$), while the E2 group had a significantly higher estradiol level ($p< 0.01$). Compared to the OVX group, the E2, and high AG groups ($p< 0.01$) and DA, and low AG groups ($p< 0.05$) had significantly higher estradiol levels, and the OA group had a higher estradiol level, but this was not statistically significant . Compared to the E2 group, the SHAM, OVX, DA, OA, low AG, and high AG groups had significantly lower levels of estradiol ($p< 0.01$). ALP was significantly lower in the E2 group than in the OVX group ($p< 0.05$). Compared to the OVX group, the DA, OA, low AG, and high AG groups had lower levels of ALP, but this was not statistically significant. AST and ALT levels did not significantly differ across the groups. Compared to the OVX group, the DA, low AG, and high AG groups ($p< 0.01$) and OA group ($p< 0.05$) had significantly higher BUN levels, and compared to the E2 group, the DA, OA, low AG, high AG groups had significantly higher BUN levels ($p< 0.01$), but there were no significant differences in creatinine level and BUN/creatinine ratio across the groups (Table 2, Fig. 2).

BMD analysis

The total femoral BMD was significantly higher in the SHAM, E2, and low AG groups than that of the OVX group ($p< 0.05$). Although the DA, OA, and high AG groups had higher BMD than that of the OVX group, this difference was not statistically significant. The high AG group had a significantly lower BMD than that of the SHAM group ($p< 0.05$). With regard to the BMD of the femoral head and neck, the SHAM, E2, OA, and low AG groups had significantly higher BMD than the OVX group ($p< 0.05$), and the DA and high AG groups had higher BMD than the OVX group, but this was not statistically significant (Table 3, Fig. 3).

Discussion

During menopause, a woman's ovarian follicle reserve is depleted and the concentration of estradiol released by the ovaries is reduced to about $1/6^{th}$ of that observed in healthy young women. Estradiol promotes bone formation by increasing the calcium concentration in bones, it helps osteoblast survival, and induces osteoclast apoptosis. Hence, the reduction of estrogen is speculated to be the major cause of postmenopausal osteoporosis [23,24].

Drug therapies for osteoporosis include, hormone supplementation (e.g., estrogen), agents such as bisphosphonates which inhibit bone resorption, active vitamin D_3 which improves bone metabolism, vitamin K which stimulates bone formation while inhibiting bone resorption, and calcitonin injection which lowers blood calcium and phosphorus concentrations.

Table 2. Effects of Deer Antler, Old Antler, and Antler Glue on Serum Parameters in Ovariectomized Rats.

	SHAM	OVX	E2	DA	OA	low-AG	high-AG
Calcium (mg/dL)	10.43 ± 0.4	10.74 ± 0.87	10.48 ± 0.23	11.92 ± 0.62	10.90 ± 0.78	10.58 ± 0.79	10.52 ± 0.79
Phosphorus (mg/dL)	7.23 ± 1.45	8.28 ± 1	6.54 ± 0.42	9.23 ± 0.79	6.96 ± 1.94	8.32 ± 1.73	6.70 ± 1.53
Estradiol (pg/mL)	3.99 ± 0.48	0.93 ± 0.55 *	6.46 ± 0.5 *‡	2.59 ± 0.61 §‖	2.23 ± 1.12 ‖	2.61 ± 0.19 §‖	3.6 ± 0.28 ‡‖
ALP (U/L)	84.67 ± 6.60	126.25 ± 18.38	79 ± 13.39 §	113 ± 7.79	112.33 ± 22.31	106.33 ± 13.7	113.33 ± 17.44
AST (U/L)	130.75 ± 25.17	149 ± 50.99	126.2 ± 39.39	201.33 ± 26.74	180.6 ± 38.82	186.25 ± 34.63	169 ± 24.52
ALT (U/L)	35 ± 5.74	46.75 ± 8.84	33.6 ± 6.34	57 ± 9.63	50 ± 9.19	44.5 ± 7.4	54.5 ± 8.44
BUN (mg/dL)	16.28 ± 1.43	19.92 ± 1.79	16.48 ± 1.49	24.5 ± 2.67 ‡‖	22.52 ± 2.64 ‡‖	22.58 ± 1.89 ‡‖	21.7 ± 1.05 ‡‖
Creatinine (mg/dL)	0.51 ± 0.09	0.5 ± 0.07	0.51 ± 0.02	0.61 ± 0.08	0.53 ± 0.07	0.54 ± 0.07	0.51 ± 0.05
BUN/creatinine Ratio	33.7 ± 8.83	40.39 ± 5.05	32.44 ± 3.84	40.55 ± 2.97	43.39 ± 9.55	42.58 ± 5.72	43.46 ± 5.79

Values are presented as mean ± SD.
*$p < 0.01$ and †$p < 0.05$ compared with SHAM group. ‡$p < 0.01$ and §$p < 0.05$ compared with OVX group. ‖$p < 0.01$ compared with E2 group
ALP, alkaline phosphatase;ALT, alanine transaminase;AST, aspartate transaminase;BUN, blood urea nitrogen;DA, ovariectomized rats with deer antler extract (5.83 mg/kg, daily, orally) for 6 weeks; E2, ovariectomized rats with estradiol (10 μg/kg, daily, orally) for 6 weeks; high-AG, ovariectomized rats with high dose of antler glue powder (37.5 mg/kg, daily, orally) for 6 weeks; low-AG, ovariectomized rats with low dose of antler glue powder (12.5 mg/kg, daily, orally) for 6 weeks; OA, ovariectomized rats with old antler extract (3.8 mg/kg, daily, orally) for 6 weeks; OVX, ovariectomized rats; SHAM, SHAM operated rats.

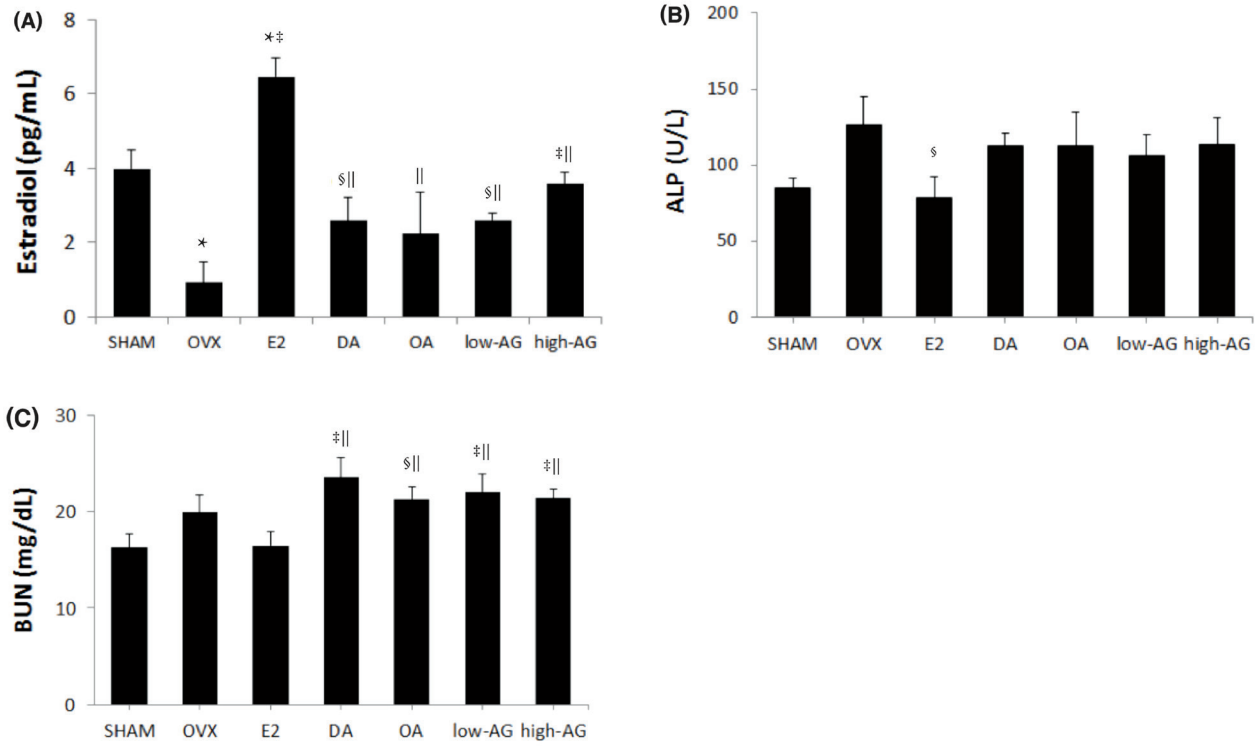

Fig. 2. Effects of deer antler, old antler, and antler glue on estradiol (A), ALP (B), and BUN (C) in ovariectomized rats.

Values are presented as mean ± SD.
*$p < 0.01$ and †$p < 0.05$ compared with SHAM group. ‡$p < 0.01$ and §$p < 0.05$ compared with OVX group. ‖$p < 0.01$ compared with E2 group.
ALP, alkaline phosphatase;BUN, blood urea nitrogen;DA, ovariectomized rats with deer antler extract (5.83 mg/kg, daily, orally) for 6 weeks; E2, ovariectomized rats with estradiol (10 μg/kg, daily, orally) for 6 weeks; high-AG, ovariectomized rats with high dose of antler glue powder (37.5 mg/kg, daily, orally) for 6 weeks; low-AG, ovariectomized rats with low dose of antler glue powder (12.5 mg/kg, daily, orally) for 6 weeks; OA, ovariectomized rats with old antler extract (3.8 mg/kg, daily, orally) for 6 weeks; OVX, ovariectomized rats; SHAM, SHAM operated rats.

Table 3. Effects of Deer Antler, Old Antler, and Antler Glue on Bone Mineral Density of Right Femur (g/cm2) in Ovariectomized Rats.

	Femur	
	Total	Head & Neck
SHAM	0.2517 ± 0.0066 ‡	0.2375 ± 0.0053 ‡
OVX	0.2236 ± 0.0066 *	0.1828 ± 0.0096 *
E2	0.2472 ± 0.0103 ‡	0.2269 ± 0.0103 ‡
DA	0.2389 ± 0.0062	0.2176 ± 0.0161
OA	0.2321 ± 0.0042	0.2236 ± 0.0025 ‡
low-AG	0.2499 ± 0.0119 ‡	0.2262 ± 0.0033 ‡
high-AG	0.2302 ± 0.0038 †	0.2186 ± 0.0082

Values are presented as mean ± SD.
*$p < 0.01$ and †$p < 0.05$ compared with SHAM group. ‡$p < 0.05$ compared with OVX group.
DA, ovariectomized rats with deer antler extract (5.83 mg/kg, daily, orally) for 6 weeks; E2, ovariectomized rats with estradiol (10 μg/kg, daily, orally) for 6 weeks; high-AG, ovariectomized rats with high dose of antler glue powder (37.5 mg/kg, daily, orally) for 6 weeks; low-AG, ovariectomized rats with low dose of antler glue powder (12.5 mg/kg, daily, orally) for 6 weeks; OA, ovariectomized rats with old antler extract (3.8 mg/kg, daily, orally) for 6 weeks; OVX, ovariectomized rats; SHAM, SHAM operated rats.

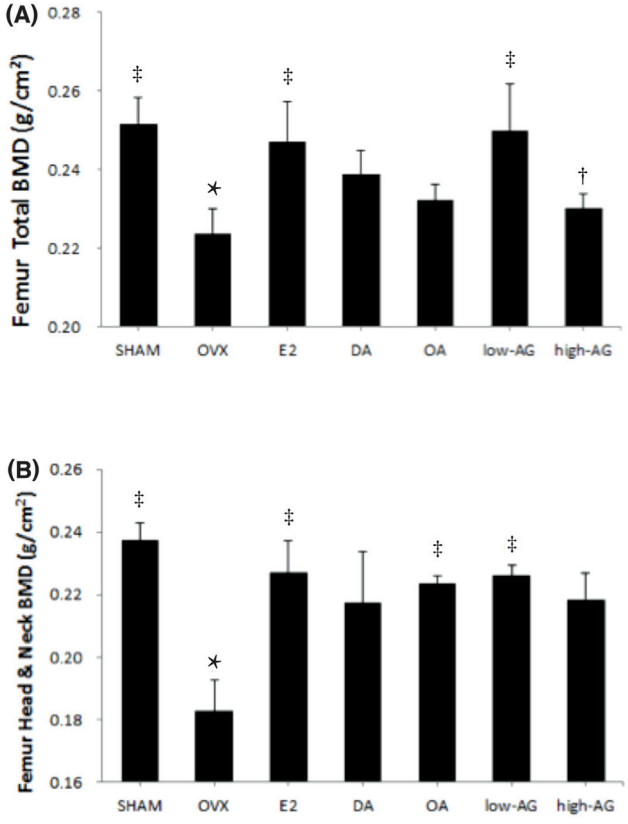

Fig. 3. Effects of deer antler, old antler, and antler glue on BMD of right femur (A) total, and (B) head & neck in ovariectomized rats.

Values are presented as mean ± SD.
*$p < 0.01$ and †$p < 0.05$ compared with SHAM group. ‡$p < 0.05$ compared with OVX group.
BMD, bone mineral density;DA, ovariectomized rats with deer antler extract (5.83 mg/kg, daily, orally) for 6 weeks; E2, ovariectomized rats with estradiol (10 μg/kg, daily, orally) for 6 weeks; high-AG, ovariectomized rats with high dose of antler glue powder (37.5 mg/kg, daily, orally) for 6 weeks; low-AG, ovariectomized rats with low dose of antler glue powder (12.5 mg/kg, daily, orally) for 6 weeks; OA, ovariectomized rats with old antler extract (3.8 mg/kg, daily, orally) for 6 weeks; OVX, ovariectomized rats; SHAM, SHAM operated rats.

Estrogen supplementation may have some side effects, such as carcinogenesis, bleeding, and thrombus formation. Bisphosphonates may induce upper gastrointestinal tract disturbance and liver dysfunction, and an active vitamin D_3 may cause hypercalcemia and hypercalciuria. Further, vitamin K may cause digestive symptoms, while calcitonin injection may induce shock [25]. Thus, efforts to discover alternative treatments with fewer side effects, when taken in the long term, are still underway.

Deer antler is taken from young antlers that have not or have mildly formed bones from *Cervus nippon Temminck, C. elaphus Linne,* and *C. canadensis Erxleben* bucks and has been reported to have pharmacological effects such as stimulating hematopoietic stem cells, improving immunity, treating osteoporosis, and promoting antifungal effects. Old antlers, which are antlers that have already formed bony tissues, can be used in replacement of deer antlers, but its efficacy is known to fall short than that of deer antlers. Antler glue is a lump of collagen made by cutting an old antler, obtaining an extract and concentrating it. It is similar to deer antler but has lower tonifying effects, so it has to be taken for prolonged periods to be efficacious [26,27].

Experimentation on ovariectomized white rat models is a simple, commonly used method to study postmenopausal osteoporosis [24], and white Sprague-Dawley rats have been reported to be an appropriate model for osteoporosis assessment [28]. After orally administering the prepared drugs for 6 weeks, the effects of deer antler, old antler, low-dose antler glue, and high-dose antler glue were examined using body weight measurement, blood test, and radiologic imaging.

In general, women gain weight as they undergo menopause [29], and ovary-removed white rats have also been known to be heavier than those that underwent sham surgery [30]. In our study, all white rats that underwent ovariectomy gained more weight than those that underwent sham surgery. The E2 group gained significantly less weight than the OVX group, while the DA, OA, low AG, and high AG groups also gained less weight than the OVX group, but this was not statistically significant, suggesting that administration of deer antler, old antler, and antler glue had no effect on the weight of ovary-removed white rats.

Calcium and phosphorus are stored in bones, and they contribute to forming a firm tissue structure. Postmenopausal women have a reduced ability to store calcium in their bones, show lower calcium absorption in the intestine, and have increased calcium excretion through the kidney, leading to increased bone loss [3]. Furthermore, calcium and phosphorus levels in the blood are known to increase when bone is rapidly destroyed, such as during bone metastasis, and when bone absorption is increased [31]. In this current study, calcium levels were lower in the E2, low AG, and high AG groups than in the OVX group, and phosphorus levels were lower in the E2, OA, and high AG groups than in the OVX group, but this was not statistically significant. Maintaining homeostasis of calcium and phosphorus levels is important because they play critical roles in cellular physiology [23]. The fact that all groups showed no significant differences in these levels compared to the SHAM group indicates that these levels were maintained

consistently even after the use of the corresponding drugs.

The DA, low AG, and high AG groups had a significantly higher estradiol level than the OVX group, and the OA group had a higher estradiol level than the OVX group, but this was not statistically significant. Furthermore, the DA, OA, low AG, and high AG groups had lower estradiol levels than the E2 group, but this was not statistically significantly different from that of the SHAM group. Therefore, we speculate that deer antler, low-dose antler glue, and high-dose antler glue contributed to treating osteoporosis, although estradiol levels were different from that of the E2 group.

ALP is an enzyme found in the liver, bones, placenta, and small intestine, and its blood concentration increases when bone is actively formed [32,33]. Blood ALP concentration is used as a biochemical indicator of bone turnover, which is high in ovary-removed white rats [9,22,30,34], presumably due to an elevation of both bone formation and resorption. In this study, the E2, DA, OA, low AG, and high AG groups showed a lower ALP concentration than the OVX group, but this was not statistically significant, and only the E2 group had a significantly lower ALP concentration compared to the OVX group.

AST and ALT are liver function indicators that are generally used in clinical practice, as they normally exist in liver cells but are released into the blood when liver cells are destroyed [33]. Our findings showed that the DA, OA, low AG, and high AG groups did not significantly differ in their AST and ALT levels from the SHAM and OVX groups, suggesting that these drugs are not significantly associated with liver damage.

BUN and creatinine are frequently used to assess renal function, although they cannot detect very early renal disorders. Creatinine levels are relatively consistent, but BUN level is easily affected by factors other than the kidney; hence, renal function assessed by BUN/creatinine ratio maybe more reliable. Problems with the excretory function of the kidney will lead to an increase in both BUN and creatinine levels, so the BUN/creatinine ratio gives consistency [31,35]. DA, OA, low AG, and high AG groups had significantly higher BUN levels than the OVX and E2 groups, which is speculated to be due to the continual protein intake, as deer antlers contain an abundant amount of amino acids that break down and eventually increase the BUN concentration. Creatinine levels and BUN/creatinine ratios did not significantly differ across groups, implying that these herbal medicines do not affect renal excretory function.

The standard method for diagnosing osteoporosis is by measuring BMD and using biochemical indicators of bone metabolism. DXA is a method of quantitative BMD measurement with low radiation exposure that is currently widely used in Korea. In general, DXA measures BMD from the lumbar and femoral areas [26]. BMD of white rats can be measured with peripheral DXA (pDEXA) [9,30,36,37] or micro-computed tomography (μCT) [22,38].

In this study the right femur of the white rats was removed to measure the overall femoral BMD and BMD of the head and neck of the femur via pDEXA. All groups had greater total femoral BMD than the OVX group, but the difference was only statistically significant in the SHAM, E2, and low AG groups. All groups had higher BMD of the femoral head and neck than the OVX group, but the difference was only significantly different in the SHAM, E2, OA, and low AG groups. Based on these results, deer antler, old antler, and antler glue all seem to be effective in preserving and increasing the overall BMD in the femur and BMD in the femoral head and neck, with low-dose antler glue having the most significant results. The differences of BMD in the femoral head and neck and overall femur may suggest that the drug administration period was not sufficiently long enough for the herbal medicines to affect the overall femur.

Meng et al [5] compared the effects of high-dose deer antler (540 mg/kg), low-dose deer antler (180 mg/kg), and antler glue (110 mg/kg) on osteoporosis and reported that all 3 were effective, but high-dose deer antler was the most effective. Compared to the doses used in this study (deer antler 62.5 mg/kg, low-dose antler glue 12.5 mg/kg, high-dose antler glue 37.5 mg/kg), Meng et al [5] used a dose of antler glue that was 3 times higher than that of the high-dose antler glue in our study, a dose of low-dose deer antler that is 3 times higher than that of the deer antler dose used in our study, and a dose of high-dose deer antler that is 9 times higher than that of the deer antler dose used in our study. When converted for a human adult weighing 60 kg, this is about 37.5 g of deer antler, which is rather excessive. Such differences of doses may have led to varying results.

Type 1 osteoporosis caused by menopause and Type 2 osteoporosis caused by aging combine and progress at similar time points [3], one shortcoming of this study was that only young white rats were used. In addition, the DA, OA, low AG, and high AG groups had lower ALP concentrations than the OVX group, but the differences were not statistically significant. Femoral BMD was also higher in the DA, OA, low AG, and high AG groups than that in the OVX group, but the differences were only statistically significant in the low AG and OA groups. An insufficient drug administration period may have resulted in a false negative result.

This study compared the effects of 2 types of doses of antler glue, in addition to deer antler and old antler, on osteoporosis. In particular, antler glue is similar to the effect of deer antler, but it is a cheaper medicine ingredient that can effectively replace deer antler. Therefore, in this study, the capacity of antler glue was set at 2 doses to derive the appropriate dose of antler glue. Although the high-dose antler glue (37.5 mg/kg) was 3 times that of the low-dose antler glue (12.5 mg/kg), its effects were not 3 times as great; in fact, low-dose antler glue had more significant effects, presumably because the low dose was closer to the appropriate dose for white rats. Future studies should investigate the effects of various doses and various drug administration periods.

Overall, we found that 6 weeks of deer antler, old antler, and antler glue administration was effective on osteoporosis in ovary-removed white rats by increasing estradiol concentration and femoral BMD, with a dose of 12.5 mg/kg of antler glue being the most effective.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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