Single oral dose toxicity test of polycalcium, a mixed composition of polycan and calcium lactate-gluconate 1:9 (*G*/*G*) in SD rat

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Abstract: The object of this study was to obtain acute oral toxicity information of Polycalcium, a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*), in Sprague-Dawely (SD) rats. In order to investigate the toxicity and identify target organs, Polycalcium were once orally administered to female and male SD rats at dose levels of 2000, 1000, 500 and 0 (control) mg/kg body weights. The mortality, changes on body weight and clinical signs were monitored during 14 days after treatment with gross observation, changes on the organ weights and histopathology of principle organs and treatment sites based on the recommendation of KFDA Guidelines [2009-116, 2009]. As the results of single oral treatment of Polycalcium, no treatment related mortalities were observed within 14 days after end of treatment up to 2000 mg/kg, the limited dosage of rodents in the both genders. In addition, no Polycalcium treatment related changes on the body and organ weights, clinical signs, necropsy and histopathological findings were detected. The results obtained in this study suggest that the Polycalcium is non-toxic in rats. The LD_{50} and approximate LD in rats after single oral dose of Polycalcium were considered over 2000 mg/kg in both female and male, respectively.

Keywords: acute oral toxicity, beta-glucan, calcium, histopathology, rat.

INTRODUCTION

Calcium (Ca) salts have been shown anti-inflammatory activities (Hendry *et al*., 1982; Piller, 1990; Smith *et al*., 1994) and it also has been reported that various Ca salts have a favorable effect on the preventive or therapeutic potentials on the osteoporosis (Sosa and Bregni, 2003; Heaney *et al*., 2010). Polycan is a purified β-glucan from *Aureobasidium. pullulans* SM-2001, and comprises mostly β-1,3/1,6-glucan and other organic materials, such as amino acids, mono- or di-unsaturated fatty acids (linoleic and linolenic acids), and fibrous polysaccharide (Seo *et al*., 2002). Recently, it has been found that Polycan has anti-osteoporotic effects; it inhibited bone losses and accelerated the bone formation (Song *et al*., 2006; Shin *et al*., 2007), and fracture healing promoting effects (Lee *et al*., 2008) with anti-inflammatory effects (Kim *et al*., 2006, 2007).

The object of this study was to obtain acute (single) oral dose toxicity information of Polycalcium, a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*), in female and male SD rats. Because there are no available toxicological data after oral treatment in female and male rats, the highest dosage used in this study were selected as 2000 mg/kg - the limited dosages of rodents - in a volume of 10 ml of distilled water, and 1000 and 500 mg/kg were selected as middle and lower dosage groups according to KFDA Guidelines (Notification No.2009-116**,** 2009). In addition each female and male controls were added.

In order to investigate the toxicity and identify target organs. The female and male SD rats received polycalcium at dose of 2000, 1000, 500 and 0 (control) mg/kg body weight by gastric gavage. The lifespan and changes in body weight and clinical signs were monitored for 14 days after treatment with gross observation, changes on the organ weight and histopathology of principle organs based on the recommendation of KFDA Guidelines (Notification No. 2009-116, 2009).

MATERIALS AND METHODS

Experimental animals and materials

Each of twenty female and male SD rats (6-wk old upon receipt, SLC, Japan) were used after acclimatization for 5 days. Animals were allocated five per polycarbonate cage under controlled conditions of temperature (20-25ºC), humidity (40-45%) and a 12-hours light/dark cycle. The animals were fed *ad libitum* a commercial laboratory diet (Samyang, Korea) and water. All animals were overnight fasted (about 18hrs) before treatment and terminal necropsy.

Polycan and Ca lactate-gluconate were supplied by Glucan corp. (Busan, Korea) as brown and white powder,

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respectively. Polycalcium were prepared as direct mixed as 1:9 (g/g) of Polycan and Ca lactate-gluconate. Polycalcium is light brown powder and well dissolved (clear solution) at least 200 mg/ml concentrations in distilled water. Polycalcium was stored at 4ºC in a refrigerator to protect from light and humidity.

Experimental design

The animals were distributed into 8 groups 5 rats per group upon receipt. The highest dosage used in this study was selected as 2000 mg/kg (M06-M10, F06-F10) in a volume of 10 ml, the limited dosages of rodents and the recommended oral dose volume in rat (Flecknell, 1996; OECD Guidelines #423, 2001; KFDA Guidelines Notification No. 2009-116, 2009) using distilled water as vehicle, and 1000 (M11-M15, F11-F15) and 500 (M16-M20, F16-F20) mg/kg were selected as middle and lower dosage groups recommended by KFDA Guidelines (Notification No. 2009-116, 2009). The test article was single orally administered using a gastric gavage attached to 5 ml syringe. Control groups (M01-M05, F01-F05) was administered distilled water.

All abnormal clinical signs were recorded before and after dosing at least twice a day base on the functional observational battery test (Irwin, 1968; Dourish, 1987).

Changes of body weight

Body weights were measured at the day of dosing (Day 0) immediately before treatment, 1, 2, 7, 13 and 14 days after dosing. In addition, to reduce individual body weight differences of animals at treatment, body weight gains during Day 0 - Day 7, Day 7 - Day 13 and Day 0 - Day 13 was also calculated based on measured body weight at each point.

Necropsy

Unexpected died animals were grossly observed (lung, heart, thymus, kidney, adrenal glands, spleen, liver, testis/ovary, pancreas, brain, epididymus/uterus, urinary bladder, gastrointestinal tracts, skins and popliteal lymph nodes) immediately during experimental period and all survived animals were subjected to terminal necropsy. Animals were asphyxiated by carbon dioxide, and gross necropsy was performed in all animals at 14 days after overnight fasting (about 18hr, water was not restricted).

Organ weight

The absolute organ weight (lung, heart, thymus, liver, left kidney, left adrenal gland, spleen, left testis/ovary, splenic lobe of pancreas, brain, left epididymus/total uterus, urinary bladder, prostate and left popliteal lymph node) was measured and then relative organ weight (% of body weight) was calculated when they were sacrificed.

Histopathology

Principle organs (lung-left lateral lobes, heart, liver-left lateral lobe, thymus, kidney-left side, adrenal gland- left side, spleen, testis/ovary-left sides, splenic lobe of pancreas, brain, epididymus-head of left side, total uterus, popliteal lymph node-left side, urinary bladder, and prostate) were sampled at terminal necropsy, and fixed in 10% neutral buffered formalin. After 18h of fixation, paraffin embedding was conducted and 3-4μm sections were prepared by routine histological methods. Representative sections of each specified organs were stained with Hematoxylin & eosin for light microscopical examination.

STATISTICAL ANALYSIS

All data were represented as mean±standard deviation. The Mann-Whitney U-Wilcoxon Rank Sum W (MW) test was conducted to determine the specific pairs of group comparison, which are significantly different. LD_{50} and 95% confidence limits were calculated by Probit method. Statistical analyses were conducted using SPSS software (Release 14.0K, SPSS Inc., USA).

In addition, clinical signs, gross and histopathological findings were graded 1+ (slight), 2+ (moderate) and 3+ (severe)

RESULTS

Lifespan

No Polycalcium treatment related mortalities were recorded up to 2000 mg/kg treated groups of the both female and male rats; all animals of Polycalcium treated rats were survived for 14 days of experimental period and all rats subjected to the terminal necropsy.

Clinical signs

No Polycalcium treatment related clinical signs were recorded up to 2000 mg/kg, the limited dosage in rodents, treated groups of the both female and male rats.

Changes on body weight

No meaningful changes in the body weight were detected in all Polycalcium treated rats compared with each control except for 1000 mg/kg treated male rats, in which significant ($p<0.05$) increases of body weight during Day 0 - 13 were detected compared with control (table 1).

Changes in organ weight

No significant changes in the organ weights were detected in all Polycalcium treated rats compared with each control, except for 500 mg/kg treated male rats, in which significant ($p<0.01$) increase of kidney relative weight was observed as compared with each control (tables 2-5).

Table 1: Changes in Body weight in female and male rats after single oral treatment of Polycalcium

Groups	Interval		
	Day 0* - Day 7	Day 7 - Day 13	Day 0 - Day 13
Control			
Male	81.00±3.61	40.80±2.28	121.80±5.54
Female	45.40±2.88	16.80±4.02	62.20±6.53
Polycalcium-treated male groups			
2000mg/kg	83.80±4.44	43.40±2.70	127.20±7.05
1000mg/kg	82.20±1.48	46.00±2.55	128.20±2.49[a]
500mg/kg	83.60±6.39	46.00±4.53	129.60±10.31
Polycalcium-treated female groups			
2000mg/kg	45.20±6.06	19.20±3.35	64.40±7.30
1000mg/kg	43.00±7.58	19.00±4.36	62.00±8.15
500mg/kg	47.80±5.93	19.40±6.50	67.20±10.52

Values are expressed as mean±S.D. of five rats, g
*Day of treatment after overnight fasted
Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)
[a] $p<0.05$ as compared with each control by MW test

Table 2: Changes on absolute organ weights in male rats after single oral treatment of Polycalcium

Groups	Principal organs						
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Testis L
Control	1.193±0.071	0.938±0.041	0.520±0.097	1.016±0.033	0.038±0.006	0.552±0.038	1.571±0.083
Polycalcium-treated groups							
2000mg/kg	1.209±0.045	0.941±0.012	0.578±0.049	1.021±0.036	0.036±0.007	0.577±0.059	1.580±0.115
1000mg/kg	1.202±0.035	0.965±0.046	0.464±0.071	1.063±0.042	0.039±0.005	0.536±0.058	1.539±0.031
500mg/kg	1.248±0.104	0.973±0.068	0.555±0.078	1.097±0.066	0.042±0.005	0.594±0.034	1.527±0.062

Groups	Liver	Pancreas S	Brain	Epididymis L	LN L	Prostate	Urinary bladder
Control	9.183±0.429	0.591±0.117	1.995±0.080	0.340±0.023	0.009±0.002	0.348±0.026	0.097±0.012
Polycalcium-treated groups							
2000mg/kg	9.441±0.301	0.669±0.063	1.975±0.022	0.311±0.021	0.009±0.003	0.341±0.056	0.098±0.020
1000mg/kg	9.391±0.337	0.655±0.021	1.957±0.052	0.309±0.043	0.008±0.005	0.303±0.042	0.097±0.015
500mg/kg	9.194±0.545	0.653±0.032	1.959±0.050	0.317±0.009	0.010±0.007	0.359±0.034	0.097±0.005

Values are expressed as mean±S.D. of five rats, g
L, left sides; S, splenic lobes; G, gland; LN, popliteal lymph node
Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

Table 3: Changes in absolute organ weights in female rats after single oral treatment of Polycalcium

Groups	Principal organs						
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Ovary L
Control	0.890±0.044	0.612±0.033	0.431±0.048	0.625±0.041	0.039±0.007	0.375±0.053	0.082±0.018
Polycalcium-treated groups							
2000mg/kg	0.899±0.065	0.632±0.036	0.428±0.069	0.657±0.015	0.037±0.006	0.365±0.090	0.062±0.011
1000mg/kg	0.903±0.094	0.625±0.064	0.434±0.071	0.659±0.070	0.039±0.005	0.394±0.067	0.077±0.016
500mg/kg	0.913±0.088	0.657±0.048	0.494±0.045	0.669±0.069	0.038±0.004	0.373±0.057	0.081±0.013

Groups	Liver	Pancreas S	Brain	Uterus	LN L	Urinary bladder
Control	5.455±0.421	0.462±0.070	1.813±0.035	0.332±0.073	0.012±0.003	0.071±0.007
Polycalcium-treated groups						
2000mg/kg	5.455±0.338	0.496±0.088	1.820±0.035	0.329±0.094	0.010±0.006	0.073±0.010
1000mg/kg	5.384±0.689	0.483±0.120	1.838±0.062	0.387±0.145	0.008±0.006	0.066±0.008
500mg/kg	5.475±0.417	0.476±0.038	1.825±0.046	0.407±0.157	0.012±0.007	0.074±0.017

Values are expressed as mean±S.D. of five rats, g
L, left sides; S, splenic lobes; G, gland; LN, popliteal lymph node.
Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

Table 4: Changes in relative organ weights in male rats after single oral treatment of Polycalcium

Groups	Principal organs						
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Testis L
Control	0.397±0.016	0.312±0.012	0.173±0.031	0.338±0.010	0.013±0.002	0.183±0.012	0.522±0.026
Polycalcium-treated groups							
2000mg/kg	0.400±0.017	0.311±0.009	0.191±0.012	0.337±0.007	0.012±0.002	0.190±0.016	0.522±0.038
1000mg/kg	0.393±0.009	0.316±0.011	0.152±0.022	0.348±0.015	0.013±0.002	0.176±0.023	0.504±0.022
500mg/kg	0.411±0.027	0.320±0.015	0.182±0.021	0.361±0.012[a]	0.014±0.002	0.196±0.008	0.503±0.020

Groups	Liver	Pancreas S	Brain	Epididymis L	LN L	Prostate	Urinary bladder
Control	3.055±0.129	0.196±0.038	0.664±0.029	0.113±0.007	0.003±0.001	0.116±0.008	0.032±0.004
Polycalcium-treated groups							
2000mg/kg	3.118±0.063	0.221±0.024	0.652±0.018	0.103±0.008	0.003±0.001	0.112±0.016	0.032±0.007
1000mg/kg	3.072±0.102	0.214±0.010	0.641±0.030	0.101±0.013	0.003±0.002	0.099±0.014	0.032±0.005
500mg/kg	3.026±0.104	0.215±0.011	0.646±0.030	0.104±0.003	0.003±0.002	0.118±0.011	0.032±0.002

Values are expressed as mean ± S.D. of five rats, % of body weights at sacrifice
L, left sides; S, splenic lobes; G, gland; LN, popliteal lymph node.
Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)
[a] $p<0.01$ as compared with each control by MW test

Table 5: Changes in relative organ weights in female rats after single oral treatment of Polycalcium

Groups	Principal organs						
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Ovary L
Control	0.506±0.025	0.348±0.017	0.246±0.032	0.356±0.026	0.022±0.004	0.214±0.034	0.047±0.011
Polycalcium-treated groups							
2000mg/kg	0.501±0.019	0.352±0.009	0.238±0.028	0.366±0.011	0.021±0.003	0.202±0.038	0.034±0.005
1000mg/kg	0.508±0.026	0.351±0.010	0.244±0.035	0.371±0.023	0.022±0.001	0.221±0.027	0.043±0.007
500mg/kg	0.498±0.027	0.359±0.010	0.270±0.016	0.365±0.023	0.021±0.002	0.203±0.023	0.044±0.006

Groups	Liver	Pancreas S	Brain	Uterus	LN L	Urinary bladder
Control	3.109±0.289	0.264±0.046	1.032±0.033	0.190±0.047	0.007±0.002	0.040±0.003
Polycalcium-treated groups						
2000mg/kg	3.037±0.096	0.275±0.036	1.015±0.050	0.183±0.054	0.006±0.003	0.041±0.006
1000mg/kg	3.019±0.167	0.270±0.056	1.039±0.090	0.220±0.089	0.004±0.003	0.037±0.002
500mg/kg	2.990±0.107	0.260±0.013	0.999±0.040	0.220±0.076	0.006±0.004	0.040±0.008

Values are expressed as mean ± S.D. of five rats, % of body weights at sacrifice
L, left sides; S, splenic lobes; G, gland; LN, popliteal lymph node.
Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

Necropsy findings

Slight congestion of lung, thymic atrophy, splenic atrophy and edematous changes of uterus were sporadically detected throughout all experimental groups tested in this study including both gender of controls. In addition, slight hypertrophy of popliteal lymph node was detected in 1 male (1/5; 20%) rat treated with 500 mg/kg of Polycalcium (tables 6-7).

Histopathological findings

Slight [1+] lung congestional spots – thickening of alveolar lung inflammatory cell infiltration with focal hemorrhages (fig. 1), decrease of lymphoid cells in the white pulps of spleen (fig. 2) and focal inflammatory cell infiltrations in the liver parenchyma were sporadically detected throughout all experimental groups tested in the present study including both gender controls. In addition, slight hyperplasia of lymphoid cells in the popliteal lymph node was detected in 1 female (1/5; 20%) rat of 500 mg/kg treated group (tables 8-9).

LD_{50}, approximate LD and target organs

The LD_{50} and the approximate LD of Polycalcium after single oral treatment is over 2000mg/kg in both female and male SD rats because no Polycalcium treatment related mortalities were recorded upto 2000 mg/kg treated female and male rats. In addition, no specific targets or clinical signs were also observed in this study (table 10).

Table 6: Necropsy findings observed in male rats after single oral treatment of Polycalcium at Sacrifice (Day 14)

Groups		Vehicle control	Polycalcium treated as		
			2000mg/kg	1000mg/kg	500mg/kg
Lung	Normal	3/5	3/5	4/5	3/5
	Congestion	2/5	2/5	1/5	2/5
Heart	Normal	5/5	5/5	5/5	5/5
Thymus	Normal	5/5	5/5	5/5	5/5
Kidney	Normal	5/5	5/5	5/5	5/5
Adrenal gland	Normal	5/5	5/5	5/5	5/5
Spleen	Normal	3/5	4/5	4/5	4/5
	Atrophy	2/5	1/5	1/5	1/5
Testis	Normal	5/5	5/5	5/5	5/5
Liver	Normal	5/5	5/5	5/5	5/5
Pancreas	Normal	5/5	5/5	5/5	5/5
Brain	Normal	5/5	5/5	5/5	5/5
Epididymis	Normal	5/5	5/5	5/5	5/5
Urinary bladder	Normal	5/5	5/5	5/5	5/5
Prostate	Normal	5/5	5/5	5/5	5/5
Lymph node[a)]	Normal	5/5	5/5	5/5	4/5
	Hypertrophy	0/5	0/5	0/5	1/5
Others	Normal	5/5	5/5	5/5	5/5

Values are expressed as observed animals/total observed animals (five rats per group) [a)] Bilateral popliteal lymph node. Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

Table 7: Necropsy findings observed in female rats after single oral treatment of Polycalcium at Sacrifice (Day 14)

Groups		Vehicle control	Polycalcium treated as		
			2000mg/kg	1000mg/kg	500mg/kg
Lung	Normal	4/5	4/5	4/5	4/5
	Congestion	1/5	1/5	1/5	1/5
Heart	Normal	5/5	5/5	5/5	5/5
Thymus	Normal	4/5	5/5	5/5	5/5
	Atrophy	1/5	0/5	0/5	0/5
Kidney	Normal	5/5	5/5	5/5	5/5
Adrenal gland	Normal	5/5	5/5	5/5	5/5
Spleen	Normal	4/5	4/5	4/5	5/5
	Atrophy	1/5	1/5	1/5	0/5
Ovary	Normal	5/5	5/5	5/5	5/5
Liver	Normal	5/5	5/5	5/5	5/5
Pancreas	Normal	5/5	5/5	5/5	5/5
Brain	Normal	5/5	5/5	5/5	5/5
Uterus	Normal	4/5	3/5	3/5	4/5
	Edematous Changes	1/5	2/5	2/5	1/5
Urinary bladder	Normal	5/5	5/5	5/5	5/5
Lymph node[a)]	Normal	5/5	5/5	5/5	5/5
Others	Normal	5/5	5/5	5/5	5/5

Values are expressed as observed animals/total observed animals (five rats per group). [a)] Bilateral popliteal lymph node. Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

Table 8: Histopathological findings observed in male rats after single oral treatment of Polycalcium Sacrifice (Day 14)

Groups		Vehicle control	Polycalcium treated as		
			2000mg/kg	1000mg/kg	500mg/kg
Lung (Fig 3)	Normal Congestional spot	3/5 2/5	3/5 2/5	4/5 1/5	3/5 2/5
Heart	Normal	5/5	5/5	5/5	5/5
Thymus	Normal	5/5	5/5	5/5	5/5
Kidney	Normal	5/5	5/5	5/5	5/5
Adrenal gland	Normal	5/5	5/5	5/5	5/5
Spleen (Fig 4)	Normal wDE*	4/5 1/5	5/5 0/5	4/5 1/5	4/5 1/5
Testis	Normal	5/5	5/5	5/5	5/5
Liver (Fig 5)	Normal Focal inflammatory cell infiltration	3/5 2/5	4/5 1/5	4/5 1/5	4/5 1/5
Pancreas splenic	Normal	5/5	5/5	5/5	5/5
Brain	Normal	5/5	5/5	5/5	5/5
Epididymis	Normal	5/5	5/5	5/5	5/5
Urinary bladder	Normal	5/5	5/5	5/5	5/5
Prostate	Normal	5/5	5/5	5/5	5/5
Lymph node[a)]	Normal	5/5	5/5	5/5	5/5

Values are expressed as observed animals/total observed animals (five rats per group)
[a)] Bilateral popliteal lymph node. *wDE: decreases of lymphoid cells in the white pulps
Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

Table 9: Histopathological findings in female rats after single oral treatment of Polycalcium (Day 14)

Groups		Vehicle control	Polycalcium treated as		
			2000mg/kg	1000mg/kg	500mg/kg
Lung	Normal Congestional spot	4/5 1/5	4/5 1/5	4/5 1/5	4/5 1/5
Heart	Normal	5/5	5/5	5/5	5/5
Thymus	Normal	5/5	5/5	5/5	5/5
Kidney	Normal	5/5	5/5	5/5	5/5
Adrenal gland	Normal	5/5	5/5	5/5	5/5
Spleen	Normal	4/5	5/5	4/5	4/5
	wDE*	1/5	0/5	1/5	1/5
Ovary	Normal	5/5	5/5	5/5	5/5
Liver	Normal	2/5	3/5	3/5	4/5
	Focal inflammatory cell infiltration	3/5	2/5	2/5	1/5
Pancreas splenic	Normal	5/5	5/5	5/5	5/5
Brain	Normal	5/5	5/5	5/5	5/5
Uterus	Normal	5/5	5/5	5/5	5/5
Urinary bladder	Normal	5/5	5/5	5/5	5/5
Lymph node[a)]	Normal	5/5	5/5	5/5	4/5
	Hyperplasia of lymphoid cells	0/5	0/5	0/5	1/5

Values are expressed as observed animals/total observed animals (five rats per group).; [a)] Bilateral popliteal lymph node; * wDE: decreases of lymphoid cells in the white pulps; Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*).

Table 10: LD_{50}, ALD, MTD and target organs detected in female and male rats after single oral treatment of Polycalcium.

Animal	Index		
	LD_{50}	Approximated LD	Targets and Clinical signs
Genders			
Male	>2000mg/kg	>2000mg/kg	Not detected upto 2000mg/kg
Female	>2000mg/kg	>2000mg/kg	

95% confidence limits of could not calculated because of no regularities of mortalities detected in the present study
LD_{50}, 50% lethal dose; LD, lethal dose. Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*)

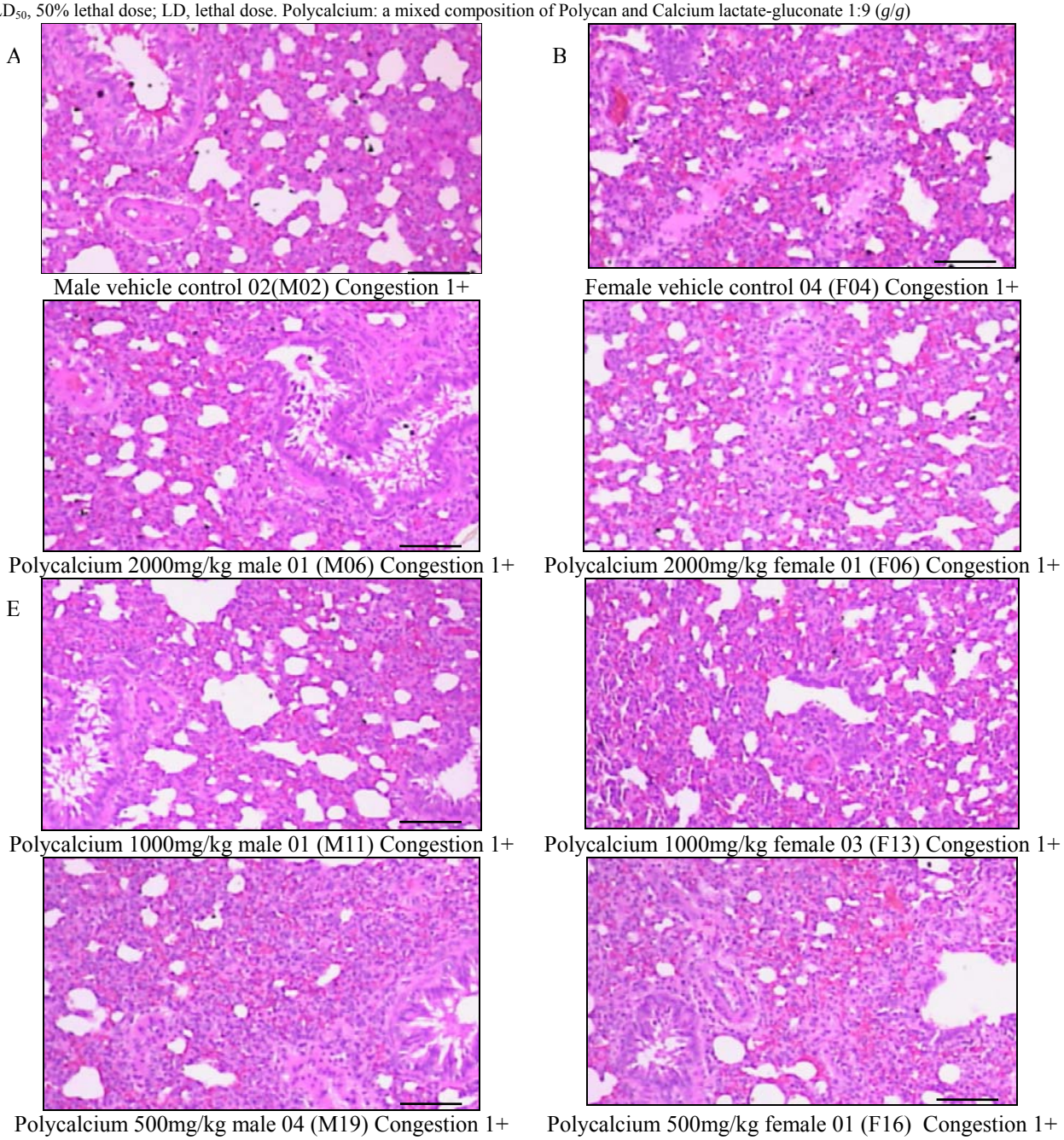

Fig. 1: Histopathological changes detected on the lung.
Note that slight [1+] lung focal congestional spots – thickening of alveolar lung inflammatory cell infiltration with/without focal hemorrhages were randomly detected throughout the all experimental groups tested regardless of genders including vehicle control as sporadic finings not Polycalcium-treatment related toxicological signs.; E, exudates; B, primary bronchiole; A, alveolar sac-respiratory bronchiole; Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*); All H&E stain, Scale bars = 160μm.

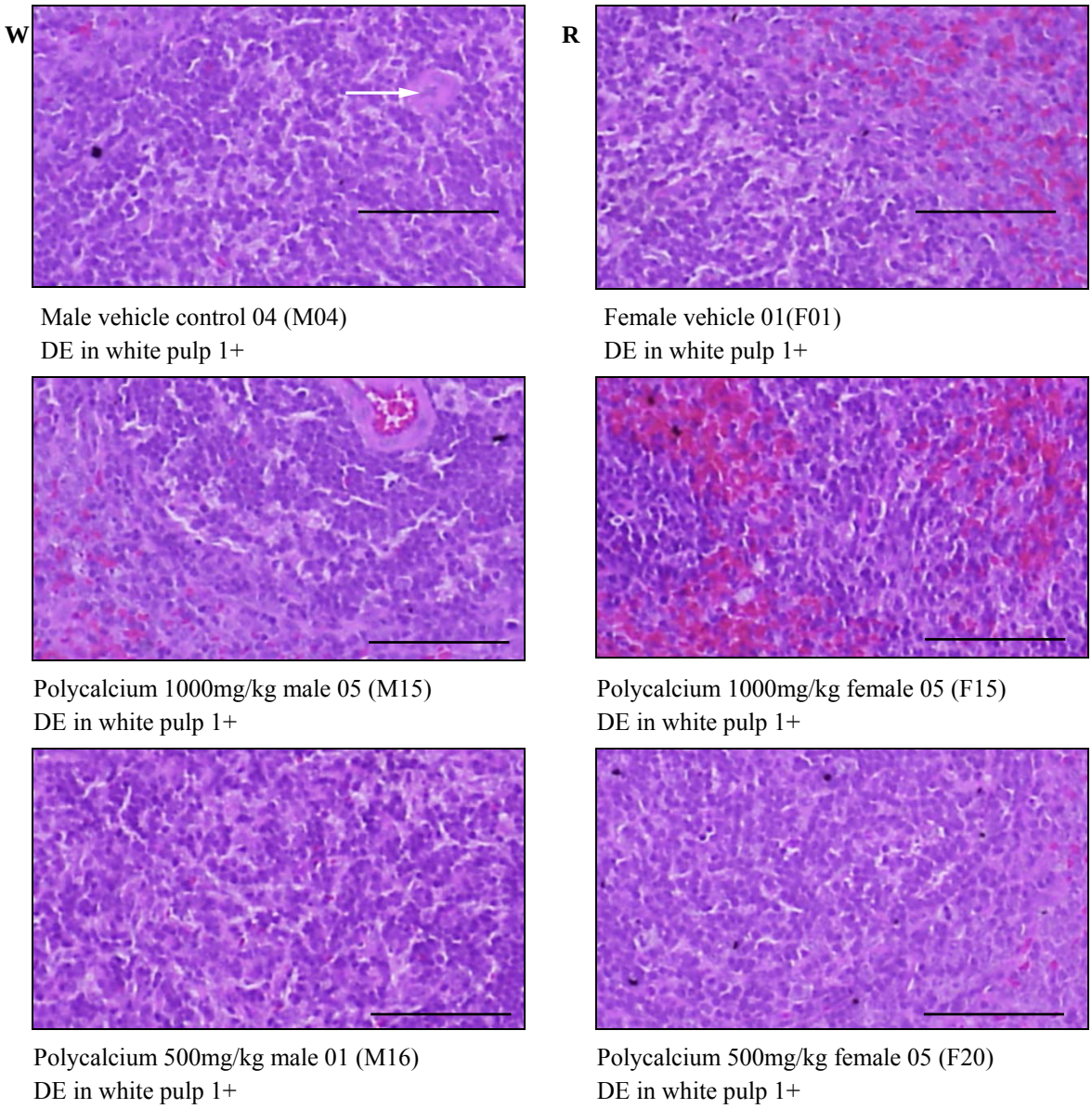

Fig 2: Histopathological changes detected on the spleen.
Note that slight [1+] decreases of lymphoid cells (DE; loosening between lymphoid cells or vacuoles) in the white pulps of spleen were randomly detected throughout the experimental groups tested regardless of genders including vehicle control as sporadic finings not Polycalcium-treatment related toxicological signs; Arrow, central arteriole ; W, white pulp; R, red pulp; Polycalcium: a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*); All H&E stain, Scale bars = 160μm.

DISCUSSION

Polycan, exopolymer of *Aureobasidium pullulans* SM-2001, is known to safe (Lee, 2005) and authorized functional food material in Korea (No. 2011-02) for bone health and approved Generally Recognized As Safe in USA (GRAS notice No. GRN 000309). But Polycalcium, a mixture of Polycan and calcium lactate-gluconate has not been investigated in the view of toxicology.

So, we investigated the single oral dose toxicity of Polycalcium, a mixed composition of Polycan and Calcium lactate-gluconate 1:9 (*g*/*g*), in female and male SD rats. In order to investigate the toxicity and identify target organs, Polycalcium was orally administered to female and male SD rats once at dose of 2000, 1000, 500 and 0 mg/kg. The mortality and changes on body weight and clinical signs were monitored during 14 days after treatment with gross observation, changes on organ weights and histopathology of 16 types of principle organs and treatment sites based on the recommendation of KFDA Guidelines (Notification No.2009-116, 2009) and some modifications.

In KFDA Guidelines (Notification No.2009-116, 2009) and OECD Guidelines (#423, 2001), the recommended highest dose of test materials were 2000 mg/kg or the maximum solubility, and they also recommended that in case of acute toxicity in rat, the dosage volume were below 10 ml/kg (Flecknell, 1996). In the present study, the highest dosage was selected as 2000 mg/kg in a volume of 10 ml, the limited dosages of rodents and the recommended oral dose volume in rat (Flecknell, 1996; OECD Guidelines #423, 2001; KFDA Guidelines Notification No. 2009-116, 2009), and 1000 and 500 mg/kg were selected as middle and lower dosage groups recommended by KFDA Guidelines (Notification No. 2009-116, 2009). Each female and male control groups were added. Test material was dosed using distilled water as vehicle in this study.

As the results of single oral treatment of Polycalcium, no treatment related mortalities were observed within 14 days after end of treatment up to 2000 mg/kg, the limited dosage of rodents in the both genders. In addition, no Polycalcium treatment related changes on the body and organ weights, clinical signs, necropsy and histopathological findings were detected in this study.

A significant ($p<0.05$) increase of body weight during Day 0 - 13 detected in Polycalcium 1000mg/kg treated male rats, and significant ($p<0.01$) increase of kidney relative weight observed in Polycalcium 500mg/kg treated male rats as compared with equal gender of control, were difficult to considered as Polycalcium treatment related toxicological signs because no obvious dosage-dependencies were detected in this study. All rats used in this study, showed normal body weight ranged in normal age-matched rats regardless of treatment in the present study including Polycalcium 1000mg/kg treated male rats (Cohen *et al*., 1984; Tanimoto, 1989).

The slight congestion of lung, thymic atrophy, splenic atrophy and edematous changes of uterus detected as gross findings, and lung congestional spots, decrease of lymphoid cells in the white pulps of spleen and focal inflammatory cell infiltrations in the liver parenchyma detected as histopathological findings were considered as accidental findings not toxicological signs related to Polycalcium treatment because they were sporadically detected throughout the whole experimental groups tested in the present study including both genders of control. Especially, the edematous changes in uterus were considered as secondary changes from different physiological estrus cycles (Banks, 1986; Pineda, 1989). In addition, most of them were also generally observed in normal rats (Montgomery *et al*., 1990; Greaves, 1990; Hasechek and Rousseaux, 1998). The hyperplasia of lymphoid cells or hypertrophy of popliteal lymph node restrictly detected in 1 female or male rats treated with Polycalcium 500 mg/kg were also difficult to consider as Polycalcium treatment related toxicological signs because no obvious dosage-dependencies were detected in this study.

The results obtained in this study suggest that the Polycalcium is non-toxic in rats and is therefore likely to be safe for clinical use. The LD_{50} and approximate LD in rats after single oral dose of Polycalcium were considered over 2000mg/kg, the limited dosage of rodents, respectively in both female and male. In addition, no specific target or clinical sings were detected in this study.

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