Oral Administration of *Saldi tierra* Containing Various Trace Elements Has Anabolic Effects on Bone Component in the Femoral Tissues of Rats

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The effects of *Saldi tierra* containing various trace elements on bone components in the femoral-diaphyseal and -metaphyseal tissues of rats was investigated. *Saldi tierra* (sodium salt or potassium salt) was obtained from natural zeolite using the method of ion exchange with sodium chloride or potassium chloride solutions. *Saldi tierra* contained more than 20 elements. Rats were orally administered a solution of *Saldi tierra* once daily for 7 days. Calcium content, alkaline phosphatase activity and DNA content in the femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues of rats were significantly increased with the administration of *Saldi tierra* sodium salt (100 mg/100 g body weight). Calcium content and alkaline phosphatase activity in the femoral-diaphyseal tissues of rats were significantly increased with the administration of *Saldi tierra* sodium salt (50 mg/100 g). *Saldi tierra* (50 mg) contained 3.155 mg of calcium. Femoral components were not significantly altered by the administration of calcium chloride (Ca 3.155 mg/100 g body weight). Moreover, the administration of *Saldi tierra* potassium salt (50 or 100 mg/100 g) caused a significant increase in bone components in the femoral-diaphyseal or -metaphyseal tissues of rats. Potassium salt of *Saldi tierra* had a potent effect on bone components as compared with its sodium salt. Bone components were not significantly increased with the administration of an elemental mixture containing sodium (26.8 mg), potassium (1.660 mg), magnesium (0.431 mg), chloride (43.86 mg), silicon (11.1 μg), calcium (6.310 mg), zinc (0.42 μg), and manganese (1.32 mg/100 g), which are contained in *Saldi tierra* (100 mg). This study demonstrates that the oral administration of *Saldi tierra* containing various elements has anabolic effects on bone component in rats. The intake of *Saldi tierra* may have a role in the prevention of bone loss with aging.

Key words —— bone metabolism, mineral, trace element, *Saldi tierra*, rat femur

INTRODUCTION

Aging induces a decrease in bone mass. Osteoporosis with its accompanying decrease in bone mass is widely recognized as a major public health problem. The most dramatic expression of the disease is represented by fractures of the proximal femurs.[1–4] A decrease in bone mass may be due to decreased bone formation and increased bone resorption. Pharmacologic and nutritional factors may prevent bone loss with aging.[5,6] Chemical compounds in food and plants which act on bone metabolism, however, have not been fully clarified.

Isoflavones, which are contained in soybean, have been shown to have stimulatory effects on bone formation and inhibitory effects on bone resorption,[7–10] thereby increasing bone mass. Menaquinone-7, an analogue of vitamin K_2 which is essential for the γ-carboxylation of osteocalcin of bone matrix protein, is abundant in fermented soybean.[11] Menaquinone-7 has been demonstrated to stimulate osteoblastic bone formation and to inhibit osteoclastic bone resorption.[12–15] Carotenoid β-cryptoxanthin, which is present in Satsuma mandarin (*Citrus unshiu* MARC.), has stimulatory effects on osteoblastic bone formation and inhibitory effects on osteoclastic bone resorption.[16–19] The supplementation of isoflavones, menaquinone-7, and β-cryptoxanthin has preventive effects on bone loss induced by ovariectomy in rats, which are an animal model

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of osteoporosis.[20–22] Cinnamic acid is present in many plants. *p*-Hydroxycinnamic acid is an intermediate-metabolic substance in plants and is synthesized from thyrosine. *p*-Hydroxycinnamic acid has been shown to have unique anabolic effects on bone components in rat femur.[23,24] Chemical factors in food and plants thus play a role in bone health and may be important in the prevention of bone loss with aging.

Saldi tierra, which is obtained from natural zeolite, contains more than 20 elements. The intake of calcium or zinc has been known to have anabolic effects on bone components in rats.[25] The effects of the intake of products with many elements on bone components are unknown. This study was undertaken to determine the effects of administration of *Saldi tierra* on bone components in rats.

MATERIALS AND METHODS

Chemicals —— Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Wako Pure Chemical Industries (Osaka, Japan). All water used was glass-distilled. *Saldi tierra* was obtained from Tokai Ever Clean Co. Ltd. (Shizuoka, Japan). *Saldi tierra* (sodium salt or potassium salt) was prepared from natural zeolite using the method of ion exchange with sodium chloride or potassium chloride solutions. The content of various mineral and trace elements in *Saldi tierra* is shown in Table 1.

Table 1. Composition of Elements in *Saldi tierra*

Element	Content	
	g or mg/kg	mol or mmol
Potassium	396.0 g	10.128 mol
Calcium	57.2 g	1.427 mol
Magnesium	1.5 g	62.0 mmol
Sulfur	97.0 mg	3.0 mmol
Sodium	21.3 g	926.5 mmol
Bromine	1.4 g	17.5 mmol
Strontium	0.57 g	6.5 mmol
Boron	0.70 mg	0.06 mmol
Silicon	55.0 mg	1.96 mmol
Zinc	3.0 mg	0.05 mmol
Iron	8.2 mg	0.15 mmol
Copper	0.3 mg	0.01 mmol
Manganese	34.0 mg	0.62 mmol
Nickel	0.5 mg	0.01 mmol
Cobalt	0.5 mg	0.01 mmol
Vanadium	0.05 mg	0.001 mmol
Selenium	0.10 mg	0.001 mmol
Barium	0.910 g	6.60 mmol
Yttrium	6.0 mg	0.07 mmol
Others	10.0 mg	0.20 mmol

Each element content indicates the amount of element per kilogram of *Saldi tierra*.

Animals —— Male Wistar rats (conventional) weighing 90–100 g (4 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 1.1% calcium and 1.1% phosphorus at room temperature of 25°C, with free access to distilled water.

Administration Procedures —— *Saldi tierra* was dissolved in distilled water. The solution of *Saldi tierra* (50 or 100 mg/ml/100 g body weight) was orally administered to rats through a stomach tube once daily for 7 days. Rats were killed 24 hr after the last administration of *Saldi tiera*, and the blood and femur were removed immediately.

Analytical Procedures —— Blood samples obtained by cardiac puncture were centrifuged 30 min after collection, and the serum was separated. Serum was frozen at –80°C until assay. Serum calcium and inorganic phosphorus concentrations were determined using an assay kit (Wako Pure Chemical Industries).

The diaphyseal or metaphyseal tissues were dried for 16 hr at 110°C. Calcium was determined using atomic absorption spectrophotometry.[26] Calcium content in bone tissues was expressed as milligrams per gram of dry bone.

To assay alkaline phosphatase activity, the diaphyseal or metaphyseal tissues were immersed in 3.0 ml of ice-cold barbital buffer 6.6 mM (pH 7.4), cut into small pieces, and disrupted for 60 sec with an ultrasonic device. The supernatant centrifuged at $600 \times g$ for 5 min was used to measure enzyme activity. Enzyme assay was carried out under optimal conditions. Alkaline phosphatase activity was determined by the method of Walter and Schutt.[27] Enzyme activity was expressed as micromols of *p*-nitrophenol liberated per minute per milligram of protein. The protein concentration was determined by the method of Lowry *et al.*[28]

To measure bone DNA content, the diaphyseal or metaphyseal tissues were shaken with 4.0 ml of ice-cold 0.1 N NaOH solution for 24 hr after the homogenization of the bone tissues.[29] After alkaline extraction, the samples were centrifuged at $1000 \times g$ for 5 min, and the supernatant was determined by the method of Ceriotti[30] and expressed as the amount

Table 2. Body Weight, Serum Calcium, and Inorganic Phosphorus Concentrations in Rats Orally Administered *Saldi tierra*

Treatment	Body weight (g)	Serum concentration (mg/dl)	
		Calcium	Inorganic phosphorus
Control	111.8 ± 3.39	10.50 ± 0.15	9.95 ± 0.31
Saldi tierra			
50 mg/100 g	120.2 ± 3.03	10.78 ± 0.13	9.83 ± 0.37
100 mg/100 g	126.7 ± 1.99	10.81 ± 0.20	9.44 ± 0.28
Calcium chloride			
3.155 mg/100 g	111.5 ± 1.52	10.62 ± 0.07	10.09 ± 0.03

Rats were orally administered *Saldi tierra* (50 or 100 mg/100 g body weight) or calcium chloride (3.155 mg/100 g) for 7 days and killed 24 hr after the last administration. Each value is the mean ± SEM of six rats. Differences were not significant.

of DNA (mg)/g wet weight of bone tissue.

Statistical Analysis ——— The significance of difference between values was estimated using Student's *t*-test. *p*-Values of less than 0.05 were considered to indicate statistically significant differences. We also used multiple analysis of variance (ANOVA) to compare the treatment groups.

RESULTS

Effects of Administration of *Saldi tierra* (Sodium Salt) on Bone Components in Rats

The solution of *Saldi tierra* (50 or 100 mg/100 g body weight) with sodium salt was orally administered to rats once daily for 7 days, and the animals were killed 24 hr after the last administration. The body weight and serum calcium and inorganic phosphorus concentrations did not changed significantly after *Saldi tierra* administration (Table 2).

Calcium content and alkaline phosphatase activity in the femoral-diaphyseal tissues were significantly increased after the administration of *Saldi tierra* (50 or 100 mg/100 g) (Fig. 1). Femoral-metaphyseal DNA content was significantly increased after the administration of *Saldi tierra* (100 mg/100 g). Calcium content, alkaline phosphatase activity, and DNA content in the femoral-metaphyseal tissues were significantly increased after the administration of *Saldi tierra* (100 mg/100 g) (Fig. 1).

Saldi tierra (50 mg) contained 3.155 mg of calcium. The administration of calcium chloride solution (Ca 3.155 mg/100 g body weight) to rats did not cause a significant increase in calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphyseal tissues (Fig. 1).

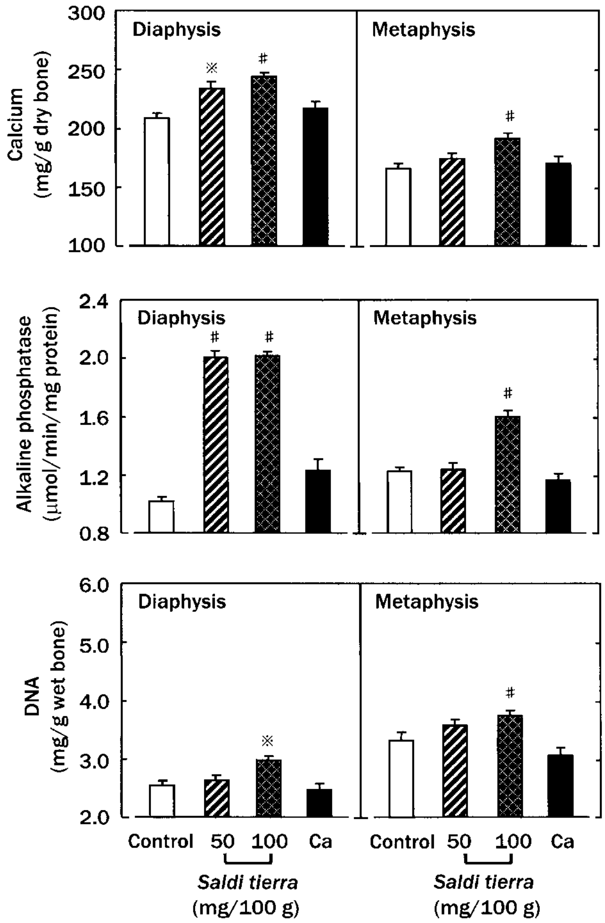

Fig. 1. Change in Calcium Content, Alkaline Phosphatase Activity, and DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats Orally Administered *Saldi tierra* (Sodium Salt) Solution

Rats were orally administered *Saldi tierra* solution (50 or 100 mg/ml/100 g body weight) or calcium chloride solution (calcium 3.155 mg/ml/100 g) once daily for 7 days and killed 24 hr after the last administration. Each value is the mean ± SEM of six rats. *$p < 0.05$ compared with the control (none) value. #$p < 0.01$ compared with the control (none) value.

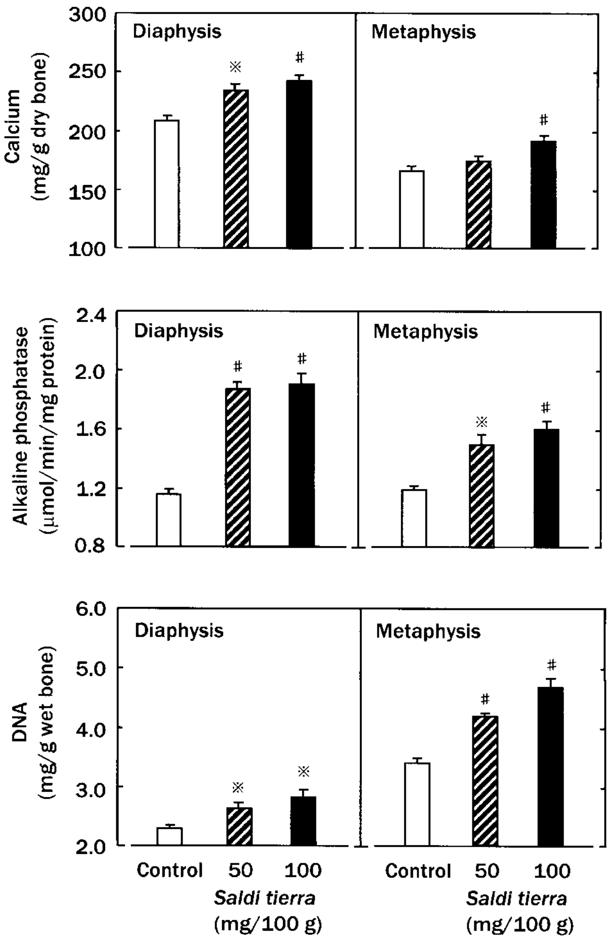

Fig. 2. Change in Calcium Content, Alkaline Phosphatase Activity, and DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats Orally Administered *Saldi tierra* (Potassium Salt) Solution

Rats were orally administered *Saldi tierra* solution (50 or 100 mg/ml/100 g body weight) once daily for 7 days and killed 24 hr after the last administration. Each value is the mean ± SEM of six rats. $*p < 0.05$ compared with the control (none) value. $\#p < 0.01$ compared with the control (none) value.

Effects of Administration of *Saldi tierra* (Potassium Salt) on Bone Components in Rats

The solution of *Saldi tierra* (50 or 100 mg/100 g body weight) with potassium salt was orally administered to rats once daily for 7 days, and the animals were killed 24 hr after the last administration. The administration of *Saldi tierra* (potassium salt) did not cause a significant alteration in the body weight, serum calcium and inorganic phosphorus concentrations in rats (data not shown).

Calcium content, alkaline phosphatase activity, and DNA content in the femoral-diaphyseal and -metaphyseal tissues of rats were significantly increased after the administration of *Saldi tierra* (potassium salt) (50 or 100 mg/100 g) (Fig. 2).

Effects of Administration of Element Composition Contained in *Saldi tierra* on Bone Components in Rats

A mixture of some elements composing *Saldi tierra* was prepared, and its composition is shown in Table 3. These element mixtures were orally administered to rats once daily for 7 days, and the animals were killed 24 hr after the last administration. The administration of element mixture A (sodium, potassium, magnesium, and chloride) solution (1.0 ml/100 g body weight) caused a significant decrease in alkaline phosphatase activity and DNA content in the femoral-diaphyseal tissues of rats (Fig. 3). Element B (silicon) administration did not cause a significant change in bone components in the femoral-diaphyseal and -metaphyseal tissues of rats. The administration of the combination of elements A, B, and C (containing calcium, zinc, and

Table 3. Element Mixture Prepared Artifically with the Identical Amounts of Elements in *Saldi tierra*

	Mineral mixture		Element content (mg or μg/ml)
A.	Sodium	(NaCl)	26.80 mg
	Potassium	(KCl)	1.660 mg
	Magnesium	($MgCl_2$)	0.431 mg
	Chloride		43.86 mg
B.	Silicon	(Na_2SiO_3)	11.1 μg
C.	Calcium	($CaCl_2$)	6.310 mg
	Zinc	($ZnSO_4$)	0.420 μg
	Manganase	($MnCl_2$)	1.320 mg

The content of each element was identical to the amount of elements contained in *Saldi tierra* (100 mg/ml).

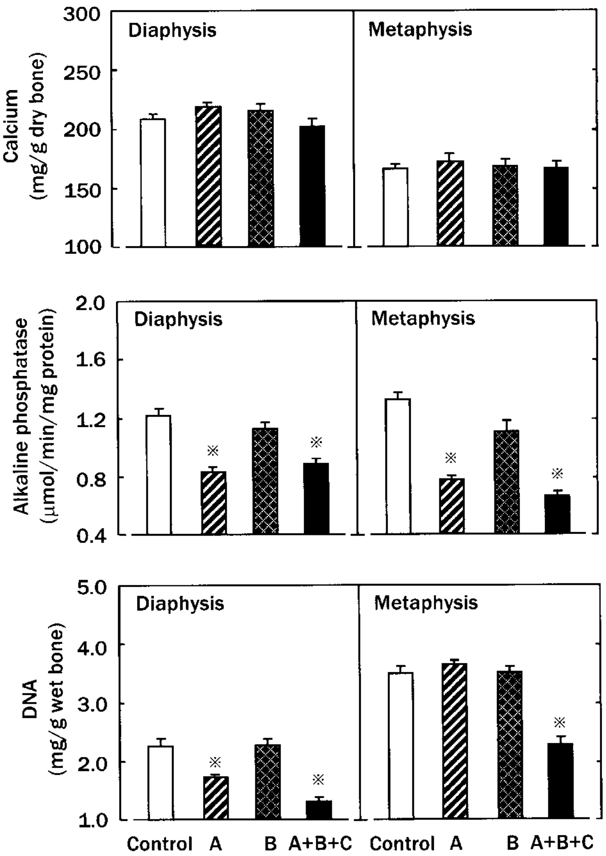

Fig. 3. Change in Alkaline Phosphatase Activity and DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats Orally Administered Elements Contained in *Saldi tierra*

Rats were orally administered an element mixture contained in *Saldi tierra* (1.0 ml/100 g body weight), as shown in Table 3, once daily for 7 days and killed 24 hr after the last administration. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value.

manganese) caused a significant decrease in alkaline phosphatase activity and DNA content in the femoral-diaphyseal and -metaphyseal tissues of rats (Fig. 3).

DISCUSSION

Recent studies have shown that the chemical factors in food and plants have anabolic effects on bone components in the femoral tissues of rats, suggesting a role in prevention of bone loss with aging.[6,31,32] Zinc, among various trace elements, has been shown to have a unique anabolic effect on bone components in rats.[25] Whether the composion of mineral and trace elements has an anabolic effect on bone components, however, is unknown. *Saldi tierra* made from natural zeolite, contains various mineral and trace elements, as shown in Table 1. We found that the oral administration of *Saldi tierra* has an anabolic effect on bone components in rats.

Saldi tierra was prepared from natural zeolite using the method of ion exchange with sodium chloride or potassium chloride. The effect of *Saldi tierra* potassium salt had a potent anabolic effect in increasing bone components as compared with that of the sodium salt. *Saldi tierra* potassium salt may thus be useful in the prevention of bone loss with supplementation as food material.

The administration of the mixture of calcium chloride or other trace elements, which are contained in *Saldi tierra*, did not cause a significant increase in bone components in the femoral-diaphyseal (cortical bone) and -metaphyseal (trabecular bone) tissues of rats. The dose of silicon, zinc, and manganese contained in *Saldi tierra* did not have a significant effect on bone components in rats. The total composition of mineral and trace elements in *Saldi tierra* may be needed for the anabolic effects on bone components in rats.

Alkaline phosphatase is a marker enzyme of osteoblasts, and the enzyme participates in bone mineralization.[33] DNA content is an index of bone growth and the number of bone cells.[34] The administration of *Saldi tierra* caused a significant increase in calcium content, alkaline phosphatase activity, and DNA content in the femoral tissues of rats. The intake of *Saldi tierra* may stimulate the proliferation and function of osteoblastic cells in bone tissues. It is speculated that the intake of *Saldi tierra* induces an increase in ion components in intracellular fluid in bone tissues in rats. This may have an effect on osteoblastic cell function.

Saldi tierra is a natural material containing many mineral and trace elements and may prevent bone loss with aging. Further study is needed to determine the preventive effect of *Saldi tierra* on osteoporosis using an *in vivo* animal model.

In conclusion, it has been shown that the intake of *Saldi tierra* containing many trace elements has an anabolic effect on bone components in the femoral tissues of rats *in vivo*.

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