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Thirteen-Week Oral Dose Toxicity Study of *G. bimaculatus* in Sprague-Dawley Rats

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Gryllus bimaculatus (Gb) was orally administered at doses of 0, 0.04, 0.2, 1 and 5 g/kg bw/day for 13 consecutive weeks. There were no observed clinical signs or deaths related to treatment in all the groups tested. Therefore, the approximate lethal oral dose of *G. bimaculatus* was considered to be higher than 5 g/kg in rats. Throughout the administration period, no significant changes in diet consumption, ophthalmologic findings, organ weight, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis) or gross pathology were detected. Minor changes were found in hematological parameters for the 5 g/kg Gb-treated group (triglyceride reduction of 35.8%), but all changes were within normal physiological ranges. Microscopic examination did not identify any treatment-related histopathologic changes in the organs of Gb-treated rats in the high dose group. From these results, one can conclude that the no-observed adverse effect level (NOAEL) of *G. bimaculatus* is higher than 5 g/kg bw/day in rats.

***Key words*:** *G. bimaculatus*, 13-Week toxicity

INTRODUCTION

Cricket (*Gryllus bimaculatus*) water extract has been used in oriental medicine as a crude drug for treating fever and hypertension and the cricket is presently reared as food (Ahn *et al*., 2005). The main components of *Gryllus bimaculatus* are protein, fat including essential fatty acids- oleic acid, linoleic acid and γ-linoleic acid, ash, and moisture (Ahn *et al.*, 2000). Recently, the extracts from *Gryllus bimaculatus*, were found to cause a significant decrease in blood ethanol concentrations by enhancing liver mitochondrial alcohol metabolizing enzymes (Ahn *et al*., 2004). In other countries, like grasshopper, the cricket (*Gryllus bimaculatus*) is eaten after roasting, but, it was not consumed as a food in Korea. Safety evaluation data on these products is limited.

The acute toxicity of *G. bimaculatus* in Sprague-Dawley rats was tested and it was practically non-toxic with an oral LD_{50} value of > 5 g/kg (Kim *et al.*, 2002). A genotoxic evaluation of the biocomponents of cricket (*Gryllus bimaculatus*) was assessed using three mutagenicity tests: the Ames test, the chromosome aberration test in Chinese hamster ovary cells *in vitro*, and the micronucleus (MN) test *in vivo*, involving different test systems (bacteria, mammalian cells and mice bone marrow; Ahn *et al*., 2005).

On the other hand, there is little safety evaluation data on these products in terms of toxicology. Therefore, the present study examined the subacute toxicity of *G. bimaculatus* extract administered orally at 0, 0.04, 0.2, 1 and 5 g/kg for 13 consecutive weeks.

MATERIALS AND METHODS

Materials. The cricket, *G. bimaculatus* was purchased from an insect farm, Jungsun City, located in Kangwon-do, South Korea. They were freezed-dried in the Department of Agricultural Biology, National Academy of Agricultural, Korea.

Test preparation of Gb. Dried G. *bimaculatus* was homogenized in a blender to a powder at 4°C, dissolved in phosphate buffered saline (Sigma-Aldrich Inc., St. Louis, MO) and then orally administered at doses of 0, 0.04, 0.2, 1 and 5 g/kg bw/day over a 13-week period.

Animals. Specific pathogen-free SD rats (4 weeks old, weighing 165 ± 5 g, male and female), purchased from Samtako Co. Ltd. (Osan, Korea), were housed in an environmentally-controlled room at 23 ± 1°C, with relative

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humidity of 55 ± 10%, air ventilation of 10~18 cycles/hr, a 12-hr light/dark cycle of 150~300 lux, with feed and water available *ad libitum* and acclimated one week before the repeat-dose toxicity study began.

All procedures were conducted in accordance with the Korean Food and Drug Administration (KFDA) "Testing Guidelines for Safety Evaluation of Drugs" (Notification No. 2005-60, issued by the KFDA on Oct 21, 2005).

Ten animals of both sexes in each group were weighed and then administered Gb at a dose of 0.04, 0.2, 1 or 5 g/kg/day or its vehicle over a 13-week period.

The parameters examined included clinical signs and mortality, body weight, food consumption, urine analysis, hematological analysis, serum biochemical analysis, organ weight, ophthalmic observation and histopathological findings (Song *et al*., 2006).

Body weight. Animals were observed three times daily for clinical signs. Changes in body weight were recorded weekly and group means were calculated.

Food consumption. Daily food consumption was determined by subtracting leftover feed from provided feed. Food consumption was measured daily for the 1st week and weekly thereafter.

Urine sampling. During the final week of testing (week 13), rats were transferred to metabolic cages for 24 hr and urine was collected to determine specific gravity, pH, leukocyte content, nitrite, protein, glucose, ketone, urobilinogen, bilirubin and hemoglobin levels using commercial kits (Roche Diagonisctics GmbH, Mannheim, Germany).

Blood sampling and plasma assay. After 13 weeks of treatment, blood (~3 m*l*) was collected from the posterior vena cava under light CO_2 inhalation and used for serum chemistry measurements. The parameters examined included total protein, albumin, total bilirubin, glucose, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), total cholesterol, blood urea nitrogen (BUN), creatinine, triglyceride, uric acid, sodium, potassium and chloride. All were evaluated using an autoanalyzer (Hitachi 7060 automatic clinical analyzer, Tokyo).

Organ weights. Absolute and relative (organ-to-body weight ratios) weights were determined after sacrifice at 13 weeks; tissues included brain, pituitary gland, adrenal glands, liver, spleen, kidneys, heart, thymus, lung, stomach, thyroid gland and testes (or ovary).

Pathology and histopathology. The organs and tissues in the cranial, thoracic, and abdominal cavities of euthanized rats, were examined grossly for ophthalmic observation. Each organ was excised and fixed in phosphate-buffered formalin. After paraffin embedding, the excised organs and tissues were prepared for microscopic examination by sectioning and staining with hematoxylin and eosin.

Statistical analysis. Mean and standard deviation of all parameters were determined for each of the 5 groups. A Student's *t*-test was used to establish the significant differences between the control and treatment groups. $p < 0.05$ was considered statistically significant.

RESULTS

Clinical signs and food consumption. No deaths or adverse clinical signs were observed due to the ingestion of *G. bimaculatus* at doses of 0.04, 0.2, 1.0 and 5.0 g/kg/day (Table 1). Food consumption was similar for all study groups (Fig. 1).

Body weight changes. There were no toxicologically significant differences in mean body weight between any of

Table 1. Mortality of Sprague-Dawley rats treated orally with *G. bimaculatus* over a 13-week period

Sex	Dosage (g/kg bw)	Weeks														Total mortality
		Start	1	2	3	4	5	6	7	8	9	10	11	12	13	
Male	CON[a]	0/10[b]	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	0.04	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	0.2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	1.0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	5.0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Female	CON	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	0.04	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	0.2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	1.0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	5.0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

[a]CON: vehicle control group treated with PBS buffer.

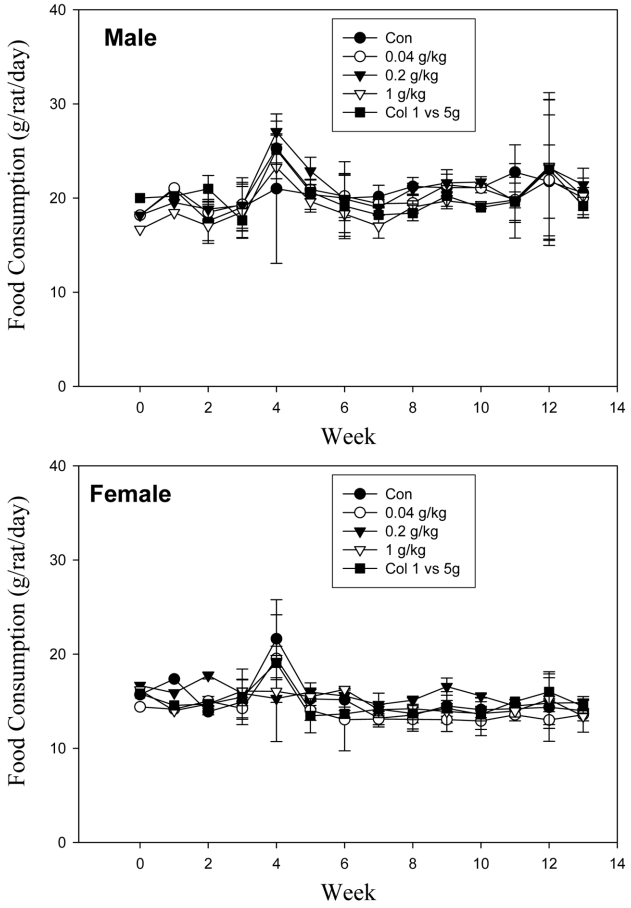

Fig. 1. Food consumption of male and female SD rats, treated orally with *G. bimaculatus* powder over a 13-week period *Significantly different from the untreated controls ($P < 0.05$).

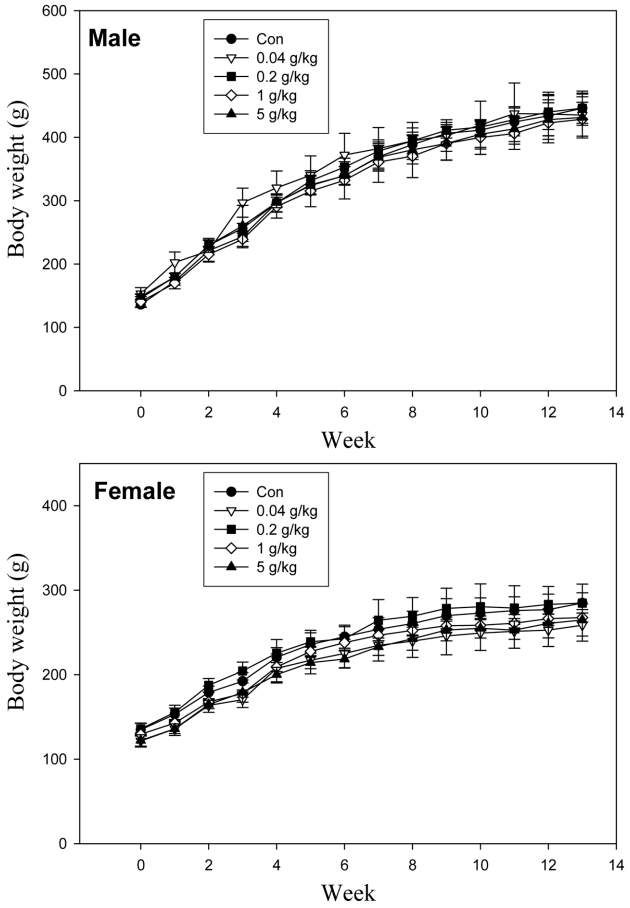

Fig. 2. Body weight increases of male and female SD rats, treated orally with *G. bimaculatus* powder over a 13-week period. *Significantly different from the untreated controls ($P < 0.05$).

the treatment groups (Fig. 2). During the 13-week administration period, the body weights of the male and female SD rats in the 3 treatment groups were comparable across the control and treated groups. The mean weekly body weights versus time are presented in Fig. 2. No statistically significant differences were observed between the 5.0 g/kg *G. bimaculatus*-treated group and the control group.

Urinalysis. No significant differences were observed between treatment and control group (Table 2).

Hematology and blood chemistry. Some dose-dependent changes were observed between the treated and control groups with respect to the hematological parameters at the end of the experiment. An increase in partial thromboplastin time count was observed in the male rats in the treated groups; (control, 118.3 ± 30.8 sec; 0.04 g/kg, 91.5 ± 44.1 sec; 0.20 g/kg, 96.7 ± 13.3 sec; 1.0 g/kg, 122.7 ± 40.4 sec; 5.0 g/kg, 132.6 ± 28.6 sec) and the same trends were seen in females (Table 3 and Table 4) but without significant differences. Hematocrit, MCV, MCHC and Factor I (indicators of RBS function and status) were significantly different between some of the treated groups versus the control group. Minor changes were found in hematological parameters (eosinophils, neutrophils, lymphocytes and basophils) for some Gb-treated (one side) male or female rat groups. But, effects of Gb were not considered adverse because all changes in hematological data were within the normal physiological range (Table 3 and Table 4).

Serum biochemistry. In the sera of the Gb-treated groups, trigyceride levels were significantly lower than in the control after 13 weeks with dose-dependent changes in both males (control, 82.7 ± 18.9 mg/d*l*; 1.0 g/kg, 60.3 ± 11.1 mg/d*l*), and females (control, 93.7 ± 59.4 mg/d*l*; 1.0 g/kg, 54.4 ± 7.1 mg/d*l*). Serum glucose levels were lower vs control in the males (control, 403.0 ± 69.6 mg/d*l*; 1.0 g/kg, 293.5 ± 81.0 mg/d*l*), and females (controls, 309.1 ± 90.2 mg/d*l*; 1.0 g/kg, 147.8 ± 53.2 mg/d*l*). A significant increase in HDL cholesterol was observed in the 0.04 and 1.0 g/kg groups for both the males and females (male: control, 12.0 ± 2.2 mg/d*l*; 0.04 g/kg, 15.5 ± 3.9 mg/d*l*; 1.0 g/kg, 16.0 ± 2.2 mg/

Table 2. Urinalysis data of *G. bimaculatus* treated groups at the end of the administration period

Item	Urinalysis values	Male					Female				
		CON[a]	0.04	0.2	1	5	CON[a]	0.04	0.2	1	5
Specific gravity	1.000	0	5	0	0	4	1	0	2	1	1
	1.005	9	0	2	5	1	2	1	0	1	1
	1.010	0	4	1	3	2	4	7	0	5	7
	1.015	0	5	0	0	3	3	2	4	4	2
	1.020	0	1	0	0	2	0	0	6	0	0
	1.025	0	0	0	0	1	0	0	0	0	0
PH	6.0	0	0	0	0	1	0	0	5	0	0
	6.5	0	5	0	0	4	1	0	2	1	1
	7.0	1	1	0	0	2	4	0	3	6	4
	7.5	9	4	8	8	3	5	10	0	3	5
	8.0	0	0	2	2	0	0	10	0	0	0
Leucocyte	10~25 mg/d*l*	10	6	10	10	10	10	10	10	10	10
	75	0	4	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0
Nitrite	-	0	0	0	0	0	0	0	0	0	0
	+	10	10	10	10	10	10	10	10	10	10
Protein	25 mg/d*l*	10	8	10	10	10	10	10	10	10	10
	50	0	2	0	0	0	0	0	0	0	0
	75	0	0	0	0	0	0	0	0	0	0
Glucose	normal	10	10	9	10	10	10	10	9	9	10
	50 mg/d*l*	0	0	1	0	0	0	0	1	1	0
	100	0	0	0	0	0	0	0	0	0	0
Ketone	-	10	10	10	10	10	10	10	10	10	10
	5 mg/d*l*	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	0	0
Urobili nogen	normal	10	10	10	10	10	10	10	10	10	0
	1 mg/d*l*	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
Bilirubin	-	10	10	10	10	10	10	10	10	10	10
	1 mg/d*l*	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0
Blood	-	10	10	10	9	9	10	10	8	10	10
	1+	0	0	0	1	0	0	0	0	0	0
	2+	0	0	0	0	1	0	0	2	0	0
Hemoglobin	-	10	10	10	10	10	10	10	10	9	10
	1+	0	0	0	0	0	0	0	0	0	0
	2+	0	0	0	0	0	0	0	0	1	0

[a]CON: vehicle control group treated with PBS buffer.
Data are number of animals presenting the value of each item of urinalysis.

d*l*; female: control, 25.5 ± 8.3 mg/d*l*; 0.04 g/kg, 29.1 ± 3.7 mg/d*l*; 1.0 g/kg, 31.0 ± 3.8 mg/d*l*).

Alkaline phosphatase (ALP) levels of the treated groups were reduced in a dose-dependent manner in males (control, 133.5 ± 16.4 mg/d*l*; 1.0 g/kg, 109.9 ± 17.5 mg/d*l*), and females (controls, 169.0 ± 121.8 mg/d*l*; 1.0 g/kg, 93.8 ± 14.0 mg/d*l*). Also, uric acid levels of the treated groups were reduced in a dose-dependent manner in males (control, 8.7 ± 1.6 mg/d*l*; 1.0 g/kg, 6.8 ± 1.0 mg/d*l*), and females (control, 6.6 ± 2.0 mg/d*l*; 1.0 g/kg, 5.4 ± 0.8 mg/d*l*).

Calcium ion levels of the treated groups were reduced in a dose-dependent manner in males (control, 13.0 ± 0.3 nmol/*l*; 1.0 g/kg, 12.2 ± 0.5 nmol/*l*) and females (control, 13.0 ± 0.7 nmol/*l*; 1.0 g/kg, 11.6 ± 0.9 nmol/*l*), whereas inorganic phosphorus levels increased (male: control, 18.9 ± 3.3 nmol/*l*; 5.0 g/kg, 14.9 ± 2.6 nmol/*l*, female: control, 17.7 ± 5.7 mg/d*l*; 1.0 g/kg, 15.0 ± 2.0 mg/d*l*) (Tables 4 and 5). However, Gb was considered non-toxic because the all

Table 3. Hematological findings of male rats treated orally with *G. bimaculatus* for 13 weeks

Item	Unit	CON[a]	0.04	0.2	1	5 (g/kg bw/day)
WBC	$10^3/mm^3$	6.4 ± 1.6	8.5 ± 2.0	10.4 ± 4.3	8.8 ± 1.3	7.4 ± 2.1
RBC	$10^6/mm^3$	8.8 ± 0.3	8.6 ± 0.4	9.0 ± 0.3	8.9 ± 0.4	9.6 ± 0.6
Hgb	g/dl	16.7 ± 0.3	16.3 ± 0.9	16.3 ± 0.6	16.5 ± 0.8	17.5 ± 1.3
Hct	%	54.1 ± 0.9	54.0 ± 2.8	54.2 ± 2.5	54.2 ± 2.7	56.8 ± 4.1
MCV	fl	61.6 ± 3.1	62.3 ± 1.4	60.2 ± 2.3	60.4 ± 1.6	58.9 ± 1.0
MCH	pg	19.0 ± 0.9	18.8 ± 0.6	18.2 ± 0.6	18.4 ± 0.6	18.2 ± 0.4
MCHC	g/d*l*	30.9 ± 0.4	30.2 ± 0.5	30.2 ± 0.5	30.5 ± 0.3	30.9 ± 0.3
PLT	$10^3/mm^3$	1023.0 ± 195.2	915.4 ± 110.2	956.8 ± 168.0	852.7 ± 155.4	849.8 ± 148.9
PTT	sec	118.3 ± 30.8	91.5 ± 44.1	96.7 ± 13.3	122.7 ± 40.4	132.6 ± 28.6
Thrombin time	sec	181.8 ± 0	74.4 ± 18.8	131 ± 0.1	136.1 ± 43.6	106.5 ± 3.7
Factor I	mg/d*l*	124.3 ± 10.7	211.4 ± 42.5	163.6 ± 60.7	169.8 ± 49.3	197.6 ± 39.3
PT	sec	3.5 ± 2.8	2.2 ± 0.8	2.1 ± 0.6	2.3 ± 0.6	2.2 ± 0.9
Neutrophil	%	9.4 ± 4.3	9.8 ± 4.2	8.6 ± 3.4	14.0 ± 5.7	19.9 ± 4.5*
Lymphocyte	%	76.0 ± 5.2	80.2 ± 2.4	82.1 ± 4.1	81.7 ± 5.4	76.1 ± 5.0
Monocyte	%	1.4 ± 0.5	1.7 ± 1.1	3.1 ± 0.6	1.4 ± 1.0	1.1 ± 0.4
Eosinophil	%	12.6 ± 9.0	7.8 ± 3.2	5.6 ± 2.4	2.2 ± 1.1*	2.2 ± 1.8
Basophil	%	0.6 ± 0.3	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1

Abbreviations: WBC, white blood cell; RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet. PTT, partial thromboplastin time; PT, prothrombin time.
[a]CON: PBS (as a vehicle) treated with murine normal diet[a].
Each value represents mean ± S.D. Statistically significant from control (*$P < 0.05$).

Table 4. Hematological findings of female rats treated orally with *G. bimaculatus* for 13 weeks

Item	Unit	CON[a]	0.04	0.2	1	5 (g/kg bw/day)
WBC	$10^3/mm^3$	6.6 ± 2.3	6.3 ± 0.7	6.6 ± 2.0	5.4 ± 1.6	4.1 ± 0.3
RBC	$10^6/mm^3$	9.0 ± 0.3	8.6 ± 0.4	8.2 ± 0.8*	8.3 ± 0.7*	7.8 ± 0.3*
Hgb	g/d*l*	16.7 ± 0.6	16.2 ± 0.9	15.2 ± 2.0	15.7 ± 1.5	14.8 ± 0.5*
Hct	%	53.4 ± 2.9	50.0 ± 3.7	48.9 ± 6.4	48.1 ± 5.2*	49.8 ± 2.4*
MCV	fl	59.3 ± 2.7	57.7 ± 1.6	58.9 ± 2.5	57.7 ± 1.6	63.3 ± 3.6
MCH	pg	18.5 ± 0.4	18.7 ± 0.4	18.4 ± 0.6	18.8 ± 0.5	18.7 ± 0.3
MCHC	g/dl	31.3 ± 1.1	32.4 ± 0.7*	31.2 ± 0.9	32.6 ± 0.9*	29.7 ± 1.8*
PLT	$10^3/mm^3$	774.7 ± 281.4	922.6 ± 215.8	812.3 ± 204.0*	884.1 ± 253.4	850.7 ± 150.2*
PTT	sec	86.1 ± 56.7	96.0 ± 17.9	118.0 ± 35.5	103.4 ± 25.1	105.5 ± 40.6
Thrombin time	sec	134.6 ± 58.0	155.0 ± 55.1	128.7 ± 56.2	122.8 ± 44.3	68.7 ± 6.0
Factor I	mg/d*l*	100.8 ± 34.6	95.4 ± 18.3*	105.0 ± 29.3	97.7 ± 36.3	110.0 ± 41.2
PT	sec	2.8 ± 0.8	4.6 ± 3.4	3.1 ± 1.3	6.9 ± 4.4	2.5 ± 1.1
Neutrophil	%	14.1 ± 5.3	12.9 ± 5.3	8.2 ± 4.5*	11.9 ± 5.4	10.9 ± 5.8
Lymphocyte	%	81.9 ± 5.9	83.0 ± 6.0*	85.9 ± 6.2*	82.0 ± 7.8	83.2 ± 4.8
Monocyte	%	1.6 ± 0.7	1.3 ± 0.6	2.3 ± 1.4	3.2 ± 3.7	2.8 ± 1.1
Eosinophil	%	1.7 ± 0.5	2.2 ± 0.8	2.0 ± 1.7	2.4 ± 1.5	1.8 ± 1.3
Basophil	%	0.4 ± 0.1	0.3 ± 0.1	1.4 ± 3.0	0.3 ± 0.10*	1.0 ± 1.1

Abbreviations: WBC, white blood cell; RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet. PTT, partial thromboplastin time; PT, prothrombin time
[a]CON :PBS (as a vehicle) treated with murine normal diet[a]
Each value represents mean±S.D. Statistically significant from control (*$P < 0.05$)

changes in serum biochemical data were within normal physiological range (Tables 5 and 6).

Pathology and organ weight. No significant treatment-related pathologies were observed. Any minor changes were few and dose-independent. At the end of the administration period, there were no treatment-related changes in absolute (Table 7) or relative organ weights (Table 8). There were some histopathological findings observed, however the histopathological alterations at the end of the adminis-

Table 5. Biochemical serum values of male rats treated orally with. *G. bimaculatus* over a 13-week period

Item	Unit	CON[a]	0.04	0.2	1	5
Toal protein	g/d*l*	7.0 ± 0.1	6.9 ± 0.3	7.05 ± 0.2	7.2 ± 0.3	7.0 ± 0.2
Bilirubin	mg/d*l*	below 0.1	below 0.1	below 0.1	below 0.1	below 0.1
ALP	IU/*l*	133.5 ± 16.4	112.7 ± 21.0	127.1 ± 34.9	109.9 ± 17.5*	134.6 ± 45.2
AST	IU*l*	81.5 ± 14.0	80.9 ± 14.8	81.8 ± 16.6	90.9 ± 18.0	75.6 ± 8.5
ALT	IU/*l*	44.5 ± 12.1	41.8 ± 7.6	41.5 ± 10.9	44.4 ± 12.4	43.2 ± 8.5
GGT	g/d*l*	below 2	below 2	below 2	below 2	below 2
CK	IU/*l*	157.9 ± 102.3	165.0 ± 102.8	137.4 ± 59.5	301.9 ± 234.9	161.9 ± 111.9
LDH	IU/*l*	528.3 ± 426.1	576.8 ± 491.0	424.5 ± 233.2	728.6 ± 369.9	329.5 ± 113.9
Na	nmol/*l*	139.6 ± 2.9	139.6 ± 5.4	140.8 ± 3.2	137.3 ± 4.3	140.6 ± 3.2
K	nmol/*l*	22.6 ± 4.1	23.5 ± 7.3	21.5 ± 5.2	24.9 ± 4.2	20.2 ± 4.3
Cl	nmol/*l*	97.4 ± 2.4	98.1 ± 2.1	97.9 ± 0.9	96.5 ± 3.3	97.5 ± 1.7*
Creatine	mg/d*l*	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.8 ± 0.1
BUN	mg/d*l*	19.4 ± 2.1	19.9 ± 3.1	19.6 ± 3.5	21.3 ± 2.8	21.1 ± 3.6
Uric acid	mg/d*l*	8.7 ± 1.6	6.9 ± 1.8	7.5 ± 1.2	6.8 ± 1.0*	7.59 ± 1.61
T.Chol	mg/d*l*	84.2 ± 11.2	94.2 ± 17.7	88.5 ± 11.3	87.2 ± 10.0	89.7 ± 15.5
H.Chol	mg/d*l*	12.0 ± 2.2	15.5 ± 3.9*	15.7 ± 2.5*	16.0 ± 2.2*	16.1 ± 3.3*
L.Chol	mg/d*l*	15.9 ± 2.3	15.9 ± 3.6	16.0 ± 2.8	14.9 ± 2.6	14.7 ± 3.8
TG	mg/d*l*	82.7 ± 18.9	65.7 ± 9.8*	68.8 ± 13.1	60.3 ± 11.1*	64.7 ± 21.5
Glucose	mg/d*l*	403.0 ± 69.6	289.4 ± 112.1*	338.7 ± 115.4	293.5 ± 81.0*	392.3 ± 100.6
Ca	nmol/*l*	13.0 ± 0.3	12.6 ± 0.6	12.8 ± 0.5	12.2 ± 0.5*	12.5 ± 0.4*
IP	mg/d*l*	18.9 ± 3.3	19.5 ± 3.9	16.5 ± 4.2	17.9 ± 2.1	14.9 ± 2.6*

Abbreviations: ALP: alkaline phosphatase; AST (GOT), glutamate oxaloacetate transaminase; ALT (GPT), glutamate pyruvate transaminase; GGT, γ-glutamyl transferase; CK: creatinine phosphokinase; LDH, lactate dehydrogenase; Na, Sodium; K, potassium; Cl, chloride; BUN, blood urea nitrogen; T. Chol: total cholesterol; H. Chol: HDL cholesterol; L. Chol: LDL cholesterol; TG, triglyceride; Ca, calcium; IP, inorganic phosphorus.
[a]CON: PBS (vehicle) treated with murine normal diet.
Each value represents mean ± S.D. Statistically significant from control (*$P < 0.05$).

Table 6. Biochemical serum values of female rats treated orally with *G. bimaculatus* over a 13-week period

Item	Unit	CON[a]	0.04	0.2	1	5 (g/kg bw/day)
Toal protein	g/d*l*	7.2±0.3	6.9±0.2*	6.88±0.3	7.0±0.2	6.6±0.3*
Bilirubin	mg/d*l*	below 0.1	below 0.1	below 0.1	below 0.1	below 0.1
ALP	IU/*l*	169.0±121.8	407.4±564.2	123.0±56.7	93.8±14.0*	135.4±43.3
AST	IU*l*	90.7±27.0	144.5±196.1	88.5±11.2	89.2±14.7	93.5±24.4
ALT	IU/*l*	34.6±6.3*	82.0±108.4	46.7±13.0	44.6±11.0	55.9±11.9
GGT	g/d*l*	below 2	below 2	below 2	below 2	below 2
CK	IU/*l*	271.1±150.2	210.0±129.9	238.3±92.7*	176.5±54.0	222.2±116.3
LDH	IU/*l*	717.2±497.3	1384.8±2538.9	634.3±152.9	634.8±289.0	819.5±582.7
Na	nmol/*l*	137.2±4.5	139.4±1.7	141.0±1.5	143.7±1.8*	136.4±7.0
K	nmol/*l*	22.0±5.3	19.4±2.6	17.4±1.5*	16.9±2.1	21.9±8.4
Cl	nmol/*l*	95.5±1.84	99.5±2.4	99.8±1.8*	102.3±1.4*	100.7±1.8*
Creatine	mg/d*l*	0.8±0.1	0.7±0.1*	0.7±0.1	0.7±0.0	0.7±0.1*
BUN	mg/d*l*	31.7±5.6	28.1±3.3*	30.6±5.3*	32.3±5.9*	31.4±5.8*
Uric acid	mg/d*l*	6.6±2.0	6.2±1.3*	5.5±1.1*	5.4±0.8*	4.7±0.9*
T.Chol	mg/d*l*	94.3±23.6	91.1±11.9	94.0±17.8	108.7±17.1*	108.9±25.8*
H.Chol	mg/d*l*	25.5±8.3	29.1±3.7*	25.9±3.6*	31.0±3.8*	29.1±6.8*
L.Chol	mg/d*l*	9.1±2.1	9.3±2.5*	9.6±1.9*	9.9±2.2*	12.7±3.1*
TG	mg/d*l*	93.7±59.4	63.2±10.3*	65.6±11.3*	54.4±7.1*	60.2±16.4*
Glucose	mg/d*l*	309.1±90.2	166.2±115.3*	160.5±68.0*	147.8±53.2*	190.6±80.3*
Ca	nmol/*l*	13.0±0.7	12.4±0.6*	12.1±0.6*	11.6±0.9*	11.4±0.8*
IP	mg/d*l*	17.7±5.7	19.4±3.5	15.6±1.7*	15.0±2.0*	15.6±3.9

Abbreviations: ALP: alkaline phosphatase; AST (GOT), glutamate oxaloacetate transaminase; ALT (GPT), glutamate pyruvate transaminase; GGT, γ-glutamyl transferase; CK: creatinine phosphokinase; LDH, lactate dehydrogenase; Na, Sodium; K, potassium; Cl, chloride; BUN, blood urea nitrogen; T. Chol: total cholesterol; H. Chol: HDL cholesterol; L. Chol: LDL cholesterol; TG, triglyceride; Ca, calcium; IP, inorganic phosphorus.
[a]CON: PBS (vehicle) treated with murine normal diet.
Each value represents mean ± S.D. Statistically significant from control (*$P < 0.05$).

Table 7. Absolute organ weight of Sprague-Dawley rats treated orally *G. bimaculatus* over a 13--week period

(g)

Sex	Organs	CON	0.04	0.2	1	5 (g/kg bw/day)
	Adernal gland R.	0.047 ± 0.012	0.050 ± 0.011	0.061 ± 0.014	0.536 ± 0.015	0.086 ± 0.082
	L	0.043 ± 0.006	0.047 ± 0.011	0.051 ± 0.018	0.053 ± 0.013	0.043 ± 0.015
	Kidney R.	1.605 ± 0.322	1.605 ± 0.133	1.610 ± 0.181	1.408 ± 0.261	1.543 ± 0.143
	L.	1.542 ± 0.279	1.476 ± 0.255	1.589 ± 0.180	1.416 ± 0.237	1.527 ± 0.191
	Heart	1.442 ± 0.149	1.370 ± 0.168	1.409 ± 0.180	1.316 ± 0.148	1.307 ± 0.110
	Liver	13.353 ± 1.069	13.030 ± 0.162	12.874 ± 1.452	9.486 ± 3.709	11.656 ± 1.078
Male	Lung	2.068 ± 1.191	2.123 ± 0.454	2.273 ± 0.434	1.972 ± 0.257	1.930 ± 0.112
	Spleen	0.822 ± 0.087	0.782 ± 0.809	0.807 ± 0.108	0.744 ± 0.154	0.684 ± 0.127
	Testis R.	1.708 ± 0.188	0.771 ± 0.195	1.743 ± 0.130	1.694 ± 0.150	1.700 ± 0.274
	L.	0.583 ± 0.280	0.795 ± 0.183	1.730 ± 0.115	1.717 ± 0.198	1.706 ± 1.706
	Stomach	0.960 ± 0.224	0.740 ± 0.430	2.395 ± 0.468	2.216 ± 0.312	2.295 ± 0.236
	Pancreas	0.611 ± 0.082	0.627 ± 0.143	0.699 ± 0.078	0.463 ± 0.101	0.554 ± 0.110
	Thymus	0.362 ± 0.097	0.382 ± 0.111	0.382 ± 0.117	0.365 ± 0.145	0.321 ± 0.079
	Adernal gland R.	0.053 ± 0.011	0.058 ± 0.012	0.046 ± 0.009	0.050 ± 0.009	0.046 ± 0.013
	L	0.053 ± 0.012	0.053 ± 0.009	0.047 ± 0.009	0.048 ± 0.010	0.043 ± 0.015
	Kidney R.	1.041 ± 0.213	1.018 ± 0.127	1.033 ± 0.101	0.973 ± 0.075	1.042 ± 0.227
	L.	1.088 ± 0.136	1.018 ± 0.089	1.016 ± 0.103	1.070 ± 0.074	0.994 ± 0.070
	Heart	0.967 ± 0.036	0.863 ± 0.059	1.030 ± 0.210	0.870 ± 0.042	0.880 ± 0.071
	Liver	8.521 ± 1.524	8.269 ± 1.000	8.742 ± 0.961	7.405 ± 0.734	7.410 ± 0.837
Female	Lung	1.636 ± 0.169	1.515 ± 0.103	1.775 ± 0.252	1.592 ± 0.283	1.569 ± 0.238
	Spleen	0.637 ± 0.107	0.585 ± 0.085	0.654 ± 0.091	0.509 ± 0.070	0.535 ± 0.074
	Ovary R.	0.088 ± 0.015	0.080 ± 0.027	0.095 ± 0.019	0.082 ± 0.014	0.081 ± 0.030
	L	0.079 ± 0.023	0.076 ± 0.009	0.088 ± 0.024	0.073 ± 0.012	0.068 ± 0.020
	Stomach	1.965 ± 0.651	1.805 ± 0.488	1.997 ± 0.520	1.916 ± 0.332	1.970 ± 0.380
	Pancreas	0.566 ± 0.144	0.509 ± 0.055	0.536 ± 0.051	0.477 ± 0.085	0.526 ± 0.080
	Thymus	0.249 ± 0.062	0.243 ± 0.097	0.282 ± 0.148	0.165 ± 0.033	0.169 ± 0.033

Each value represents mean ± S.D.
Statistically significant from control ($^{*}P < 0.05$).

tration period were not related to treatment as finding were dose independent. There were no detectable pathological findings for either sex. Furthermore, there were no adverse findings that presented in the 13-week repeated toxicity test (Table 9, Table 10).

DISCUSSION

G. bimaculatus belongs to Gryllidae, Ortoptera and is used as a natural food for humans and reptiles in Southeast Asia and Africa (Cui *et al*., 2002). Owing to the develop-

Table 8. Relative organ weight of Sprague-Dawley rats treated orally with *G. bimaculatus* over a 13-week period

(g)

Sex	Organs / Dosage (g/kg)	CON	0.04	0.2	1	5
	Adernal gland R.	0.011 ± 0.003	0.012 ± 0.003	0.014 ± 0.004	0.014 ± 0.036	0.023 ± 0.024
	L	0.009 ± 0.001	0.012 ± 0.003	0.012 ± 0.005	0.014 ± 0.003	0.011 ± 0.005
	Kidney R.	0.367 ± 0.074	0.394 ± 0.022	0.379 ± 0.042	0.381 ± 0.065	0.403 ± 0.047
	L.	0.353 ± 0.063	0.364 ± 0.069	0.374 ± 0.037	0.383 ± 0.054	0.400 ± 0.046
	Heart	0.330 ± 0.032	0.337 ± 0.041	0.332 ± 0.039	0.357 ± 0.035	0.342 ± 0.029
	Liver	3.052 ± 0.120	3.174 ± 0.276	3.029 ± 0.282	2.555 ± 0.976	3.059 ± 0.326
Male	Lung	0.473 ± 0.038	0.518 ± 0.091	0.533 ± 0.079	0.535 ± 0.059	0.507 ± 0.046
	Spleen	0.188 ± 0.020	0.191 ± 0.021	0.199 ± 0.043	0.199 ± 0.043	0.178 ± 0.026
	Testis R.	0.391 ± 0.040	0.434 ± 0.037	0.410 ± 0.025	0.466 ± 0.047	0.445 ± 0.066
	L.	0.365 ± 0.072	0.440 ± 0.029	0.408 ± 0.030	0.408 ± 0.030	0.447 ± 0.035
	Stomach	1.960 ± 0.224	0.180 ± 0.096	0.561 ± 0.092	0.601 ± 0.080	0.605 ± 0.095
	Pancreas	0.611 ± 0.082	0.154 ± 0.034	0.165 ± 0.015	0.132 ± 0.031	0.015 ± 0.032
	Thymus	0.083 ± 0.036	0.095 ± 0.031	0.090 ± 0.030	0.102 ± 0.047	0.085 ± 0.025

Table 8. Continued

Sex	Organs / Dosage (g/kg)	CON	0.04	0.2	1	5
Female	Adernal gland R.	0.021 ± 0.005	0.249 ± 0.005	0.019 ± 0.004	0.023 ± 0.005	0.020 ± 0.005
	L	0.021 ± 0.005	0.023 ± 0.004	0.018 ± 0.007	0.022 ± 0.005	0.019 ± 0.007
	Kidney R.	0.411 ± 0.077	0.438 ± 0.067	0.422 ± 0.044	0.440 ± 0.041	0.462 ± 0.095
	L.	0.431 ± 0.052	0.439 ± 0.054	0.415 ± 0.041	0.439 ± 0.039	0.442 ± 0.035
	Heart	0.384 ± 0.024	0.371 ± 0.027	0.426 ± 0.121	0.393 ± 0.035	0.392 ± 0.040
	Liver	3.354 ± 0.422	3.551 ± 0.441	3.563 ± 0.258	3.342 ± 0.243	3.290 ± 0.367
	Lung	0.649 ± 0.076	0.651 ± 0.050	0.724 ± 0.091	0.720 ± 0.131	0.695 ± 0.086
	Spleen	0.254 ± 0.051	0.251 ± 0.036	0.267 ± 0.032	0.230 ± 0.022	0.237 ± 0.027
	Ovary R.	0.035 ± 0.006	0.035 ± 0.012	0.039 ± 0.009	0.037 ± 0.007	0.036 ± 0.011
	L	0.031 ± 0.009	0.033 ± 0.006	0.036 ± 0.011	0.033 ± 0.006	0.030 ± 0.008
	Stomach	0.776 ± 0.237	0.771 ± 0.195	0.816 ± 0.212	0.864 ± 0.126	0.873 ± 0.159
	Pancreas	0.222 ± 0.048	0.219 ± 0.028	0.220 ± 0.024	0.216 ± 0.040	0.234 ± 0.041
	Thymus	0.098 ± 0.022	0.142 ± 0.013	0.159 ± 0.101	0.075 ± 0.015	0.075 ± 0.015

Relative organ weight is calculated as organ weight/body weight (%).
Each value represents of mean ± S.D.
Statistically significant from control ($^{*}P < 0.05$).

Table 9. Histopathological findings of organs in male rats treated orally with *G. bimaculatus* for 13 weeks

Histopathological findings	0 (CON[a])				0.04				0.2				1				5 (g/kg bw/day)			
	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Kidney																				
Chronic nephritis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Pyelonephritis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Microcalcification	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Nuclear enlargement (cellular atypia)	10	0	0	0	10	0	0	0	0	10	0	0	10	0	0	0	10	0	0	0
Liver																				
Vasculitis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Lung																				
Epithelioid granulomas	10	0	0	0	0	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Foamy cell collection	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Spleen																				
Hemosiderosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Extramedullary hematopoiesis	10	0	0	0	0	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Testis																				
Necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Stomach																				
Necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Pancreas																				
Fat necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Thymus																				
Agonal hemorrhage	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Lymphocytic necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0

[a]CON: treated with PBS buffer.
-: Normal, +: Mild, ++: Moderate, +++: Severe.
Values are number of animals presenting the histopathological lesions.
The value of 0 of animals means non-significant finding.

ment of artificial rearing techniques, the number of insect rearing farms is increasing, suggesting that insect protein might be a future food resource because of its safety, economical cost, easy management and small area require-

Table 10. Histopathological findings of organs in female rats treated orally with *G. bimaculatus* for 13 weeks

Histopathological findings	0 (CON[a])				0.04				0.2				1				5 (g/kg bw/day)			
	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Kidney																				
Chronic nephritis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Pyelonephritis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Microcalcification	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Nuclear enlargement (cellular atypia)	10	0	0	0	10	0	0	0	0	10	0	0	10	0	0	0	10	0	0	0
Liver																				
Vasculitis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Lung																				
Epithelioid granulomas	10	0	0	0	0	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Foamy cell collection	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Spleen																				
Hemosiderosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Extramedullary hematopoiesis	10	0	0	0	0	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Testis																				
Necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Stomach																				
Necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Pancreas																				
Fat necrosis	0	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Thymus																				
Agonal hemorrhage	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Lymphocytic necrosis	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0

[a]CON: treated with PBS buffer.
-: Normal, +: Mild, ++: Moderate, +++: Severe.
Values are number of animals presenting the histopathological lesions.
The value of 0 of animals means non-significant finding.

ments. Accordingly, last year, an industrial entomology rearing law was announced. Therefore, more safety data will be needed for insect food approval in the future.

Recently, it was reported that the primary pharmacological activities of *G. bimaculatus* include selective hepatoprotective activities (Ahn *et al*., 2002; Kwon *et al*., 2004) and alcohol metabolizing enzyme induction in rats (Ahn *et al*., 2004).

The safety of Gb has been evaluated systematically in a series of acute and sub-acute toxicological tests showing only slight acute (single dose) toxicity with an oral LD_{50} value of > 5 g/kg (Kim *et al*., 2002). Another safety study on the extract of cricket was reported. *G. bimaculatus* at doses of 0 (vehicle), 0.025, 0.05, 0.1 and 0.2 mg/kg for 2 weeks (Hwang *et al*., 2004) were examined. On the other hand, a previous report showed that a dose of 0.2 mg/kg of a water/methanol cricket extract had protective effects on acute hepatic damage in ICR-mice induced by the administration of CCl_4 (Ahn *et al*., 2002). The same extract dosage (0.2 mg/kg) was used to evaluate the alcohol-induced toxicity in Gb as an alcohol metabolizing enhancer via liver mitochondrial alcohol dehydrogenase and acetaldehyde dehydrogenase (Ahn *et al*., 2004). Therefore, the repeated upper oral dose in subchronic toxicity study would need to be more than 0.2 mg/kg, such as 0.5 g/kg or 1 g/kg for calculating of NOAEL. In this acute oral toxicity study of Gb in SD rats, Gb did not induce any remarkable toxic responses and the LD_{50} was previously reported as > 5 g/kg (Kim *et al.*, 2002). After a dose range finding (DRF), the safety of Gb at doses of 0, 0.04, 0.2, 1 and 5 g/kg was examined over 13 consecutive weeks. Treatment at 5.0 g/kg/day with Gb resulted in a decrease in serum triglycerides and glucose in a dose-dependent manner in both sexes but all changes were within normal physiological range. There were no clinical signs or deaths related to treatment in any of the groups tested. Therefore, the approximate lethal oral dose of *G. bimaculatus* was considered to be > 5 g/kg/day in rats. Throughout the administration period, no significant changes in diet consumption, ophthalmologic findings, organ weight, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis) and gross pathology were detected. Minor changes were noted in hematological and biochemical serum parameters for the 0.04. 0.2, 1, 5 g/kg/day Gb-treated groups, but all changes were within the normal phys-

iological range. A microscopic examination did not identify any treatment-related histopathology changes in the organs of the Gb-treated groups.

Overall, the no-observed adverse effect level (NOAEL) of *G. bimaculatus* is higher than 5.0 g/kg bw/day in rats.

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