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Comparative Studies on Velvet Deer Antler and Ossified Deer Antler on the Contents of Bioactive Components and on the Bone Mineral Density Improving Activity for Oophorectomized Rat

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Abstract – Velvet deer antler (VDA) is well known oriental medicine claimed to have tonic activities as improving bone mineral density (BMD), immune-enhancing, rejuvenating and many other medicinal activities. Ossified deer antler (ODA) is bony product produced by over-calcification of deer antler due to late harvesting. The extraction efficiency of ODA by conventional boiling in water must be very poor due to bony nature, hence the reputations for the medicinal efficacies of ODA has been highly under-evaluated compared to that of VDA without any experimental evidences. Employing our new efficient water extraction process (135 °C), the extracts of ODA and VDA were analysed to compare the contents of bioactive components and the potencies of pharmacological activities. The results showed that; 1) The 135 °C extraction (autoclaving) of ODA gave highly increased amount of biomass, 120% more than the conventional extraction by 100-boiling, whereas the same treatment for VDA showed only 15% increased amount of biomass. 2) Feeding the ODA- or VDA-extracts to oophorectomized rats showed very potent BMD-recovering activity. 3) During the ossification of deer antler, the total collagen content was found to be increased by addition of type-1 to pre-existing type-2 collagen, but not replacement of type-2 to type-1 collagen. High titer of peptide hormones like growth hormone and IGF-1 were detected in the ODA- and VDA-extracts and also in the serum of ODA- or VDA-treated oophorectomized animals dose-dependently. Present experimental data will give a conclusion that folkloric poor reputations on ODA must be concerned only with poor extraction efficiency of conventional 100 °C water extraction and not based on the composition of bioactive substances of ODA.
Keywords – Ossified deer antler, Deer antler, Bone mineral density, Growth hormone, IGF-1

Introduction

Velvet deer antler (VDA) is oriental premium medicine claimed to have tonic activities, such as wound healing (Pnudden *et al*., 1965), growth-promoting for infants (Kim *et al*., 2006), improving bone mineral density (Shim *et al.*, 1999; Hwang *et al.*, 2010; Yook *et al.*, 2006), immune-enhancing (Suh *et al.*, 2001), anti-rheumatic (Trentham *et al.*, 1993; Barnet *et al.*, 1996; Kalden *et al.*, 1998), anti-anemic (Yong *et al.*, 1964), performance enhancing (Church *et al.*, 1999), rejuvenating (Mineshita *et al.*, 1935) and many other medicinal activities. Since the earliest scientific researches on velvet antler, several hundred scientific articles have been published on the biochemical compositions and pharmacological activities of VDA, resulting in today the recognition of most versatile multipurpose natural remedies even in the Western world.

Ossified deer antler (ODA) is bony product produced by over-calcification of deer antler due to the late harvesting. Due to the bony natures of ODA, traditional water extraction of ODA is very difficult, hence few research paper is available on the biochemical composition and on the pharmacological activities. This facts induced serious under-evaluation of ODA for the pharmacological efficacies without any experimental evidences (Pravin *et al*., 2010). Rajaran et al reported that type-2 collagen, an important anti-component of VDA, was replaced into inactive type-1 collagen during ossification. (Rajaram *et al*., 2004). He found under

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Table 1. The extraction yields of VDA and ODA depending on temperature changes

Temperature (°C)	90	100	110	120	130	135	139
ODA yield (%)	16.50 ± 1.01	19.50 ± 0.54	22.70 ± 0.98	28.60 ± 0.78	35.7 ± 0.21	42.70 ± 0.34	42.40 ± 0.29
VDA yield (%)	25.40 ± 0.99	29.40 ± 1.51	32.70 ± 0.09	34.80 ± 2.21	34.90 ± 2.87	35.40 ± 0.89	35.50 ± 1.58

microscopical observation of VDA- and ODA-slices that gradual transition of chondrocyte-pattern on VDA-slice into osteocyte-pattern during ossification process, and was derived the conclusion without any chemical assay for the type-2 and type-1 collagen composition. Furthermore, other paper (Klaus *et al*., 1977) described that the cell cultures of chondrocytes produced type-2 collagen during the early stage of culture, but transition occurred to type-1 collagen in the later stage of cell culture. This report induced also erroneous conclusion that type-2 collagen in VDA will be replaced to type-1 during the ossification of VDA. Present paper is describing 1) on a new efficient extraction method for ODA established by simply elevating the temperature to 135 °C, 2) on the compositional analysis of the ODA- and VDA-extracts on the types of collagens and peptide hormones as growth hormone and insuline-like growth factor and 3) on bone mineral density-enhancing activities of ODA-extract and VDA-extract for oophorectomized rats.

Experimental

Materials – Velvet deer antler (VDA) and ossified deer antler (ODA) were purchased from Kyung-dong herbal medicine market in Seoul, Korea as the commercial products imported from Russia. Standard type-1 and type-2 collagen were purchased from Chondrex Co., ELISA-kits for growth hormone and IGF-1 assay were purchased from R & D. Co.

Preparation of sample extracts – 50%-Acetic acid extraction (Extraction method-1); Ten grams of powdered VDA and ODA were extracted with 200 mL 50%-acetic acid by magnetic stirring at 4 °C for 24 hours to avoid heat denaturation of collagen components, and continuously dialysed against distilled water overnight and freeze-dried to obtain SDS-PAGE samples for the analysis of type-1 and type-2 collagen contents in VDA and ODA. Freeze dried samples were obtained 0.371 g of undenatured ODA-extract and 1.0786 g of VDA-extract.

High-temperature extraction (135 °C Extraction method-2); VDA (100 g) or ODA (100 g) was extracted with 500 mL water by heating to 135 °C for 3 hours. The extractions were repeated three times for the complete extraction. The extracts were pooled and filtered through filter paper, concentrated by vacuum evaporation and finally subjected to freeze drying to obtain VDA-extract and ODA-extract (refer to Table 1).

Table 2. Chemical composition of VDA- and ODA-extraxts

	Extraction method-2 (135°C, 3 × 3 hr)	
	VDA-extract	ODA-extract
Powdered extract used	100 g	100 g
dryed extract	31.3 g	42.6 g
Total collagen content (hydroxy-proline assay)	30.67 g	41.31 g
other components	0.63 g	1.29 g
Ash content*	44.7 g	50.6 g

*: Ash content was assayed by the general test methods included in 8th edition of Korea pharmacopoeia.

Conventional extraction (below 100 °C; Extraction method-3); VDA and ODA were extracted by heating to 100 °C or 90 °C and subsequent treatment followed as the way of Extraction method-2.

Analysis for total collagen content of VDA and ODA – Total collagen contents of VDA- and ODA-extract were assayed by the analysis of hydroxy-proline contents in the extraction process (Neuman *et al*., 1950). One gram of VDA-, or ODA-extract was dissolved in 20 mL of 6N-HCl and heated 16 hours in 118 °C-autoclave to hydrolyze the collagen components completely. After cooling the hydrolysate was filtered and one ml of the filtrate was mixed with 1 ml of 0.01 M-$CuSO_4$-solution, 1 mL of 2.5 N-NaOH and 1 mL of 6%-H_2O_2 and heated to 80 °C for 5 minutes to produce pyrrole. After cooling for 3 minutes, 4 mL of 3 N-H_2SO_4 and 2 mL of 5%-DBA (*p*-dimethylaminbenzaldehyde in propanol) were added to the above reaction mixture and warmed for 16 min in 70 °C water-bath to develop colour reaction. After cooling to room temperature the absorbancy at 540 nm were recorded. Parallel reaction was conducted with standard hydroxyproline to obtain calibration curve. The total collagen contents could be obtained by multiplication of 100/13.7 (refer to Table 2).

SDS-PAGE analysis of collagen composition (type-1 and type-2) of VDA and ODA;

Preparation of sample-solution for SDS-PAGE loading: Twelve mg of VDA-extract or ODA-extract prepared by extraction method-1 were dissolved in 1 mL

0.1 M-HAc and 10 μL of the resulting sample solution were mixed well with 5 uL of 2x-SDS-PAGE sample buffer (1 M Tris-HCl 1.25 mL, SDS 0.4 g, glycerol 2 mL, 2-mecaptoethanol, bromophenol 0.02 g, DW 5.33 mL are mixed in tube) and loaded on polyacrylamide gel plate.

Preparation of polyacrylamide gel (29 : 1) plate and electrophoresis: 12%-gel (1.5 M-tris base 2,600 uL. pH 8.8, 10%-SDS 100 uL, 10%-ammonium-persulfate 100 uL, tetramethylethylene-diamine 4 uL, D.W 3,200 uL), electrophoresis was run on 30 mA current for 2 hours.

Staining for analysis; PAGE-gel plate was stained by immersing in 0.25% CBB (coomassie brilliant blue)-solution for 30 min. and washed with distilled water. The PAGE-gel plate was scanned by using UN-SCAN-IT gel Version 6.1 program to give densitogram (Fig. 1). Parallel experiments were conducted with standards type-1 and type-2 collagens for the assignment of band. The intensities are expressed on identified band of Fig. 1. The polyacrylamide gel-electrophoregrams of VDA and ODA were converted to densitogram by employing UN-SCAN-IT gel Version 6.1-program and illustrated in Fig. 2.

Animal bleeding; Bleeding room conditions were set to constant temperature of 24 ºC (± 1.0), constant humidity 45% (± 5.0) and 12 hour alternative lightning and carefully controlled to maintain stress-free. Feeding was at libidum.

Oophorectomy of rat; Seventy female SD-rats of 4-weeks age weighing 220 g (± 10 g) were fed at libidum for 2-weeks and subjected to surgical operation for oophorectomy under isoflurane sleeping anesthetization.

Feeding experiments for bone mineral density determination; Animals were randomly allocated to nine groups. Group-1 is normal control group (no oophorectomy), Group-2 is sham-group (surgical operation but not oophorectomy), Group-3 is negative control group to which casein were fed instead of ODA- or VDA-extract to oophorectomized rats, Group-4 is positive control group to which VDA-extract (extraction method-2) 150 mg/kg. were fed to oophorectomized rats. Group-5~9 are experimental groups consisting of oophorectomized rats to which ODA-extracts were administered with increasing

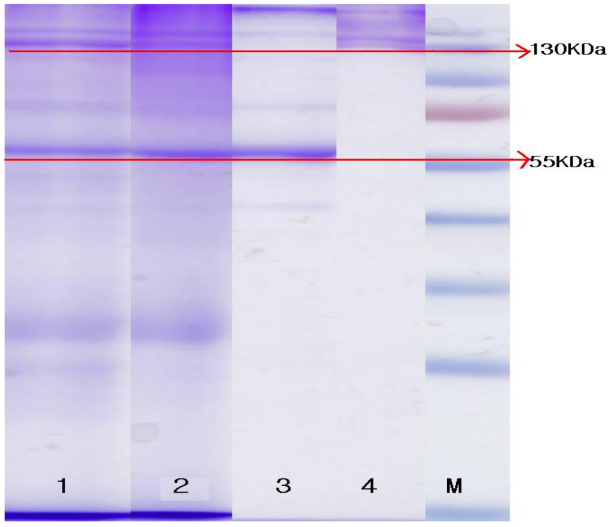

Fig. 1. SDS-PAGE of VDA- and ODA-extracts (12% gel, 80 Volt, 30 mA, 2 hour). 1; Ossified antler extract, 2; Antler extract, 3; Type-2 collagen, 4; Type-1 collagen, M; Size marker, VDA and ODA were extracted by 50%-acetic acid.

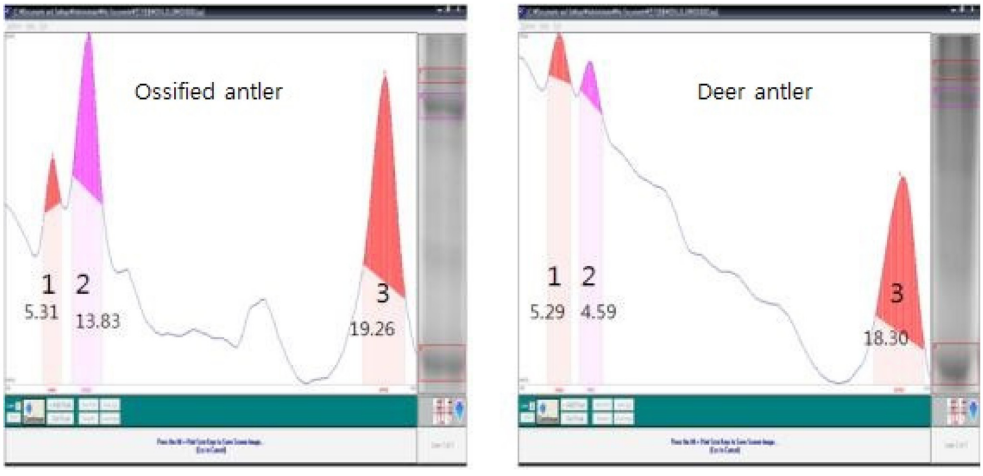

Fig. 2. Densitograms of ODA and VDA obtained by UN-SCAN-IT gel Version 6.1; Pixel-1 and pixel-2 were identified as Type-1 collagen and pixel-3 as type-2 collagen. Peak intensities are shown on each pixels. The peak intensity of type-1 collagen appears as 9.88 (5.29 + 4.59) and type-2 collagen appears as 18.3. Hence type-2 collagen content is almost twice compared to type-1. However, after the ossification of deer antler, the ratio of type-1 to type-2 changed to 19.14/19.26. This data means that due to ossification of deer antler to ossified deer antler, type-1 collagen is significantly increased but not replacement of type-2 to type-1 is occurred, but rather smaller increase of type-2 from 18.30 to 19.26.

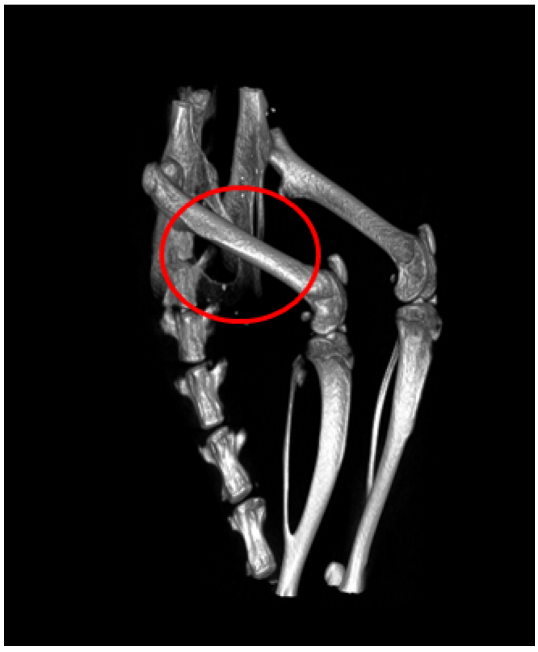

Fig. 3. Circled part is the position of measuement bone mineral density.

dose differences as 50, 100, 150, 200, 300 mg/kg. All samples were dissolved in distilled water and administered orally by using oral-sonde every day once for 16 weeks.

Measurement of bone mineral density and bone growth; Bone mineral densities of all experimental animals including 63 rats were measured individually on starting day and additional four times every four weeks by micro-CT (Inveon™, Siemens Preclinical Solution, USA). Operation condition for CT is as followings; x-ray voltage 80 kV, current 500 μA, exposure time 250 ms and filed of view (FOV) 56.97 * 71.21), CT-image was obtained by reconstruction by Shepp-Logan Filter Method from acquired images and image analysis were conducted by Inveon Research Workplace Programme (Siemens Preclinical Solution, USA). To determine the bone mineral density (BMD), region of interest assigned on certain point of femur (refer to Fig. 3) and the scanning were conducted from neck to patellar surface and the average values were assigned as BMD (Fig. 4 and 5). In this regults, the initial BMD (g/cm^3) was assigned as 100%. Fig. 4 shows the effect of ODA as dose-dependent change of BMD-curve. Bone length were measured on tibia (refer to Fig. 6).

Assay of peptide hormones;

1) Peptide hormone assay for VDA-extract and ODA-extract; VDA-extract and ODA-extract obtained by extraction method-1 or method-2 were dissolved in distilled water (10 mg/mL). Growth hormone and IGF-1

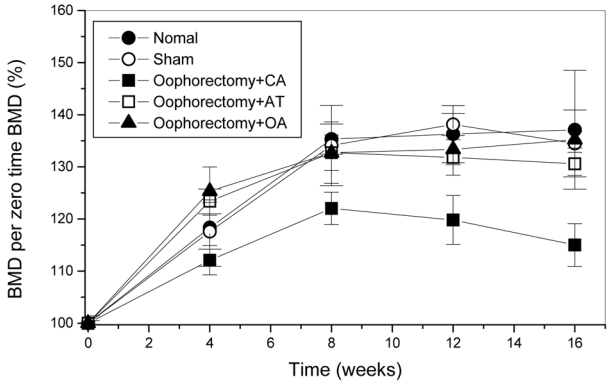

Fig. 4. Time-course data of bone mineral density change of ODA-extract on oophorectomized rats, CA; Casein 150 mg/kg, AT; VDA-extract 150 mg/kg, OA; ODA-extract 150 mg/kg, All time course data of ODA-extract, VDA-extract and Sham-group are showing almost same level of BMD with that of normal group. Only casein group is showing highly decreased BMD data all through the time course.

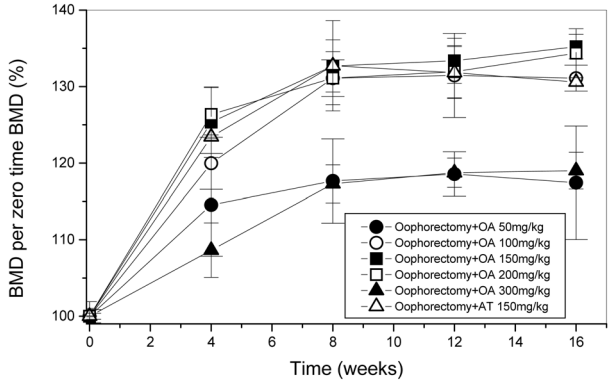

Fig. 5. Time course dose-dependency curve of ODA-extract on oophorectomized rats.; Dose dependencies are shown with upper limit of BMD, however, extra-higher dose (300 mg/kg) of ODA seems to have negative effect.

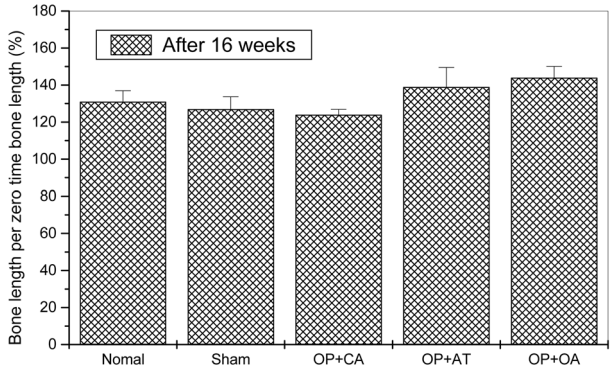

Fig. 6. Growth effect on bone length; Tibia bone lengths were measured by CT at the starting day of experiment and also at last day of experiment. The bone length of starting day was adopted as 100% and the ratio of final length (after 16-weeks) to initial length were shown as percentage growth. OP; Oophorectomy CA; Casein 150 mg/kg, AT; Antler extract 150 mg/kg, OA; Ossified antler extract 150 mg/kg.

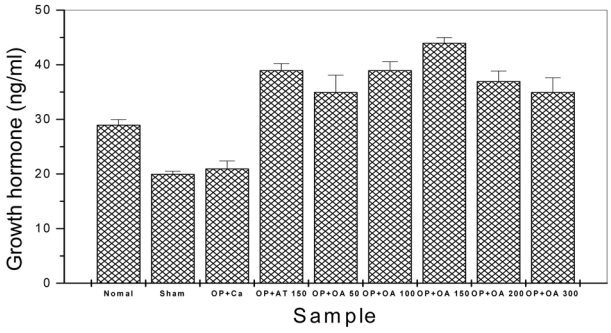

Fig. 7. Growth hormone level in the serum of experimental animals; Legends are same with Fig. 4.

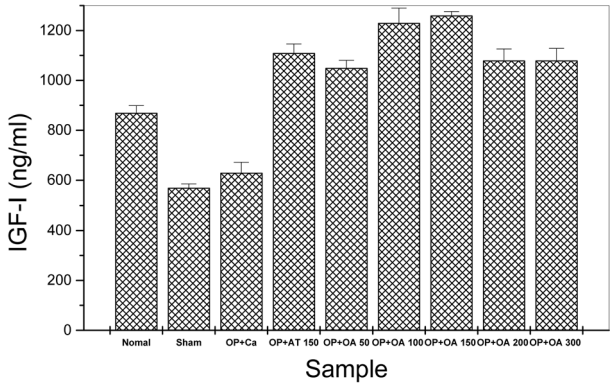

Fig. 8. The serum level of Insulin-like growth factor-1, The legends are same with Fig. 7.

were assayed by ELISA-kit (refer to Fig. 7 and Fig. 8).

2) Peptide hormone assay in the serum of experimental animals; At the end of 16 weeks experiment on BMD-measurement, bloods were taken and set aside for a while and centrifuged 15 minutes at 6,000 rpm to get light yellow serum. The serum level of growth hormone or IGF-1 was assayed by ELISA (refer to Fig. 9 and Fig. 10)

Statistical analysis; Experimental data were analyzed by original software (ver 8.0, Microcal Software Inc., USA). T-value was calculated with confidence limit of $p < 0.05$.

Results and Discussion

The Effect of extraction temperature on the extraction efficiency – As illustrated in Table 1, the extraction efficiencies of VDA and ODA have greatly different patterns depending on the extraction temperatures. VDA shows smaller increase of extraction rate depending on the elevation of extraction temperature from 100 °C to 135 °C, resulting in extraction efficiencies from 29.4% to 35.4%, however, ODA showed dramatic increase of

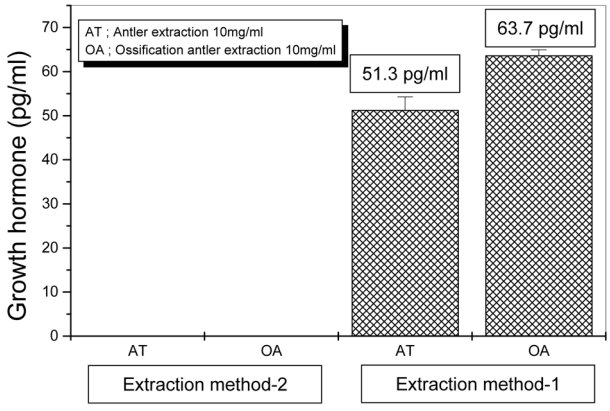

Fig. 9. Growth hormone contents in VDA- or ODA-extract; Both the extracts of antler and ossified antler extracted by 50%-acetic acid.

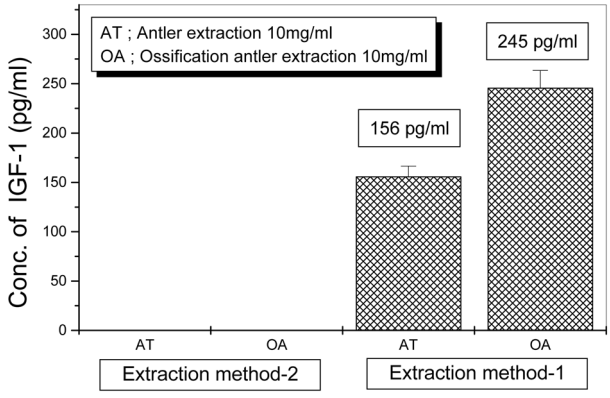

Fig. 10. IGF-1 contents in VDA- or ODA-extract; Both the extracts of antler and ossified antler extracted by 50%-acetic acid.

extraction yield from 19.5% to 42.7% by same temperature change.

These results denote that most of water soluble substances (83%) in VDA is extracted in water by boiling (100 °C), however, only 45.6% of the same substances in ODA is extracted by same treatment. These results suggest that the conventional extraction method must have influenced badly to the pharmacological potencies and hence to the serious under-evaluation of ODA in the market.

Analysis of total collagen contents in VDA- and ODA-extract (analysis of hydroxy-proline) – The total collagen contents of VDA- and ODA-extract were determined through the analysis of hydroxy-proline content in the hydrolysates of the extracts. As shown in Table 2, ODA-extract has higher content than VDA in respect to the total collagen, ash, and other organic substances. This facts imply that the ossification process of VDA increases not only insoluble inorganic calcium salt, but also water-soluble substances including total

collagen, calcium salt and other unidentified organic substances.

In other words, during ossification process, not only the increase of insoluble calcium salt, but also the increase of total collagen content must be occurred from 30.67% to 41.31%. Here, we had a question on the compositions of type-1 and type-2 collagens of ODA and VDA. It is well known facts that type-1 collagen is major component of hardened bone, however, type-2 collagen is major collagen component of cartilage. The type-2 collagen has been known as orally active anti-rheumatic substance (Kalden *et al.*, 1998), hence, we have deep interest on the higher content of type-2 collagen in the extract of VDA and ODA. In this respect, the composition of type-1 and type-2 collagen components in the VDA- and ODA-extracts were analyzed.

Assay for collagen compositions type-1 and -2 of VDA and ODA – Type-1 and type-2 collagen composition of VDA- and ODA-extract obtained by extraction method-1 were determined by combination of SDS-polyacrylamide gel electrophoresis and density scanning of electrophoregrams as shown in Fig. 1 and Fig. 2.

Fig. 1 is SDS-PAGE-electrophoregram of VDA- and ODA-extracts obtained by method-1 which enable to extract collagen components without any heat denaturation. Fig. 2 are densitograms obtained by UN-SCAN-IT gel Version 6.1. As shown in the densitogram, all of peak-1, -2, and -3 are appearing in both densitograms of VDA and ODA. These peaks were identified by parallel running of SDS-PAGE on standard type-1 and type-2 collagen, i.e. peak-1 and peak-2 were assigned as type-1 collagen, and peak-3 as type-2 collagen. The peak intensities are shown on the peaks of densitograms (refer to Fig. 2). Peak-1 plus peak-2 is corresponding to the concentration of type-1 collagen and peak-3 to type-2 collagen. These results show that type-1 collagen is highly increased during the ossification process of deer antler and type-2 collagen is also showing a smaller increase. This result shows clearly that the replacement of type-2 collagen to type-1 collagen suggested by Rajaram *et al*., is practically not occurring, rather a smaller increase of type-2 is occurred during ossification of antler. We conclude that Rajaram's erroneous conclusion could be derived from the lack of chemical analysis for the composition of collagen types of VDA- and ODA-extracts.

The authors are paying special interest to the type-2 collagen concentrations in VDA and ODA, since a number of scientific paper are describing the type-2 collagen from chicken as the orally active anti-rheumatic agent through oral tolerance mechanism to the auto-immune diseases as rheumatic arthritis. This oral tolerance activity of type-2 collagen has been shown to be active even when the type-2 collagen is denatured or partially hydrolyzed by proteolytic enzyme treatment (Kalden *et al*., 1998; Joachim *et al*., 1996; Barnet *et al*., 1998; Moskowitz *et al*., 2000; Tsuji *et al*., 2001).

Bone mineral density and bone growth enhancing activity of ODA- and VDA-extract – Bone mineral density enhancing activity of ODA-extract is illustrated in Fig. 4. ODA-extract (150 mg/kg. body weight) were administered orally once per day for 16 weeks to oophorectomiced rats, and measured BMD by CT (computer tomography) five times including starting time and every four weeks during 16 weeks. In this result the BMDs of normal-group, sham-group, ODA-group and VDA-group animals are showing gradual increases untill 8 weeks almost in parallel manner reaching to upper limitting plateau until the end of experiment, however, casein group showed seriously decreased BMD through out the total experimental periods. Statistically the BMD-differences between ODA- and VDA-groups are not significant, however, the BMD-difference between casein group and all other groups are highly significant. This results are opposing the classical concept that pharmacological activities of ODA-extract may be very weak than that of VDA.

Fig. 5 shows the dose-dependency curve of ODA-extract on BMD enhancing activities. The dose dependency (50 mg - 200 mg) were shown within dose range from 50 mg/kg to 200 mg/kg. However, overdose (300 mg/kg) showed clearly negative effect on BMD increasing tendencies. This unusual phenomena will be acceptable, when we assume that the BMD-increasing activity of ODA or VDA may be mediated via growth hormone activity, since there are a number of papers (Nam *et al*., 2001; Bremanert *et al*., 2003; Sunwoo *et al*., 1996; Fisher *et al*., 1998; Elliott *et al*., 1992; Jang *et al*., 2006; Mohan *et al*., 2005; Kim *et al*., 1999) describing that over-doses of growth hormone to enhance the BMD will reduce BMD and increase fat accumulation on the contrary. This results suggest strongly that BMD-enhancing activity of ODA or VDA may be demonstrated through the gastro-intestinal absorption of growth hormone (Mattheus *et al*., 1975; Heyman *et al*., 1992; Pang *et al*., 1981; Jakobsson *et al*., 1986; Husby *et al*., 1986; Mikiko *et al*., 2008; Michael *et al*., 1988) or growth hormone releasing factors in the extract of ODA or VDA. It is well known fact that growth hormone will bind to receptors on liver cells to produce insulin-like growth factor (IGF-1) (Elliott *et al*.,

1992; Jang *et al*., 2006; Mohan *et al*., 2005; Kim *et al*., 1999) and the IGF-1 is triggering osteocytes to increase bone mineral density. Based on this references, it was necessary to assay growth hormone and IGF-1 in the ODA and VDA-extracts and also in the serum of experimental animals.

Growth effect on bone length – Tibia bone length were measured by CT (computed tomography). Initial bone length adopted as 100% on Fig. 6. Normal group and sham group showed 30%-growth of tibia-bone length during 16-weeks experimental period. Casein group rats, which were oophorectomised, showed some decreased growth of 20% while VDA-group showed 39% growth and ODA-group showed the highest growth promoting activity by 41% growth. The ODA- and VDA-extracts showed non-disputable results of growth promoting activities to the oophorectomized rats. However, ODA-group seems to be numerically superior to VDA-group when the extracts were prepared by Extraction method-2 (135 °C), although statistically unsignificant.

Assay of growth hormone and IGF-1 – After the completion of the experiments on BMD-assay, the animals were sacrificed to get serum for the analysis of growth hormone (Fig. 7) and IGF-1 (Fig. 8) by using ELISA kits.

As shown in Fig. 7 and Fig. 8, the serum levels of growth hormone and IGF-1 are showing very similar pattern. i.e., sham group and casein group animals showed least serum level of growth hormone and IGF-1. However, ODA-group and VDA-group showed higher level of both hormones and further partial dose dependency in the case of ODA-treateded group. In the case of 200 - 300 mg/kg dosage groups of ODA treatment it showed some decrease of both hormones, possibly because some over-doses mechanism may be played.

As a whole, BMD-enhancing and bone length growing activity induced by feeding of VDA- or ODA-extract to oophorectomized rats may have direct concern with growth hormone and/or IGF-1 components or their precursor substances or their releasing factors contained in the VDA or ODA.

Based on this background, the contents of growth hormone and IGF-1 in the VDA- and ODA-extract were assayed by ELISA kits. As shown in Fig. 9 and Fig. 10, the undenatured extracts of ODA and VDA prepared by extraction method-1 showed higher titers of growth hormone and IGF-1 than those of VDA prepared by method-1, as shown on the Figs 9 and 10. However, VDA- and ODA-extract prepared by extraction method-2 (135 °C) showed completely no reactions to the ELISA-kits due to heat denaturation of proteins. This heat denatured extracts of ODA and VDA were administered to oophorectomized rats every days during 16 weeks long. Finally it was found that every parameters including BMD and serum level of growth hormone and IGF-1 at oophorectomized rats by oral administation of ODA-extract showed higher values than those data of VDA-extract-administered rats.

On the other hand, it will be very valuable for the understanding of somewhat controversial aspects of our present data mentioned above when we have deep consideration to the recent progress on the studies of intestinal absorption of intact protein molecule or trans-mucosal absorption of HMW-proteins (Mattheus *et al*., 1975; Heyman *et al*., 1992; Pang *et al*., 1981; Jakobsson *et al*., 1986; Husby *et al*., 1986; Mikiko *et al*., 2008; Michael *et al*., 1988; Wolf *et al*., 1984). These pioneering studies on intestinal absorption of intact protein molecules were actually started on 1975 years around. However, now it is widely accepted in the academic societies and furthermore growth hormone is now appearing in a form of nasal spray in market as a healthcare product various peptide hormones are appearing as macro-molecular complex (MMC-2000 is patented name) (Mikiko *et al*., 2008). Actually it is well known fact that wide spectrum of intact macro-molecules including various peptide hormones, antigenic proteins and anti-inflammatory plant enzymes are absorbed in the small intestine through M-cell pathway (Wolf *et al*., 1984). The treatment of rheumatic arthritis by using oral tolerance mechanism of type-2 collagen will belong to same category. It is well known fact that this intestinal absorption rate of intact macromolecules through M-cell pathway will be increased when the intact molecules are mixed with such denatured protein as gelatine (Mikiko *et al*., 2008) and the absorption rate will be decreased by aging.

These facts are greatly assisting us to understand the pharmacological aspects of deer antler's macro-molecular components.

Conclusion

Ossified deer antler (ODA) and velvet deer antler (VDA) were compared with their contents of some biologically active components as type-2 collagen and peptide hormones as growth hormone and IGF-1 and also on their bone mineral density enhancing activity to oophorectomized rats. The results show that decoction of ODA in 100 °C water is insufficient for the complete extraction of bio-active components. However, our new

extraction method heating at 135 ºC in an autocrave give us complete extraction of ODA. ODA-extract prepared by our new process showed higher contents of bioactive components as type-2 collagen, growth hormone and IGF-1 and higher potency of bone mineral density enhancing activity than those of VDA.

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