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Single and Five-Week Oral Dose Toxicity Studies of Calcitriol and Alendronate Mixtures in Rats

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ABSTRACT. The purpose of this study was to assess the single and 5 week oral dose toxicity of calcitriol and alendronate combination (1 : 10,000) treatment for osteoporosis or Paget's disease in male and female rats. In single dose oral toxicity study, the values of LD_{50} of calcitriol and alendronate mixture were 750.075 mg/kg in male rats and 775.0775 mg/kg in female rats, respectively. Body weight and food consumption were continuously increased after adminstration of calcitriol and alendronate mixtures, and there was no significant changes in body weight and food consumption in all groups. In five-week oral toxicity study of calcitriol and alendronate mixture at a dose of 0.2 μg + 2 mg, 1 μg + 10 mg, 5 μg + 50 mg and 25 μg + 250 mg, respectively, there was no mortality, abnormal behavior and appearance in all groups throughout the administration period (5 weeks) and recovery period (2 weeks). Dose-dependent changes in parameters of urinalysis and hematological analysis were not observed in male and female rats treated with calcitriol and alendronate mixtures. All the values of the parameters appeared to be in the normal range. These data indicate that both calcitriol and alendronate are drugs having low toxicity in rats. NOAEL of calcitriol and alendronate mixtures were 50.005 mg/kg in 5-week oral toxicity.

Keywords: Alendronate, Calcitriol, Single oral dose toxicity, LD_{50}, Five-week oral dose toxicity.

INTRODUCTION

Calcitriol, 1,25-dihydroxy cholecalciferol, 1,25-$(OH)_2$-vitamine D_3 (Kiriyama *et al.*, 1989; Nakatsuka *et al.*, 1992; Caniggia and Vattimo, 1979; Need *et al.*, 1985; Caniggia *et al.*, 1996; Gallagher, 1990) and alendronate, 4-amino-1-hydroxy butylidene-1,1-bisphosphonic acid (Azuma *et al.*, 1995; Sahni *et al.*, 1993; James and Reynolds, 1993; Boonekamp *et al.*, 1986; Nagao *et al.*, 1990; Carano *et al.*, 1990; Lowic *et al.*, 1988) have been used for therapeutic agents of osteoporosis. Recently many studies showed that alendronate and calcium or calcitriol combination treatment for osteoporosis and Paget's disease are clearly greater than those achieved by calcium monotherapy. Malavolta *et al.* (1999) reported that continuous treatment for 9 months with calcitriol or calcium in combination with alendronate significantly increases both vertebral and femoral neck density (from 3.8% to 4.5% and from 0.61% to 2.36%, respectively) in osteopenic postmenopausal women. The effects of both combinations on bone mass are clearly greater than those achieved by calcium monotherapy. In addition, Wendlova *et al.* (1999) showed that Paget's disease treated with alendronate, calcium and calcitriol combination treatment reduced the number of osteoplastic foci on the x-ray image of vertebrae and pelvis, regression of the vertebrogenic algic syndrome and improved mobility of the patient. Frediani *et al.* (1998) recently reported that combined treatment with calcitriol and alendronate was more effective than therapy with alendronate alone. The toxicity of combined treatment with calcitriol and alendronate for osteoporosis remains unclear.

Therefore, in this study, we investigated single dose and five-week oral toxicity of calcitriol and alendronate combination (1 : 10,000) in male and female rats.

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MATERIALS AND METHODS

Animals

The Sprague-Dawley rats with 5-week old were purchased from Han-Lym Laboratory Animal Ltd. Co. and acclimated for one week in environmentally controlled rooms (temperature: 23 ± 2°C, relative humidity: 60 ±

Table 1. Mortality and LD_{50} of calcitriol and alendronate mixture (1 : 10,000) in rats

	Doses		Mortality	LD_{50}[1]
	Calcitriol (μg/kg)	Alendronate (mg/kg)		Calcitriol + Alendronate (mg/kg)
Male	0.2	2.0	0/10	750.075
	1.0	10.0	0/10	
	5.0	50.0	0/10	
	25.0	250.0	0/10	
	50.0	500.0	0/10	
	75.0	750.0	6/10	
	100.0	1,000.0	9/10	
	125.0	1,250.0	10/10	
Female	0.2	2.0	0/10	775.0775
	1.0	10.0	0/10	
	5.0	50.0	0/10	
	25.0	250.0	0/10	
	50.0	500.0	0/10	
	75.0	750.0	5/10	
	100.0	1,000.0	9/10	
	125.0	1,250.0	10/10	

[1]LD_{50} were calculated by Behrens-Kärber method.

2%, air ventilation: 13~15 times/hr, artificial light: 300 Lux from 7 am to 7 pm) and were fed with sterilized regular laboratory chow and water *ad libitum* in animal SPF center in Chung-Ang University.

Preparation of test compounds

Calcitriol and alendronate were obtained from YuYu Industrial Co., Seoul. Calcitriol and alendronate were mixed in various ratios (as described in Tables 1 and 2) in 0.5% carboxylmethylcellulose (CMC). The mixture was prepared every week in brown bottle and stored in a refrigerator until use. The volume of administration was adjusted to 5 ml/kg body weight.

Acute single oral toxicity

All experiments were conducted according to "Guidelines for Toxicity Testing of Pharmaceuticals" (KFDA, 1999-61). To investigate single dose oral toxicity and LD_{50}, both sex animals were randomly divided into 9 groups, respectively (male: 10 rats/group, female: 10 rats/group), and orally administered with calcitriol + alendronate mixtures at the ratio (1 : 10,000) : 0.2 μg + 2 mg, 1 μg + 10 mg, 5 μg + 50 mg, 25 μg + 250 mg, 50 μg + 500 μg, 75 μg + 750 mg, 100 μg + 1000 mg, 125 μg + 1250 mg, respectively. The value of LD_{50} for 2 weeks was calculated by Brehrens-Kärber method. Clinical signs, food and water consumption, and body weight change were observed everyday after administration. After 2 weeks, major organs and tissues including brain, liver, kidney, heart, spleen, adrenal gland, testis, ovary and femoral bone were fixed in 10% neutral buffered formalin solution, and processed for microscopic examination.

5-Week oral dose toxicity

To investigate 5-week oral dose toxicity of calcitirol + alendronate mixtures, both sex animals were randomly divided into 5 groups, respectively (male: 20 rats/group, female: 20 rats/group) and orally administered with calcitirol + alendronate mixtures at the ratio (1 : 10,000) for

Table 2-1. Urinalysis in male and female rats treated orally with test drugs for 5 weeks

Sex	Dose	n	pH					Protein[1]			Ketone body[2]			Glucose[3]	Occult blood[4]			Bilirubin[5]	Urobilinogen[6]		Color[7]	Nitrite[8]	Urine sediment							
																							RBC		WBC		SEC	SREC		Cast
			6.0	6.5	7.0	7.5	8.0	-	±	+	-	±	+	-	-	±	+	-	±	+	Y	-	-	±	-	±	±	-	±	-
Male	Control	10	0	0	0	1	9	9	1	0	9	1	0	10	9	1	0	10	10	0	10	10	9	1	10	0	10	10	0	10
	25+250	10	0	0	0	2	8	8	1	1	9	0	1	10	8	1	1	10	10	0	10	10	10	0	9	1	10	10	0	10
	5+50	10	0	0	0	2	8	9	1	0	10	0	0	10	9	0	1	10	10	0	10	10	9	1	10	0	10	9	1	10
	1+10	10	0	0	1	2	7	10	0	0	10	0	0	10	9	1	0	10	10	0	10	10	9	1	10	0	10	9	1	10
	0.2+2	10	0	0	3	3	4	9	1	0	9	1	0	10	10	0	0	10	9	1	10	10	10	0	10	0	10	9	1	10
Female	Control	10	0	0	0	1	9	10	0	0	10	0	0	10	10	0	0	10	9	1	10	10	10	0	9	1	10	10	0	10
	25+250	10	0	0	0	2	8	9	1	0	9	1	0	10	10	0	0	10	9	1	10	10	10	0	10	0	10	9	1	10
	5+50	10	0	4	4	2	0	10	0	0	10	0	0	10	10	0	0	10	10	0	10	10	9	1	10	0	10	10	0	10
	1+10	10	2	5	3	0	0	9	1	0	9	1	0	10	9	1	0	10	10	0	10	10	10	0	10	0	10	10	0	10
	0.2+2	10	3	4	3	0	0	10	0	0	10	0	0	10	10	0	0	10	10	0	10	10	10	0	10	0	10	10	0	10

[1]– : 0 mg/dl, ± : 10~20 mg/dl, + : 30~70 mg/dl.
[2]– : 0 mg/dl, ± : 5 mg/dl, + : 10~20 mg/dl.
[3]– : 0~10 mg/dl.
[4]– : 0 mg/dl, ± : 0.03 mg/dl, + : 0.06~0.1 mg/dl.
[5]– : 0 mg/dl.
[6]± : 0.2~1.0 mg/dl, + : 30~70 mg/dl.
[7]Y : yellow.
[8]– : below 10^5 bacteria/ml. SEC : Squamous epithelial cell, SREC : Small round epithelial cell, PS : Phosphate salts, CO : Calcium oxalate.

Table 2-1. Continued

Sex	Dose	n	Urine sediment: Crystallization: PS -	PS ±	PS +	CO -	Water intake (ml/24 hr)	Urine volume (ml/24 hr)	Sp.G 1.01	Sp.G 1.02	Sp.G 1.03	Leucocyte -	Leucocyte ±	Na (mEq/24 hr)	K (mEq/24 hr)	Cl (mEq/24 hr)
Male	Control	10	1	8	1	10	17.4 ± 3.4[a)]	7.8 ± 2.1	8	2	0	10	0	1.51 ± 0.21	2.67 ± 0.35	2.20 ± 0.44
	25+250	10	0	9	1	10	20.5 ± 2.1	8.4 ± 1.5	6	3	1	9	1	1.57 ± 0.24	2.70 ± 0.36	2.31 ± 0.32
	5+50	10	1	9	0	10	16.7 ± 3.8	7.3 ± 1.7	5	5	0	10	0	1.62 ± 0.32	2.81 ± 0.28	2.18 ± 0.35
	1+10	10	0	9	1	10	17.3 ± 2.3	8.2 ± 2.3	5	5	0	10	0	1.57 ± 0.27	2.77 ± 0.38	2.26 ± 0.27
	0.2+2	10	1	8	1	10	19.5 ± 6.2	8.4 ± 1.7	6	4	0	10	0	1.49 ± 0.33	2.72 ± 0.41	2.32 ± 0.19
Female	Control	10	1	9	0	10	16.2 ± 1.2	6.2 ± 1.5	9	1	0	9	1	1.54 ± 0.19	1.85 ± 0.24	1.44 ± 0.24
	25+250	10	0	9	1	10	17.5 ± 2.6	5.9 ± 1.4	3	7	0	10	0	1.56 ± 0.31	1.94 ± 0.31	1.51 ± 0.22
	5+50	10	1	9	0	10	18.1 ± 2.3	6.9 ± 1.4	2	8	0	10	0	1.47 ± 0.28	1.92 ± 0.32	1.43 ± 0.31
	1+10	10	1	9	0	10	17.3 ± 1.5	6.6 ± 2.1	3	7	0	10	0	1.51 ± 0.35	1.89 ± 0.24	1.37 ± 0.21
	0.2+2	10	0	9	1	10	17.3 ± 5.3	6.4 ± 2.3	5	5	0	10	0	1.54 ± 0.34	1.87 ± 0.37	1.48 ± 0.26

[a)]Mean ± S.E. All of data were not significantly different from control.

Table 2-2. Urinalysis in male and female rats after 2-week recovery period following 5-week treatment with test drugs

Sex	Dose	n	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	Protein[1)] -	Protein ±	Protein +	Ketone body[2)] -	Ketone body ±	Ketone body +	Glucose[3)] -	Occult blood[4)] -	Occult blood ±	Occult blood +	Bilirubin[5)] -	Urobilinogen[6)] ±	Urobilinogen +	Color[7)] Y	Nitrite[8)] -	Urine sediment: RBC -	RBC ±	WBC -	WBC ±	SEC ±	SREC -	SREC ±	Cast -
Male	Control	10	0	2	8	0	0	9	1	0	9	1	0	10	9	1	0	9	10	0	10	10	10	0	9	1	10	9	1	10
	25+250	10	0	4	6	0	0	9	0	1	9	0	1	10	9	1	0	10	10	0	10	10	9	1	9	1	10	10	0	10
	5+50	10	4	6	0	0	0	9	1	0	9	1	0	10	9	0	1	9	9	1	10	10	10	0	9	1	10	10	0	10
	1+10	10	6	3	1	0	0	9	1	0	10	0	0	10	10	1	0	10	10	0	10	10	10	0	10	0	10	10	0	10
	0.2+2	10	9	1	0	0	0	10	0	0	10	0	0	10	10	1	0	10	10	0	10	10	9	1	10	0	10	9	1	10
Female	Control	10	3	7	0	0	0	10	0	0	10	0	0	10	10	1	0	10	10	0	10	10	9	1	10	0	10	10	0	10
	25+250	10	2	8	0	0	0	9	1	0	10	0	0	10	9	1	0	10	10	0	10	10	10	0	9	1	10	9	1	10
	5+50	10	7	3	0	0	0	10	0	0	9	1	0	10	10	0	0	9	10	0	10	10	9	1	10	0	10	9	1	10
	1+10	10	3	4	2	0	1	10	0	0	10	0	0	10	10	0	0	10	10	0	10	10	9	1	9	1	10	9	1	10
	0.2+2	10	10	0	0	0	0	10	0	0	10	0	0	10	9	1	0	9	10	0	10	10	9	1	10	0	10	10	0	10

[1)]– : 0 mg/dl, ± : 10~20 mg/dl, + : 30~70 mg/dl.
[2)]– : 0 mg/dl, ± : 5 mg/dl, + : 10~20 mg/dl.
[3)]– : 0~10 mg/dl.
[4)]– : 0 mg/dl, ± : 0.03 mg/dl, + : 0.06~0.1 mg/dl.
[5)]– : 0 mg/dl.
[6)]± : 0.2~1.0 mg/dl, + : 30~70 mg/dl.
[7)]Y : yellow.
[8)]– : below 10^5 bacteria/ml. SEC : Squamous epithelial cell, SREC : Small round epithelial cell, PS : Phosphate salts, CO : Calcium oxalate.

Table 2-2. Continued

Sex	Dose	n	Urine sediment: Crystallization: PS -	PS ±	PS +	CO -	Water intake (ml/24 hr)	Urine volume (ml/24 hr)	Sp.G 1.01	Sp.G 1.02	Sp.G 1.03	Leucocyte -	Leucocyte ±	Na (mEq/24 hr)	K (mEq/24 hr)	Cl (mEq/24 hr)
Male	Control	10	0	9	1	10	17.0 ± 0.8[a)]	10.1 ± 2.5[a)]	8	2	0	10	0	1.49 ± 0.28[a)]	2.57 ± 0.32[a)]	2.24 ± 0.24[a)]
	25+250	10	1	9	0	10	17.5 ± 1.3	11.2 ± 1.8	6	3	1	10	0	1.54 ± 0.31	2.64 ± 0.21	2.25 ± 0.35
	5+50	10	0	9	1	10	19.0 ± 1.1	13.2 ± 1.4	6	4	0	9	1	1.52 ± 0.24	2.72 ± 0.23	2.21 ± 0.31
	1+10	10	0	9	1	10	20.5 ± 2.1	9.8 ± 2.1	7	3	0	10	0	1.47 ± 0.20	2.70 ± 0.31	2.31 ± 0.22
	0.2+2	10	1	9	0	10	15.1 ± 3.2	9.2 ± 1.3	8	2	0	10	0	1.59 ± 0.32	2.69 ± 0.24	2.24 ± 0.25
Female	Control	10	0	9	1	10	15.6 ± 2.1	5.2 ± 0.7	9	1	0	9	1	1.48 ± 0.23	1.91 ± 0.31	1.52 ± 0.21
	25+250	10	0	9	1	10	16.8 ± 1.4	8.4 ± 1.4	2	8	0	10	0	1.56 ± 0.24	1.90 ± 0.22	1.55 ± 0.27
	5+50	10	0	9	1	10	17.5 ± 1.4	7.3 ± 2.1	3	7	0	10	0	1.51 ± 0.30	1.89 ± 0.24	1.56 ± 0.30
	1+10	10	1	9	0	10	18.3 ± 2.2	11.1 ± 3.2	3	7	0	10	0	1.54 ± 0.32	1.93 ± 0.22	1.43 ± 0.26
	0.2+2	10	1	9	0	10	15.2 ± 2.5	8.7 ± 1.8	5	5	0	10	0	1.50 ± 0.32	1.92 ± 0.24	1.52 ± 0.22

[a)]Mean ± S.E. All of data were not significantly different from control.

35 consecutive days : 0.2 μg + 2 mg, 1 μg + 10 mg, 5 μg + 50 mg, 25 μg + 250 mg, respectively. Clinical signs were observed everyday after administration and further observed for 2 weeks as recovery period. Food and water consumption, and body weight were observed twice per week for 7 weeks. Urine was collected for 4 hr and 20 hr at the time of 35th day (5 weeks) and 49th day (2 weeks recovery), respectively. In the urinalysis, contents of glucose, protein, ketone body, leukocytes, urobilinogen, pH, specific gravity, nitrite and bilirubin in 4 hr-urine were determined by N-Multistix™ SG-L and contents of Na^+, K^+ and Cl^- concentration in 20 hr-urine were determined by spectrophotometer using Stanbio Lab. kit.

Blood was collected in Vacutainer™ (Becfon Dickinson Vacutainer Systems Europe, England) from abdominal aorta under light ether anesthesia at the time of 35th day (5 weeks) and 49th day (2 weeks recovery), respectively. For hematological examination, white blood cells, red blood cells, hematocrit, hemoglobin, platelet, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), prothrombin time (PT) and differential leucocyte count were determined by electric resistance change method. The serum chemistry parameters included total protein, total bilirubin (azobarbin method), glucose (Hexokinase PD method), transaminases (UV rate method), alkaline phosphatase (Bessey-Lowry method), total cholesterol (CEH-COD-POD method), blood urea nitrogen (Urease-GLDH method), creatinine and electrolytes, Na^+, K^+, Cl^- (ion-selective electrode method), Ca^{2+} (OCPC method), PO_4^{3-} (molybdenic acid method) using reagents (Stanbio Laboratory, Inc., U.S.A., Texas). All major organs and tissues including brain, liver, kidney, heart, spleen, adrenal gland, testis, ovary, and sternum were examined grossly and the weight was measured. The organs and tissues were fixed in 10% neutral buffered formalin solution, and processed for microscopic examination by hematoxylin-eosin (H&E) staining.

Statistical analysis

All data were expressed as mean ± SE and analyzed statistically by analysis of variance (ANOVA) and differences between groups were determined with Newmann-Keuls test. The level of significance was set at 5%.

RESULTS

Single dose oral toxicity of calcitriol and alendronate mixture

Mortality and clinical signs. In male rats, mortality of calcitriol + alendronate mixture were 6/10 at a dose of 750.075 mg/kg, 9/10 at a dose of 1,000.1 mg/kg, and 10/10 at a dose of 1,250.125 mg/kg. In female rats, motarity of calcitriol + alendronate mixture were 5/10 at a dose of 750.075 mg/kg, 9/10 at a dose of 1,000.1 mg/kg, and 10/10 at a dose of 1,250.125 mg/kg. The values of LD_{50} of calcitriol + alendronate mixture were 750.075 mg/kg in male rats and 775.0775 mg/kg in female rats, respectively (Table 1). The groups treated

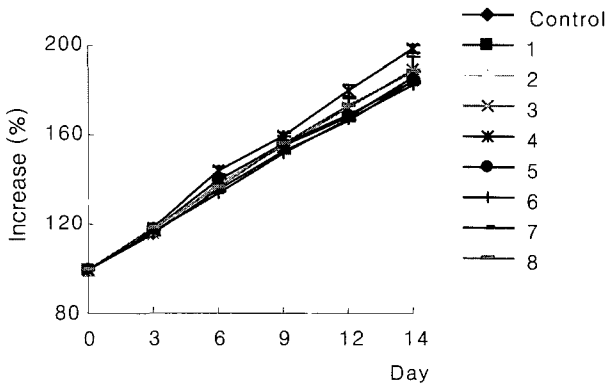

Fig. 1. Increase of body weight in female rats treated orally with calcitriol and alendronate. 1, calcitriol 125 μg/kg + alendronate 1250 mg/kg; 2, calcitriol 100 μg/kg + alendronate 1000 mg/kg; 3, calcitriol 75 μg/kg + alendronate 750 mg/kg; 4, calcitriol 50 μg/kg + alendronate 500 mg/kg; 5, calcitriol 25 μg/kg + alendronate 250 mg/kg; 6, calcitriol 5 μg/kg + alendronate 50 mg/kg; 7, calcitriol 1 μg/kg + alendronate 10 mg/kg; 8, calcitriol 0.2 μg/kg + alendronate 2 mg/kg. All of data were not significantly diferrent from control.

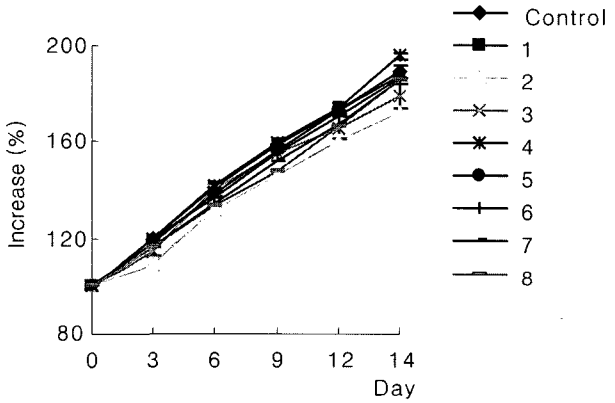

Fig. 2. Increase of body weight in male rats treated orally with calcitriol and alendronate. 1, calcitriol 125 μg/kg + alendronate 1250 mg/kg; 2, calcitriol 100 μg/kg + alendronate 1000 mg/kg; 3, calcitriol 75 μg/kg + alendronate 750 mg/kg; 4: calcitriol 50 μg/kg + alendronate 500 mg/kg; 5, calcitriol 25 μg/kg + alendronate 250 mg/kg; 6, calcitriol 5 μg/kg + alendronate 50 mg/kg; 7, calcitriol 1 μg/kg + alendronate 10 mg/kg; 8, calcitriol 0.2 μg/kg + alendronate 2 mg/kg. All of data were not significantly diferrent from control.

with more than LD_{50} values showed clinical symptoms like scratched head, convulsion and dyspnea, and died within 24 hr. The group treated with calcitriol (50 μg/kg) + alendronate (500 mg/kg) mixture showed a similar symptom like scratched head in 3 rats/group but such symptom was disappeared 1 day after administration.

Body weight and food consumption. Body weight and food consumption were continuously increased after adminstration of calcitriol + alendronate mixtures, but there was no significant changes in body weight and food consumption in all groups except dead rats (Fig. 1 and Fig. 2).

Autopsy and organ weight. The dead rats treated with calcitriol + alendronate mixtures at a dose of more than LD_{50} showed marked congestion and hemorrhage in spleen, but any pathological changes in other organs were not observed (Table 2). Also there were no changes in gross/histological examination and organ weight 2 weeks after treatment with calcitriol + alendronate mixtures at a dose of less than LD_{50}.

Five-week oral dose toxicity of calcitriol and alendronate mixture

Mortality and clinical signs. Mortality and abnormal clinical signs were observed for 5 weeks during administration of calcitriol + alendronate mixtures and for 2 weeks after cessation of administration. There was no dead rat for 7 weeks. Abnormal behavior such as scratched head was observed for 3 days starting from

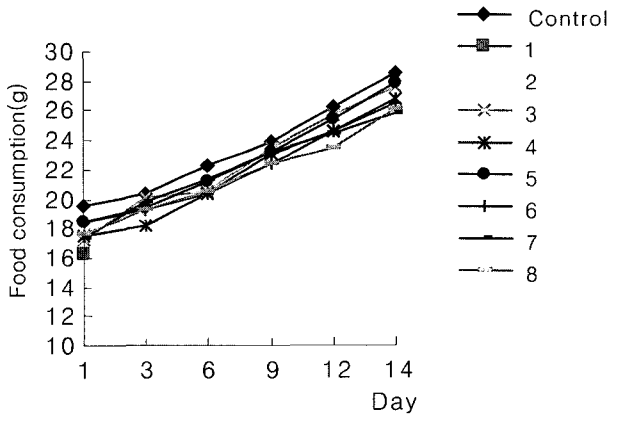

Fig. 3. Food consumption in female rats treated orally with calcitriol and alendronate. 1, calcitriol 125 μg/kg + alendronate 1250 mg/kg; 2, calcitriol 100 μg/kg + alendronate 1000 mg/kg; 3, calcitriol 75 μg/kg + alendronate 750 mg/kg; 4, calcitriol 50 μg/kg + alendronate 500 mg/kg; 5, calcitriol 25 μg/kg + alendronate 250 mg/kg; 6, calcitriol 5 μg/kg + alendronate 50 mg/kg; 7, calcitriol 1 μg/kg + alendronate 10 mg/kg; 8, calcitriol 0.2 μg/kg + alendronate 2 mg/kg. All of data were not significantly diferrent from control.

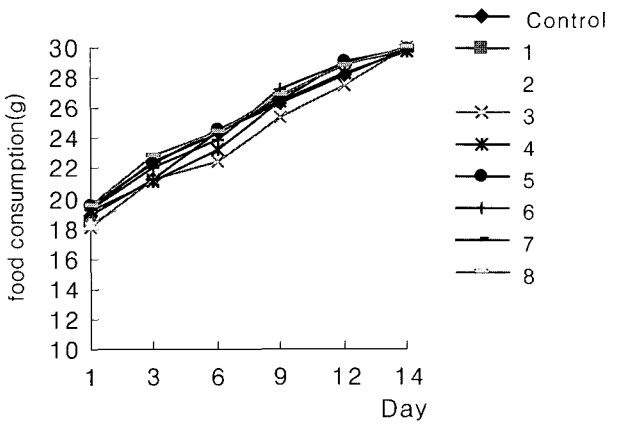

Fig. 4. Food consumption in male rats treated orally with calcitriol and alendronate. 1, calcitriol 125 μg/kg + alendronate 1250 mg/kg; 2, calcitriol 100 μg/kg + alendronate 1000 mg/kg; 3, calcitriol 75 μg/kg + alendronate 750 mg/kg; 4, calcitriol 50 μg/kg + alendronate 500 mg/kg; 5, calcitriol 25 μg/kg + alendronate 250 mg/kg; 6, calcitriol 5 μg/kg + alendronate 50 mg/kg; 7, calcitriol 1 μg/kg + alendronate 10 mg/kg; 8, calcitriol 0.2 μg/kg + alendronate 2 mg/kg. All of data were not significantly diferrent from control.

administration of calcitriol + alendronate mixtures but disappeared 5th day after administration. NOAEL (no observed adversed effects level) of calcitriol + alendronate mixtures were 50.005 mg/kg in 5-week oral dose toxicity.

Body weight and food consumption. Body weight in male and female rats treated with calcitriol + alendronate mixtures was continuously increased from 90 g to 290 g for 7 weeks and food consumption was also gradually increased. However, there were no significant changes in body weight and food consumption between control group and the groups treated with calcitriol + alendronate mixtures (Fig. 3 and Fig. 4).

Urinalysis. There were no significant changes in urinalysis parameters between control group and the groups treated with calcitriol + alendronate mixtures (Table 2-1 and Table 2-2).

Hematology. There were no significant changes in hematological examination between control group and the groups treated with calcitriol + alendronate mixtures (Table 3-1 and Table 3-2).

Serum chemistry. Among the parameters measured in the serum biochemical analysis at the time of 5th week, the value of GPT in male rats treated with calcitriol + alendronate mixtures at a dose of more than 5 μg/kg + 50 mg/kg was slightly increased as compared with the control group. In all experimental groups including control, the value of GOT was slightly higher than the reference value, which was not dose-dependent. All other parameters were within the normal

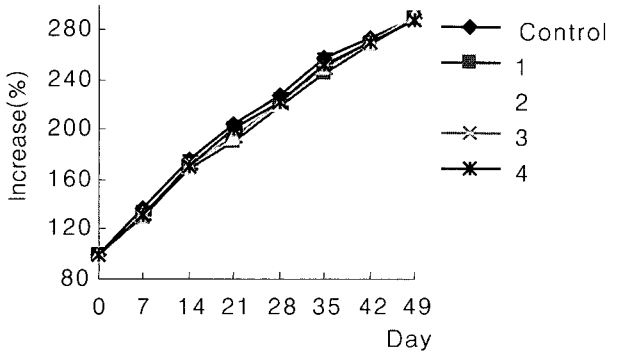

Fig. 5. Body weight in female rats treated orally with calcitriol and alendronate for 7 weeks. 1, calcitriol 25 μg/kg + alendronate 250 mg/kg; 2, calcitriol 5 μg/kg + alendronate 50 mg/kg; 3, calcitriol 1 μg/kg + alendronate 10 mg/kg; 4, calcitriol 0.2 μg/kg + alendronate 2 mg/kg. All of data were not significantly different from control.

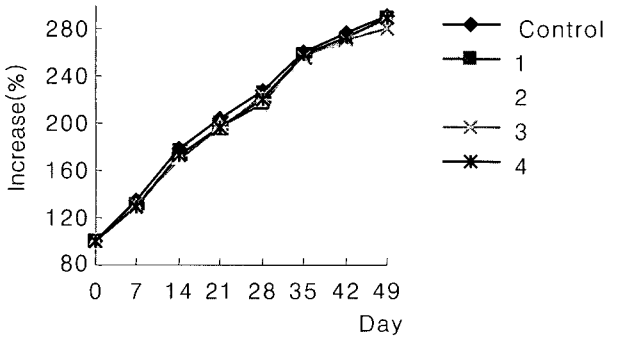

Fig. 6. Body weight in male rats treated orally with calcitriol and alendronate for 7 weeks. 1, calcitriol 25 μg/kg + alendronate 250 mg/kg; 2, calcitriol 5 μg/kg + alendronate 50 mg/kg; 3, calcitriol 1 μg/kg + alendronate 10 mg/kg; 4, calcitriol 0.2 μg/kg + alendronate 2mg/kg. All of data were not significantly different from control.

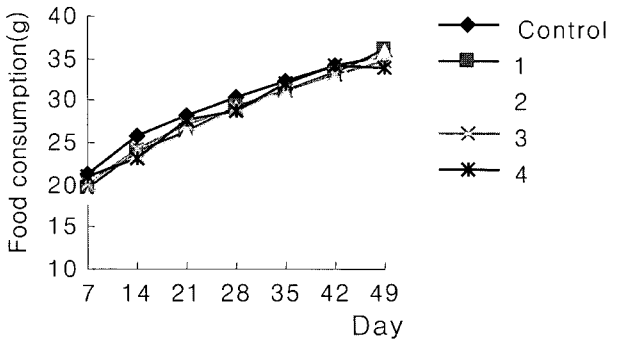

Fig. 7. Food consumption in female rats treated orally with calcitriol and alendronate for 7 weeks. 1, calcitriol 25 μg/kg + alendronate 250 mg/kg; 2, calcitriol 5 μg/kg + alendronate 50 mg/kg; 3, calcitriol 1 μg/kg + alendronate 10 mg/kg; 4, calcitriol 0.2 μg/kg + alendronate 2 mg/kg. All of data were not significantly different from control.

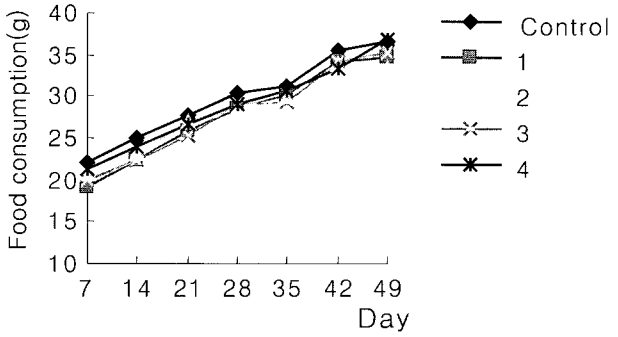

Fig. 8. Food consumption in male rats treated orally with calcitriol and alendronate for 7 weeks. 1, calcitriol 25 μg/kg + alendronate 250 mg/kg; 2, calcitriol 5 μg/kg + alendronate 50 mg/kg; 3, calcitriol 1 μg/kg + alendronate 10 mg/kg; 4, calcitriol 0.2 μg/kg + alendronate 2mg/kg. All of data were not significantly different from control.

ranges. After recovery period of 2 weeks, all parameters of serum biochemical analysis did not shown any significant changes between control group and the groups treated with calcitriol + alendronate mixtures (Table 4-1 and Table 4-2).

Autopsy and organ weight. In the rats treated with calcitriol + alendronate mixtures, no lesions in all organs were observed at the doses tested. In both male and female rats treated with calcitriol (25 μg/kg) + alendronate (250 mg/kg) mixtures, spleen exhibited slight hypertrophy, but other organs did not show any changes (Table 5-1.1, Table 5-1.2, Table 5-2.1, and Table 5-2.2).

Histopathology. In both male and female rats treated with calcitriol (25 μg/kg) + alendronate (250 mg/kg) mixtures at the time of 5th-week and 7th-week, slight hypertrophy, edema and congestion were observed in lung, spleen, liver, kidney and stomach (Table 6), but these pathological changes could be also observed in the control group. There were not dependent on the doses of calcitriol + alendronate mixtures. Other organs including brain, endocrine glands (thyroid, thymus, adrenal gland, pancreas, testis and ovary), gastrointestinal tract (salivary gland, small intestine and large intestine) and bone marrow did not show any changes.

DISCUSSION

In this study, single oral dose and five-week oral dose toxicity of calcitriol + alendronate mixtures were examined in male and female rats. In single oral toxicity study, the values of LD_{50} of calcitriol + alendronate mixture were 750.075 mg/kg in male rats and 775.0775

Table 3-1. Hematological examinations in male and female rats treated orally with test drugs for 5 weeks

Sex	Dose[1)]	n	RBC (10^4/μl)	Hb (g/dl)	Ht (%)	MCV (μl^3)	MCH (pg)	MCHC (%)	Plts (10^3/μl)	PT (sec)	WBC (10^2/μl)	Differential leucocyte counting (%)					
												Lympho	Seg	Eosino	Baso	Mono	Others
Male	Control	10	792 ± 5	15.3 ± 0.2	45 ± 2	56.8 ± 1.2	19.3 ± 0.3	34.0 ± 0.2	312 ± 21	10.1 ± 0.8	85 ± 2	96.2 ± 3.1	0.6 ± 0.1	0.4 ± 0.2	0.2 ± 0.4	2.6 ± 0.8	0.0 ± 0.0
	25 + 250	10	756 ± 7	14.2 ± 0.3	45 ± 3	59.5 ± 1.3	18.8 ± 0.5	31.6 ± 0.3	342 ± 18	9.8 ± 0.4	75 ± 2	95.4 ± 2.5	0.6 ± 0.3	0.5 ± 0.1	0.2 ± 0.2	2.4 ± 0.5	0.0 ± 0.0
	5 + 50	10	724 ± 3	15.7 ± 0.2	44 ± 2	60.7 ± 0.9	21.7 ± 0.2	35.7 ± 0.1	399 ± 12	9.7 ± 0.5	83 ± 3	96.7 ± 1.9	0.5 ± 0.3	0.7 ± 0.2	0.8 ± 0.3	1.3 ± 0.5	0.0 ± 0.0
	1 + 10	10	740 ± 9	14.9 ± 0.7	43 ± 2	58.1 ± 0.5	20.1 ± 0.5	34.7 ± 0.2	334 ± 15	11.0 ± 0.7	79 ± 2	94.8 ± 2.4	0.6 ± 0.3	0.5 ± 0.1	0.9 ± 0.4	3.2 ± 0.8	0.0 ± 0.0
	0.2 + 2	10	753 ± 7	15.4 ± 0.3	46 ± 3	61.1 ± 1.2	20.5 ± 0.6	33.5 ± 0.3	346 ± 15	9.5 ± 0.9	89 ± 3	95.8 ± 3.4	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.3	1.8 ± 0.6	0.0 ± 0.0
Female	Control	10	768 ± 6	15.4 ± 0.6	44 ± 2	57.3 ± 1.0	19.7 ± 0.3	35.0 ± 0.4	354 ± 11	9.6 ± 1.1	77 ± 2	94.9 ± 2.1	0.4 ± 0.2	0.6 ± 0.2	0.9 ± 0.3	3.2 ± 0.8	0.0 ± 0.0
	25 + 250	10	744 ± 8	14.7 ± 0.3	44 ± 3	59.1 ± 0.9	19.8 ± 0.2	33.4 ± 0.2	326 ± 13	9.8 ± 0.8	81 ± 3	96.2 ± 2.3	0.5 ± 0.3	0.4 ± 0.1	0.2 ± 0.2	2.7 ± 0.5	0.0 ± 0.0
	5 + 50	10	716 ± 4	15.3 ± 0.2	44 ± 3	61.4 ± 1.3	21.4 ± 0.5	34.8 ± 0.1	319 ± 16	10.1 ± 0.7	75 ± 2	94.2 ± 3.2	0.4 ± 0.2	0.3 ± 0.1	0.8 ± 0.4	4.3 ± 1.0	0.0 ± 0.0
	1 + 10	10	720 ± 5	14.3 ± 0.5	43 ± 2	59.7 ± 0.7	19.9 ± 0.5	33.3 ± 0.3	361 ± 24	9.9 ± 0.7	80 ± 3	95.5 ± 2.7	0.5 ± 0.3	0.3 ± 0.1	0.9 ± 0.2	2.8 ± 0.6	0.0 ± 0.0
	0.2 + 2	10	734 ± 9	14.9 ± 0.3	45 ± 1	61.3 ± 1.5	20.3 ± 0.3	33.1 ± 0.2	335 ± 15	9.8 ± 0.8	84 ± 2	94.5 ± 1.8	0.6 ± 0.2	0.4 ± 0.2	0.7 ± 0.3	3.8 ± 0.9	0.0 ± 0.0

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
All of data were not significantly different from control.

Table 3-2. Hematological examinations in male and female rats after 2-week recovery period following 5-week treatment with test drugs

Sex	Dose[1)]	n	RBC (10^4/μl)	Hb (g/dl)	Ht (%)	MCV (μl^3)	MCH (pg)	MCHC (%)	Plts (10^3/μl)	PT (sec)	WBC (10^2/μl)	Differential leucocyte counting (%)					
												Lympho	Seg	Eosino	Baso	Mono	Others
Male	Control	10	772 ± 8	16.7 ± 0.2	46 ± 3	59.6 ± 1.1	21.6 ± 0.6	36.3 ± 0.2	864 ± 15	10.5 ± 0.7	82 ± 2	94.1 ± 2.4	0.4 ± 0.1	0.4 ± 0.1	5.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	25 + 250	10	755 ± 12	17.2 ± 0.5	44 ± 1	58.3 ± 1.2	22.8 ± 0.2	39.1 ± 0.1	814 ± 21	9.7 ± 0.6	77 ± 4	96.1 ± 3.1	0.3 ± 0.2	0.5 ± 0.2	3.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	5 + 50	10	749 ± 9	16.6 ± 0.4	45 ± 2	60.1 ± 0.9	22.2 ± 0.3	36.9 ± 0.1	826 ± 14	10.0 ± 0.5	80 ± 2	95.8 ± 2.1	0.4 ± 0.1	0.4 ± 0.1	3.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
	1 + 10	10	731 ± 5	15.9 ± 0.3	42 ± 3	57.5 ± 1.2	21.8 ± 0.5	37.9 ± 0.3	830 ± 10	10.2 ± 0.8	76 ± 3	96.7 ± 1.8	0.3 ± 0.1	0.3 ± 0.1	2.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	0.2 + 2	10	782 ± 11	16.1 ± 0.4	45 ± 3	57.5 ± 0.8	20.6 ± 0.3	35.8 ± 0.2	813 ± 20	9.9 ± 0.7	78 ± 2	95.1 ± 2.4	0.5 ± 0.1	0.4 ± 0.2	4.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Female	Control	10	763 ± 9	17.1 ± 0.3	44 ± 3	57.7 ± 0.8	22.4 ± 0.5	38.9 ± 0.1	798 ± 12	9.8 ± 0.3	91 ± 4	96.5 ± 1.6	0.4 ± 0.2	0.6 ± 0.2	2.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	25 + 250	10	745 ± 12	16.5 ± 0.2	43 ± 5	57.7 ± 1.3	22.2 ± 0.2	38.4 ± 0.3	817 ± 13	9.5 ± 0.5	85 ± 4	94.2 ± 0.9	0.4 ± 0.1	0.4 ± 0.1	5.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	5 + 50	10	757 ± 10	16.9 ± 0.6	46 ± 4	60.8 ± 0.8	22.3 ± 0.3	36.7 ± 0.2	822 ± 21	9.9 ± 0.8	78 ± 5	96.2 ± 1.7	0.3 ± 0.1	0.3 ± 0.2	3.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	1 + 10	10	722 ± 8	15.3 ± 0.2	45 ± 3	62.3 ± 1.0	21.2 ± 0.3	34.0 ± 0.1	835 ± 23	10.2 ± 0.4	76 ± 2	95.7 ± 2.7	0.5 ± 0.2	0.6 ± 0.3	3.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	0.2 + 2	10	732 ± 11	15.7 ± 0.7	44 ± 2	60.1 ± 1.2	21.5 ± 0.4	35.7 ± 0.2	847 ± 24	9.8 ± 0.7	73 ± 2	94.7 ± 2.1	0.3 ± 0.1	0.5 ± 0.2	4.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
All of data were not significantly different from control.

Table 4-1. Blood biochemical examinations in male and female rats treated orally with test drugs for 5 weeks

Sex	Dose[1)]	n	GOT (I.U/l)	GPT (I.U/l)	ALP (I.U/l)	T-PRO (g/ml)	BUN (mg/dl)	Creatine (mg/dl)	GLU (g/ml)	T-CHOL (mg/dl)	Bilirubin (mg/dl)	Ca^{++} (mg/dl)	Na^{+} (mEq/l)	K^{+} (mEq/l)	Cl^{-} (mEq/l)	Phosphorus (mg/dl)
Male	Control	10	66.1 ± 12.2	43.8 ± 6.5	101.4 ± 8.7	6.7 ± 0.1	15.8 ± 2.1	0.6 ± 0.2	112.4 ± 6.5	36.4 ± 4.54	0.12 ± 0.02	10.2 ± 0.3	144.3 ± 0.6	4.7 ± 0.2	102.1 ± 0.6	7.6 ± 1.7
	25+250	10	61.9 ± 13.4	45.1 ± 7.8	95.7 ± 7.0	9.1 ± 1.2	13.9 ± 0.9	0.5 ± 0.1	121.5 ± 8.9	43.1 ± 5.44	0.16 ± 0.02	9.4 ± 0.2	142.8 ± 0.4	4.3 ± 0.1	103.1 ± 0.4	7.2 ± 2.1
	5+50	10	62.9 ± 13.4	45.1 ± 7.8	95.7 ± 8.0	9.1 ± 1.2	13.9 ± 0.9	0.6 ± 0.2	121.4 ± 8.8	43.4 ± 5.44	0.16 ± 0.02	9.7 ± 0.1	147.8 ± 0.2	4.2 ± 0.1	100.3 ± 0.2	7.2 ± 2.0
	1+10	10	65.5 ± 13.1	45.3 ± 6.4	82.5 ± 12.3	6.7 ± 0.3	11.3 ± 0.4	0.5 ± 0.2	117.2 ± 3.5	41.6 ± 7.10	0.14 ± 0.02	9.1 ± 0.3	143.2 ± 0.3	4.6 ± 0.2	102.1 ± 0.2	8.8 ± 0.7
	0.2+2	10	65.1 ± 20.3	43.8 ± 9.8	89.4 ± 15.9	5.9 ± 0.2	12.8 ± 1.6	0.6 ± 0.1	113.2 ± 6.5	49.2 ± 4.27	0.16 ± 0.02	9.8 ± 0.3	142.1 ± 0.2	4.8 ± 0.1	99.3 ± 0.4	7.9 ± 2.9
Female	Control	10	65.3 ± 11.4	42.6 ± 5.6	101.1 ± 9.1	7.4 ± 0.4	15.1 ± 1.3	0.6 ± 0.2	118.2 ± 7.2	39.2 ± 2.85	0.18 ± 0.03	9.3 ± 0.4	140.1 ± 0.4	4.4 ± 0.1	100.3 ± 0.3	7.7 ± 1.9
	25+250	10	50.2 ± 12.7	43.1 ± 4.0	88.4 ± 10.1	5.8 ± 0.3	15.3 ± 0.6	0.5 ± 0.2	117.5 ± 9.8	41.4 ± 4.69	0.12 ± 0.02	9.6 ± 0.2	142.1 ± 0.2	4.2 ± 0.1	98.7 ± 0.3	7.9 ± 2.2
	5+50	10	60.0 ± 12.7	43.1 ± 4.0	88.5 ± 10.1	5.9 ± 0.3	15.3 ± 0.6	0.6 ± 0.2	117.2 ± 9.1	41.4 ± 4.69	0.14 ± 0.02	9.7 ± 0.3	144.9 ± 0.5	4.8 ± 0.1	103.2 ± 0.6	7.9 ± 2.2
	1+10	10	68.9 ± 15.6	44.1 ± 8.0	101.4 ± 10.0	7.9 ± 0.3	15.1 ± 0.4	0.5 ± 0.1	121.4 ± 4.6	39.4 ± 4.04	0.22 ± 0.03	9.8 ± 0.3	146.2 ± 0.4	4.3 ± 0.1	101.2 ± 0.5	8.5 ± 1.0
	0.2+2	10	67.7 ± 9.1	41.5 ± 4.2	97.2 ± 11.6	6.8 ± 0.5	17.1 ± 0.6	0.4 ± 0.2	115.2 ± 8.0	40.4 ± 7.51	0.14 ± 0.02	9.1 ± 0.3	143.5 ± 0.5	4.2 ± 0.1	99.8 ± 0.3	8.3 ± 2.2

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
All of data were not significantly different from control.

Table 4-2. Blood biochemical examinations in male and female rats after 2-week recovery period following 5-week treatment with test drugs

Sex	Dose[1)]	n	GOT (I.U/l)	GPT (I.U/l)	ALP (I.U/l)	T-PRO (g/ml)	BUN (mg/dl)	Creatine (mg/dl)	GLU (g/ml)	T-CHOL (mg/dl)	Bilirubin (mg/dl)	Ca^{++} (mg/dl)	Na^{+} (mEq/l)	K^{+} (mEq/l)	Cl^{-} (mEq/l)	Phosphorus (mg/dl)
Male	Control	10	58.2 ± 8.8	36.3 ± 2.4	85.2 ± 3.4	6.7 ± 0.2	20.6 ± 0.7	0.5 ± 0.2	119.1 ± 3.9	56.8 ± 3.4	0.20 ± 0.03	9.9 ± 0.1	143.5 ± 0.5	4.2 ± 0.1	99.8 ± 0.5	6.0 ± 0.5
	25+250	10	65.8 ± 6.4	35.1 ± 2.4	95.3 ± 6.0	6.8 ± 0.3	22.9 ± 2.3	0.6 ± 0.2	123.2 ± 2.5	69.3 ± 4.0	0.15 ± 0.02	10.0 ± 0.4	141.2 ± 0.3	4.2 ± 0.1	101.1 ± 0.1	5.9 ± 0.3
	5+50	10	62.1 ± 7.3	36.1 ± 2.2	93.7 ± 5.6	7.1 ± 0.2	19.1 ± 1.8	0.7 ± 0.1	120.3 ± 10.2	59.8 ± 3.4	0.18 ± 0.03	9.9 ± 0.8	144.2 ± 0.3	4.0 ± 0.1	103.2 ± 0.3	6.0 ± 0.4
	1+10	10	61.0 ± 5.8	34.6 ± 2.1	109.4 ± 9.4	7.7 ± 0.4	15.7 ± 2.6	0.7 ± 0.3	97.2 ± 3.0	51.5 ± 2.4	0.18 ± 0.03	9.7 ± 0.2	145.3 ± 0.6	4.2 ± 0.3	100.2 ± 0.7	6.7 ± 0.2
	0.2+2	10	68.1 ± 5.0	31.7 ± 0.1	109.6 ± 7.4	7.0 ± 0.3	21.8 ± 1.7	0.6 ± 0.1	123.4 ± 5.7	59.8 ± 4.1	0.16 ± 0.02	9.4 ± 0.3	140 ± 0.3	4.5 ± 0.1	102.3 ± 0.3	6.2 ± 0.4
Female	Control	10	54.3 ± 4.7	36.9 ± 2.1	83.9 ± 8.0	7.2 ± 0.2	22.1 ± 0.9	0.5 ± 0.2	116.1 ± 3.2	62.1 ± 4.5	0.18 ± 0.03	9.7 ± 0.4	145.6 ± 0.5	4.3 ± 0.1	100.2 ± 0.3	6.5 ± 1.1
	25+250	10	71.8 ± 6.2	31.8 ± 1.1	91.7 ± 6.8	7.5 ± 0.3	16.1 ± 2.0	0.5 ± 0.1	113.7 ± 3.2	79.3 ± 5.7	0.13 ± 0.02	9.5 ± 0.2	145.2 ± 0.4	4.1 ± 0.1	100.2 ± 0.3	5.8 ± 0.4
	5+50	10	74.9 ± 7.4	40.6 ± 5.9	100.6 ± 11.1	7.1 ± 0.4	19.9 ± 2.4	0.6 ± 0.2	89.5 ± 11.3	69.2 ± 5.7	0.16 ± 0.03	9.7 ± 0.6	141.2 ± 0.6	4.3 ± 0.1	102.1 ± 0.3	6.2 ± 1.4
	1+10	10	69.4 ± 6.5	30.1 ± 1.3	89.8 ± 13.8	7.5 ± 0.3	16.3 ± 1.3	0.5 ± 0.1	96.3 ± 8.1	69.8 ± 3.1	0.26 ± 0.04	9.1 ± 0.3	140.2 ± 0.4	4.2 ± 0.2	102.3 ± 0.2	5.9 ± 0.6
	0.2+2	10	64.1 ± 6.0	29.9 ± 1.7	101.3 ± 4.8	7.0 ± 0.3	21.7 ± 0.8	0.6 ± 0.2	118.4 ± 5.7	60.8 ± 8.6	0.14 ± 0.02	9.6 ± 0.3	142.1 ± 0.3	4.2 ± 0.1	99.7 ± 0.6	6.4 ± 0.3

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
All of data were not significantly different from control.

Table 5-1.1. Absolute and relative organ weight in male rats treated orally with test drugs for 5 weeks

	Drug[1)]	Body wt. (g)	Spleen (g,g%)	Thymus (g,g%)	Adrenal (mg,mg%)		Liver (g,g%)	Brain (g,g%)	Heart (g,g%)
					Right	Left			
Absolute	Control	258.23 ± 1.72	0.93 ± 0.81	0.36 ± 0.24	27.7 ± 1.02	26.4 ± 1.02	9.71 ± 1.21	2.19 ± 0.12	1.01 ± 0.07
	25+250	252.11 ± 2.81	1.21 ± 1.71	0.31 ± 0.12	22.5 ± 1.00	24.0 ± 0.31	9.77 ± 0.87	2.07 ± 0.08	0.96 ± 0.02
	5+50	254.23 ± 1.09	0.81 ± 1.24	0.38 ± 0.16	23.6 ± 1.03	22.7 ± 0.54	10.2 ± 0.74	0.21 ± 0.04	1.04 ± 0.10
	1+10	253.92 ± 3.56	0.79 ± 1.87	0.36 ± 0.24	25.9 ± 0.87	24.6 ± 0.74	8.3 ± 0.32	2.06 ± 0.12	1.07 ± 0.08
	0.2+2	255.24 ± 2.16	0.71 ± 0.12	0.33 ± 0.34	28.1 ± 0.74	26.1 ± 0.65	10.2 ± 0.41	0.12 ± 0.01	0.94 ± 0.05
Relative	Control	-	0.36 ± 0.51	0.14 ± 0.59	10.72 ± 0.25	10.22 ± 0.42	3.76 ± 1.01	0.85 ± 0.04	0.39 ± 0.05
	25+250	-	0.48 ± 0.37*	0.12 ± 0.28	8.92 ± 0.17	9.51 ± 0.21	3.87 ± 0.21	0.82 ± 0.01	0.38 ± 0.04
	5+50	-	0.32 ± 0.64	0.15 ± 0.12	9.27 ± 0.36	8.94 ± 0.34	4.02 ± 0.31	0.79 ± 0.06	0.41 ± 0.01
	1+10	-	0.35 ± 1.02	0.14 ± 0.89	10.21 ± 0.52	9.67 ± 0.74	3.27 ± 0.74	0.81 ± 0.18	0.42 ± 0.02
	0.2+2	-	0.28 ± 0.47	0.13 ± 0.51	11.02 ± 0.41	10.24 ± 0.64	3.98 ± 0.41	0.83 ± 0.07	0.37 ± 0.01

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
Significant difference from control : *$p<0.05$.

Table 5-1.1. Continued

	Drug[1)]	Thyroid (mg,mg%)		Lung (g,g%)	Kidney (g,g%)		Stomach (g,g%)	Salivary gland (g,g%)		Testis (g,g%)	
		Right	Left		Right	Left		Right	Left	Right	Left
Absolute	Control	7.72 ± 0.14	7.18 ± 0.41	1.01 ± 0.04	1.01 ± 0.24	0.75 ± 0.01	1.91 ± 0.04	1.24 ± 0.12	1.39 ± 0.14	0.80 ± 0.04	0.93 ± 0.04
	25+250	7.49 ± 0.31	7.61 ± 0.31	1.03 ± 0.01	0.93 ± 0.01	0.76 ± 0.02	1.36 ± 0.01	1.56 ± 0.08	1.18 ± 0.21	1.18 ± 0.14	1.06 ± 0.05
	5+50	9.69 ± 0.24	9.51 ± 0.16	0.99 ± 0.03	0.97 ± 0.05	0.79 ± 0.12	1.55 ± 0.14	1.45 ± 0.13	1.58 ± 0.16	1.55 ± 0.16	1.47 ± 0.12
	1+10	7.29 ± 0.25	8.81 ± 0.21	0.89 ± 0.05	1.02 ± 0.12	0.81 ± 0.05	1.46 ± 0.09	1.24 ± 0.12	1.19 ± 0.18	0.99 ± 0.11	1.04 ± 0.04
	0.2+2	8.04 ± 0.21	9.32 ± 0.31	0.94 ± 0.01	0.97 ± 0.11	0.79 ± 0.07	1.81 ± 0.15	0.89 ± 0.04	1.45 ± 0.17	1.33 ± 0.05	1.45 ± 0.18
Relative	Control	2.99 ± 0.10	2.78 ± 0.11	0.39 ± 0.01	0.39 ± 0.04	0.29 ± 0.01	0.74 ± 0.05	0.48 ± 0.07	0.54 ± 0.02	0.96 ± 0.01	0.36 ± 0.01
	25+250	2.97 ± 0.12	3.02 ± 0.12	0.41 ± 0.02	0.37 ± 0.01	0.30 ± 0.03	0.60 ± 0.04	0.62 ± 0.04	0.47 ± 0.04	1.11 ± 0.15	0.42 ± 0.02
	5+50	3.81 ± 0.13	3.74 ± 0.31	0.39 ± 0.04	0.38 ± 0.02	0.31 ± 0.02	0.61 ± 0.07	0.57 ± 0.02	0.62 ± 0.05	1.50 ± 0.24	0.58 ± 0.01
	1+10	2.87 ± 0.21	3.47 ± 0.45	0.35 ± 0.05	0.40 ± 0.01	0.32 ± 0.01	0.57 ± 0.01	0.49 ± 0.01	0.47 ± 0.04	0.99 ± 0.18	0.41 ± 0.03
	0.2+2	3.15 ± 0.24	3.65 ± 0.14	0.37 ± 0.02	0.38 ± 0.02	0.31 ± 0.02	0.71 ± 0.12	0.35 ± 0.03	0.57 ± 0.11	1.33 ± 0.06	0.57 ± 0.04

mg/kg in female rats, respectively. These LD_{50} of calcitriol and alendronate were as about 2,000 and 75 times greater that the orally common dose of human adult (Fawcett *et al.*, 1999: Frediani *et al.*, 1998), respectively. Although group treated with calcitriol (50 μg/kg) + alendronate (500 mg/kg) mixture showed a similar symptom like scratched head in 3 rats/group, such symptom disappeared 24 hr after administration of drugs. There was no significant changes in body weight and food consumption in all groups.

In subchronic toxicity study, five-week oral dose toxicity of calcitriol + alendronate mixture was examined. There were no mortality, abnormal behavior and appearance in all groups throughout the administration period (5 weeks) and recovery period (2 weeks). There were no significant changes in body weight and food

Table 5-1.2. Absolute and relative organ weight in female rats treated orally with test drugs for 5 weeks

	Drug[1)]	Body wt. (g)	Spleen (g,g%)	Thymus (g,g%)	Adrenal (mg,mg%)		Liver (g,g%)	Brain (g,g%)	Heart (g,g%)
					Right	Left			
Absolute	Control	260.21 ± 1.56	0.81 ± 0.02	0.29 ± 0.01	25.7 ± 3.21	26.7 ± 2.54	9.29 ± 1.04	1.95 ± 0.24	0.88 ± 0.14
	25+250	253.27 ± 3.54	0.99 ± 0.11	0.30 ± 0.02	23.5 ± 2.18	26.9 ± 2.14	10.20 ± 1.24	2.05 ± 0.45	2.05 ± 0.10
	5+50	258.15 ± 2.58	0.75 ± 0.24	0.39 ± 0.01	25.9 ± 2.17	24.0 ± 2.11	10.30 ± 0.87	1.91 ± 0.17	1.91 ± 0.07
	1+10	253.02 ± 3.24	0.79 ± 0.14	0.33 ± 0.03	24.8 ± 1.54	20.7 ± 2.15	8.63 ± 0.67	2.25 ± 0.15	2.25 ± 0.07
	0.2+2	256.26 ± 4.12	0.77 ± 0.05	0.31 ± 0.02	25.8 ± 2.65	23.9 ± 1.25	8.41 ± 1.78	2.13 ± 0.16	2.13 ± 0.10
Relative	Control	-	0.31 ± 0.01	0.11 ± 0.01	9.87 ± 1.25	10.28 ± 1.02	3.57 ± 0.27	0.75 ± 0.08	0.34 ± 0.02
	25+250	-	0.39 ± 0.03*	0.11 ± 0.02	9.28 ± 2.14	10.64 ± 2.12	4.01 ± 0.45	0.81 ± 0.15	0.38 ± 0.04
	5+50	-	0.29 ± 0.05	0.15 ± 0.01	10.02 ± 1.54	9.28 ± 1.25	3.98 ± 0.35	0.74 ± 0.01	0.39 ± 0.01
	1+10	-	0.31 ± 0.02	0.13 ± 0.01	9.81 ± 1.64	8.18 ± 1.24	3.39 ± 0.14	0.89 ± 0.02	0.41 ± 0.07
	0.2+2	-	0.31 ± 0.10	0.12 ± 0.02	10.08 ± 2.08	9.34 ± 0.89	3.28 ± 0.47	0.83 ± 0.03	0.38 ± 0.03

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
Significant difference from control : *$p<0.05$.

Table 5-1.2. Continued

	Drug[1)]	Thyroid (mg,mg%)		Lung (g,g%)	Kidney (g,g%)		Stomach (g,g%)	Salivary gland (g,g%)		Ovary (g,g%)
		Right	Left		Right	Left		Right	Left	Right
Absolute	Control	7.83 ± 0.21	7.18 ± 0.15	1.09 ± 0.01	1.04 ± 0.01	1.07 ± 0.03	2.13 ± 0.10	1.35 ± 0.13	0.96 ± 0.03	0.39 ± 0.02
	25+250	7.19 ± 0.31	7.55 ± 1.21	0.86 ± 0.02	0.96 ± 0.02	0.99 ± 0.01	1.80 ± 0.09	1.24 ± 0.11	1.11 ± 0.11	0.43 ± 0.05
	5+50	7.64 ± 1.02	8.44 ± 1.32	0.96 ± 0.02	1.01 ± 0.02	0.98 ± 0.04	2.17 ± 0.13	1.32 ± 0.05	1.15 ± 0.24	0.39 ± 0.01
	1+10	7.44 ± 2.05	9.29 ± 2.45	0.91 ± 0.01	1.04 ± 0.01	0.99 ± 0.04	1.70 ± 0.11	1.44 ± 0.08	1.19 ± 0.31	0.46 ± 0.05
	0.2+2	9.92 ± 1.78	7.35 ± 0.24	1.05 ± 0.06	0.97 ± 0.03	0.95 ± 0.02	1.69 ± 0.09	1.23 ± 0.04	1.31 ± 0.24	0.54 ± 0.01
Relative	Control	3.01 ± 0.47	2.76 ± 0.04	0.42 ± 0.04	0.40 ± 0.01	0.41 ± 0.04	0.82 ± 0.04	0.52 ± 0.02	0.37 ± 0.02	0.15 ± 0.02
	25+250	2.84 ± 0.15	2.98 ± 0.11	0.34 ± 0.01	0.38 ± 0.02	0.39 ± 0.07	0.71 ± 0.11	0.49 ± 0.07	0.44 ± 0.03	0.17 ± 0.02
	5+50	2.88 ± 0.24	3.27 ± 0.12*	0.37 ± 0.02	0.39 ± 0.04	0.38 ± 0.06	0.84 ± 0.08	0.51 ± 0.04	0.44 ± 0.04	0.15 ± 0.01
	1+10	3.13 ± 0.12	3.67 ± 0.06*	0.36 ± 0.01	0.41 ± 0.01	0.39 ± 0.01	0.67 ± 0.01	0.57 ± 0.01	0.47 ± 0.01	0.18 ± 0.03
	0.2+2	3.26 ± 0.034	2.87 ± 0.03	0.41 ± 0.03	0.38 ± 0.05	0.37 ± 0.02	0.66 ± 0.02	0.48 ± 0.02	0.51 ± 0.02	0.21 ± 0.01*

consumption between control group and the groups treated with calcitriol + alendronate mixtures.

The parameters of urinalysis and hematological analysis were no changed in male and female rats treated with calcitriol + alendronate mixtures. All the values of the parameters were within the normal range. In biochemical analysis of serum, however, the value of GPT in male rats treated with calcitriol + alendronate mixtures at a dose of more than 5 μg/kg + 50 mg/kg was slightly increased as compared with the control group, and the value of GOT was slightly higher than the reference value in all experimental groups including control. But such a result was not dependent on doses of calcitriol + alendronate mixtures, which may be due to the hemolysis in the process of blood sampling. At autopsy, although spleen slightly increased in both male and female rats treated with calcitriol (25 μg/kg) + alendronate (250 mg/kg) mixtures, no significant lesions in

Table 5-2.1. Absolute and relative organ weight in male rats after 2-week recovery period following 5-week treatment with test drugs

	Drug[1)]	Body wt. (g)	Spleen (g,g%)	Thymus (g,g%)	Adrenal (mg,mg%)		Liver (g,g%)	Brain (g,g%)	Heart (g,g%)
					Right	Left			
Absolute	Control	290.24 ± 4.21	0.90 ± 0.12	0.35 ± 0.02	28.1 ± 2.14	28.1 ± 3.02	12.0 ± 1.02	2.15 ± 0.05	1.19 ± 0.17
	25+250	284.51 ± 3.28	1.17 ± 0.08	0.30 ± 0.01	28.1 ± 1.87	29.2 ± 2.11	10.7 ± 1.11	2.25 ± 0.04	1.19 ± 0.08
	5+50	287.34 ± 2.57	0.98 ± 0.04	0.40 ± 0.05	25.8 ± 1.08	26.0 ± 1.56	11.3 ± 2.03	2.33 ± 0.11	1.09 ± 0.17
	1+10	279.05 ± 2.46	0.81 ± 0.02	0.47 ± 0.01	28.6 ± 3.11	24.5 ± 2.12	9.15 ± 0.57	2.32 ± 0.03	1.03 ± 0.06
	0.2+2	286.34 ± 3.24	0.94 ± 0.01	0.34 ± 0.05	28.3 ± 2.15	26.5 ± 2.10	10.7 ± 0.79	2.26 ± 0.05	1.20 ± 0.05
Relative	Control	-	0.31 ± 0.03	0.12 ± 0.01	9.68 ± 1.54	9.68 ± 1.07	4.12 ± 1.02	0.74 ± 0.01	0.41 ± 0.01
	25+250	-	0.41 ± 0.02*	0.11 ± 0.01	9.87 ± 1.44	10.25 ± 1.45	3.75 ± 0.84	0.79 ± 0.02	0.42 ± 0.03
	5+50	-	0.34 ± 0.01	0.14 ± 0.03	8.99 ± 2.11	9.05 ± 1.22	3.92 ± 0.71	0.81 ± 0.01	0.38 ± 0.02
	1+10	-	0.29 ± 0.02	0.17 ± 0.02	10.24 ± 1.09	8.79 ± 0.89	3.28 ± 0.54	0.83 ± 0.05	0.37 ± 0.01
	0.2+2	-	0.33 ± 0.01	0.12 ± 0.01	9.89 ± 1.05	9.26 ± 1.23	3.75 ± 0.09	0.78 ± 0.04	0.42 ± 0.03

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
Significant difference from control : *$p<0.05$.

Table 5-2.1. Continued

	Drug[1)]	Thyroid (mg,mg%)		Lung (g,g%)	Kidney (g,g%)		Stomach (g,g%)	Salivary gland (g,g%)		Testis (g,g%)	
		Right	Left		Right	Left		Right	Left	Right	Left
Absolute	Control	9.03 ± 0.17	8.07 ± 0.14	1.10 ± 0.11	1.19 ± 0.02	0.98 ± 0.01	1.65 ± 0.01	1.51 ± 0.21	1.19 ± 0.04	1.13 ± 0.11	1.12 ± 0.11
	25+250	9.30 ± 0.42	8.48 ± 0.16	1.17 ± 0.21	1.08 ± 0.12	0.83 ± 0.05	1.93 ± 0.24	1.65 ± 0.13	1.11 ± 0.14	1.17 ± 0.12	1.15 ± 0.12
	5+50	8.56 ± 0.54	8.99 ± 0.05	1.09 ± 0.05	1.03 ± 0.31	0.81 ± 0.01	2.13 ± 0.15	1.75 ± 0.18	1.58 ± 0.06	1.09 ± 0.22	1.10 ± 0.08
	1+10	8.01 ± 0.43	8.51 ± 0.09	0.61 ± 0.01	1.17 ± 0.05	0.86 ± 0.02	1.53 ± 0.21	1.87 ± 0.06	1.14 ± 0.12	1.09 ± 0.11	1.07 ± 0.04
	0.2+2	8.91 ± 0.28	8.56 ± 0.21	1.17 ± 0.42	1.12 ± 0.41	0.95 ± 0.02	1.98 ± 0.19	1.69 ± 0.09	1.35 ± 0.17	1.20 ± 0.09	1.19 ± 0.03
Relative	Control	3.11 ± 0.08	2.78 ± 0.08	0.38 ± 0.03	0.41 ± 0.01	0.34 ± 0.03	0.57 ± 0.08	0.52 ± 0.05	0.41 ± 0.05	0.39 ± 0.01	0.38 ± 0.02
	25+250	3.27 ± 0.12	2.98 ± 0.09	0.41 ± 0.01	0.38 ± 0.05	0.29 ± 0.01	0.68 ± 0.04	0.58 ± 0.01	0.39 ± 0.01	0.41 ± 0.06	0.40 ± 0.01
	5+50	2.98 ± 0.06	3.13 ± 0.12	0.38 ± 0.05	0.36 ± 0.02	0.28 ± 0.02	0.74 ± 0.02	0.61 ± 0.02	0.55 ± 0.02	0.38 ± 0.05	0.39 ± 0.03
	1+10	2.87 ± 0.11	3.05 ± 0.11	0.22 ± 0.01	0.42 ± 0.01	0.31 ± 0.04	0.55 ± 0.01	0.67 ± 0.03	0.41 ± 0.03	0.39 ± 0.01	0.38 ± 0.02
	0.2+2	3.11 ± 0.03	2.99 ± 0.21	0.41 ± 0.03	0.39 ± 0.03	0.33 ± 0.11	0.69 ± 0.03	0.59 ± 0.04	0.47 ± 0.01	0.42 ± 0.03	0.41 ± 0.01

Table 5-2.2. Absolute and relative organ weight in female rats after 2-week recovery period following 5-week treatment with test drugs

	Drug[1)]	Body wt. (g)	Spleen (g,g%)	Thymus (g,g%)	Adrenal (mg,mg%)		Liver (g,g%)	Brain (g,g%)	Heart (g,g%)
					Right	Left			
Absolute	Control	287.81 ± 4.01	1.01 ± 0.24	0.35 ± 0.01	29.5 ± 2.11	28.4 ± 1.54	11.6 ± 1.02	2.50 ± 0.08	0.83 ± 0.05
	25+250	285.14 ± 3.11	1.08 ± 0.22	0.30 ± 0.02	27.8 ± 1.04	29.2 ± 2.11	11.0 ± 0.71	2.20 ± 0.11	1.08 ± 0.06
	5+50	290.18 ± 2.41	0.96 ± 0.11	0.36 ± 0.03	28.0 ± 2.03	32.0 ± 1.65	10.0 ± 0.64	2.58 ± 0.15	0.99 ± 0.01
	1+10	281.12 ± 3.45	0.82 ± 0.23	0.31 ± 0.01	24.9 ± 1.02	27.4 ± 0.98	10.1 ± 0.87	2.22 ± 0.07	1.10 ± 0.02
	0.2+2	280.64 ± 1.24	0.95 ± 0.08	0.38 ± 0.02	25.6 ± 2.01	27.1 ± 2.11	9.2 ± 0.54	2.27 ± 0.10	1.01 ± 0.03
Relative	Control	-	0.35 ± 0.02	0.12 ± 0.04	10.24 ± 0.71	9.87 ± 1.08	4.02 ± 0.12	0.87 ± 0.05	0.29 ± 0.01
	25+250	-	0.38 ± 0.01*	0.14 ± 0.02	9.74 ± 0.51	10.24 ± 2.03	3.87 ± 0.22	0.77 ± 0.04	0.38 ± 0.02
	5+50	-	0.33 ± 0.03	0.12 ± 0.08	9.64 ± 0.73	11.02 ± 0.89	3.46 ± 0.09	0.89 ± 0.09	0.34 ± 0.03
	1+10	-	0.29 ± 0.01	0.11 ± 0.02	8.87 ± 1.02	9.74 ± 1.24	3.59 ± 0.05	0.79 ± 0.10	0.39 ± 0.02
	0.2+2	-	0.34 ± 0.02	0.14 ± 0.02	9.12 ± 0.95	9.67 ± 0.89	3.28 ± 0.04	0.81 ± 0.04	0.36 ± 0.03

[1)]Calcitriol (μg/kg) + Alendronate (mg/kg).
Data are shown mean ± S.E.
Significant difference from control : *$p<0.05$.

Table 5-2.2. Continued

	Drug[1)]	Thyroid (mg,mg%)		Lung (g,g%)	Kidney (g,g%)		Stomach (g,g%)	Salivary gland (g,g%)		Ovary (g,g%)
		Right	Left		Right	Left		Right	Left	Right
Absolute	Control	8.69 ± 1.02	7.45 ± 0.98	0.98 ± 0.06	1.09 ± 0.24	1.09 ± 0.02	1.67 ± 0.21	1.93 ± 0.04	1.18 ± 0.15	0.49 ± 0.10
	25+250	8.50 ± 1.22	8.18 ± 1.02	1.25 ± 0.12	1.06 ± 0.04	1.05 ± 0.01	1.88 ± 0.07	1.65 ± 0.12	1.23 ± 0.23	0.60 ± 0.11
	5+50	7.20 ± 0.98	8.73 ± 0.84	1.10 ± 0.13	1.04 ± 0.06	1.19 ± 0.03	1.98 ± 0.31	1.92 ± 0.24	1.51 ± 0.04	0.64 ± 0.04
	1+10	8.46 ± 0.54	8.77 ± 0.54	0.90 ± 0.08	1.18 ± 0.11	1.18 ± 0.12	1.96 ± 0.05	1.63 ± 0.18	1.24 ± 0.05	0.53 ± 0.03
	0.2+2	8.11 ± 0.74	7.83 ± 0.43	1.09 ± 0.21	0.98 ± 0.07	1.09 ± 0.21	1.99 ± 0.22	1.88 ± 0.08	1.49 ± 0.11	0.51 ± 0.02
Relative	Control	3.02 ± 0.21	2.59 ± 0.25	0.34 ± 0.04	0.38 ± 0.02	0.38 ± 0.02	0.58 ± 0.04	0.67 ± 0.05	0.41 ± 0.07	0.17 ± 0.02
	25+250	2.98 ± 0.64	2.87 ± 0.34	0.44 ± 0.02	0.37 ± 0.04	0.37 ± 0.01	0.66 ± 0.03	0.58 ± 0.01	0.43 ± 0.01	0.21 ± 0.05
	5+50	2.48 ± 0.24	3.01 ± 0.05	0.38 ± 0.01	0.36 ± 0.03	0.41 ± 0.02	0.68 ± 0.07	0.66 ± 0.02	0.52 ± 0.05	0.22 ± 0.02
	1+10	3.01 ± 0.31	3.12 ± 0.17	0.32 ± 0.02	0.42 ± 0.02	0.42 ± 0.05	0.70 ± 0.04	0.58 ± 0.03	0.44 ± 0.03	0.19 ± 0.01
	0.2+2	2.89 ± 0.25	2.79 ± 0.23	0.39 ± 0.05	0.35 ± 0.04	0.39 ± 0.01	0.71 ± 0.01	0.67 ± 0.02	0.53 ± 0.02	0.18 ± 0.03

Table 6. Summary of toxicological findings in rats given the mixture of calcitriol and alendronate for 5-week

Observation Items	Calcitriol (μg/kg) + Alendronate (mg/kg)			
	0.2 + 2	1 + 10	5 + 50	25 + 250
Sign				
Mortality	–	–	–	–
Insomnia	–	–	–	±
Irritation of esophagus-stomach	–	–	–	+
Biochemistry				
Increased GOT	–	–	–	±
Increased in glucose	–	–	–	±
Histopathological findings				
Lung				
Thickeness of alveolar wall	–	–	–	±
Infiltration of inflammatory cells	–	–	–	±
Spleen				
Congestion in red pulp	–	–	–	±
Liver				
Congestion in central vein	–	–	–	±
Kupffer cell mobilization	–	–	–	±
Kidney				
Congestion in medullary ray	–	–	–	±
Esophagus and Stomach				
Edema	–	–	–	±

– : no changed, ± : minimal change, + : mild change.

other organs were grossly observed. The changes in spleen weight might not be related to doses of calcitriol + alendronate mixtures. Histopathological examination did not indicate any abnormal findings associated with doses of calcitriol + alendronate mixtures in major organs and tissues at the time of 35th day (5th-week) and 49th day (7th-week). In both male and female rats treated with calcitriol (25 μg/kg) + alendronate (250 mg/kg) mixtures, slight hypertrophy, edema and congestion were observed in lung, spleen, liver, stomach and kidney. Considering that these changes were also observed in the control group, these changes was not due to the administration of calcitriol + alendronate mixtures.

In conclusion, combined treatment of calcitriol + alendronate mixtures at the doses of less than 250.025 mg/kg showed no signs in the acute toxicity and 5-week oral dose toxicity, and NOAEL of calcitriol + alendronate mixtures were 50.005 mg/kg in 5-week oral dose toxicity.

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