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Studies on the Effects of Antler Extract in Osteoporosis-Induced Rats
Ⅱ. Effect of Antler Extract on Body Weight, Femur Weight, Bone Ash Quantity, Organ Weight and Histological Changes in Osteoporosis-Induced Rats

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녹용 추출물 투여가 골다공증 유발 Rat에 미치는 효과에 관한 연구
Ⅱ. 녹용 추출물 투여가 골다공증 유발 Rat의 체중, 골회분량, 대퇴 및 장기중량 및 조직상의 변화에 관한 연구

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ABSTRACT

In this study, we investigated the preventive and therapeutic effects of antler-extract for osteoporosis. Rats were ovariectomized bilaterally and were fed up with Ca- and P-free diet in order to induce osteoporosis. Body weight, organ weight, the weight of.femur and bone ash quantity were examined for 5 weeks. We also performed histological and electronical microscopic examinations.

1. After adminstration of female and male antler extract to osteoporosis-induced rats at the doses of 625 and 1250 mg/kg, respectively, the body weights were significantly increased compared with those of normal control group's which was 230.2±2.3~281.0±2.5g ($p<0.05$).
2. The weights of both right and left femur of osteoporosis-induced rats, administered with female or male antler-extract, little decreased compared with those of normal control group.
3. The bone ash quanties of femur of osteoporosis-induced rats, administered with female or male antler-extract, little decreased compared with those of normal control group.
4. The weights of liver, spleen, and kidney of osteoporosis-induced rats, administered with female or male antler-extract, decreased compared with those of normal control group.
5. Histological and electronic microscopical findings were (1) that in normal control rats the connections of lacunae appeared well and were without loss of bone mineral, (2) that in ovariectomized rats the connections of lacunae were mostly broken and were with loss of bone mineral compared with those of normal control rats, (3) that in osteoporosis-induced rats,

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administrated with female or male antler-extract, the shape of lacunae and the connections of them were similar to those of normal control rats.

These findings suggest a possible protective and therapeutic effects of female or male antler extract against bone loss in ovariectomized rats.

(Key words : Osteoporosis, Estradiol, Calcitonin, Osteocalcin, Ca, P, ALP)

Ⅰ. INTRODUCTION

Osteoporosis is a metabolic disturbance of bone. The main symptom of osteoporosis is the decrease of bone density. People who are suffering from osteoporosis are more susceptible to fractures than others. Osteoporosis occurs in cats or dogs (Dachshund) as an inherited disease. Sometimes it occurs by nonspecific causes such as aging, non-use, nutritional imbalance (Ohta et al., 1992a,b; Brunelli and Einhorn, 1998; Hauselmann et al., 1998; Bemben, 1999).

In animals, osteoporosis arise from the decrease in bone anabolism when nutritionally deficient state was sustained. In lamb osteoporosis occurs from functional disorders of osteoblasts in the status of copper deficiency or disturbances in osteoid formation in the status of chronic lead poisoning. The pharmaceutical effects and compositions of antlers are different among these according to the portions of the antler and the habitats where the deer live.

Antlers contain several essential amino acids, Ca, Mg, saccharides, squalene, mucoploysaccharide, triglyceride, uracil, uridine, hypoxanthine (metabolites of nucleic acids) prostaglandin, lipopolysaccharides, phospholipids, cholesterol- derivatives, epidermal growth factor (EGF). And the receptor of insuline-like growth factor was found at the end of an antler. The effects of antler-extract are growth-promoting of white rats, promotion of hepatic function, promotion of haematopoietic function of bone marrow, promotion of antibody production, phagocytic activity, and overall strengthening of immunity from the reticuloendotherial system, prevention of aging-process related osteoporosis (Suttie et al., 1985; Hattori et al., 1989; Elliott et al., 1992; Zhang et al., 1992).

Clinical treatments of osteoporosis are differentiated into the prevention of bone resorption and the use of estrogen, calcitonin biphosphonate, Ca, vitamin D-derivatives to induce bone formation. The use of estrogen has several side effects. It is hard to keep administration time appropriately. It is contraindication the use of estrogen to whom are suffering breast cancer, metrocarcinoma, hepatic disorder, hypertension, migrane headache etc. The risk factors of osteoporosis are ages, sex, race or genetic inheritance, endocrinal disorders such as abnormal secretion of estrogen, thyroidal and parathyroidal hormones, nutritional imbalance, long term uses of anti-epileptics or steroid-derivatives, smoking, alcholism etc.

During the 5 weeks administration period, we observed the changes of the weights of body, organs and femurs and bone mineral quantities of osteoporosis-induced rats to elucidate the preventative and therapeutic effects of antler extract. We also carried out light and electronic microscopic observations.

Ⅱ. MATERIALS AND METHODS

1. Animals

10 week-old female Sprague-Dawley 200 rats were used. Animals were acclimatized for 2 weeks before use, and had free access to feed and water.

The cycles of dark and light were 12h/12h. The other animal husbandry were followed according to the methods of Chungnam National University. Experimental groups were allocated to control (Control) group, ovariectomized (OG) group, sham operation (SO) group, female antler-extract (FA) administeed group, male antler-extract (MA) administrated group. SO rats were laparotomized without ovariectomy. The FA and MA groups were supplied with Ca- and P-deficient diet for 5 weeks after ovariectomy, and then they were administered with the respective antler-extract.

2. Preparation and Administration of Antler-extracts

The antlers of Elk deer were obtained from the Department of Livestock Improvement, National Livestock Research Institute (Kim et al., 1996). The distal one third of each antler (11.25g) was boiled with 10 times water (v/v) for 4 h, according to the prescription of Oriental medicine. Thereafter, the antler was extracted, filtrated, and concentrated into a volume of 60 ml. The female antlers were obtained by the method of NLRI. The antler extracts were administered per oraly with stomach tube at the doses of 625, 1,250 mg/kg body weight in every other day.

3. Induction of Osteoporosis

The rats were anesthesized i.p. with 0.01 ml/g of Avertin solution (Aldrich Co., USA) and were incised at one third of midline according to the method of Waynforth and Flecknell (Waynforth and Flecknell, 1996). Osteoporosis was determined by analysis of serum concentrations of estradiol, calcitonin, osteocalcin, Ca, P, and ALP activity. In addtion, osteoporosis was confirmed by light and electron microscopies.

4. Measurements of Body Weight

After ovariectomizing the animals, the body weight of the animals were measured with electronic balance (Shimadzu, Japan) just before the blood samples were collected from the animals. The body weight were measured for 5 weeks.

5. Measurements of Organ Weights

The weights of liver, spleen, both kidneys were measured. Before the measurement, each organ was trimmed off connective tissues and fat and then removed blood and body fluid with filter paper. The weights of organ were measured with electronic balance (Shimadzu, Japan). After the measurement, the organs was dipped in 10% formaline for microscopic observations.

6. Measurements of Bone Weight and Mineral

The weights of both left or right femur were measured. The femurs were trimmed off other tissues except bone and epiphyseal cartilage before measured. After being measured its weight, the femur was put into melting pot, and then 6N-HCl was poured into the pot. The pot was put into a oven and heated at 600°C for 24 hours. The weight of the ash which was collected from the pot was measured after storing at room temperature for 30 minutes.

7. Histological and Electronic Microscopic Observations

For light microscopical observations (Nikon, Japan), the fragments of femurs were decalcified with 5% HNO_3 solution for 3 days, and then dehydrated by alcohol series. The dehydrated bone tissues were embedded in paraffin. The paraffin blocks were sectioned by micrtome (each 4~5 μm), and then stained with Hematoxylin- Eosin.

For scanning electron microscopical observations (SEM, Hitachi-100, Japan), the fragments of femurs were fixed with 2.5% glutaraldehyded (Merk,

Germany) diluted with 0.1M cacodylate buffer (pH 7.3) for two hours at room temperature. And then fixed again with 1% osmium tetroxide (Merk, Germany) diluted with 0.1M cacodylate buffer (pH 7.3) for two hours.

8. Statistical Analysis

Statistical significances among groups were determined by Duncan's multiple range test with General Linerars Model (GLM) Procedure (SAS ver. 6.12, SAS Institute, 1996).

Ⅲ. RESULTS AND DISCUSSION

1. Changes in Body Weight

The changes in body weight of each group are represented in Table 1. Both groups which were administered antler-extracts showed significant increases in body weight compared to other groups ($p<0.05$). Ovariectomized group which weren't administered antler-extract showed less increase in body weight compared to control group.

Ovariectomized group showed decrease in their body weight after the operation. But after recovered from the post-operational stress, oariectomized group started to gain weight significantly. These were almost coincident with Tarttelin and Gorski (1971, 1973), Bagi et al. (1997), Mueller and Hsiao (1980), and Li et al. (1997). Also, Geiselman and Almli (1978) reported the increases in body weight and water intake after ovariectomy.

2. Changes in Femur Weight

The changes in the weight of femur after administration of antler-extract to osteoporosis-induced rats are represented in Table 2.

The weight of femur of both antler-extract administered group decreased a little compared to normal control group's. Between antler-extract administrated groups, the weights of femur from female antler-extract administered group was heavier than thoes of male antler-extract administered group's but without any significance.

These are similar with Li et al. (1997) and Kinny et al. (1995) who reported that the bone loss from the epiphyses, metaphyses, and the decreases of diaphyseal diameters of tibiae is aggravating with time. It is also coincident with Li et al. (1997) and Kinny et al. (1995), and Wronski et al. (1988). Li et al. (1997) and Kinny et al. (1995) reported bone losses after 2 weeks from ovariectomy. And Wronski et al. (1988) reported bone losses and the increases of porosity and resorption of the bone after 2 weeks from ovariectomy. In the bending test

Table 1. Effects of female or male antler extracts on body weights in ovariectomized rats

Experimental group	Body weight (g ± SD)				
	1	2	3	4	5(w)
Control[a]	230.2±2.3	241.5±2.8	262.8±2.9	270.3±3.3	281.0±2.5
SO	220.9±3.3	230.0±2.9	248.0±2.8	256.8±3.3	278.0±2.7
OG	208.2±2.2	225.4±2.4	245.0±2.7	250.8±4.3	270.0±3.9
FA 625	213.2±3.5	242.2±3.1	261.4±3.4	282.5±3.8	311.0±3.0
FA1250[b]	234.8±3.3	255.7±4.5	270.3±3.9	290.9±3.2	314.3±3.4
MA 625	227.6±3.2	241.2±3.2	279.6±2.9	296.2±3.0	307.5±3.2
MA1250[b]	216.3±3.4	252.3±4.1	293.5±3.7	298.0±3.2	304.0±3.4

* Values with different superscripts within column were significantly different($p<0.05$)

** OG : Ovariectomized group, SO : Sham operation, FA : Female antler, MA : Male antler

Table 2. Effects of female or male antler extracts on femur weights in ovariectomized rats

Experimental group	Femur weight (g ± SD)									
	1		2		3		4		5(w)	
	R	L	R	L	R	L	R	L	R	L
Control	0.69±0.1	0.69±0.1	0.86±0.2	0.88±0.1	0.78±0.1	0.76±0.1	0.77±0.3	0.79±0.1	0.83±0.3	0.85±0.1
SO	0.69±0.1	0.67±0.1	0.72±0.2	0.73±0.1	0.73±0.1	0.75±0.2	0.69±0.1	0.68±0.1	0.83±0.2	0.83±0.1
OG	0.70±0.1	0.69±0.1	0.70±0.2	0.70±0.2	0.68±0.2	0.68±0.2	0.78±0.1	0.67±0.1	0.65±0.2	0.65±0.2
FA 625	0.72±0.2	0.71±0.2	0.70±0.1	0.70±0.1	0.67±0.1	0.68±0.1	0.69±0.1	0.70±0.2	0.81±0.2	0.83±0.2
FA1250	0.62±0.1	0.84±0.2	0.71±0.1	0.74±0.2	0.73±0.2	0.78±0.2	0.77±0.2	0.78±0.2	0.87±0.2	0.88±0.2
MA 625	0.68±0.1	0.71±0.2	0.65±0.1	0.64±0.1	0.72±0.1	0.74±0.2	0.75±0.2	0.75±0.2	0.84±0.2	0.84±0.2
MA1250	0.70±0.2	0.69±0.1	0.76±0.2	0.75±0.1	0.78±0.1	0.77±0.1	0.74±0.2	0.75±0.2	0.79±0.1	0.78±0.1

of Kinny et al. (1995), the skeleton of ovariectomized rats showed reduction in the thickness of compact and trabecular bone, and showed increased bone loss rate time-dependently. Bagi et al. (1992) and Peng et al. (1997) inferred from the result of torsion test of overloading and unloading group that the increase of bone density and volume in overloading group was resulted from increased physical load when compared with unloading group's.

3. Changes in Bone Ash Quantities

The changes in bone ash quantities of osteoporosis-induced rats are represented in Table 3.

Both antler-extract administered groups showed significant decreases in bone ash quantities of femur compared to normal control group's. The decreases in bone ash quantities of femurs from both antler-extract administered groups' are closely related to the decrease of estrogen concentration in serum, and it is coincident with the report of Ammann et al. (1992) and Wronski et al. (1988) which the bone loss was more prominent in femurs (7% of total bone ash quantity) than any other bone after 4 weeks from ovariectomy. The bone ash quantities of femurs were decreased significantly compared to shame operation group's, and it is coincident with the reports of Yamazaki and Yamaguchi (1989).

4. Changes in Organ Weights

The changes in organ weight of osteoporosis-

Table 3. Effects of female or male antler extracts on bone ash quantity in ovariectomized rats

Experimental group	Bone ash quantity (g ± SD)				
	1	2	3	4	5(w)
Control[a]	0.34±0.01	0.34±0.02	0.33±0.01	0.34±0.01	0.34±0.01
SO	0.33±0.01	0.34±0.02	0.33±0.01	0.34±0.01	0.34±0.01
OG[b]	0.30±0.01	0.28±0.01	0.27±0.01	0.25±0.01	0.24±0.01
FA625	0.29±0.01	0.29±0.00	0.30±0.01	0.31±0.01	0.31±0.01
FA1250[b]	0.30±0.01	0.31±0.01	0.31±0.01	0.32±0.01	0.32±0.01
MA625	0.29±0.01	0.29±0.01	0.30±0.01	0.30±0.01	0.30±0.01
MA1250	0.29±0.01	0.29±0.01	0.30±0.01	0.30±0.01	0.30±0.01

* Values with different superscripts within column were significantly different($p<0.05$)

Table 4. Effects of female or male antler extract on liver and spleen weights in ovariectomized rats

Experimental group	Liver and spleen weight (g ± SD)									
	1		2		3		4		5(w)	
	L	S	L	S	L	S	L	S	L	S
Control	10.4±0.40	0.49±0.15	10.3±0.45	0.46±0.13	9.4±0.33	0.52±0.13	12.3±0.33	0.87±0.23	12.3±0.31	0.69±0.12
SO	8.7±0.25	0.54±0.13	11.7±0.38	0.58±0.14	9.0±0.32	0.49±0.24	12.3±0.33	0.63±0.27	11.2±0.42	0.54±0.13
OG	7.8±0.34	0.55±0.12	9.7±0.25	0.58±0.12	10.1±0.26	0.55±0.18	10.5±0.37	0.57±0.21	10.8±0.33	0.50±0.21
FA 625	10.2±0.32	0.67±0.21	11.4±0.43	0.59±0.26	10.1±0.41	0.49±0.26	11.6±0.47	0.86±0.23	13.6±0.65	0.97±0.24
FA1250	8.1±0.28	0.43±0.17	12.9±0.55	0.72±0.27	10.4±0.45	0.55±0.19	10.8±0.34	0.68±0.18	12.3±0.52	0.83±0.26
MA 625	10.5±0.43	0.56±0.15	9.7±0.44	0.63±0.23	12.9±0.52	0.71±0.24	10.6±0.47	0.58±0.18	12.6±0.44	0.68±0.19
MA1250	9.5±0.27	0.65±0.20	12.1±0.49	0.65±0.28	12.6±0.46	0.64±0.27	12.4±0.53	0.60±0.23	12.6±0.52	0.60±0.21

*L : Liver, S : Spleen

Table 5. Effects of female or male antler extract on kidney weights in ovariectomized rats

Experimental group	Kidney weight (g ± SD)									
	1		2		3		4		5(w)	
	R	L	R	L	R	L	R	L	R	L
Control[a]	0.90±0.21	0.93±0.15	0.89±0.21	0.87±0.16	0.93±0.21	0.96±0.13	1.17±0.22	1.12±0.13	0.98±0.32	0.93±0.17
SO	0.84±0.19	0.91±0.18	1.02±0.17	1.04±0.20	0.87±0.12	0.92±0.14	0.97±0.18	1.06±0.13	1.03±0.24	0.92±0.15
OG	0.82±0.22	0.88±0.20	0.82±0.25	0.84±0.21	0.87±0.22	0.88±0.18	0.90±0.18	0.91±0.12	0.92±0.22	0.90±0.21
FA 625[b]	0.92±0.17	0.93±0.19	0.99±0.22	0.96±0.17	0.99±0.15	0.94±0.15	0.96±0.19	1.09±0.16	0.96±0.18	0.93±0.19
FA1250[b]	0.82±0.15	0.87±0.17	1.08±0.21	1.10±0.12	1.01±0.24	1.06±0.21	1.02±0.22	0.98±0.19	0.97±0.19	0.98±0.16
MA 625[b]	1.00±0.20	1.02±0.21	1.07±0.19	1.03±0.13	1.05±0.20	1.09±0.18	1.12±0.21	1.08±0.22	1.30±0.22	1.29±0.21
MA1250[b]	1.07±0.16	1.05±0.21	1.01±0.18	0.97±0.10	1.02±0.13	0.95±0.17	1.02±0.16	0.97±0.18	1.16±0.21	1.19±0.24

* LK : Left kidney, RK : Right kidney

induced rats after administration of female or male antler-extracts are represented in Table 4 and 5.

After administration of antler-extract at the doses of 625 and 1,250 ± mg/kg, the weight of liver were 10.1±0.41~13.6±0.65g, 8.1±0.28~12.3±0.52g and 9.7±0.44~12.9±0.52g, 9.5±0.27~12.6±0.52g, respectively for each dose of group. These showed decreases compared to that of normal control group's 9.4±0.33~12.3±0.33g. The weights of spleen of antler-extract administered groups were 0.49±0.26~0.97±0.24g, 0.43±0.17~0.83±0.21g and 0.56±0.15~0.71±0.24g, 0.60±0.21~0.65±0.28g, so showed increase compared to normal control group's. The weight of kidneys of antler-extract administered groups showed decrease compared to normal control. By the way, kidneys of female antler-extract administered group were heavier than those of male antler-extract administered group whithout significance (Seo et al., 1998).

5. Light and Electronic Microscopic Observations

In the cases of normal control group, the connections between trabecular bone were sustained well, so it was hard to find bone loss. But in the cases of ovariectomized group, the connections between trabecular bone were weakened or broken

almost, so bone losses were significantly increased in this case. In contrast to the cases of ovariectomized group, the shapes of trabecular bones and connections between trabecular bone were preserved well in the cases of antler-extract administered groups (Luis et al., 1986). These observations are similar with those of normal control group. There was no difference between female or male antler-extract administered groups, and between different doses (Fig. 1).

Under electron scanning microscopic observation, the number of lacunae were numbered 16 (normal control), 26 (ovariectomized group), 18 (antler-extract administrated group). The number of lacunae of antler-extract adm inistered group was similar to that of normal control group and decreased prominently compared to that of ovariectomized group (Table 6).

Fig. 1. Electronic microscopical findings of the transverse section of femur in normal group (SEM, 60μg×500).

Fig. 2. Electronic microscopical findings of the transverse section of femur in ovariectomized group (SEM, 60μg×500).

Fig. 3. Electronic microscopical findings of the transverse section of femur in female antler extract group (SEM, 60μg×500).

Table 6. Electronic microscopical findings on femur bone in ovariectomiz rats administered with female or male antler extract

Treatment	Electronic microscopical findings	
	No. of lacunae	Loss of bone mineral
Control	16	Normal
OG	26	Mostly broken, loss
FA	18	Similar to control
MA	19	Similar to control

Ⅳ. 요 약

본 연구는 골다공증유발 rat에 녹용추출물의 투여가 예방 및 치료효과에 미치는 영향을 구명하고자, 양측 난소를 적출한 후 골다공증이 유발된 rat에 암, 수녹용 추출물 525, 1,250 mg/kg을 투여했을 때 체중, 대퇴골의 중량, 장기중량, 골회분량의 측정 및 조직학적, 전자현미경적 검사 등을 실시하였다.

1. 골다공증유발 rat에 암, 수녹용 추출물을 투여했을 때 체중은 정상대조군에 비하여 유의한 증가를 나타냈다 ($p<0.05$).
2. 골다공증유발 rat에 암, 수녹용 추출물을 투여했을 때 우측, 좌측의 femur중량은 정상대조군에 비하여 감소하였다.
3. 골다공증유발 rat에 암, 수녹용 추출물을 투여했을 때 골회분량은 정상대조군에 비하여 약간 감소된 치를 나타냈으나 유의성은 인정되지 않았다.
4. 골다공증유발 rat에 암, 수녹용 추출물을 투여했을 때 간과 비장 및 신장의 각각 정상대조군에 비하여 감소된 치를 나타냈다.
5. 골다공증유발 rat에 암, 수녹용 추출물을 투여했을 때 조직학적 및 전자현미경적 소견은 정상대조군에서는 소주골간의 연결이 비교적 잘 유지되어 있고 골소실을 발견할 수 없었으나 난소적출군은 정상군에 비하여 소주골이 가늘어졌거나 소주골간 연결부분의 대다수가 끊어져 골양이 많이 소실되었음이 관찰되었으며, 녹용처리군에서는 소주골의 형태가 굵고 연결부분이 잘 유지되어 정상군과 유사한 소견이 관찰되었다.

위의 결과들에서 암, 수녹용 추출액은 난소제거 후의 골소실을 억제하므로 골다공증의 예방과 치료에 효과가 있는 것으로 판단된다.

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