Preventive Effects of *Saldi tierra* Containing Various Trace Elements on Bone Loss in Rats with Diabetes or Adjuvant Arthritis

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The preventive effect of *Saldi tierra* containing various trace elements on bone loss which is induced in rats with diabetes or adjuvant arthritis were investigated. Rats received a single subcutaneous administration of streptozotocin (STZ) (6.0 mg/100 g body weight), and the animals were orally administered a solution of *Saldi tierra* (25 or 50 mg/100 g) once daily for 14 days. STZ-administered rats caused a significant increase in serum glucose, triglyceride, or calcium levels and a significant decrease in body weight or serum inorganic phosphorus levels. These alterations were significantly restored after the oral administration of *Saldi tierra*. The femoral-diaphyseal and -metaphyseal calcium contents were significantly decreased in STZ-diabetic rats. This decrease was significantly inhibited after the administration of *Saldi tierra* (25 or 50 mg/100 g). The decreases in diaphyseal and metaphyseal alkaline phosphatase activity and metaphyseal DNA content were significantly prevented after the administration of *Saldi tierra* (50 mg/100 g). Meanwhile, rats received an injection of 1% *Mycobacterium butyricum* suspension into the subplantar surface of the right hind paw, and the animals were orally administered *Saldi tierra* (50 mg/100 g) once daily for 18 days. Calcium content, alkaline phosphatase activity, and DNA content in the femoral-metaphyseal tissues were significantly decreased in rats with adjuvant arthritis. These decreases were significantly inhibited after the administration of *Saldi tierra* (50 mg/100 g). This study demonstrates that the intake of *Saldi tierra* has preventive effects against bone loss in rats with diabetes or adjuvant arthritis *in vivo*.

Key words —— bone, diabetes, adjuvant arthritis, osteoporisis, *Saldi tierra*, mineral

INTRODUCTION

Osteoporosis with its accompanying loss of bone mass is widely recognized as a major public health problem. Osteoporosis induces bone fracture. A decrease in bone mass with increasing age may be due to decreased bone formation and increased bone resorption. Pharmacologic and nutritional factors may prevent bone loss with aging.[1–3] The role of chemical compounds in food and plants in preventing bone loss with aging is worthy of note.

Isoflavones,[4–7] which are contained in soybeans, menaquinone-7,[8–11] an analogue of vitamin K_2 abundant in fermented soybeans, and carotenoid β-cryptoxanthin,[12–15] which is present in Satsuma mandarins (*Citrus unshiu*, MARC), have been demonstrated to have stimulatory effects on osteoblastic bone formation and inhibitory effects on osteoclastic bone resorption, thereby increasing bone mass. The supplemental intake of isoflavones, menaquinone-7, and β-cryptoxanthin has a role in maintaining bone mass in humans.[16–18] 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.[19] The effects of the intake of products with many elements on bone metabolism are unknown. We have reported recently that the oral administration of *Saldi tierra* has anabolic effects on bone components in the femoral

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tissues of normal rats.[20]

This study was undertaken to determine whether the intake of *Saldi tierra* has preventive effects on bone loss seen in rats with diabetes or adjuvant arthritis *in vivo*.

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* (potassium salt) was prepared from natural zeolite using the method of ion exchange with potassium chloride solutions. The content of various mineral and trace elements in *Saldi tierra* is shown in Table 1.[20] Streptozotocin (STZ) was purchased from Sigma Chemical Co. STZ was dissolved in 50 mM of sodium citrate (pH 4.5) solution containing NaCl 150 mM.[21] *Mycobacterium butyricum*, from Difco Laboratories (Detroit, MI, U.S.A.). were dissolved in liquid paraffin to 1%.[22]

Animals —— Male Wistar rats (conventional) weighing 105–115 g (5 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 and housed at room temperature of 25°C, with free access to distilled water.

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*.

Administration Procedures —— STZ (6.0 mg/0.5 ml/100 g body weight) was subcutaneously administered in rats, and 14 days later the animals were killed by exsanguination. At 3 hr after a single subcutaneous administration of STZ, the solution of *Saldi tierra* (25 or 50 mg/ml/100 g body weight) was orally administered to the rats through a stomach tube once daily for 14 days.

Arthritis was induced in male rats with injection of 50 μl of a suspension of 1% *Mycobacterium butyricum* (MB) into the subplantar surface of the right hind paw of rats three times at 72 hr intervals. The control rats received the vehicle alone. At 3 hr after the first administration of MB, the solution of *Saldi tierra* (50 mg/ml/100 g) was orally administered to the rats once daily for 18 days.

Rats were killed 24 hr after the last administration of *Saldi tierra*, 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 glucose, triglyceride, 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.[23] The 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 × *g* for 5 min was used to measure enzyme activity. Enzyme assay was carried out under optimal conditions. Alkaline phosphatase activity was determined using the method of Walter and Schutt.[24] Enzyme activity was expressed as micromoles of *p*-nitrophenol liberated per minute per milligram of protein. The protein concentration was determined using the method of Lowry *et al.*[25]

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 ho-

mogenization of the bone tissues.[26] After alkaline extraction, the samples were centrifuged at 1000 × *g* for 5 min, and the supernatant was determined using the method of Ceriotti[27] and expressed as the amount of DNA (milligrams) per gram of bone tissue.

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

RESULTS

Effects of *Saldi tierra* Administration in STZ-Diabetic Rats

Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight) and the animals were orally administered *Saldi tierra* (25 or 50 mg/100 g) once daily for 14 days. The decrease in body weight (Fig. 1) or the increases in serum glucose and triglyceride concentrations (Fig. 2) was induced in STZ-administered rats, indicating that the administration induces a diabetic state. These alterations were significantly restored after the oral administration of *Saldi tierra* (25 or 50 mg/100 g).

The serum calcium levels were significantly increased in STZ-diabetic rats and the serum inorganic phosphorus levels were significantly decreased (Fig. 3). These alterations were significantly inhibited after the oral administration of *Saldi tierra* (25 or 50 mg/100 g).

Calcium content in the femoral-diaphyseal and -metaphyseal tissues was significantly decreased in STZ-diabetic rats (Fig. 4). This reduction was significantly inhibited after the oral administration of *Saldi tierra* (25 or 50 mg/100 g).

Alkaline phosphatase activity in the femoral-diaphyseal and -metaphyseal tissues was significantly decreased in STZ-diabetic rats (Fig. 5). This decrease was significantly inhibited after the oral

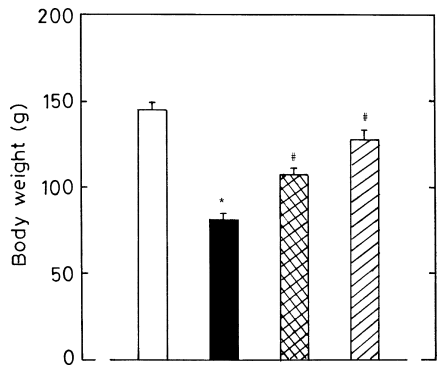

Fig. 1. Effects of *Saldi tierra* Administration on the Change in Body Weight in STZ-Diabetic Rats

Rats received a single subcutaneous administration of STZ (6.0 mg/100 g body weight), and 3 hr later the animals were orally administered a solution of *Saldi tierra* (25 or 50 mg/100 g) once daily for 14 days. The animals were killed 24 hr after the last administration. Each value is the mean ± standard error of the mean (SEM) of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value with STZ treatment. White bar: control, black bar: STZ treatment, double-hatched bar: STZ plus *Saldi tierra* (25 mg/100 g), hatched bar: STZ plus *Saldi tierra* (50 mg/100 g).

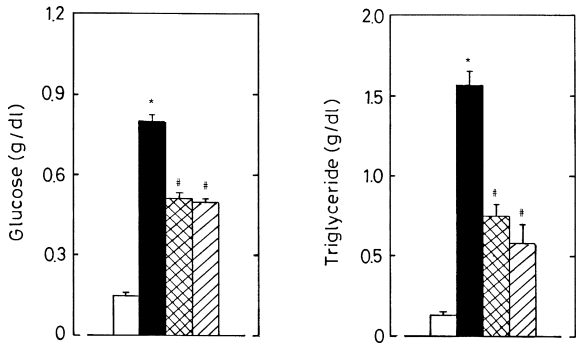

Fig. 2. Effects of *Saldi tierra* Administration on the Change in Serum Glucose and Triglyceride Concentrations in STZ-Diabetic Rats

The procedure of administration is described in the legend to Fig. 1. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value with STZ treatment. White bars: control, black bars: STZ treatment, double-hatched bars: STZ plus *Saldi tierra* (25 mg/100 g), hatched bars: STZ plus *Saldi tierra* (50 mg/100 g).

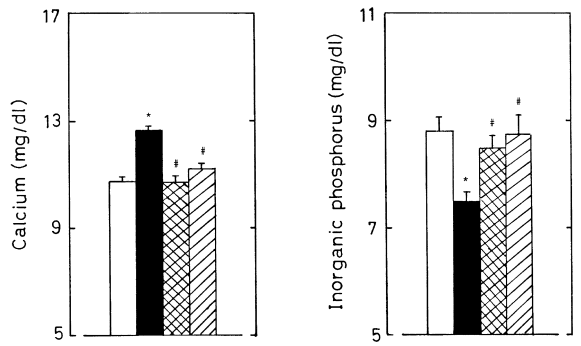

Fig. 3. Effects of *Saldi tierra* Administration on the Change in Serum Calcium and Inorganic Phosphorus Concentrations in STZ-Diabetic Rats

The procedure of administration is described in the legend to Fig. 1. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value with STZ treatment. White bars: control, black bars: STZ treatment, double-hatched bars: STZ plus *Saldi tierra* (25 mg/100 g), hatched bars: STZ plus *Saldi tierra* (50 mg/100 g).

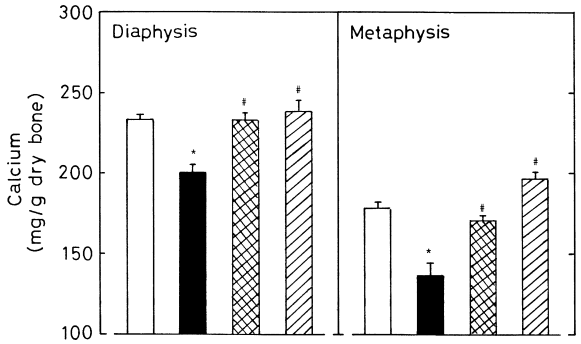

Fig. 4. Effects of *Saldi tierra* Administration on the Change in Calcium Content in the Femoral-Diaphyseal and -Metaphyseal Tissues in STZ-Diabetic Rats

The procedure of administration is described in the legend to Fig. 1. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value with STZ treatment. White bars: control, black bars: STZ treatment, double-hatched bars: STZ plus *Saldi tierra* (25 mg/100 g), hatched bars: STZ plus *Saldi tierra* (50 mg/100 g).

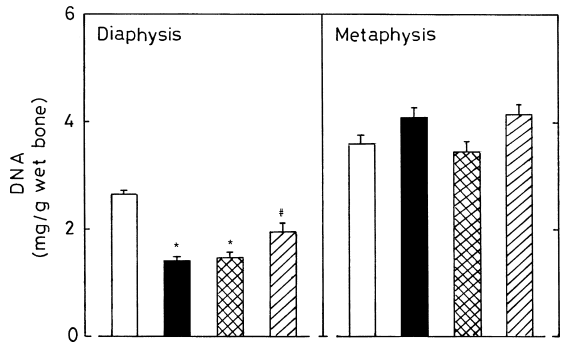

Fig. 6. Effects of *Saldi tierra* Administration on the Change in DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues in STZ-Diabetic Rats

The procedure of administration is described in the legend to Fig. 1. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value from STZ treatment. White bars: control, black bars: STZ treatment, double-hatched bars: STZ plus *Saldi tierra* (25 mg/100 g), hatched bars: STZ plus *Saldi tierra* (50 mg/100 g).

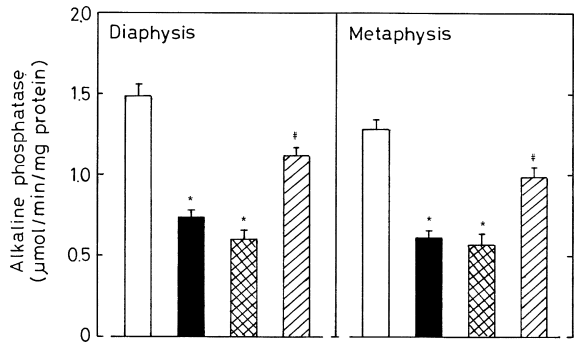

Fig. 5. Effects of *Saldi tierra* Administration on the Change in Alkaline Phosphatase Activity in the Femoral-Diaphyseal and -Metaphyseal Tissues in STZ-Diabetic Rats

The procedure of administration is described in the legend to Fig. 1. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value from STZ treatment. White bars: control, black bars: STZ treatment, double-hatched bars: STZ plus *Saldi tierra* (25 mg/100 g), hatched bars: STZ plus *Saldi tierra* (50 mg/100 g).

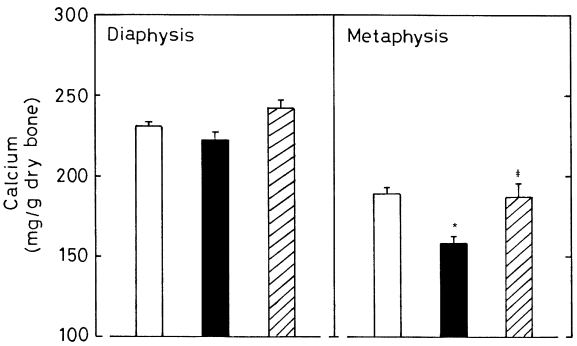

Fig. 7. Effects of *Saldi tierra* Administration on the Change in Calcium Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats with Adjuvant Arthritis

The arthritics rats received an oral administration of *Saldi tierra* (50 mg/100 g body weight) once daily for 18 days. The animals were killed 24 hr after the last administration. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value for arthritic rats. White bars: control, black bars: arthritic rats, hatched bars: *Saldi tierra*-administered arthritic rats.

administration of *Saldi tierra* (50 mg/100 g).

DNA content in the femoral-diaphyseal tissues was significantly decreased in STZ-diabetic rats (Fig. 6). The decrease was significantly inhibited after the oral administration of *Saldi tierra* (50 mg/100 g).

Effects of *Saldi tierra* Administration in Rats with Adjuvant Arthritis

Rats received an injection of 1% MB suspension into the subplantar surface of the right hind paw three times at 72 hr intervals, and the animals were orally administered *Saldi tierra* (50 mg/100 g) once daily for 18 days. The body weight was significantly decreased in rats with adjuvant arthritis (data not shown). This decrease was significantly inhibited after the oral administration of *Saldi tierra* (50 mg/100 g). Serum calcium and inorganic phosphorus concentrations were not significantly changed in rats with adjuvant arthritis (data not shown).

Calcium content (Fig. 7), alkaline phosphatase activity (Fig. 8), and DNA content (Fig. 9) in the femoral-metaphyseal tissues were significantly de-

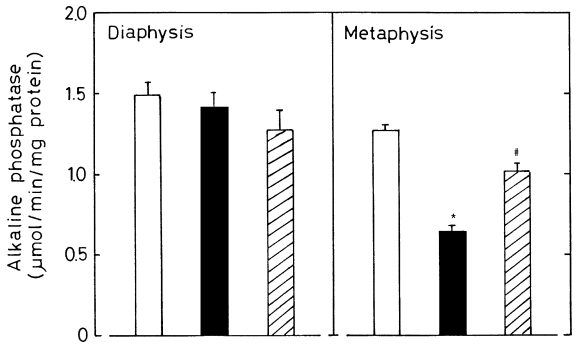

Fig. 8. Effects of *Saldi tierra* Administration on the Change in Alkaline Phosphatase Activity in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats with Adjuvant-Arthritis

The procedure of administration is described in the legend to Fig. 7. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value for arthritic rats. White bars: control, black bars: arthritic rats, hatched bars: *Saldi tierra* (50 mg/100 g)-administered arthritic rats.

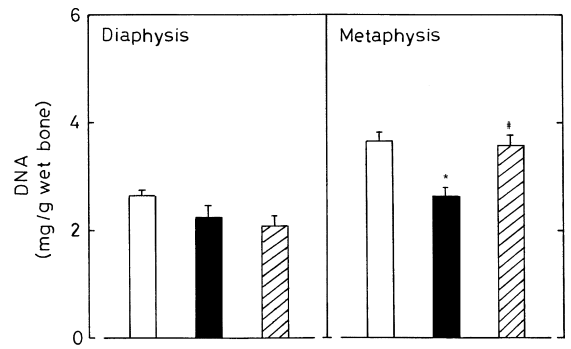

Fig. 9. Effects of *Saldi tierra* Administration on the Change in DNA Content in the Femoral-Diaphyseal and -Metaphyseal Tissues of Rats with Adjuvant-Arthritis

The procedure of administration is described in the legend to Fig. 7. Each value is the mean ± SEM of six rats. $^{*}p < 0.01$ compared with the control value. $\#p < 0.01$ compared with the value for arthritic rats. White bars: control, black bars: arthritic rats, hatched bars: *Saldi tierra* (50 mg/100 g)-administered arthritic rats.

creased in rats with adjuvant arthritis. These decreases were not seen in the femoral-diaphyseal tissues of rats with adjuvant arthritis (Figs. 7–9). The decreases in these bone components were significantly inhibited after the oral administration of *Saldi tierra* (50 mg/100 g) (Figs. 7–9).

DISCUSSION

Nutritional factors may play a role in the prevention of bone loss with increasing age. Recent studies have shown that *Saldi tierra* containing various trace elements as a nutrient factor has anabolic effects on the bone component in the femoral tissues of normal rats.[20] This study was undertaken to determine whether the oral administration of *Saldi tierra* has preventive effects against bone loss in rats with diabetes or adjuvant arthritis, which is known to induce bone loss.[22,28–31] We found that the supplemental intake of *Saldi tierra* has preventive effects against bone loss in the pathophysiologic state.

Diabetes has been shown to induce bone loss.[28,29] The oral administration of *Saldi tierra* to STZ-diabetic rats was found to have preventive effects against bone loss with diabetes *in vivo*. The diabetic state induces impairment of intestinal calcium absorption.[32,33] The serum calcium levels were found to increase in STZ-diabetic rats, suggesting that this increase results from the release of calcium from bone tissues. The femoral calcium content was found to decrease markedly in STZ-diabetic rats. The oral administration of *Saldi tierra* may have an inhibitory effect on bone resorption *in vivo*.

Alkaline phosphatase participates in mineralization in osteoblastic cells in bone tissues.[34] The DNA content in bone tissues may be an index of bone growth.[35] Femoral alkaline phosphatase activity and DNA content were significantly decreased in STZ-diabetic rats. These decreases were significantly inhibited after the oral administration of *Saldi tierra*. Presumably, the administration of *Saldi tierra* has stimulatory effects on osteoblastic bone formation and bone growth *in vivo*.

Interestingly, the oral administration of *Saldi tierra* to STZ-diabetic rats had significant restorative effects on the decrease in body weight and the increase in serum glucose and triglyceride levels induced in the diabetic state. This is a novel finding. The intake of *Saldi tierra* has been demonstrated to have preventive effects against bone loss, hyperglycemia, and hyperlipidemia in diabetic rats.

Bone metabolism is impaired in rats during the inflammatory reaction following a local inflammatory stimulus.[30,31] It has been demonstrated that a transient inhibition of bone formation occurs during acute inflammation in the rat and that changes in osteoblast function are part of the acute-phase response following local inflammation.[31] Also, the inflammation induces osteoclastic bone resorption.[36] The oral administration of *Saldi tierra* was found to have restorative effects on the decrease in body weight of rats with adjuvant arthritis, which induces local inflammation. The administration was also found to have preventive effects against the de-

creases in calcium content, alkaline phosphatase activity, and DNA content in the femoral-metaphyseal tissues (trabecular bone) in rats with adjuvant arthritis *in vivo*. Presumably, the oral administration of *Saldi tierra* has a stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption in rats with adjuvant arthritis *in vivo*. The present findings demonstrate that the intake of *Saldi tierra* has preventive effects against the acute-phase response following local inflammation.

Bone loss was interestingly observed in the femoral-metaphyseal tissues of rats with adjuvant arthritis, while it was not seen in the femoral-diaphyseal tissues (contical bone). The femoral-metaphyseal tissues are close to the joint. Bone loss in this area may be unique in rats with arthritis.

In conclusion, it was demonstrated that the intake of *Saldi tierra* containing various trace elements has preventive effects against bone loss in rats with diabetes or adjuvant arthritis *in vivo*.

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