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Mouse Single Oral Dose Toxicity Studies of PGB-1, a Novel Polyglucosamine Polymer Produce from *Enterobacter sp.* BL-2

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This study was conducted to obtain acute information of the oral dose toxicity of PGB-1, a novel polyglucosamine polymer produced from a new strain *Enterobacter* sp. BL-2 in male and female mice. In order to calculated 50% lethal dose (LD_{50}) and approximate lethal dose (LD), test material was once orally administered to male and female ICR mice at dose levels of 2000, 1000, 500, 250, 125 and 0 (vehicle control) ml/kg (body wt.). The mortality and changes on body weight, clinical signs, gross observation and organ weight and histopathology of principle organs were monitored 14 days after dosing with PGB-1. We could not find any mortalities, clinical signs, body weight changes and gross findings. In addition, significant changes in the organ weight and histopathology of principal organs were not observed except for some sporadic findings. The results obtained in this study suggest that PGB-1 may not be toxic in mice and may be therefore safe for clinical use. The LD_{50} and approximate LD in mice after single oral dose of PGB-1 were considered over 2000 mg/kg in both female and male mice.

Key words: Polyglucosamine, PGB-1, Polymer, *Enterobacter*, Single oral dose toxicity, Mice, Histopathology.

INTRODUCTION

The deacetylated form of chitin, chitosan (polyglucosamine), has unique properties, which make it useful for a variety of industrial applications such as a viscosity control agent, adhesive, paper-strengthening agent, and flocculating aid. The traditional industrial source of chitin is shellfish waste from shrimp, crab, and lobster processing. However, problems with seasonal and limited supply, confined production locations, product variability, and high processing costs associated with the chemical conversion of chitin to chitosan appear to have limited the potential industrial acceptance of this polymer (White *et al.*, 1979).

Recent advances in fermentation technology suggest that large-scale culturing of an organism that contains chitosan might be an attractive route to the production of this polymer and microbial biopolymers have received considerable attention as flocculating agents; e.g., those from *Bacillus* (Yoon *et al.*, 1998; Deng *et al.*, 2003), *Phodovulum* (Watanabe *et al.*, 1999), *Rhodococcus* (Kurane *et al.*, 1986), *Klebsiella* (Dermlim *et al.*, 1999), *Paenibacillus* (Oh *et al.*, 2001), *Alcaligenes* (Toeda and Kurane, 1991), *Enterobacter* (Yokoi *et al.*, 2001) and *Enterobacter* (Fujita *et al.*, 2001; Jang *et al.*, 2001). The polysaccharide-type bioflocculants are more effective and stable than protein and glycoprotein-type bioflocculants. Also the cationic flocculants are known to be more powerful for treatment of negatively charged organic

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waste in sewage or the recovery of microbial cells. Most microbial polysaccharides are produced as a less effective anionic or natural type; nevertheless, the microbial cationic bioflocculant, except for the natural biopolymer chitosan, has rarely been developed.

A novel polyglucosamine polymer, PGB-1, was produced extracellularly from a new strain *Enterobacter sp.* BL-2 using pH-stat fed batch cultivation. It was composed of glucosamine, rhamnose, and galactose at a molar ratio of 86.4 : 1.6 : 1.0, respectively, indicating a rarely found novel high glucosamine-containing biopolymer and its average molecular weight was 106 kDa. FT-IR and 1H NMR spectra of PGB-1 revealed a close identity with chitosan from crab shells (Son *et al.*, 2005). However, there are no reports dealing the toxicological aspects of microbial biopolymers, even if the basic single dose toxicity in rodents except for our previous mouse single oral dose study about PGB-2, was produced extracellularly from a new strain *Citrobacter* sp. BL-4 using pH-stat fed batch cultivation (Lee *et al.*, 2007).

The objective of the present study, therefore, was to obtain the primary safety information about PGB-1, a novel polyglucosamine polymer and further clarify their safety for clinical use.

MATERIALS AND METHODS

Experimental animals. Each of thirty female and male ICR mice (6-wk old upon receipt, SLC, Japan) was used after acclimatization for 8 days. Five animals were allocated per a polycarbonate cage in a temperature (20~25°C) and humidity (40~45%) controlled room. Light : dark cycle was 12 h : 12 h and feed (Samyang, Korea) and water were supplied free to access. All animals were overnight fasted (about 18 h) before dosing and terminal necropsy. Animals were marked by picric acid. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as directed in the Korea Food and Drug Administration (KFDA) guidelines.

Purification of PGB-1, grouping and dosing. The strain *Enterobacter sp.* BL-2 was cultivated in pH-stat fed-batch culture in a 5 l jar fermenter (KoBiotech Co., Korea) containing 3 l of a liquid medium composed of 1.5% (w/v) sodium acetate, 0.1% $(NH_4)_2SO_4$, 0.1% yeast extract, 2.0% $MgSO_4 \cdot 7H_2O$, 0.5% NaCl, and 0.05% trace elements, at 30°C, 5000 rpm, and 0.3 vvm for 96h. A 2 M acetic acid solution was fed intermittently to maintain the pH at 8.0. as previously (Son *et al.*, 2005).

The extracellular polymer excreted from the *Enterobacter sp.* BL-2 was mixed with three volumes of absolute ethanol and precipitated at 4°C for 12 h (Yu *et al.*, 2004). The precipitant was then treated twice with 2 M NaOH at 121°C for 10 min to remove the residual protein and then neutralized with 2 M HCl to near neutrality. The recovered biopolymer was lysophilized after dialysis against distilled water (Son *et al.*, 2005).

PGB-1 is light yellow powder. PGB-1 was stored in a refrigerator at -20°C to protect from light and degeneration. The appearance of PGB-1 in vehicle (distilled water) is light yellow suspension at 100 and 50 mg/ml concentrations, but it is well soluble at 25, 12.5 and 6.25 mg/ml concentrations and observed as clear light yellow solution. The test article was single orally administered at a dosage volume of 20 ml/kg using distilled water as vehicle.

The animals were distributed into 12 groups (5 mice per group) upon receipt. The fixed highest dosage level was 2000 mg/kg according to the recommendation of KFDA (2005) and Organization for Economic Co-Operation and Development (OECD) (2001) guidelines, and the doses of 1000, 500, 250 and 125 mg/kg were selected using common ratio 2. In addition, a vehicle control group was added. Animal was once orally dosed using a sonde attached to a syringe of 1ml after overnight fasting (about 18 h, water was not restricted). Food and water were further restricted for about 3 h after dosing.

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

Body weight changes. 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 the erratum originated from individual body weight differences of animals at initial dosing, body weigh gains during Day 0~Day 7, Day 7~Day 13 and Day 0~Day 14 were also calculated based on measured body weight at each day.

Necropsy. All unscheduled died animals were grossly observed immediately after finding them and all survived animals were subjected to terminal necropsy. Animals were asphyxiated by carbon dioxide and gross necropsy was performed in all animals at Day 14 after overnight fasting (about 18 h, water was not restricted).

Specific organs grossly observed: lung, heart, kidney, spleen, testis, liver, pancreas, epididymis, popliteal lymph node, ovary, brain, and uterus.

Organ weight measurement. The absolute organ weight was measured and then relative organ weight (% of body weight) was calculated for the following organs of all experimental animals when they were sacrificed.

Measured organs: lung, heart, kidney (left), spleen, testis (left), liver, pancreas (splenic lobes), epididymis (left), popliteal lymph node (left), ovary (left), brain, and uterus.

Histopathology. Principle organs listed below were sampled at terminal necropsy, and fixed in 10% NBF (neutral buffered formalin). After 18 h of fixation, paraffin embedding was conducted and 3~4 μm sections were prepared by routine histological methods. Representative sections of each specified organs were stained with Hematoxylin & Eosin for light microscopical examination.

Specific organs sampled: lung, heart, kidney (left), spleen, testis (left), liver, pancreas (splenic lobes), epididymis (left), popliteal lymph node (left), ovary (left), brain, and uterus.

Table 1. Body weight gains after oral dose of PGB-1

Group		Intervals		
		Day 0[a]~Day 7	Day 7~Day 13	Day 0~Day 14
Male	G0M	9.68 ± 1.84	3.32 ± 0.94	10.08 ± 1.32
	G1M	9.44 ± 1.04	2.78 ± 0.16	9.66 ± 1.03
	G2M	8.60 ± 1.78	1.92 ± 0.36*	8.40 ± 1.34
	G3M	8.92 ± 1.00	1.52 ± 0.40**	7.48 ± 1.25*
	G4M	7.84 ± 1.59	2.00 ± 1.25	7.96 ± 2.43
	G5M	8.56 ± 0.82	1.46 ± 0.43**	8.14 ± 1.20*
Female	G0F	5.32 ± 0.73	1.40±2.33	4.44 ± 2.42
	G1F	4.92 ± 1.20	1.02±1.26	3.80 ± 1.11
	G2F	4.96 ±0.45	2.38±1.37	4.80 ± 0.82
	G3F	5.48 ± 1.05	2.12±1.34	5.48 ± 1.26
	G4F	6.10 ± 0.64	1.94±1.46	5.80 ± 1.14
	G5F	5.40 ± 1.16	0.98±1.72	4.60 ± 1.59

Values are expressed as mean ± S.D., g (n = 5); [a]Day of dosing; *p < 0.05 compared to that of vehicle control by MW test; **p < 0.01 compared to that of vehicle control by MW test.

Statistical analyses. Changes of body weight were analyzed by Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) compared to those of vehicle controls.

Table 2. Changes on the absolute organ weights after oral dose of PGB-1

Group	Organs: Male											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis L	Liver	Pancreas S	Brain	Epididymis L	Lymph node L[a]
G0M	0.218 ± 0.006	0.180 ± 0.013	0.068 ± 0.016	0.365 ± 0.030	0.009 ± 0.003	0.143 ± 0.042	0.135 ± 0.021	1.620 ± 0.811	0.220 ± 0.026	0.495 ± 0.023	0.042 ± 0.006	0.035 ± 0.010
G1M	0.214 ± 0.012	0.181 ± 0.017	0.068 ± 0.009	0.355 ± 0.039	0.009 ± 0.002	0.161 ± 0.032	0.117 ± 0.008	1.737 ± 0.201	0.217 ± 0.015	0.481 ± 0.018	0.038 ± 0.002	0.027 ± 0.010
G2M	0.211 ± 0.017	0.172 ± 0.020	0.051 ± 0.009	0.360 ± 0.066	0.008 ± 0.002	0.120 ± 0.018	0.125 ± 0.017	1.663 ± 0.149	0.201 ± 0.022	0.496 ± 0.018	0.039 ± 0.006	0.026 ± 0.003
G3M	0.206 ± 0.018	0.165 ± 0.010	0.067 ± 0.015	0.314 ± 0.013*	0.010 ± 0.006	0.118 ± 0.021	0.121 ± 0.008	1.615 ± 0.117	0.201 ± 0.036	0.492 ± 0.010	0.041 ± 0.004	0.031 ± 0.010
G4M	0.231 ± 0.035	0.179 ± 0.014	0.062 ± 0.019	0.323 ± 0.047	0.007 ± 0.003	0.129 ± 0.061	0.119 ± 0.006	1.680 ± 0.235	0.205 ± 0.024	0.472 ± 0.013	0.038 ± 0.005	0.039 ± 0.017
G5M	0.216 ± 0.013	0.186 ± 0.019	0.066 ± 0.017	0.330 ± 0.025	0.009 ± 0.003	0.128 ± 0.034	0.112 ± 0.013*	1.717 ± 0.123	0.235 ± 0.012	0.494 ± 0.006	0.037 ± 0.004	0.037 ± 0.015
	Organs: Female											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Ovary L	Liver	Pancreas S	Brain	Uterus	Lymph node L
G0F	0.185 ± 0.025	0.140 ± 0.007	0.076 ± 0.026	0.220 ± 0.014	0.008 ± 0.002	0.145 ± 0.041	0.034 ± 0.012	1.363 ± 0.183	0.184 ± 0.023	0.484 ± 0.025	0.172 ± 0.056	0.025 ± 0.005
G1F	0.179 ± 0.009	0.135 ± 0.006	0.062 ± 0.012	0.209 ± 0.032	0.009 ± 0.001	0.124 ± 0.012	0.042 ± 0.013	1.254 ± 0.125	0.179 ± 0.021	0.470 ± 0.014	0.164 ± 0.036	0.027 ± 0.007
G2F	0.181 ± 0.015	0.147 ± 0.016	0.069 ± 0.010	0.214 ± 0.030	0.008 ± 0.002	0.122 ± 0.014	0.042 ± 0.008	1.334 ± 0.123	0.186 ± 0.014	0.483 ± 0.028	0.210 ± 0.062	0.026 ± 0.013
G3F	0.194 ± 0.028	0.143 ± 0.020	0.060 ± 0.011	0.223 ± 0.034	0.007 ± 0.001	0.151 ± 0.035	0.045 ± 0.021	1.407 ± 0.237	0.184 ± 0.039	0.474 ± 0.022	0.239 ± 0.090	0.026 ± 0.010
G4F	0.179 ± 0.007	0.144 ± 0.009	0.069 ± 0.026	0.219 ± 0.009	0.010 ± 0.002	0.135 ± 0.040	0.064 ± 0.016*	1.389 ± 0.100	0.195 ± 0.042	0.485 ± 0.018	0.146 ± 0.035	0.038 ± 0.015
G5F	0.180 ± 0.009	0.150 ± 0.014	0.069 ± 0.018	0.195 ± 0.016*	0.008 ± 0.003	0.125 ± 0.024	0.042 ± 0.018	1.314 ± 0.129	0.193 ± 0.010	0.467 ± 0.021	0.155 ± 0.041	0.029 ± 0.015

Values are expressed as mean ± S.D., g (n = 5); L, left sides; S, splenic lobes; [a]Popliteal lymph node; *p < 0.05 compared to that of vehicle control by MW test.

LD_{50} was calculated by Probit method. Statistical analyses were conducted using SPSS for Windows (Release 6.1.3., SPSS Inc., USA). In addition, degrees of clinical signs, gross and histopathological findings were subdivided into 3 degrees: 3+ Severe, 2+ moderate, 1+ slight.

RESULTS

Mortalities. No unscheduled or PGB-1-treatment-related mortalities were detected at all dose levels tested in this study. At the end of the treatment, all of animals were survived at all dose levels tested including vehicle control.

Clinical signs. In this study, no PGB-1-treatment-related abnormal clinical signs were observed during observation periods regardless of gender.

Changes in body weights. No meaningful changes on body weight and gains were detected in all dosing groups tested compared to that of vehicle control in all dose levels tested except for significant ($p < 0.01$ or $p < 0.05$) decrease of body weight gains during day 7~14 of dosing of PGB-1 1000 mg/kg-dosing male group, and during day 7~14 of dosing periods and whole experimental periods (Day 0~14) of PGB-1 500 and 250 mg/kg-dosing male groups, respectively (Table 1).

Changes in organ weight. No meaningful changes on the absolute and relative organ weight of 11 principle organs were observed in all dosing groups tested compared to that of vehicle control except for significant ($p < 0.01$ or $p < 0.05$) decrease of absolute kidney weight in 500 mg/kg-dosing male group, of absolute weight of testis in 125 mg/kg-dosing male group and of relative weight of kidney in 125 mg/kg-dosing female group, and increase of relative weight of heart in 125 mg/kg-dosing male, of absolute weight of ovary in 500 mg/kg-dosing female and of relative weight of ovary in 250 mg/kg-dosing female group, respectively (Table 2, 3).

Table 3. Changes on the relative organ weights after oral dose of PGB-1

Group	Organs: Male											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Testis L	Liver	Pancreas S	Brain	Epididymis L	Lymph node L[a]
G0M	0.526 ± 0.029	0.435 ± 0.014	0.164 ± 0.038	0.883 ± 0.078	0.022 ± 0.007	0.343 ± 0.092	0.325 ± 0.051	3.869 ± 1.920	0.529 ± 0.051	1.195 ± 0.073	0.102 ± 0.012	0.085 ± 0.023
G1M	0.533 ± 0.029	0.453 ± 0.032	0.166 ± 0.026	0.880 ± 0.078	0.023 ± 0.006	0.410 ± 0.087	0.290 ± 0.018	4.257 ± 0.330	0.525 ± 0.013	1.185 ± 0.037	0.093 ± 0.005	0.068 ± 0.025
G2M	0.538 ± 0.039	0.436 ± 0.034	0.128 ± 0.024	0.913 ± 0.130	0.019 ± 0.004	0.297 ± 0.035	0.325 ± 0.048	4.245 ± 0.218	0.504 ± 0.043	1.265 ± 0.098	0.099 ± 0.016	0.068 ± 0.008
G3M	0.535 ± 0.043	0.433 ± 0.013	0.182 ± 0.027	0.819 ± 0.070	0.029 ± 0.011	0.295 ± 0.045	0.318 ± 0.034	4.219± 0.165	0.535 ± 0.073	1.288 ± 0.069	0.107 ± 0.009	0.088 ± 0.019
G4M	0.611 ± 0.164	0.465 ± 0.040	0.147 ± 0.026	0.821 ± 0.068	0.018 ± 0.005	0.284 ± 0.061	0.309 ± 0.019	4.354 ± 0.225	0.535 ± 0.076	1.240 ± 0.166	0.097 ± 0.012	0.106 ± 0.033
G5M	0.534 ± 0.027	0.480 ± 0.017*	0.162 ± 0.044	0.824 ± 0.056	0.025 ± 0.006	0.304 ± 0.071	0.286 ± 0.032	4.295 ± 0.254	0.583 ± 0.034	1.238 ± 0.036	0.093 ± 0.012	0.095 ± 0.034
	Organs: Female											
	Lung	Heart	Thymus	Kidney L	Adrenal gland L	Spleen	Ovary L	Liver	Pancreas S	Brain	Uterus	Lymph node L
G0F	0.612 ± 0.047	0.464 ± 0.029	0.248 ± 0.065	0.729 ± 0.062	0.026 ± 0.006	0.472 ± 0.107	0.110 ± 0.033	4.489 ± 0.204	0.607 ± 0.052	1.606 ± 0.121	0.574 ± 0.198	0.083 ± 0.009
G1F	0.605 ± 0.034	0.461 ± 0.022	0.205 ± 0.044	0.707 ± 0.101	0.032 ± 0.003	0.423 ± 0.032	0.153 ± 0.036	4.251 ± 0.281	0.629 ± 0.034	1.602 ± 0.088	0.551 ± 0.108	0.090 ± 0.022
G2F	0.605 ± 0.031	0.492 ± 0.054	0.223 ± 0.032	0.704 ± 0.098	0.024 ± 0.005	0.408 ± 0.043	0.137 ± 0.028	4.361 ± 0.243	0.612 ± 0.025	1.569 ± 0.093	0.667 ± 0.180	0.088 ± 0.044
G3F	0.626 ± 0.074	0.459 ± 0.041	0.196 ± 0.033	0.725 ± 0.111	0.023 ± 0.003	0.481 ± 0.093	0.125 ± 0.038	4.565 ± 0.373	0.603 ± 0.088	1.535 ± 0.107	0.691 ± 0.157	0.075 ± 0.021
G4F	0.567 ± 0.029	0.452 ± 0.046	0.207 ± 0.076	0.686 ± 0.041	0.032 ± 0.004	0.385 ± 0.063	0.195 ± 0.043*	4.359 ± 0.257	0.599 ± 0.067	1.511 ± 0.064	0.434 ± 0.084	0.132 ± 0.036
G5F	0.583 ± 0.025	0.486 ± 0.034	0.215 ± 0.042	0.622 ± 0.029	0.025 ± 0.010	0.383 ± 0.030	0.124 ± 0.043	4.276 ± 0.243	0.622 ± 0.060	1.517 ± 0.110	0.523 ± 0.156	0.092 ± 0.043

Values are expressed as mean ± S.D., g (n = 5); L, left sides; S, splenic lobes; [a]Popliteal lymph node; *$p < 0.05$ compared to that of vehicle control by MW test.

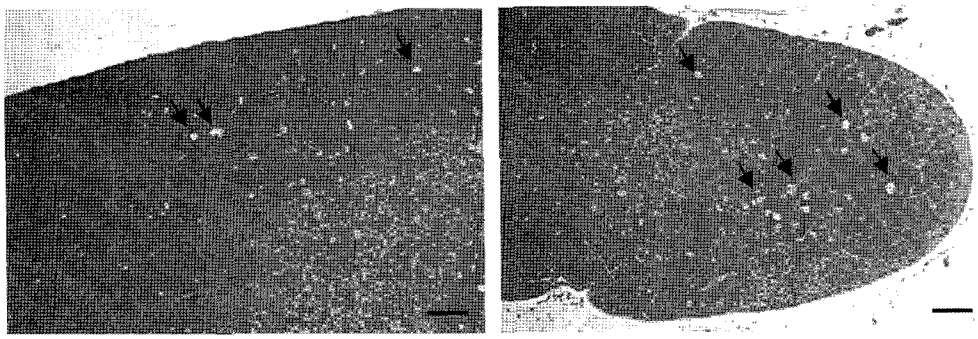
Table 4. Necropsy findings after oral dose of PGB-1

Group	Male						Female					
	G0M	G1M	G2M	G3M	G4M	G5M	G0F	G1F	G2F	G3F	G4F	G5F
Lung												
Normal	3/5	4/5	4/5	2/5	4/5	3/5	3/5	4/5	5/5	3/5	4/5	4/5
Congestion	2/5	1/5	1/5	3/5	1/5	2/5	2/5	1/5	0/5	2/5	1/5	1/5
Heart												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Thymus												
Normal	3/5	4/5	3/5	5/5	4/5	4/5	4/5	4/5	4/5	5/5	4/5	4/5
Atrophy	2/5	1/5	2/5	0/5	1/5	1/5	1/5	1/5	1/5	0/5	1/5	1/5
Kidney												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Adrenal gland												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Spleen												
Normal	2/5	3/5	4/5	4/5	3/5	3/5	2/5	5/5	5/5	4/5	3/5	5/5
Atrophy	2/5	2/5	1/5	1/5	2/5	2/5	3/5	0/5	0/5	1/5	2/5	0/5
Hypertrophy	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis/Ovary												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Liver												
Normal	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Atypical Foci	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Pancreas												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Brain												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Epididymis/Uterus												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Lymph node[a]												
Normal	3/5	5/5	4/5	4/5	4/5	4/5	2/5	4/5	2/5	3/5	4/5	4/5
Hypertrophy	2/5	0/5	1/5	1/5	1/5	1/5	3/5	1/5	3/5	2/5	1/5	1/5
Others												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Observed animals/total observed animals (n = 5); [a]Bilateral popliteal lymph node.

Necropsy findings. In this study, no meaningful changes on the gross findings of 12 principle organs were observed in all dosing groups tested compared to that of vehicle control except for some sporadic findings such as congestion spots of lung, atrophy of thymus, spleen atrophy or hypertrophy, atypical white foci in liver and hypertrophy of popliteal lymph node (Table 4).

Histopathological findings. No PGB-1 treatment-related changes on the histopathological findings of 12



G3F-01 G3M-05

Fig. 1. Histopathological changes in the thymus. Note that depletion of thymocytes in the cortex (arrows) was restricted to one animal of 500 mg/kg-dosing female and male groups, respectively. All Hematoxylin & Eosin staining, Scale bars = 100 μm.

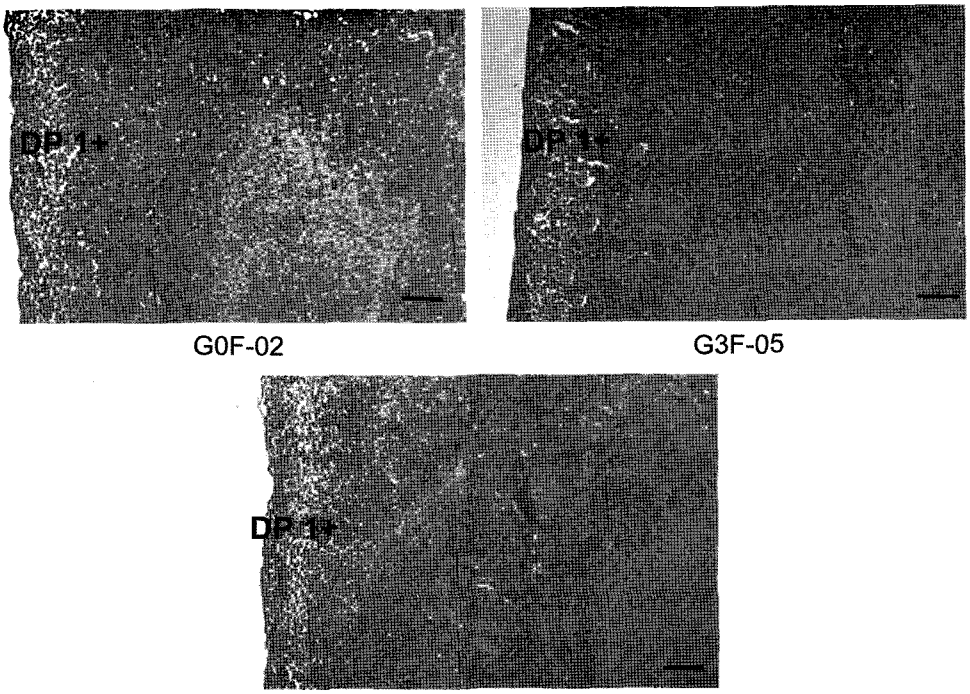
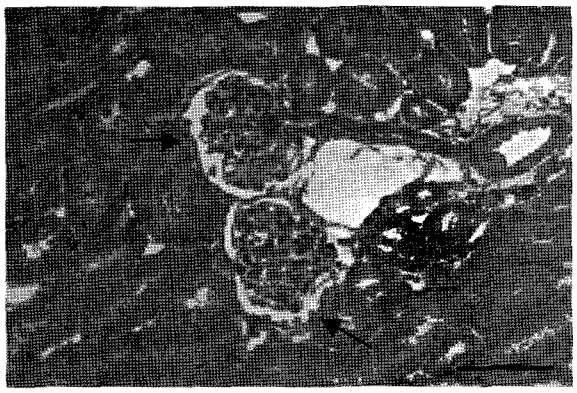


Fig. 2. Histopathological changes in the spleen. Note that depletion of lymphoid cells (DP) in the red pulps were randomly detected dispersed throughout the all tested groups including vehicle control. They did not show any dose-dependency. All Hematoxylin & Eosin staining, Scale bars = 100 μm.

principle organs were observed in all dosing groups tested compared to that of vehicle control except for some accidental findings such as depletion of lymphoid cells in the cortex of thymus (Fig. 1) and red pulps of spleen (Fig. 2), focal atrophy of glomerulus in kidney (Fig. 3), focal inflammatory cell infiltration in liver with necrosis (Fig. 4), edematous changes on the uterus (Fig. 5), and hyperplasia of lymphoid cells in the popliteal lymph node (Fig. 6, Table 5).

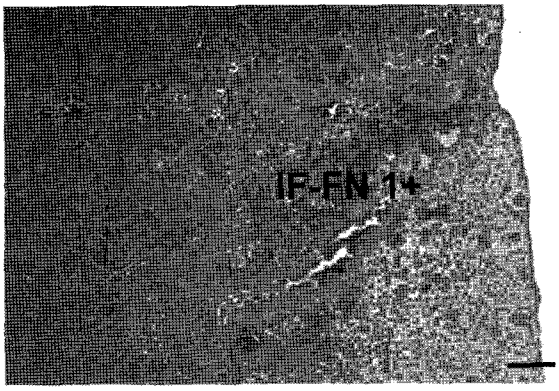
DISCUSSION

In the present study, we examined the acute toxicity of single oral dose with PGB-1, a novel polyglu-



G4M-01

Fig. 3. Histopathological changes in the kidney. Note that focal atrophy of glomerulus (arrows) was restricted to only one animal of PGB-1 250 mg/kg-dosing male group. All Hematoxylin & Eosin staining, Scale bars = 100 μm.



G2M-04

Fig. 4. Histopathological changes in the liver. Note that infiltration of mononuclear inflammatory cells in the hepatic lobules with focal necrosis (IF-FN) was restricted to only one animal of PGB-1 1000 mg/kg-dosing male group. All Hematoxylin & Eosin staining, Scale bars = 100 μm.

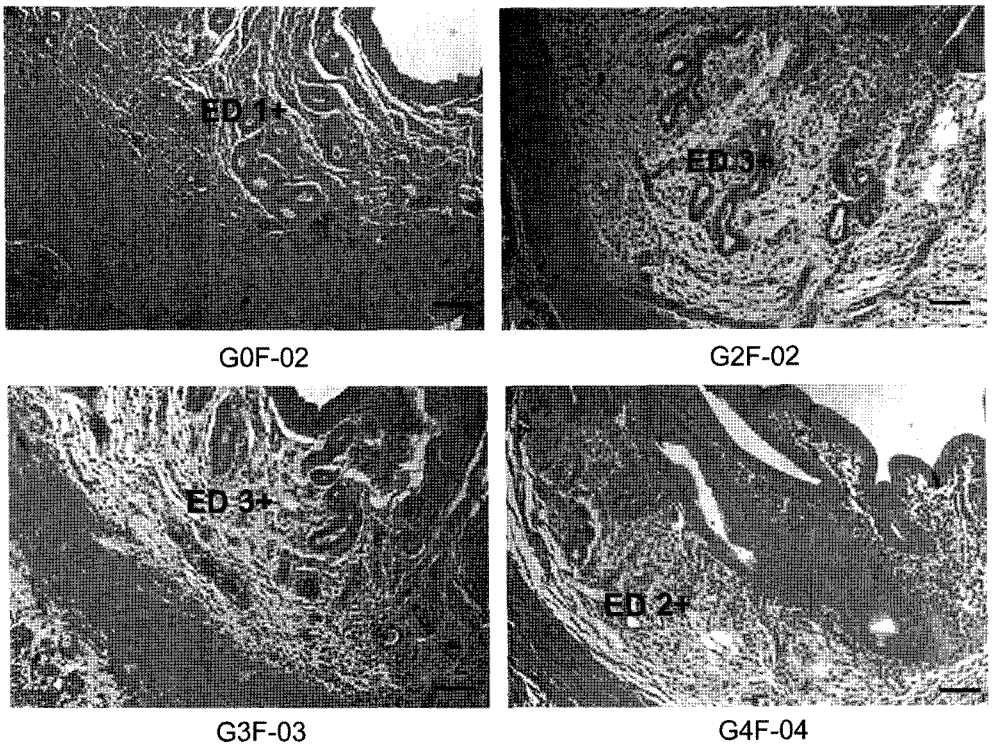


Fig. 5. Histopathological changes in the uterus. Note that various degrees of edematous changes (ED) were randomly detected dispersed throughout all tested groups including vehicle control female. They did not show any dose-dependency. All Hematoxylin & Eosin staining, Scale bars = 100 μm.

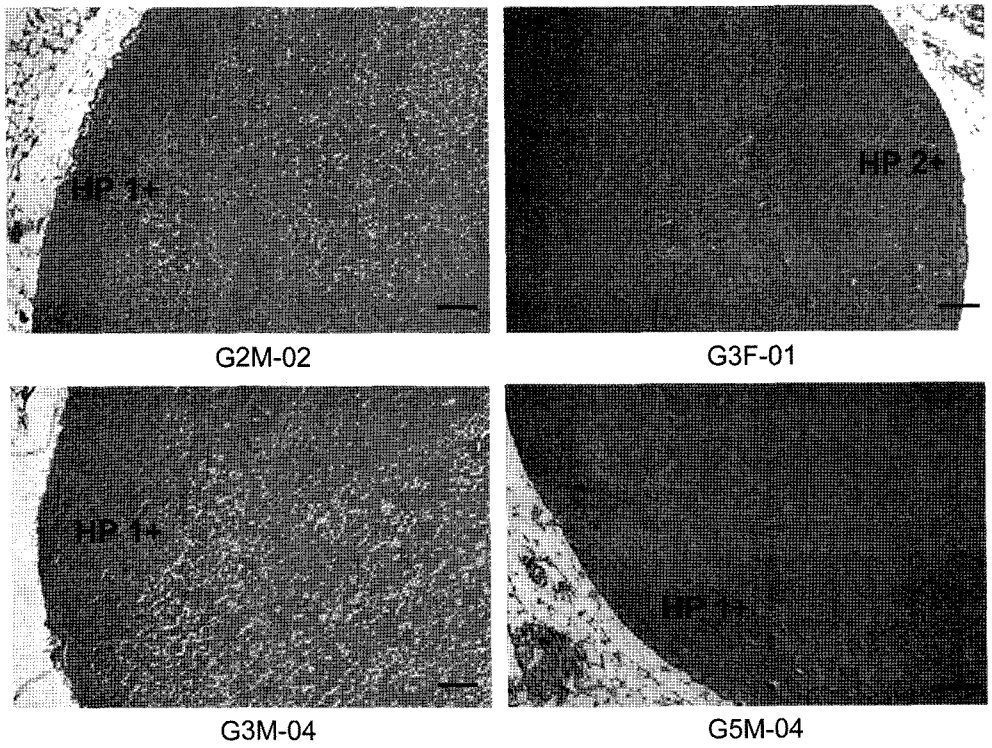


Fig. 6. Histopathological changes in the popliteal lymph node. Note that hyperplasia of lymphoid cells (HP) in the cortex were randomly detected dispersed throughout all tested groups including vehicle control. They did not show any dose-dependency. All Hematoxylin & Eosin staining, Scae bars = 100 μm.

Table 5. Histopathological findings after oral dose of PGB-1

Group	Male						Female					
	G0M	G1M	G2M	G3M	G4M	G5M	G0F	G1F	G2F	G3F	G4F	G5F
Lung												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Heart												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Thymus												
Normal	5/5	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5
DC*	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Kidney												
Normal	5/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Glomerulus atrophy	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Adrenal gland left												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Spleen												
Normal	4/5	5/5	5/5	5/5	3/5	5/5	2/5	4/5	5/5	4/5	5/5	5/5
DC*	1/5	0/5	0/5	0/5	2/5	0/5	3/5	1/5	0/5	1/5	0/5	0/5
Testis/Ovary left												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Liver												
Normal	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
IF-NE*	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Pancreas splenic												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Brain												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Epididymis left/Uterus												
Normal	5/5	5/5	5/5	5/5	5/5	5/5	4/5	5/5	3/5	3/5	3/5	5/5
Edematous changes	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	2/5	2/5	2/5	0/5
Lymph node[a]												
Normal	4/5	4/5	4/5	4/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5
Lymphoid hyperplasia	1/5	1/5	1/5	1/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5

Observed animals/total observed animals (n = 5); [a]Bilateral popliteal lymph node; *IF-NE: Focal inflammatory cell infiltration with necrosis, DC: Depletion of lymphoid cells.

cosamine polymer in female and male mice as part of the safety test and tried to further clarify their safety for clinical use.

Well corresponded with our previous mouse single oral dose study about PGB-2, was produced extracellularly from a new strain *Citrobacter* sp. BL-4 (Lee *et al.*, 2007), we could not find any mortalities, clinical signs, changes in the body weight and gross findings. In addition, no PGB-1-treatment related abnormal changes on the organ weight and histopathology of principle organs except for some sporadic findings.

In KFDA (2005) and OECD (2001) guidelines, 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 mice, the dosage volume were below 20 ml/kg. In the present study, the highest dose of PGB-1 was selected as 2000 mg/kg because it was relatively well suspended or dissolved upto 100 mg/ml concentration in distilled water, and 1000, 500, 250 and 125 mg/kg was selected. PGB-1 was dosed at 20 ml/kg dosage levels in the present study.

Significant ($p < 0.01$ or $p < 0.05$) decreases of body weight gains during day 7~14 of dosing of PGB-1 1000 mg/kg-dosing male group, and during day 7~14 of dosing periods with whole experimental periods (Day 0~14) of PGB-1 500 and 250 mg/kg-dosing male groups were considered as not meaningful changes, because no dose dependency was observed, it was detected that no changes on the body weight gains in the same periods in the highest dosage group compared to that of vehicle control.

Significant ($p < 0.01$ or $p < 0.05$) decrease of absolute kidney weight in 500 mg/kg-dosing male group, of absolute weight of testis in 125 mg/kg-dosing male group and of relative weight of kidney in 125 mg/kg-dosing female group, and increase of relative weight of heart in 125 mg/kg-dosing male, of absolute weight of ovary in 500 mg/kg-dosing female and of relative weight of ovary in 250 mg/kg-dosing female group were also

considered as not PGB-1-treat related abnormal changes. They did not show any dose-dependency. The ovary weights in the mice were generally changed with estrus cycles (Pineda, 1989). These changes were restricted to the some animals in some dosing groups only.

Congestion spots of lung, atrophy of thymus, spleen atrophy or hypertrophy, atypical white foci in liver and hypertrophy of popliteal lymph node detected in the present study as gross findings, and depletion of lymphoid cells in the cortex of thymus and red pulps of spleen, focal atrophy of glomerulus in kidney, focal inflammatory cell infiltration in liver with necrosis, edematous changes on the uterus, and hyperplasia of lymphoid cells in the popliteal lymph node detected as histopathological findings were considered as accidental findings because they were restricted in some dosing groups and in some case, they were also observed in vehicle control. In addition, they did not show clear dose-dependency and most of them were rarely observed in normal mice (Lee *et al.*, 2005, 2006). The edematous changes of uterus are general signs related to the estrus cycles (Banks, 1986). The atypical white foci detected in one animal of PGB-1 1000 mg/kg-dosing male group was revealed as focal necrosis and infiltration of inflammatory cells, but not being PGB-1 treatment related toxicological signs.

Although the Hodge and Sterner (1949) classify the LD_{50} of non-toxic materials, as those of were 5000~15000 mg/kg as indicated by US Environmental Protection Agency (1998), recently notified guidelines by KFDA (2005) and OECD (2001) recommended that the highest oral dose of test materials was 2000 mg/kg. In the present study, the LD_{50} and approximate LD in mice after single oral dose of PGB-1 were considered over 2000 mg/kg, respectively in both male and female.

From these results, oral gavage of PGB-1 caused no serious toxic effect to the male and female mice upto 2000 mg/kg - the highest dosage tested in this study. Therefore, PGB-1 has relatively favorable toxicological profiles.

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