OPEN ACCESS Freely available online

ISSN: 2471-2663
Clinical & Medical Biochemistry

Research Article

Toxicity of House Cricket (*Acheta domesticus*) in Mice

Matano Yasuki, Sakagami Kiyo, Nojiri Yuuto, Nomura Kenta, Masuda Akira, Moriike Yuuki, Yamamoto Akane, Nagai Nobuo*, Ogura Atsushi

Department of Animal Bioscience, Nagahama Institute of Bio-Science and Technology, Tamura 1266, Nagahama, Shiga 526-0829, Japan

ABSTRACT

There is an urgent need to address the shortage of animal protein due to food shortages caused by the global population growth. Crickets contain an abundance of proteins in their exoskeleton and muscles and have attracted attention as a new protein source; however, their safety as a food source has not been confirmed. We evaluated the toxicity of the House cricket (*Acheta domesticus*), on cells and mammals. In genotoxicity *in vitro*, cricket powder was added to Chinese hamster lung CHL-IU cells at concentrations of 5,000 μg/mL, and the rate of chromosomal aberrations was assessed. In genotoxicity *in vivo*, mice were orally administered up to 2,000 mg/kg of cricket powder for 2 days. In both tests, cricket powder did not show any toxic effect. A repeated oral toxicity study was performed administering up to 3,000 mg/kg of cricket powder or control (saline) for 14 or 90 consecutive days and measuring body weight changes, blood biochemistry, blood properties, and organ weights. In each time course, there were no differences in there parameters between the control and cricket powder treated groups. These results suggest that House crickets (≤ 3,000 mg/kg) are not toxic to cells and organisms.

Keywords: *Acheta domesticus*; *In vitro* chromosome aberration test; *In vivo* micronucleus test; Repeated oral toxicity study

Abbreviations: ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; ARRIVE: Animal Research: Reporting of *In Vivo* Experiments; AST: Aspartate Aminotransferase; BUN: Blood Urea Nitrogen; BW: Body Weight; Ca: Calcium; CHO: Total Cholesterol; Cl: Chloride; CPA: Cyclophosphamide Monohydrate; CPK: Creatine Phosphokinase; CRE: Creatinine; GLU: Glucose; IP: Inorganic Phosphorus; K: Potassium; LDH: Lactate Dehydrogenase; MCH: Mean Corpuscular Hemoglobin; Mg: Magnesium; MN: Micronucleated; Na: Sodium; NCEs: Normochromatic Erythrocytes; OECD: Organization for Economic Cooperation and Development; PCEs: Polychromatic Erythrocytes; RBC: Red Blood Cell; T-BIL: Total Bilirubin; TG: Triglycerides; TP: Total Protein; UA: Uric Acid; WBC: White Blood Cell

INTRODUCTION

The world's population is predicted to grow to 9 billion by 2030 and solutions to the problems of food shortage caused by this population growth are required urgently. There are concerns that the demand for animal protein will increase rapidly and lead to deforestation due to increased production and overgrazing of livestock, such as cattle and pigs, and exacerbation of environmental problems caused by greenhouse gases, such as methane and carbon dioxide, emitted from livestock [1]. One possible solution to address these food problems is an insect diet, which is high in protein, rich in minerals, and has high nutritional value. Insects are already used as food in many parts of the world, including Asia, North and South America, and Africa, and it is estimated that 1,400 species of insects are used as food worldwide. Furthermore, insects are considered to have a lower environmental impact than livestock, such as cattle and pigs, because they can be raised on smaller areas of land, require less food and water, and emit less methane and carbon dioxide gas [2]. It has been reported that insects contain many bioactive substances beneficial to humans, such as vitamin C, polyphenols, and glycosaminoglycans, which have antioxidant and anti-inflammatory properties [3-5].

The House cricket (*Acheta domesticus*) is an insect belonging to the family of crickets in the order Grasshopperae. It is commercially bred for use as food for amphibians, birds, and reptiles. Crickets are composed of protein and fat (e.g., in the exoskeleton and

Correspondence to: Nagai Nobuo, Department of Animal Bioscience, Nagahama Institute of Bio-Science and Technology, Tamura 1266, Nagahama, Shiga 526-0829, Japan, Tel: +81-749-64-8176; Fax: +81-749-64-8140; E-mail: n_nagai@nagahama-i-bio.ac.jp

Received: 05-Jul-2022, Manuscript No. CMBO-22-17324; **Editor assigned:** 11-Jul-2022, PreQC No. CMBO-22-17324(PQ); **Reviewed:** 25-Jul-2022, QC No CMBO-22-17324; **Revised:** 01-Aug-2022, Manuscript No. CMBO-22-17324(R); **Published:** 08-Aug-2022, DOI:10.35841/2471-2663.22.8.128.

Citation: Yasuki M, Kiyo S, Yuuto N, Kenta N, Akira M, Yuuki M, et al. (2022) Toxicity of House Cricket (*Acheta domesticus*) in Mice. Clin Med Bio Chem. 8:128.

muscle) and are considered to be a high-quality food source of animal protein [6]. However, the toxicity and safety of insects as food remains unclear [7]. Therefore, we evaluated the toxicity of dried house cricket powder in cells and mammals. Genotoxicity was evaluated by an *in vitro* chromosome aberration test using Chinese hamster lung-derived CHL-IU cells and by an *in vivo* micronucleus test using mice. In addition, a repeated dose 14 and 90 consecutive days oral toxicity study was performed in mice.

EFSA Panel on Nutrition, Novel Food and Food Allergens (NDA) concludes that the house crickets (*Acheta domesticus*) are safe under the three formulations: frozen, dried, and ground. Following this conclusion, the European Commission authorised the marketing of house crickets (*Acheta domesticus*) as novel food in the EU as of 11 February 2022. The Panel, however, only discusses toxicological information based on various aspects, but does not test for genotoxicity and just referred that toxicological test on the *G. bimaculatus*. However, they notes that *G. bimaculatus* belongs to the same family as *A. domesticus* (Gryllidae), but these are different species and also the rearing conditions of the insects used in the study are not known. Therefore, the addition of toxicological tests in house cricket in this paper is very meaningful in supporting the marketing of house cricket.

MATERIALS AND METHODS

Preparation of cricket powder

House cricket powder was provided by Ecologgie Inc. (Tokyo, Japan) and used for all safety studies. Two-day fasted House crickets were euthanized in ice and washed thoroughly in running water. Next, 100 g-200 g of dried crickets was boiled in water at 100˚C for 15 min, dried at 70˚C for 12 hr. to a moisture content of 5%-7%, and crushed for 30 seconds five times to produce dried cricket powder. The cricket powder was also sterilized (KOGA ISOTOPE Inc. Shiga, Japan) using 30 Gy γ-rays and used for experiments. For the animal experiments, cricket powder was suspended in 0.9% saline and administered orally at various concentrations.

Animals

All animal experimental procedures were approved by the Committee on Animal Care and Use of the Nagahama Institute of Bio-Science and Technology (Permit Number: 099). The animal studies were performed in accordance with the institutional and national guidelines and regulations, and with the ARRIVE (Animal Research: Reporting of *in vivo* Experiments) guidelines (https://www.nc3rs.org.uk/arrive-guidelines). An *in vivo* micronucleus test and 14 and 90 consecutive days oral toxicity study with a repeated dose were performed according to the Organization for Economic Cooperation and Development (OECD) guidelines No. 474 (https://doi.org/10.1787/9789264264762-en) and 408 (https://doi.org/10.1787/9789264070707-en), respectively. JcI:ICR female and male mice (CLEA Japan, Tokyo, Japan) aged 5 weeks were used. These animals were maintained at approximately 23˚C, 50% humidity, 18.6 m^3/min on charge and 17.1 m^3/min on return in air ventilation, and a 12-hr light/dark cycle. Mice were fed a rodent diet ad libitum (CE-2; CLEA Japan, Tokyo, Japan). The number of animals used in each experiment is described in each figure legend.

Genotoxicity analysis

Mammalian chromosomal aberration test: A mammalian chromosomal aberration test for cricket power was performed according to OECD guideline 473 for testing chemicals (https://doi.org/10.1787/9789264264649-en). CHL-IU cells were plated in E-MEM (Fujifilm, 051-07615, Tokyo, Japan) containing heat-inactivated 10% fetal bovine serum at a density of 4 × 104/6 cm dish and incubated for 48 hr. Positive control cells were treated with a combination of metabolic activator S9-mix (Ieda boeki, Tokyo, Japan) and 200 ng/mL benzo(a)pyrene (MilliporeSigma, B1790, Saint Louis, USA). Negative control cells were treated without metabolic activator and/or benzo(a)pyrene. The toxicity of house cricket powder was examined under three conditions as follows: 5,000 μg of cricket powder/mL without S9-mix for 6 hr; 5,000 μg of cricket powder/mL with S9-mix for 22 hr; and 5,000 μg of cricket powder/mL without S9-mix for 22 hr.

For the 6-hr treatment and 16-hr recovery incubation, cells were rinsed and the media were replaced with fresh media after the 6 hr. treatment, followed by culture for an additional 16 hr. Colcemid (Fijifilm 47253, Tokyo, Japan) at final 100 ng/μL was added for the last 2 hr and then cells were harvested with 0.25% trypsin/1 mM EDTA (Nacalai Tesque, 32777, Kyoto, Japan). Single cells were treated with 75 mM KCl hypotonic solution for 20 min at 37˚C and fixed with Carnoy's solution (3:1=methanol: acetic acid). Giemsa staining was performed in specimens after fixation with methanol for 3 min. Staining was performed using 2% Giemsa solution (Merck, 1.109204, Kenilworth, USA)/Giemsa buffer (pH6.8) for 5 min at room temperature followed by washing in running water for 2 min. Air-dried samples were mounted in Multi Mount 480 (Matsunami Glass, Osaka, Japan) and more than 100 cells were observed under a microscope (Olympus CKX53, Tokyo, Japan).

***In vivo* micronucleus test:** ICR mice were acclimated for 1 week in the breeding room and then checked for health and weight. Mice were divided into five groups (5-7 animals/sex/group) and treated with 500 mg/kg, 1,000 mg/kg, or 2,000 mg/kg of cricket powder, 0.9% saline (control), or cyclophosphamide monohydrate (CPA; Sigma Chemical Company, Saint Louis, USA) as a positive control [8]. Cricket powder and control groups received two oral doses at 24-hr intervals, while the CPA group received a single intraperitoneal dose of 70 mg/kg CPA. Under deep anesthesia by intraperitoneal administration of 200 mg/kg sodium secobarbital solution (Nichi-Iko Pharmaceutical Company, Toyama, Japan) 24 hr after the last dose, both femur were removed. Then bone marrow fluid was extracted from the femur and centrifuged at 1,200 rpm for 5 min to isolate bone marrow cells, and their smears were prepared on glass slides [9]. After drying at room temperature, smears were fixed with methanol and stained for nucleic acids using acridine orange solution (Fujifilm, Osaka, Japan). The percentage of Micro-nucleated (MN) Polychromatic Erythrocytes (PCEs) in 2,000 juvenile erythrocytes per mouse, including PCEs and Normochromatic Erythrocytes (NCEs), was calculated. The ratio of PCEs per 500 erythrocytes {PCEs/(PCEs+NCEs)} was measured to assess genotoxicity and cytotoxicity.

Oral toxicity studies for 14-day and 90-day repeated dosing

In the repeated dose 14-day oral toxicity study, ICR mice were acclimated for 1 week in the breeding room, weighed, and divided into four groups of 4-9 animals/sex/group to receive treatment of 300 mg/kg, 1,000 mg/kg, or 3,000 mg/kg cricket powder or 0.9% saline (control). Mice were orally administered cricket powder or saline for 14 days, weighed, checked daily for abnormalities in

condition and death. Mice were fasted for no more than 16 hr after last administration on the 14th day. Under anesthesia by intraperitoneal administration of 200 mg/kg sodium secobarbital solution, blood characterization, blood biochemical tests, and organ weights were assessed.

Blood biochemistry tests were performed by serum prepared from blood collected from a posterior vena cava in each animal, coagulated at 37°C for at least 2 hr., and centrifuged at 3,300 rpm (4°C) for 15 min to extract the serum. The serum was analyzed using Fuji Drychem 7000 (Fujifilm, Osaka, Japan) to measure levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Total Bilirubin (T-BIL), Blood Urea Nitrogen (BUN), Creatinine (CRE), Uric Acid (UA), Glucose (GLU), Total Cholesterol (CHO), Triglycerides (TG), and Total Protein (TP), Albumin (ALB), Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CPK), Calcium (Ca), Inorganic Phosphorus (IP), Magnesium (Mg), Sodium (Na), Potassium (K), and Chloride (Cl).

Blood characterization was performed by collecting approximately 100 µL of blood from each animal via vena cava using EDTA as an anticoagulant. The numbers of White Blood Cell (WBC), Red Blood Cell (RBC), and total blood cell counts together with hemoglobin concentration, hematocrit ratio, mean corpuscular volume, Mean Corpuscular Hemoglobin (MCH), RBC distribution width, platelets, and leukocytes (lymphocyte, monocyte, granulocyte, eosinocyte count and ratio) were performed using an LC-662 blood cell counter (FUKUDA DENSHI, Shiga, Japan).

Mice were euthanized and the brain, pituitary gland, submandibular gland, thymus, heart, lung, liver, spleen, pancreas, adrenal gland (right and left), kidney (right and left), ovary (right and left), testis (right and left), and seminal vesicle/coagulating gland were collected and weighed using an electronic balance. Each organ weight was converted to percentage of total body weight after fasting. These results were used to determine the dose for the 90-day oral toxicity study.

In the repeated dose 90-day oral toxicity study, mice were orally administered cricket powder or saline then weighed, checked daily for abnormalities in condition and death for 90 days. The mice were then fasted for 16 hr. after the 90th day dose, deeply anesthetized by intraperitoneally administration of 200 mg/kg of sodium secobarbital solution. Blood measurements were made as per the 14-day oral toxicity study. Blood biochemistry was assessed for ALT, BUN, CRE, CHO, TG, ALB, CPK, Ca, Mg, and Cl. In addition to the organs in the 14-day oral toxicity study, uterus, visceral fat (right and left), subcutaneous fat, gastrocnemius muscle (right and left), and soleus muscle (right and left) were measured and expressed as percentage of total body weight after fasting.

Statistical analysis

Statistical analyses were performed using Fisher's exact test for *in vitro* chromosomal aberration test, and one-way analysis of variance followed by Dunnet test for the analysis of the *in vivo* micronucleus test and oral toxicity study. P-values<0.05 were considered significant.

RESULTS

In vitro chromosomal aberration test

The effect of cricket powder at 5,000 µg/mL on CHL-IU cells was analyzed on *in vitro* chromosomal aberration test. Negative control without Benzo(a)pyrene and/or S9-mix showed 3%~4% numerical aberration. Positive control with Benzo(a)pyrene at 200 ng/mL resulted in 15.7% of numerical aberration which is polyploid, endoreduplicated or hyperdiploid cells, which showed a significant increase (P<0.01) compared to the negative control. In contrast, there was not significant difference in numerical aberration between cells treated with 5,000 µg/mL cricket powder compared with the negative control (Table 1). These results indicate that 5,000 µg/mL house cricket powder has no cytotoxicity on CHL-IU cells.

In vivo micronucleus analysis

There was no difference in the ratio of MNPCEs per 2,000 PCEs and PCEs per 500 erythrocytes in the 500, 1,000, and 2,000 mg/kg cricket powder treatment groups compared with the control group for both sexes. On the other hand, there was a significant increase (P<0.01) in the percentage of MNPCEs per 2,000 PCEs in the CPA group compared with the control group, but no difference in the percentage of PCEs per 500 erythrocytes (Table 2). These results suggest that ≤ 2,000 mg/kg House cricket powder is not genotoxic or cytotoxic in mice.

Table 1: Mammalian chromosomal aberration test.

Substances	Dose	Treatment-recovery time (hours)	S9 mix	Frequency of cells with numerical aberration (%)	Number of metaphase
DMSO	n/a	6-16	-	3.3	122
DMSO	n/a	6-16	+	3.4	116
Benzo(a)pyrene	200 ng/mL	6-16	-	3.1	126
Benzo(a)pyrene	200 ng/mL	6-16	+	12.7**	126
Cricket powder	5000 µg/mL	6-16	-	5.4	128
Cricket powder	5000 µg/mL	22-0	+	8.6	116
Cricket powder	5000 µg/mL	22-0	+	4.5	111

Note: **P<0.01 vs. DMSO without S9 mix.

Table 2: *In vivo* micronucleus test.

Group	Dose (mg/kg)	MNPCEs/PCEs (%)	PCEs/(PCEs+NCEs) (%)
		Female	
Control	0	0.15 ± 0.07	0.56 ± 0.08
Cricket powder treatment	500	0.18 ± 0.06	0.51 ± 0.07
	1000	0.12 ± 0.07	0.52 ± 0.08
	2000	0.23 ± 0.12	0.53 ± 0.05
CPA	70	4.03 ± 0.30**	0.52 ± 0.06
		Male	
Control	0	0.13 ± 0.05	0.48 ± 0.07
Cricket powder treatment	500	0.18 ± 0.08	0.52 ± 0.07
	1000	0.14 ± 0.06	0.49 ± 0.18
	2000	0.17 ± 0.08	0.52 ± 0.07
CPA	70	4.60 ± 0.74**	0.39 ± 0.04

Note: Values represent mean ± SD. MNPCEs: Micronucleated Polychromatic Erythrocytes; PCEs: Polychromatic Erythrocytes; NCEs: Normochromatic Erythrocytes; CPA: Cyclophosphamide Monohydrate. Females: Control, 1,000 mg/kg and 2,000 mg/kg groups, n=6; 500 mg/kg group, n=7; CPA group, n=5. Males: Control and 1,000 mg/kg groups, n=5; 2,000 mg/kg group, n=6; 500 mg/kg and CPA groups, n=7. *P<0.05, **P<0.01 vs. control group.

Repeated dose for 14-day and 90-day oral toxicity studies

In the 14-day oral toxicity study, there were no differences in body weight changes or blood biochemistry tests during the 14-day treatment period in the 300, 1,000, and 3,000 mg/kg cricket powder groups compared with the control group for both males and females (Figure 1 and Table 3). Blood biochemistry tests revealed a significant increase (P<0.05) in MCH in the female 1,000 mg/kg group compared with the control group, but no difference in males in the cricket powder group compared with the control group (Table 4). In addition, there was no difference in the ratio of organ to body weight after fasting in male and female mice treated with cricket powder compared with the control group (Table 5).

In the 90-day oral toxicity study, there was no difference in body weight change during the 90-day treatment period in the 300 mg/kg, 1,000 mg/kg, and 3,000 mg/kg cricket powder groups compared with the control group for both males and females (Figure 2). Furthermore, there were no differences in blood biochemistry (Table 6), blood parameters (Table 7), or organ weights as a percentage of total body weight (Table 8) in the 300, 1000, and 3,000 mg/kg cricket powder groups compared with the control group. These results suggest that there is no acute or chronic toxicity to individual mice following 14 and 90-day consumption of House cricket powder (≤ 3,000 mg/kg).

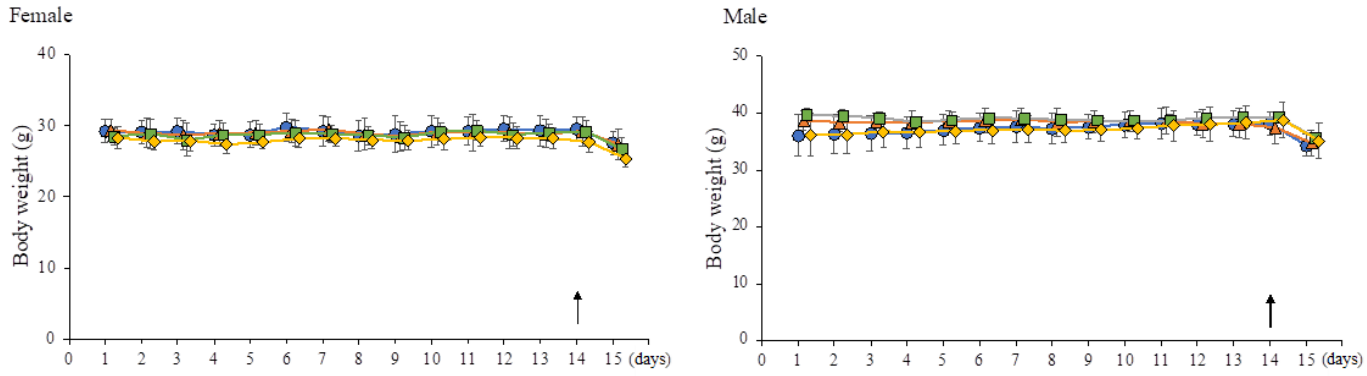

Figure 1: Body weight changes in male and female mice in the 14-day oral dose toxicity study. **Note:** Values represent mean ± SD. Arrow indicates start of fasting. Females: n=5 for all groups. Males: control group, n=8; 300 mg/kg group, n=5; 1,000 mg/kg group, n=4; and 3,000 mg/kg group, n=9; (—●—) 0 mg/kg; (—▲—) 300 mg/kg; (—■—) 1000 mg/kg; (—◆—) 3000 mg/kg.

Table 3: Serum biochemical values of mice treated with House cricket powder for 14 days.

Group	Control	300 mg/kg	1000 mg/kg	3000 mg/kg
		Female		
AST[1] (IU/L)	66 ± 31	68 ± 9	76 ± 25	60 ± 33
ALT[2] (IU/L)	23 ± 4	24 ± 6	24 ± 6	23 ± 8
ALP[3] (IU/L)	76 ± 18	100 ± 28	106 ± 32	81 ± 3
T-BIL[4] (mg/dL)	0.6 ± 0.11	0.7 ± 0.10	0.5 ± 0.04	0.5 ± 0.05

BUN[5] (mg/dL)	26.6 ± 3.91	24.4 ± 6.72	24.2 ± 6.31	23.2 ± 5.01
CRE[6] (mg/dL)	0.13 ± 0.04	0.14 ± 0.04	0.11 ± 0.03	0.12 ± 0.03
UA[7] (mg/dL)	1.4 ± 0.26	2.1 ± 1.17	1.4 ± 0.22	1.8 ± 0.75
GLU[8] (mg/dL)	149 ± 49	172 ± 83	131 ± 39	155 ± 38
CHO[9] (mg/dL)	73 ± 7	81 ± 15	77 ± 15	81 ± 18
TG[10] (mg/dL)	98 ± 32	128 ± 48	101 ± 24	46 ± 21
TP[11] (g/dL)	5.3 ± 0.11	5.5 ± 0.10	5.0 ± 0.23	5.3 ± 0.31
ALB[12] (g/dL)	2.4 ± 0.11	2.6 ± 0.12	2.5 ± 0.17	2.6 ± 0.28
LDH[13] (IU/L)	585 ± 241	814 ± 163	761 ± 172	624 ± 200
CPK[14] (U/L)	86 ± 25	136 ± 49	175 ± 50	120 ± 63
Ca[15] (mg/dL)	10.1 ± 0.42	10.3 ± 0.64	9.6 ± 0.48	10.3 ± 1.28
IP[16] (mg/dL)	13.1 ± 1.77	14.2 ± 1.49	13.5 ± 0.83	14.4 ± 0.76
Mg[17] (mg/dL)	3.5 ± 0.58	4.0 ± 0.71	3.6 ± 0.29	3.7 ± 0.68
Na[18] (mmol/L)	150 ± 1	150 ± 2	151 ± 1	152 ± 1
K[19] (mmol/L)	5.8 ± 1.30	7.7 ± 2.36	5.4 ± 0.89	6.4 ± 1.51
Cl[20] (mmol/L)	113 ± 4	115 ± 3	114 ± 4	117 ± 3
		Male		
AST[1] (IU/L)	66 ± 17	47 ± 22	54 ± 12	40 ± 6
ALT[2] (IU/L)	29 ± 10	19 ± 4	22 ± 4	22 ± 4
ALP[3] (IU/L)	73 ± 18	68 ± 23	69 ± 16	65 ± 21
T-BIL[4] (mg/dL)	0.6 ± 0.12	0.6 ± 0.15	0.5 ± 0.08	0.4 ± 0.10
BUN[5] (mg/dL)	30.2 ± 7.68	25.8 ± 3.72	24.1 ± 3.98	30.6 ± 7.88
CRE[6] (mg/dL)	0.16 ± 0.07	0.11 ± 0.02	0.10 ± 0.01	0.14 ± 0.04
UA[7] (mg/dL)	2.0 ± 0.79	1.5 ± 0.28	1.9 ± 0.77	1.3 ± 0.29
GLU[8] (mg/dL)	156 ± 46	119 ± 28	184 ± 41	162 ± 25
CHO[9] (mg/dL)	111 ± 16	124 ± 23	115 ± 4	101 ± 19
TG[10] (mg/dL)	69 ± 20	77 ± 23	100 ± 24	62 ± 34
TP[11] (g/dL)	5.4 ± 0.16	5.3 ± 0.64	5.2 ± 0.11	4.8 ± 0.19
ALB[12] (g/dL)	2.5 ± 0.10	2.5 ± 0.44	2.5 ± 0.11	2.2 ± 0.12
LDH[13] (IU/L)	729 ± 210	586 ± 263	517 ± 116	405 ± 111
CPK[14] (U/L)	194 ± 60	159 ± 111	107 ± 23	122 ± 31
Ca[15] (mg/dL)	10.2 ± 0.39	10.1 ± 0.63	10.3 ± 0.72	9.7 ± 0.32
IP[16] (mg/dL)	14.1 ± 1.32	14.0 ± 1.20	14.1 ± 0.25	12.3 ± 2.27
Mg[17] (mg/dL)	4.0 ± 0.81	4.2 ± 1.29	3.7 ± 0.27	3.6 ± 0.26
Na[18] (mmol/L)	151 ± 0.4	151 ± 1	150 ± 2	151 ± 1
K[19] (mmol/L)	7.2 ± 1.69	5.5 ± 0.89	6.5 ± 1.41	5.0 ± 0.79
Cl[20] (mmol/L)	113 ± 1	112 ± 2	113 ± 2	111 ± 2

Note: Values represent mean ± SD. 1: Aspartate aminotransferase, 2: Alanine aminotransferase, 3: Alkaline phosphatase, 4: Total bilirubin, 5: Blood urea nitrogen, 6: Creatinine, 7: Uric acid, 8: Glucose, 9: Total cholesterol, 10: Triglycerides, 11: Total protein, 12: Albumin, 13: Lactate dehydrogenase, 14: Creatine phosphokinase, 15: Calcium, 16: Inorganic phosphorus, 17: Magnesium, 18: Sodium, 19: Potassium, and 20: Chloride. Females: Control group, n=4; 300 mg/kg, 1,000 mg/kg, and 3,000 mg/kg groups, n=5. Males: Control and 1,000 mg/kg groups, n=4; 300 and 3,000 mg/kg groups, n=5.

Table 4: Hematological values of mice treated with House cricket powder for 14 days.

Group	Control	300 mg/kg	1000 mg/kg	3000 mg/kg
		Female		
WBC[1] (10^3/μL)	7.05 ± 2.25	9.65 ± 2.07	7.05 ± 3.37	7.65 ± 3.50
RBC[2] (10^6/μL)	6.77 ± 1.83	7.88 ± 1.23	5.96 ± 0.78	7.49 ± 1.10
Hemoglobin (g/dL)	10.10 ± 2.54	12.83 ± 1.77	10.25 ± 1.47	12.40 ± 2.03
Hematocrit (%)	32.98 ± 9.67	40.15 ± 6.09	30.78 ± 5.96	37.73 ± 6.53
MCV[3] (μm[3])	48.43 ± 1.27	50.98 ± 0.39	51.23 ± 3.58	50.20 ± 1.71
MCH[4] (pg)	15.03 ± 1.11	16.30 ± 0.37	17.20 ± 0.78*	16.55 ± 0.52
MCHC[5] (g/dL)	31.00 ± 2.39	31.95 ± 0.59	33.75 ± 2.41	33.00 ± 0.61
RDW[6] (%)	14.05 ± 0.51	13.53 ± 0.47	15.05 ± 2.36	14.00 ± 0.46
Platelets (10^3/μL)	420.8 ± 340.5	146.0 ± 79.6	304.0 ± 35.4	113.5 ± 83.7

Lymphocyte (10^3/μL)	3.18 ± 1.33	4.83 ± 0.64	3.65 ± 1.47	4.00 ± 2.33
Monocyte (10^3/μL)	0.23 ± 0.08	0.30 ± 0.07	0.20 ± 0.07	0.23 ± 0.11
Granulocyte (10^3/μL)	3.65 ± 1.87	4.53 ± 1.63	3.20 ± 1.87	3.43 ± 1.16
Eosinocyte (10^3/μL)	0.90 ± 0.63	1.40 ± 0.79	0.98 ± 0.63	1.33 ± 0.36
		Male		
WBC[1] (10^3/μL)	5.85 ± 2.36	3.94 ± 1.92	6.15 ± 2.25	9.24 ± 5.12
RBC[2] (10^6/μL)	6.07 ± 2.01	6.73 ± 1.30	7.11 ± 0.28	6.33 ± 2.17
Hemoglobin (g/dL)	9.88 ± 3.36	10.88 ± 2.03	11.40 ± 0.48	10.31 ± 3.55
Hematocrit (%)	30.86 ± 10.90	33.32 ± 7.64	35.43 ± 1.38	33.05 ± 11.14
MCV[3] (μm^3)	50.68 ± 1.63	49.14 ± 2.89	49.85 ± 0.32	52.38 ± 1.60
MCH[4] (pg)	16.21 ± 0.28	16.16 ± 0.48	16.05 ± 0.38	16.26 ± 0.59
MCHC[5] (g/dL)	32.01 ± 1.14	33.04 ± 1.65	32.23 ± 0.75	31.03 ± 0.57
RDW[6] (%)	14.26 ± 0.55	14.36 ± 1.80	13.60 ± 0.39	13.58 ± 0.61
Platelets (10^3/μL)	324.6 ± 171.9	256.4 ± 181.5	310.5 ± 253.1	172.0 ± 210.7
Lymphocyte (10^3/μL)	2.90 ± 1.50	1.58 ± 1.15	3.05 ± 1.02	4.16 ± 2.03
Monocyte (10^3/μL)	0.19 ± 0.08	0.12 ± 0.04	0.15 ± 0.05	0.24 ± 0.12
Granulocyte (10^3/μL)	2.76 ± 1.19	2.24 ± 0.80	2.95 ± 1.24	4.84 ± 3.60
Eosinocyte (10^3/μL)	1.05 ± 0.80	0.90 ± 0.34	0.98 ± 0.49	1.96 ± 1.68

Note: Values represent mean ± SD. 1: White blood cell, 2: Red blood cell, 3: Mean corpuscular volume, 4: Mean corpuscular hemoglobin, 5: Mean corpuscular hemoglobin concentration, and 6: RBC distribution width. Females: n=4 for all groups. Males: Control group, n=8; 300 and 1,000 mg/kg groups, n=4; 3,000 mg/kg group, n=9. †P<0.05 vs. control group.

Table 5: Relative organ weight of mice treated with House cricket powder for 14 days.

OW/BW (%)	Control	300 mg/kg	1000 mg/kg	3000 mg/kg
		Female		
Brain	1.5408 ± 0.1810	1.6776 ± 0.1124	1.5705 ± 0.1279	1.7254 ± 0.1105
Pituitary gland	0.0079 ± 0.0023	0.0073 ± 0.0015	0.0087 ± 0.0060	0.0118 ± 0.0051
Submandibular gland	0.6025 ± 0.1620	0.5426 ± 0.0936	0.6426 ± 0.0988	0.6511 ± 0.0831
Thymus	0.2887 ± 0.0499	0.3076 ± 0.0730	0.2984 ± 0.0464	0.2982 ± 0.1014
Heart	0.4688 ± 0.0315	0.5179 ± 0.0419	0.5022 ± 0.0497	0.5229 ± 0.0334
Lung	0.6220 ± 0.0195	0.6373 ± 0.0996	0.6548 ± 0.1077	0.6353 ± 0.0701
Liver	5.0887 ± 0.3949	4.9985 ± 0.5928	5.5440 ± 0.9749	4.8743 ± 0.1564
Spleen	0.3862 ± 0.1518	0.4001 ± 0.0849	0.3359 ± 0.0682	0.3257 ± 0.0481
Pancreas	0.9261 ± 0.3614	0.9658 ± 0.2838	1.0538 ± 0.6274	0.8304 ± 0.1981
Adrenal gland (R)	0.0161 ± 0.0055	0.0202 ± 0.0133	0.0152 ± 0.0047	0.0291 ± 0.0218
Kidney (R)	0.6978 ± 0.0461	0.7007 ± 0.0826	0.7403 ± 0.0685	0.7397 ± 0.0648
Adrenal gland (L)	0.0197 ± 0.0084	0.0190 ± 0.0054	0.0199 ± 0.0045	0.0256 ± 0.0052
Kidney (L)	0.6631 ± 0.0356	0.6592 ± 0.1161	0.6972 ± 0.0647	0.7382 ± 0.0592
Ovary (R)	0.0182 ± 0.0087	0.0157 ± 0.0018	0.0251 ± 0.0094	0.0285 ± 0.0101
Ovary (L)	0.0219 ± 0.0074	0.0247 ± 0.0125	0.0240 ± 0.0063	0.0290 ± 0.0113
		Male		
Brain	1.3045 ± 0.0905	1.3570 ± 0.2318	1.2107 ± 0.0751	1.3151 ± 0.1686
Pituitary gland	0.0058 ± 0.0020	0.0092 ± 0.0051	0.0060 ± 0.0029	0.0063 ± 0.0019
Submandibular gland	0.6856 ± 0.0703	0.7845 ± 0.2069	0.6229 ± 0.0958	0.6671 ± 0.0953
Thymus	0.1517 ± 0.0398	0.1302 ± 0.0452	0.1608 ± 0.0306	0.1317 ± 0.0484
Heart	0.4863 ± 0.0468	0.4786 ± 0.0717	0.4877 ± 0.0355	0.4936 ± 0.0628
Lung	0.6517 ± 0.0631	0.5867 ± 0.0724	0.6279 ± 0.1379	0.5776 ± 0.0547
Liver	4.7717 ± 0.3678	4.5986 ± 0.7985	4.8139 ± 0.2388	4.7760 ± 0.4950
Spleen	0.2756 ± 0.0921	0.2320 ± 0.0358	0.2433 ± 0.0463	0.2770 ± 0.0594
Pancreas	0.6780 ± 0.2863	0.7191 ± 0.3076	0.9481 ± 0.2257	0.6499 ± 0.1727
Adrenal gland (R)	0.0078 ± 0.0032	0.0100 ± 0.0051	0.0087 ± 0.0076	0.0100 ± 0.0046
Kidney (R)	0.8610 ± 0.0523	0.8884 ± 0.1110	0.8878 ± 0.0844	0.9230 ± 0.0603
Adrenal gland (L)	0.0109 ± 0.0035	0.0104 ± 0.0066	0.0062 ± 0.0034	0.0101 ± 0.0037
Kidney (L)	0.8228 ± 0.0724	0.8228 ± 0.0716	0.8421 ± 0.0540	0.8888 ± 0.0693

Testis (R)	0.4301 ± 0.0369	0.4551 ± 0.0466	0.3894 ± 0.0161	0.4015 ± 0.0500
Testis (L)	0.3926 ± 0.0498	0.4496 ± 0.0402	0.3767 ± 0.0087	0.3795 ± 0.0564
Seminal vesicle/Coagulating gland	0.7160 ± 0.1280	0.7586 ± 0.1687	0.7665 ± 0.0398	0.7614 ± 0.1505

Note: Values represent mean ± SD. OW: Organ Weight; BW: Body Weight. Females: All groups, n=5. Males: Control group, n=8; 300 mg/kg group, n=5; 1,000 mg/kg group, n=4; 3,000 mg/kg group, n=9.

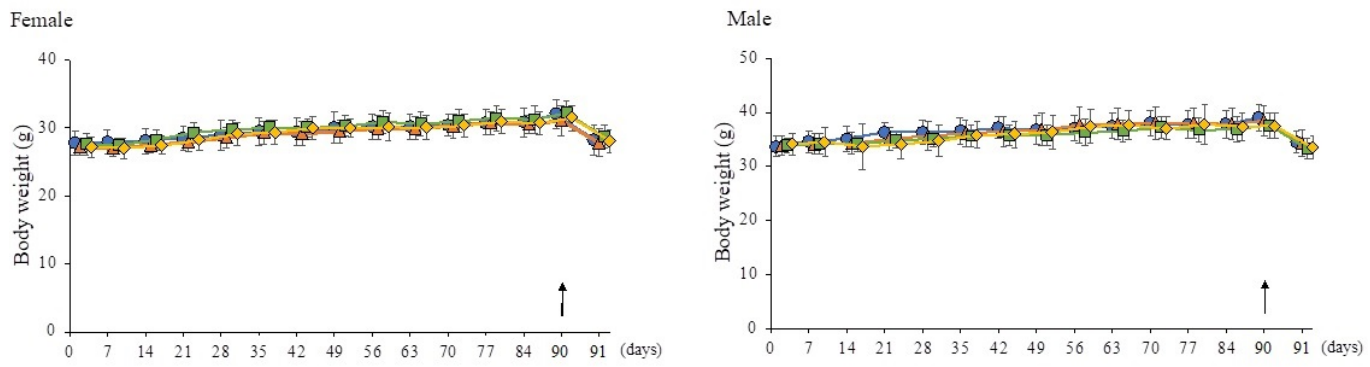

Figure 2: Body weight changes in male and female mice in the 90-day oral dose toxicity study. **Note:** Values represent mean ± SD. Arrow indicates start of fasting. Females: control and 300 mg/kg groups, n=12; 1,000 mg/kg group, n=9; 3,000 mg/kg group, n=10. Males: control, 300 mg/kg, and 3,000 mg/kg groups, n=11; 1,000 mg/kg group, n=10; (—●—) 0 mg/kg; (—▲—) 300 mg/kg; (—■—) 1000 mg/kg; (—◆—) 3000 mg/kg.

Table 6: Serum biochemical values of mice treated with House cricket powder for 90 days.

Group	Control	300 mg/kg	1000 mg/kg	3000 mg/kg
		Female		
ALT[1] (IU/L)	44 ± 14	40 ± 13	35 ± 9	38 ± 15
BUN[2] (mg/dL)	25.9 ± 4.6	21.8 ± 5.9	19.5 ± 3.5	24.6 ± 7.7
CRE[3] (mg/dL)	0.47 ± 0.28	0.87 ± 0.39	0.71 ± 0.42	0.74 ± 0.36
CHO[4] (mg/dL)	73 ± 29	62 ± 7	64 ± 9	73 ± 14
TG[5] (mg/dL)	72 ± 42	47 ± 20	56 ± 20	48 ± 23
ALB[6] (g/dL)	4.5 ± 0.9	4.7 ± 0.8	4.8 ± 0.5	4.2 ± 0.5
CPK[7] (U/L)	124 ± 61	128 ± 81	121 ± 52	99 ± 63
Ca[8] (mg/dL)	6.1 ± 2.2	4.9 ± 0.5	5.0 ± 0.5	5.3 ± 0.7
Mg[9] (mg/dL)	3.4 ± 0.6	2.9 ± 0.4	2.9 ± 0.5	3.0 ± 0.4
Cl[10] (mmol/L)	101 ± 4	99 ± 5	96 ± 4	100 ± 4
		Male		
ALT1 (IU/L)	35 ± 11	37 ± 19	32 ± 7	33 ± 8
BUN[2] (mg/dL)	24.4 ± 4.1	27.5 ± 9.3	22.8 ± 4.0	24.1 ± 6.8
CRE[3] (mg/dL)	0.51 ± 0.22	0.66 ± 0.36	0.74 ± 0.20	0.77 ± 0.22
CHO[4] (mg/dL)	90 ± 12	87 ± 17	83 ± 17	77 ± 13
TG[5] (mg/dL)	83 ± 32	79 ± 25	73 ± 27	59 ± 20
ALB[6] (g/dL)	3.5 ± 1.0	3.7 ± 0.9	4.5 ± 0.5	4.4 ± 0.7
CPK[7] (U/L)	106 ± 28	178 ± 146	108 ± 63	87 ± 46
Ca[8] (mg/dL)	7.1 ± 1.8	7.1 ± 2.2	5.7 ± 1.2	5.4 ± 0.8
Mg[9] (mg/dL)	3.6 ± 0.7	3.7 ± 0.8	3.2 ± 0.8	3.0 ± 0.4
Cl[10] (mmol/L)	104 ± 5	104 ± 3	102 ± 3	100 ± 4

Note: Values represent mean ± SD. 1: Aspartate aminotransferase, 2: Blood urea nitrogen, 3: Creatinine, 4: Total cholesterol, 5: Triglycerides, 6: Albumin, 7: Creatine phosphokinase, 8: Calcium, 9: Magnesium, and 10: Chloride. Females: Control, 300 mg/kg, and 3,000 mg/kg groups, n=10; 1,000 mg/kg group, n=9. Males: Control and 300 mg/kg groups, n=11; 1,000 mg/kg and 3,000 mg/kg groups, n=10.

Table 7: Hematological values of mice treated with House cricket powder for 90 days.

Group	Control	300 mg/kg	1000 mg/kg	3000 mg/kg
		Female		
WBC[1] (10^3/μL)	3.78 ± 1.99	4.48 ± 2.86	3.29 ± 1.65	5.91 ± 4.22
RBC[2] (10^6/μL)	8.28 ± 0.81	8.53 ± 0.68	8.15 ± 0.89	8.19 ± 0.67
Hemoglobin (g/dL)	12.66 ± 1.31	13.01 ± 0.95	12.51 ± 1.15	12.60 ± 1.19
Hematocrit (%)	39.95 ± 4.23	41.78 ± 3.70	40.11 ± 3.90	39.76 ± 3.29
MCV[3] (μm^3)	48.25 ± 1.37	48.93 ± 1.68	49.24 ± 1.30	48.57 ± 1.89
MCH[4] (pg)	15.30 ± 0.46	15.25 ± 0.55	15.39 ± 0.33	15.41 ± 0.73
MCHC[5] (g/dL)	31.73 ± 0.35	31.18 ± 0.66	31.24 ± 0.57	31.69 ± 0.60
RDW[6] (%)	13.66 ± 0.65	13.64 ± 0.63	13.57 ± 0.44	13.76 ± 0.51
Platelets (10^3/μL)	69.5 ± 42.0	170.1 ± 109.0	74.4 ± 25.5	221.1 ± 195.5
Lymphocyte (10^3/μL)	1.58 ± 0.79	1.60 ± 0.78	1.23 ± 0.62	2.44 ± 1.48
Monocyte (10^3/μL)	0.09 ± 0.06	0.11 ± 0.08	0.07 ± 0.05	0.14 ± 0.13
Granulocyte (10^3/μL)	2.11 ± 1.60	2.76 ± 2.01	1.99 ± 1.04	3.33 ± 2.73
Eosinocyte (10^3/μL)	1.04 ± 0.91	1.39 ± 1.07	0.97 ± 0.61	1.33 ± 1.04
		Male		
WBC[1] (10^3/μL)	6.13 ± 2.31	5.68 ± 2.72	4.90 ± 3.01	6.83 ± 3.97
RBC[2] (10^6/μL)	8.23 ± 1.94	8.44 ± 0.52	8.97 ± 0.76	8.64 ± 0.43
Hemoglobin (g/dL)	12.54 ± 3.02	12.95 ± 0.32	13.36 ± 1.12	13.07 ± 0.65
Hematocrit (%)	39.94 ± 9.47	41.33 ± 1.83	43.21 ± 3.95	41.53 ± 2.11
MCV[3] (μm^3)	48.45 ± 1.49	49.04 ± 1.73	48.18 ± 1.77	48.07 ± 1.09
MCH[4] (pg)	15.19 ± 0.47	15.39 ± 0.59	14.93 ± 0.43	15.10 ± 0.35
MCHC5 (g/dL)	31.35 ± 0.36	31.38 ± 0.81	30.99 ± 0.46	31.43 ± 0.41
RDW6 (%)	13.47 ± 0.23	13.75 ± 0.43	13.98 ± 0.75	13.84 ± 0.53
Platelets (10^3/μL)	72.6 ± 31.9	195.1 ± 146.8	165.5 ± 108.9	172.9 ± 74.8
Lymphocyte (10^3/μL)	2.34 ± 0.96	2.19 ± 0.99	1.98 ± 0.78	2.54 ± 1.34
Monocyte (10^3/μL)	0.17 ± 0.06	0.15 ± 0.09	0.09 ± 0.08	0.14 ± 0.10
Granulocyte (10^3/μL)	3.62 ± 1.50	3.34 ± 2.02	2.84 ± 2.20	4.14 ± 2.56
Eosinocyte (10^3/μL)	1.65 ± 0.76	1.43 ± 1.10	1.33 ± 1.09	1.99 ± 1.23

Note: Values represent mean ± SD. 1: White blood cell, 2: RBC, 3: Mean corpuscular volume, 4: Mean corpuscular hemoglobin, 5: Mean corpuscular hemoglobin concentration, and 6: RBC distribution width. Females: Control and 300 mg/kg groups, n=9; 1,000 mg/kg and 3,000 mg/kg groups, n=7. Males: Control group, n=11, 300 mg/kg, 1,000 mg/kg, and 3,000 mg/kg groups, n=8.

Table 8: Relative organ weight of mice treated with House cricket powder for 90 days.

OW/BW (%)	Control	300 mg/kg	1000 mg/kg	3000 mg/kg
		Female		
Brain	1.6022 ± 0.1735	1.6414 ± 0.1326	1.6377 ± 0.0935	1.5923 ± 0.1642
Pituitary gland	0.0101 ± 0.0046	0.0079 ± 0.0037	0.0113 ± 0.0028	0.0102 ± 0.0034
Submandibular gland	0.5154 ± 0.0903	0.5234 ± 0.1173	0.5625 ± 0.1004	0.5635 ± 0.0482
Thymus	0.1514 ± 0.0515	0.1637 ± 0.0465	0.1506 ± 0.0310	0.1386 ± 0.0491
Heart	0.4782 ± 0.0339	0.5683 ± 0.2267	0.4736 ± 0.0396	0.4872 ± 0.0468
Lung	0.5884 ± 0.1012	0.7293 ± 0.2419	0.6528 ± 0.0718	0.5974 ± 0.1049
Liver	4.3728 ± 0.3460	4.1870 ± 0.3891	4.4669 ± 0.2441	4.4709 ± 0.2109
Spleen	0.3267 ± 0.0822	0.2943 ± 0.0859	0.2648 ± 0.0353	0.2893 ± 0.0329
Pancreas	0.6797 ± 0.1466	0.6700 ± 0.1521	0.6249 ± 0.2381	0.6598 ± 0.0824
Adrenal gland (R)	0.0159 ± 0.0035	0.0147 ± 0.0038	0.0146 ± 0.0031	0.0146 ± 0.0029
Kidney (R)	0.6733 ± 0.0843	0.6967 ± 0.0729	0.7045 ± 0.0420	0.6751 ± 0.0693
Adrenal gland (L)	0.0186 ± 0.0046	0.0156 ± 0.0028	0.0173 ± 0.0056	0.0150 ± 0.0042
Kidney (L)	0.6620 ± 0.0741	0.6494 ± 0.0464	0.6676 ± 0.0370	0.6579 ± 0.0343

Ovary (R)	0.0269 ± 0.0121	0.0387 ± 0.0141	0.0294 ± 0.0081	0.0324 ± 0.0138
Ovary (L)	0.0269 ± 0.0102	0.0375 ± 0.0119	0.0284 ± 0.0110	0.0349 ± 0.0130
Uterus	0.5445 ± 0.2399	0.7079 ± 0.3909	0.6093 ± 0.1383	0.5654 ± 0.2798
Visceral fat (R)	0.3507 ± 0.3456	0.1484 ± 0.1894	0.4934 ± 0.2432	0.2581 ± 0.2209
Visceral fat (L)	0.3005 ± 0.2602	0.1341 ± 0.1394	0.4212 ± 0.2342	0.2226 ± 0.1945
Subcutaneous fat	0.4042 ± 0.1881	0.4060 ± 0.1888	0.4911 ± 0.2221	0.3361 ± 0.1402
Gastrocnemius muscle (R)	0.5669 ± 0.1381	0.5793 ± 0.1788	0.4767 ± 0.0718	0.5529 ± 0.1666
Gastrocnemius muscle (L)	0.5645 ± 0.1223	0.5040 ± 0.1073	0.4865 ± 0.0642	0.5733 ± 0.1061
Soleus muscle (R)	0.0308 ± 0.0150	0.0277 ± 0.0153	0.0319 ± 0.0116	0.0362 ± 0.0194
Soleus muscle (L)	0.0300 ± 0.0116	0.0322 ± 0.0157	0.0292 ± 0.0105	0.0299 ± 0.0107
		Male		
Brain	1.3297 ± 0.1145	1.3096 ± 0.1000	1.2957 ± 0.1230	1.3680 ± 0.0878
Pituitary gland	0.0074 ± 0.0032	0.0068 ± 0.0022	0.0059 ± 0.0023	0.0061 ± 0.0027
Submandibular gland	0.7523 ± 0.1030	0.7338 ± 0.0868	0.7665 ± 0.0566	0.6927 ± 0.1325
Thymus	0.0915 ± 0.0238	0.0965 ± 0.0289	0.0912 ± 0.0168	0.0948 ± 0.0372
Heart	0.5079 ± 0.0551	0.5067 ± 0.0560	0.5127 ± 0.0667	0.5176 ± 0.0433
Lung	0.5324 ± 0.0428	0.6463 ± 0.1577	0.6136 ± 0.0963	0.6110 ± 0.1748
Liver	4.3486 ± 0.1905	4.2449 ± 0.1357	4.3052 ± 0.1660	4.2010 ± 0.3310
Spleen	0.2115 ± 0.0426	0.2421 ± 0.0512	0.2444 ± 0.0378	0.2030 ± 0.0357
Pancreas	0.6455 ± 0.1626	0.5703 ± 0.2285	0.6348 ± 0.1322	0.5597 ± 0.2207
Adrenal gland (R)	0.0080 ± 0.0035	0.0108 ± 0.0067	0.0087 ± 0.0030	0.0092 ± 0.0022
Kidney (R)	0.8807 ± 0.0724	0.9136 ± 0.0690	0.8607 ± 0.1123	0.8933 ± 0.1368
Adrenal gland (L)	0.0097 ± 0.0040	0.0103 ± 0.0038	0.0096 ± 0.0030	0.0099 ± 0.0025
Kidney (L)	0.8264 ± 0.0784	0.8369 ± 0.0871	0.8130 ± 0.0921	0.8502 ± 0.1138
Testis (R)	0.4466 ± 0.0595	0.4663 ± 0.0681	0.4660 ± 0.1003	0.4642 ± 0.0609
Testis (L)	0.4335 ± 0.0593	0.4474 ± 0.0658	0.4801 ± 0.1513	0.4312 ± 0.0669
Seminal vesicle/Coagulating gland	1.0256 ± 0.1143	0.9737 ± 0.1812	0.8970 ± 0.1625	0.9878 ± 0.1792
Visceral fat (R)	0.4935 ± 0.2093	0.4655 ± 0.1832	0.3933 ± 0.1142	0.3776 ± 0.1858
Visceral fat (L)	0.5074 ± 0.2108	0.4579 ± 0.1760	0.3720 ± 0.1363	0.4263 ± 0.2491
Subcutaneous fat	0.4686 ± 0.1671	0.4224 ± 0.2143	0.4585 ± 0.1960	0.4046 ± 0.1345
Gastrocnemius muscle (R)	0.5347 ± 0.1792	0.4786 ± 0.1687	0.5424 ± 0.0954	0.5091 ± 0.0999
Gastrocnemius muscle (L)	0.5173 ± 0.0840	0.5163 ± 0.1147	0.5051 ± 0.1234	0.4697 ± 0.0649
Soleus muscle (R)	0.0417 ± 0.0226	0.0474 ± 0.0213	0.0460 ± 0.0259	0.0870 ± 0.1450
Soleus muscle (L)	0.0386 ± 0.0174	0.0472 ± 0.0235	0.0362 ± 0.0207	0.0772 ± 0.1127

Note: Values represent mean ± SD. OW: Organ Weight; BW: Body Weight. Females: Control and 300 mg/kg groups, n=12; 1,000 mg/kg group, n=9; 3,000 mg/kg group, n=10. Males: Control, 300 mg/kg, and 3,000 mg/kg groups, n=11; 1,000 mg/kg group, n=10.

DISCUSSION

Insects are used as food in many parts of the world, including Asia, North and South America, and Africa. In Japan, bee larvae and locusts are also used as food and it is estimated that 1,400 species of insects are used as food worldwide. Other insects, such as yellow mealworm (*Tenebrio molitor* larva) and *Gryllus bimaculatus*, have been evaluated for their safety as food [8,10,11]. Although house crickets (*Acheta domesticus*) have been reported to be safe as food and their risk has been evaluated [12,13], their cytotoxicity and toxicity to organisms have not been evaluated in accordance with the OECD guidelines. The present study evaluated the cellular genotoxicity of cricket powder using an *in vitro* chromosome aberration test and its genetic, acute, and chronic biological toxicity in ICR mice in accordance with the OECD guidelines.

In vitro chromosomal aberration test showed no clear cytotoxicity under treatment of 5,000 μg/mL cricket powder on CHL-IU cells (Table 1). *In vivo* micronucleus test results showed no genotoxic effects of ingestion of ≤ 2,000 mg/kg cricket powder on erythrocytes in mouse bone marrow (Table 2). However, the present study was unable to determine the genotoxicity of >2,000 mg/kg cricket powder due to ingestion; therefore, further studies are required to evaluate the toxicity of ingestion of higher concentrations of cricket powder. In the repeated oral toxicity study, no significant changes in body weight were observed in males and females following 14 and 90-day continuous administration studies of cricket powder (≤ 3,000 mg/kg) (Figures 1 and 2). Furthermore, 14-day administration of cricket powder did not result in weight gain over time in females and males in any of the groups. This lack of weight gain may result from the stress of repeated forced

oral administration. In addition, no abnormalities were observed in blood properties, blood biochemistry tests, or organ weights in both males and females after administration of cricket powder (≤ 3,000 mg/kg) for 14 and 90 days (Tables 3-8) [14].

CONCLUSION

In conclusion, the present study found no cellular or biological toxicity from consumption of House crickets at concentrations ≤3,000 mg/kg, indicating that House crickets may be a safe and high-quality animal protein foodstuff. This study is useful evidence for evaluating the toxicity of House crickets *in vivo*. Future studies are planned to re-evaluate AST, ALP, T-BIL, UA, GLU, TP, LDH, IP, Na, and K, which could not be measured in the blood biochemistry analysis in the 90-day oral toxicity study since the blood samples were hemolyzed. In addition, there is concern that adult crickets, such as the House cricket and Desert locust, contain substantial amounts of purines, which may aggravate diseases, such as gout; therefore, we will conduct component analysis and safety evaluation using House crickets before sexual maturity to determine the most suitable crickets to harvest for use as foodstuffs.

ACKNOWLEDGEMENT

This work was supported by Cabinet Office, Government of Japan, Cross-ministerial Moonshot Agriculture, Forestry and Fisheries Research and Development Program, "Technologies for Smart Bio-industry and Agriculture" (JPJ009237).

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

REFERENCES

1. Oonincx DG, van Itterbeeck J, Heetkamp MJ, van den Brand H, van Loon JJ, van Huis A. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLoS One. 2010;5(12):e14445.
2. Nowakowski AC, Miller AC, Miller ME, Xiao H, Wu X. Potential health benefits of edible insects. Crit Rev Food Sci Nutr. 2022;62(13):3499-3508.
3. Di Mattia C, Battista N, Sacchetti G, Serafini M. Antioxidant activities *in vitro* of water and liposoluble extracts obtained by different species of edible insects and invertebrates. Front Nutr. 2019;6:106.
4. Zielińska E, Baraniak B, Karaś M. Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. Nutrients. 2017;9(9):970.
5. Ahn MY, Kim BJ, Kim HJ, Jin JM, Yoon HJ, Hwang JS, et al. Anti-diabetic activity of field cricket glycosaminoglycan by ameliorating oxidative stress. BMC Complement Med Ther. 2020 Jul 22;20(1):232.
6. Van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, et al. Edible insects: Future prospects for food and feed security. Food and agriculture organization of the United Nations; 2013.
7. Klunder HC, Wolkers-Rooijackers J, Korpela JM, Nout MR. Microbiological aspects of processing and storage of edible insects. Food control. 2012;26(2):628-631.
8. Han SR, Yun EY, Kim JY, Hwang JS, Jeong EJ, Moon KS. Evaluation of genotoxicity and 28-day oral dose toxicity on freeze-dried powder of *Tenebrio molitor* larvae (Yellow mealworm). Toxicol Res. 2014;30(2):121-130.
9. Schmid W. The micronucleus test. Mutat Res. 1975;31(1):9-15.
10. Turck D, Castenmiller J, de Henauw S, Hirsch -Ernst KI, Kearney J, Maciuk A, et al. Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 2021;19(1):e06343.
11. Ryu HY, Lee S, Ahn KS, Kim HJ, Lee SS, Ko HJ, et al. Oral toxicity study and skin sensitization test of a cricket. Toxicol Res. 2016;32(2):159-173.
12. Turck D, Bohn T, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Maciuk A, et al. Safety of frozen and dried formulations from whole house crickets (*Acheta domesticus*) as a Novel food pursuant to Regulation (EU) 2015/2283. EFSA J. 2021;19(8):e06779.
13. Fernandez-Cassi X, Supeanu A, Vaga M, Jansson A, Boqvist S, Vagsholm I. The house cricket (*Acheta domesticus*) as a novel food: A risk profile. J Insects Food Feed. 2019;5(2):137-157.
14. Sabolová M, Kulma M, Kouřimská L. Sex-dependent differences in purine and uric acid contents of selected edible insects. J Food Compos Anal. 2021;96:103746.