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# Safety evaluation of cricket(*Gryllus bimaculatus*) extract in Sprague-Dawley rats

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## Abstract

Recently, research investment in the improvement of food safety as a food source and specializing of nutritional source of edible insects is being actively conducted. Cricket especially has been attracting considerable interest in entomophagy; however, research on the safety assessment of cricket is limited. This study investigated the effects of cricket ethanol extract when orally administrated in Sprague-Dawley rats. Here, we performed a 4 wk repeated oral dose toxicity test in Sprague-Dawley rats following the Organization for Economic Cooperation and Development test guidelines 407 under Good Laboratory Practice regulation. Rats were randomly allocated 4 groups: vehicle control, 250, 500, 1,000 mg/kg test groups and administrated based on body weight for 28 d. The animals were observed for mortalities and clinical signs, body weight changes, food and water consumption. At the end of treatment period, blood and urine were collected and analyzed. Subsequently, the animals were sacrificed and subjected to gross pathological examination and organ weight measurement. The organs were preserved for histopathological examination. The results showed that there were no systemic toxicological effects related with the cricket ethanol extract in the 4 wk oral repeated dose toxicity study. It is considered that NOAEL of cricket ethanol extract is greater than 1,000 mg/kg/d and there was no target organ detected.


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NOAEL.

## Introduction

Since increase in consumption of grain and volatility in grain supply due to global warming, it has highlighted the importance for the food supply as world population is expected to be increase to 9.6 billion in 2050 (UN, 2013). The production of sufficient protein from bovine, poultry meat, and demersal fish represents a serious challenge for the future (van Huis *et al*., 2015). As an alternative of animal protein, an edible insect is one of the breakthroughs that could solve the food crisis (Rumpold and Schlüter, 2015). According to increased interest in the edible insects, an establishment of the foundation for human or animal food based on a variety of insect

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resource is ongoing (Belluco *et al*., 2015). In 2015, cricket has been newly approved as a temporal food ingredient with mealworm beetle, and protaetia brevitarsis seulensis by Korea ministry of food and drug safety. Among the insects, cricket, which is known as high contents of chitin and unsaturated fatty acid, traditionally consumed as a medicine for fever, diarrhea, kidney stone or hypertension as well as a food source (Park, 2001; Ahn *et al*., 2005). Despite its various aspects, safety evaluation of processed cricket is limited. These characteristics of crickets have motivated the toxicological test herein a 4 wk repeated oral dose toxicity test in rats. Here, we determined that cricket ethanol extract causes no toxicological issues as part of the diet and can serve as an excellent food source, resolving the scarcity of food, in addition to being a possible health supplement in the future.

## Materials and Methods

***Preparation of test materials.*** Cricket, *G. bimaculatus* collected in the insect farm in Joungsun, South Korea. Crickets were subjected to 3 d defecation period then washed 3 times with distilled water, and then freeze-dried. 1 kg of freeze-dried cricket was homogenized. Sample was soaked into 70% ethanol and extracted by ultrasonicator over 3 times. Extracted sample was filtered using Whatman filter and concentrated by freeze-drying and complete evaporation of solvent. Powder type of sample was dissolved in saline prior to administration.

***Animals.*** Animal experiments were designed and conducted under the Organization for Economic Cooperation and Development (OECD) test guidelines No. 407 'Repeated dose 28 d oral toxicity study in rodents', Good Laboratory Practice (GLP), and the Korean Ministry of Food and Drug Safety (KMFDS) notice no. 2014-136 'Toxicity test standards of medicine and medical supplies' (OECD, 2008). Methods were approved by the Animal Care and Use Committee at the Korea Conformity Laboratory (IA14-01047). Specific pathogen free Crl:CD (Sprague-Dawley) rats were obtained from ORIENTBIO (Sungnam, Korea). Animals were maintained at a standard temperature of 23.3±0.8°C and a relative humidity of 47.4±5.1 % RH under a 12 h light/dark cycle. Rats were fed a rodent diet (Harlan Teklad, USA). All animals were provided with tap water purified by a reverse osmosis filtering system.

***A 4 wk repeated oral dose toxicity study in SD rats.*** 5-wk-old male and female rats were acclimated for 5 to 7 d prior to administration. When the rats became 6-wk-old, they were exposed to cricket ethanol extract by gavage for 28 d. According to OECD guideline, it is suggested that 1,000 mg/kg as a limit dose of repeated oral dose toxicity study in rodent. Therefore, in the present study, the dose of 1,000 mg/kg was set to high dose level. Individual dosing volumes were calculated based on 10 mL/kg body weight. During the study, general clinical signs of all treated animals were observed once a day right after administration during the exposure period. Individual animal weight was recorded at acquisition, grouping, right before administration, once a week during the study and before necropsy. Food consumption also measured once a week. On the last week of the study, urine samples were collected from randomly selected 5 animals per each groups. Fresh urine used for analysis using urine test strip (SIEMENS, Germany) and the urine auto-analyzer, Clinitek advantus (SIMENS). Leukocyte, epithelial cell, and cast were counted under microscope.

At necropsy, all animals were laparotomized under isoflurane anesthesia. Blood samples were withdrawn from the abdominal aorta and aliquoted into EDTA-K2 Tube, 3.2 % Sodium citrate Tube, Serum separating tube and Heparin Tube ABGA Syringe(20~30 IU/1 mL).

Hematology analyzer, ADVIA 2120 (SIEMENS), blood coagulation analyzer, ACL7000 (Instrumentation Laboratory, USA), biochemistry analyzer, Hitachi7180 (Hitachi, Japan) were used for blood analysis as described by Sung *et al*. (2014). After complete-mortem examination, organs were weighted and preserved in 10% neutral buffered formalin for histopathological examination.

***Statistical analysis.*** Statistical analysis was carried out using SPSS (Version 12). Statistical evaluation was performed using a two-tailed Student's t-test or an analysis of variance (ANOVA) following multiple comparison tests with Duncan's method. Asterisks (*) indicate statistically significant differences compared with the control groups. On day 48, one of the male rats in the 5,000 mg/kg test group was sacrificed at the study

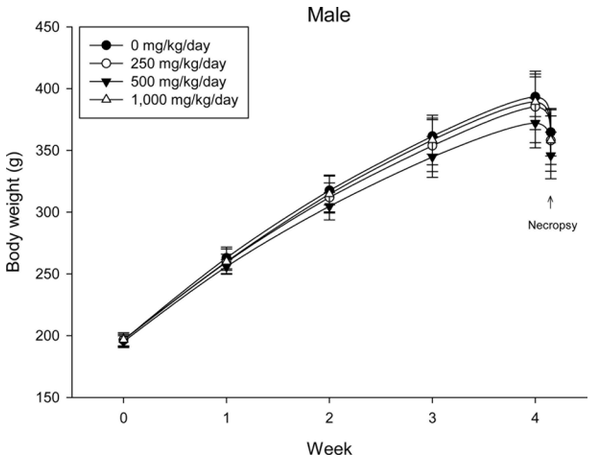


**Fig 1-1. Body weight changes in male rats.** Body weight changes of male rats of vehicle control and cricket ethanol extract treated group (n=10 per each groups). Error bar represents standard deviation.

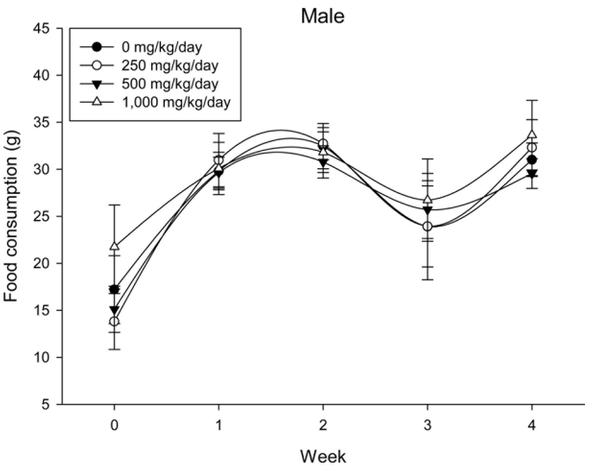


**Fig 2-1. Food consumption in male rats.** Food consumption of male rats (n=10 per each groups). Error bar represents standard deviation.

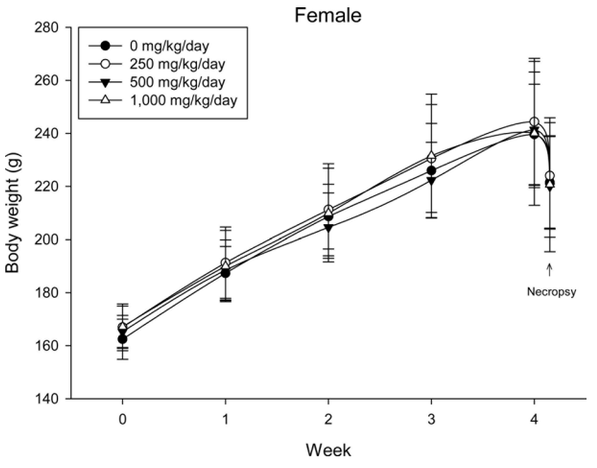


**Fig 1-2. Body weight changes in female rats.** Body weight changes of female rats of vehicle control and cricket ethanol extract treated group (n=10 per each groups). Error bar represents standard deviation.

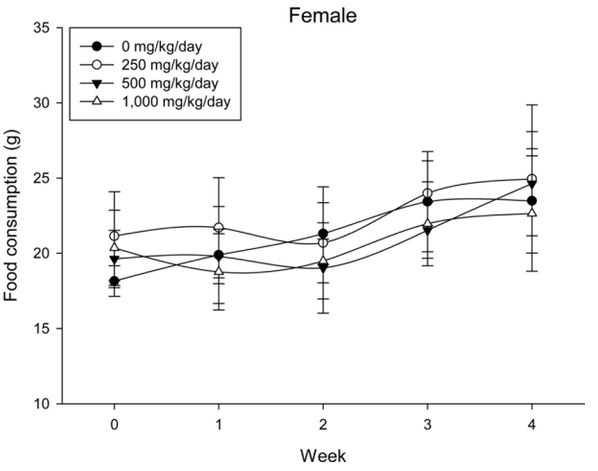


**Fig 2-2. Food consumption in female rats.** Food consumption of female rats (n=10 per each groups). Error bar represents standard deviation.

director's instruction due to a technical error by the technical assistant. Only 9 values were used in the male 5,000 mg/kg test group for the statistical analysis of hematology, plasma coagulation, serum biochemistry and absolute/relative organ weight.

## Results

During the 4 wk oral repeated test substance administration of 0, 250, 500 and 1,000 mg/kg doses, body weight changes (Figs. 1-1 and 1-2) and food consumptions (Figs. 2-1 and 2-2) for each sex were measured. There were no significant difference was detected on each week across the vehicle control groups. Morbidity or mortality was not observed in all test groups of both sexes and there were no abnormal clinical signs observed throughout the experimental period. In urinalysis and urine sediment analysis, parameters of treatment and recovery groups of both sexes were found to be comparable with the corresponding control group (Tables 1 and 2). The result of hematological and the plasma coagulation analysis showed no significant difference

**Table 1.** Urinalysis for the rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF URINALYSIS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Male | | | | Female | | | |
| TEST ITEM | | GROUP(mg/kg/d) | | | | GROUP(mg/kg/d) | | | |
| | | 0 | 250 | 500 | 1,000 | 0 | 250 | 500 | 1,000 |
| Glucose | - | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Bilirubin | - | 5/5 | 5/5 | 4/5 | 5/5 | 5/5 | 4/5 | 5/5 | 5/5 |
| | 1+ | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |
| Ketones | - | 0/5 | 0/5 | 0/5 | 1/5 | 5/5 | 3/5 | 5/5 | 5/5 |
| | +/- | 3/5 | 3/5 | 1/5 | 2/5 | 0/5 | 2/5 | 0/5 | 0/5 |
| | 1+ | 2/5 | 2/5 | 4/5 | 2/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Specific gravity | ≤ 1.005 | 0/5 | 0/5 | 0/5 | 0/5 | 4/5 | 0/5 | 0/5 | 0/5 |
| | 1.010 | 3/5 | 1/5 | 0/5 | 1/5 | 0/5 | 0/5 | 2/5 | 1/5 |
| | 1.015 | 2/5 | 3/5 | 3/5 | 3/5 | 1/5 | 2/5 | 3/5 | 3/5 |
| | 1.020 | 0/5 | 0/5 | 1/5 | 1/5 | 0/5 | 1/5 | 0/5 | 1/5 |
| | 1.025 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |
| | ≥ 1.030 | 0/5 | 1/5 | 1/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |
| Occult blood | - | 5/5 | 5/5 | 5/5 | 4/5 | 5/5 | 5/5 | 5/5 | 4/5 |
| | +/- | 0/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 | 0/5 | 1/5 |
| pH | 6 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |
| | 6.5 | 0/5 | 1/5 | 1/5 | 0/5 | 1/5 | 0/5 | 0/5 | 0/5 |
| | 7 | 0/5 | 0/5 | 0/5 | 2/5 | 1/5 | 2/5 | 0/5 | 1/5 |
| | 7.5 | 2/5 | 2/5 | 0/5 | 2/5 | 2/5 | 1/5 | 2/5 | 3/5 |
| | 8 | 1/5 | 2/5 | 4/5 | 1/5 | 1/5 | 0/5 | 2/5 | 0/5 |
| | 8.5 | 2/5 | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 1/5 | 1/5 |
| Protein | - | 0/5 | 0/5 | 0/5 | 0/5 | 5/5 | 1/5 | 5/5 | 3/5 |
| | +/- | 1/5 | 1/5 | 1/5 | 2/5 | 0/5 | 2/5 | 0/5 | 2/5 |
| | 1+ | 4/5 | 4/5 | 3/5 | 3/5 | 0/5 | 1/5 | 0/5 | 0/5 |
| | 2+ | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |
| Urobilinogen | 0.2 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Nitrate | - | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Leukocytes | - | 4/5 | 5/5 | 4/5 | 5/5 | 5/5 | 5/5 | 5/5 | 4/5 |
| | +/- | 1/5 | 0/5 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Color | colorless | 0/5 | 1/5 | 0/5 | 1/5 | 1/5 | 1/5 | 0/5 | 0/5 |
| | straw | 5/5 | 4/5 | 4/5 | 4/5 | 4/5 | 3/5 | 5/5 | 5/5 |
| | yellow | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 |

Number of animals with the sign / Number of animals examined, -: Negative, +/-: Trace.

**Table 2.** Urine sediment for the rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF URINE SEDIMENTS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Male | | | | Female | | | |
| TEST ITEMS | Grade | Group (mg/kg/d) | | | | Group (mg/kg/d) | | | |
| | | 0 | 250 | 500 | 1,000 | 0 | 250 | 500 | 1,000 |
| RBC | 0 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| WBC | 0 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Epithelial cell | 0 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |
| Casts | 0 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 | 5/5 |

Number of animals with the sign / Number of animals examined.
Grade : 0 ; 0

**Table 3-1.** Hematological analysis for the male rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF HEMATOLOGICAL VALUES | | | | |
|---|---|---|---|---|
| | | | | SEX : MALE |
| TEST ITEM | GROUP(mg/kg/d) | | | |
| (Unit) | 0 | 250 | 500 | 1,000 |
| WBC[1](K/μL) | 8.36±1.17 | 9.42±2.01 | 9.34±2.45 | 9.02±1.85 |
| NE[2](K/μL) | 1.52±0.53 | 1.21±0.34 | 1.33±0.56 | 1.13±0.32 |
| EO[3](K/μL) | 0.06±0.03 | 0.06±0.02 | 0.05±0.01 | 0.05±0.03 |
| BA[4](K/μL) | 0.01±0.01 | 0.01±0.01 | 0.01±0.00 | 0.01±0.01 |
| LY[5](K/μL) | 6.52±1.21 | 7.90±2.09 | 7.71±2.18 | 7.58±1.80 |
| MO[6](K/μL) | 0.17±0.08 | 0.18±0.05 | 0.17±0.07 | 0.16±0.07 |
| LUC[7](K/μL) | 0.08±0.04 | 0.07±0.02 | 0.08±0.04 | 0.09±0.03 |
| NEP[8](%) | 18.4±6.9 | 13.5±5.4 | 14.7±5.7 | 13.0±4.2 |
| EOP[9](%) | 0.7±0.4 | 0.7±0.2 | 0.5±0.2 | 0.6±0.3 |
| BAP[10](%) | 0.1±0.1 | 0.1±0.1 | 0.1±0.0 | 0.1±0.1 |
| LYP[11](%) | 77.8±7.1 | 83.1±5.5 | 82.1±5.9 | 83.7±4.4 |
| MOP[12]((%) | 2.1±1.0 | 1.9±0.5 | 1.8±0.5 | 1.8±0.7 |
| LUP[13](%) | 1.0±0.4 | 0.7±0.2 | 0.9±0.3 | 0.9±0.3 |
| RBC[14](M/μL) | 7.71±0.18 | 7.72±0.52 | 7.78±0.40 | 7.88±0.33 |
| Hb[15](g/dl) | 16.2±0.8 | 16.5±0.8 | 16.7±0.7 | 17.1±0.8 |
| RDW[16](%) | 11.6±0.6 | 11.5±0.3 | 11.4±0.5 | 11.3±0.4 |
| HCT[17](%) | 45.1±2.2 | 46.0±1.8 | 46.4±1.8 | 47.4±2.2 |
| MCV[18](fL) | 58.5±2.1 | 59.7±1.9 | 59.7±2.2 | 60.1±1.4 |
| MCH[19](pg) | 21.0±0.7 | 21.5±0.9 | 21.5±0.8 | 21.6±0.5 |
| MCHC[20](g/dl) | 35.9±1.1 | 36.0±0.8 | 36.0±0.4 | 36.0±0.4 |
| Reti[21](%) | 3.01±0.88 | 2.70±0.50 | 2.63±0.51 | 2.38±0.45 |
| PLT[22](K/μL) | 1071±85 | 1228±143 | 1120±130 | 1116±71 |
| MPV[23](fL) | 4.7±0.3 | 4.8±0.3 | 4.7±0.2 | 4.8±0.3 |

Mean±SD (n=10 per each groups). 1: White blood cells, 2: Neutrophils, 3: Eosinophils, 4: Basophils, 5: Lymphocytes, 6: Monocytes, 7: Large unstained cells, 8: Percentage of neutrophils, 9: Percentage of eosinophils, 10: Percentage of basophils, 11: Percentage of lymphocytes, 12: Percentage of monocytes, 13: Percentage of large unstained cells, 14: Red blood cells, 15: Hemoglobin, 16: Red blood cell distribution width, 17: Hematocrit, 18: Mean corpuscular volume, 19: Mean corpuscular hemoglobin, 20: Mean corpuscular hemoglobin concentration, 21: Reticulocytes, 22: Platelets, 23: Mean platelet volume.

in either sex in the test groups compared with the control group (Tables 3-1, 3-2, and 4). In serum biochemical analysis, the aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase of the male and female rats in 1,000 mg/kg test group was decreased compared with control group (Tables 5-1 and 5-2). However, we did not find any statistical significance in the other test items in the test groups compared with the vehicle control group.

At necropsy, enlargement of spleen was shown in one female rats in 500 mg/kg test group. There was no abnormal gross finding was shown in the other rats. In organ weights, absolute and relative organ weight of prostate in male 1,000 mg/kg test group was statistically significantly decreased compared with the vehicle control group ($p<0.01$). Relative organ weight of spleen was statistically significantly increased ($p<0.05$) (Tables 6-1 and 6-2). However, there is no toxicological meaning on these findings: because a) the values were within the normal

**Table 3-2.** Hematological analysis for the female rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF HEMATOLOGICAL VALUES | | | | |
|---|---|---|---|---|
| | | | | SEX : FEMALE |
| TEST ITEM | GROUP(mg/kg/d) | | | |
| (Unit) | 0 | 250 | 500 | 1,000 |
| WBC[1](K/μL) | 8.93±2.41 | 8.43±1.62 | 8.48±2.75 | 8.39±2.02 |
| NE[2](K/μL) | 1.16±0.64 | 1.02±0.38 | 1.14±0.49 | 0.82±0.51 |
| EO[3](K/μL) | 0.08±0.03 | 0.09±0.03 | 0.06±0.03 | 0.12±0.18 |
| BA[4](K/μL) | 0.01±0.01 | 0.01±0.01 | 0.01±0.01 | 0.01±0.01 |
| LY[5](K/μL) | 7.40±2.08 | 7.02±1.27 | 6.97±2.28 | 7.19±1.58 |
| MO[6](K/μL) | 0.18±0.06 | 0.17±0.07 | 0.20±0.08 | 0.16±0.07 |
| LUC[7](K/μL) | 0.11±0.03 | 0.13±0.05 | 0.11±0.04 | 0.09±0.05 |
| NEP[8](%) | 12.9±5.3 | 12.0±3.3 | 13.4±3.0 | 9.4±4.0 |
| EOP[9](%) | 0.8±0.4 | 1.0±0.3 | 0.7±0.3 | 1.3±1.5 |
| BAP[10](%) | 0.1±0.0 | 0.1±0.0 | 0.1±0.1 | 0.1±0.1 |
| LYP[11](%) | 82.7±5.2 | 83.5±3.9 | 82. 1±3.4 | 86.2±5.4 |
| MOP[12]((%) | 2.1±0.6 | 2.0±0.7 | 2.4±0.8 | 1.9±0.5 |
| LUP[13](%) | 1.3±0.4 | 1.5±0.4 | 1.3±0.3 | 1.1±0.4 |
| RBC[14](M/μL) | 7.95±0.25 | 7.86±0.31 | 7.65±0.72 | 7.96±0.41 |
| Hb[15](g/dl) | 17.2±0.6 | 17.1±0.6 | 16.9±1.2 | 17.3±0.8 |
| RDW[16](%) | 10.5±0.4 | 10.7±0.6 | 10.8±1.3 | 10.5±0.4 |
| HCT[17](%) | 45.7±1.9 | 45.5±1.5 | 44.7±3.7 | 46.0±1.7 |
| MCV[18](fL) | 57.5±2.2 | 57.9±2.1 | 58.4±1.4 | 57.8±1.9 |
| MCH[19](pg) | 21.6±0.6 | 21.8±0.8 | 22.1±0.7 | 21.7±0.7 |
| MCHC[20](g/dl) | 37.6±0.6 | 37.7±0.4 | 37.9±0.8 | 37.6±0.9 |
| Reti[21](%) | 2.05±0.47 | 2.26±0.53 | 2.66±1.48 | 2.00±0.35 |
| PLT[22](K/μL) | 1096±119 | 1021±162 | 1184±152 | 1011±167 |
| MPV[23](fL) | 4.5±0.2 | 4.4±0.2 | 4.4±0.1 | 4.7±1.0 |

Mean±SD (n=10 per each groups). 1: White blood cells, 2: Neutrophils, 3: Eosinophils, 4: Basophils, 5: Lymphocytes, 6: Monocytes, 7: Large unstained cells, 8: Percentage of neutrophils, 9: Percentage of eosinophils, 10: Percentage of basophils, 11: Percentage of lymphocytes, 12: Percentage of monocytes, 13: Percentage of large unstained cells, 14: Red blood cells, 15: Hemoglobin, 16: Red blood cell distribution width, 17: Hematocrit, 18: Mean corpuscular volume, 19: Mean corpuscular hemoglobin, 20: Mean corpuscular hemoglobin concentration, 21: Reticulocytes, 22: Platelets, 23: Mean platelet volume.

biological range, b) there was no dose-dependency, and c) the results did not correlate with other analysis results.

During histopathological examination of test and vehicle control groups in each sex (Tables 7-1 and 7-2), enlargement of spleen in female 500 mg/kg test group was confirmed as a congestion. However, it is not considered as a toxicological meaning because it does not show dose-dependency and it is a sporadic lesion. In male and female 1,000 mg/kg test and vehicle control group, focal mononuclear cell infiltration in liver, cyst in inner strip of kidney, tubule dilatation in

**Table 4.** Plasma coagulation values for the rats.

| SUMMARY OF PLASMA COAGULATION TESTS | | | | |
|---|---|---|---|---|
| Unit : sec | | | | SEX : MALE |
| TEST ITEM | GROUP (mg/kg/d) | | | |
| | 0 | 250 | 500 | 1,000 |
| PT[1] | 9.44±0.76 | 9.65±0.54 | 9.99±0.52 | 9.62±0.43 |
| APTT[2] | 15.10±1.5 | 15.0±1.2 | 15.8±1.2 | 16.3±1.2 |
| | | | | SEX : FEMALE |
| TEST ITEM | GROUP (mg/kg/d) | | | |
| | 0 | 250 | 500 | 1,000 |
| PT | 9.18±0.57 | 9.45±0.79 | 9.33±0.26 | 9.47±0.48 |
| APTT | 17.5±1.8 | 17.6±2.0 | 16.9±2.3 | 16.5±1.7 |

Mean±SD(n=10 per each groups). 1: Prothrombin time, 2: Active partial thromboplastin time.

**Table 5-1.** Serum biochemical analysis for the male rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF SERUM BIOCHEMICAL TESTS | | | | |
|---|---|---|---|---|
| | | | | SEX : MALE |
| TEST ITEM | GROUP(mg/kg/d) | | | |
| (Unit) | 0 | 250 | 500 | 1,000 |
| AST[1] (IU/L) | 130±32 | 137±33 | 133±21 | 119±17 |
| ALT[2] (IU/L) | 41±7 | 40±5 | 39±8 | 39±5 |
| ALP[3] (IU/L) | 490±117 | 517±83 | 473±40 | 522±93 |
| GGT[4] (IU/L) | 0.00±0.00 | 0.00±0.00 | 0.10±0.32 | 0.00±0.00 |
| T-BIL[5] (mg/dl) | 0.03±0.03 | 0.02±0.02 | 0.03±0.02 | 0.01±0.01 |
| BUN[6] (mg/dl) | 13.7±1.2 | 13.3±1.0 | 13.9±1.9 | 14.4±1.7 |
| CRE[7] mg/dl) | 0.44±0.07 | 0.44±0.08 | 0.41±0.08 | 0.44±0.13 |
| UA[8] (mg/dl) | 2.1±0.5 | 2.3±0.7 | 1.8±0.5 | 2.1±0.6 |
| GLU[9] (mg/dl) | 183±25 | 153±26 | 180±22 | 175±51 |
| CHO[10] (mg/dl) | 64±21 | 58±15 | 58±15 | 58±11 |
| TG[11] (mg/dl) | 24±12 | 28±20 | 26±16 | 30±10 |
| TP[12] (g/dl) | 5.9±0.2 | 5.9±0.2 | 5.8±0.2 | 6.0±0.3 |
| ALB[13] (g/dl) | 2.2±0.2 | 2.3±0.1 | 2.2±0.1 | 2.3±0.1 |
| A/G ratio[14] | 0.59±0.06 | 0.62±0.04 | 0.62±0.03 | 0.61±0.06 |
| LDH[15] (IU/L) | 1206±783 | 1466±781 | 1366±621 | 996±367 |
| CPK[16] (U/L) | 655±332 | 796±435 | 790±371 | 559±219 |
| Ca[17] (mg/dl) | 9.8±0.5 | 10.3±1.0 | 10.1±1.4 | 10.1±1.1 |
| IP[18] (mg/dl) | 10.6±0.8 | 11.0±1.0 | 10.7±0.8 | 10.9±1.2 |
| Mg[19] (mg/dl) | 2.9±0.2 | 2.8±0.1 | 2.8±0.1 | 2.7±0.1 |
| Na[20] (mmol/L) | 142±3 | 141±1 | 141±3 | 140±2 |
| K[21] (mmol/L) | 5.8±0.9 | 6.2±0.6 | 5.6±0.5 | 5.8±0.9 |
| Cl[22] (mmol/L) | 104±3 | 104±2 | 105±3 | 104±3 |

Mean±SD (n=10 per each groups). 1: Aspartate aminotransferase, 2: Alanine aminotransferase, 3: Alkaline phosphatase, 4: Gamma(γ)-glutamyl transferase, 5: Total bilirubin, 6: Blood urea nitrogen, 7: Creatinine, 8: Uric acid, 9: Glucose, 10: Total cholesterol, 11: Triglycerides, 12: Total protein, 13: Albumin, 14: Albumin/Globulin ratio, 15: Lactate dehydrogenase, 16: Creatine phosphokinase, 17: Calcium, 18: Inorganic phosphorus, 19: Magnesium, 20: Sodium, 21: Potassium. , 22: Chloride

**Table 5-1.** Serum biochemical analysis for the female rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF SERUM BIOCHEMICAL TESTS | | | | |
|---|---|---|---|---|
| | | | | SEX :FEMALE |
| TEST ITEM | GROUP(mg/kg/d) | | | |
| (Unit) | 0 | 250 | 500 | 1,000 |
| AST[1] (IU/L) | 114±23 | 120±31 | 106±19 | 101±28 |
| ALT[2] (IU/L) | 34±9 | 32±5 | 33±5 | 30±6 |
| ALP[3] (IU/L) | 310±40 | 284±80 | 266±30 | 279±85 |
| GGT[4] (IU/L) | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| T-BIL[5] (mg/dl) | 0.04±0.03 | 0.04±0.03 | 0.05±0.05 | 0.06±0.03 |
| BUN[6] (mg/dl) | 14.0±2.5 | 14.7±2.7 | 17.6±4.3 | 15.7±2.9 |
| CRE[7] mg/dl) | 0.44±0.04 | 0.44±0.05 | 0.45±0.06 | 0.44±0.06 |
| UA[8] (mg/dl) | 1.8±0.4 | 1.7±0.3 | 1.9±0.4 | 1.8±0.4 |
| GLU[9] (mg/dl) | 136±29 | 157±31 | 160±20 | 166±20 |
| CHO[10] (mg/dl) | 73±10 | 69±16 | 66±10 | 69±12 |
| TG[11] (mg/dl) | 17±9 | 21±14 | 25±17 | 18±8 |
| TP[12] (g/dl) | 6.2±0.3 | 6.1±0.4 | 6.1±0.3 | 6.2±0.3 |
| ALB[13] (g/dl) | 2.5±0.1 | 2.5±0.2 | 2.5±0.1 | 2.5±0.2 |
| A/G ratio[14] | 0.69±0.03 | 0.68±0.03 | 0.71±0.04 | 0.67±0.05 |
| LDH[15] (IU/L) | 857±614 | 952±622 | 673±437 | 630±538 |
| CPK[16] (U/L) | 530±346 | 549±314 | 420±214 | 403±299 |
| Ca[17] (mg/dl) | 9.5±0.3 | 9.4±0.2 | 9.4±0.4 | 9.3±0.3 |
| IP[18] (mg/dl) | 9.9±0.6 | 9.8±0.5 | 10.0±0.5 | 10.1±0.6 |
| Mg[19] (mg/dl) | 2.7±0.3 | 2.6±0.2 | 2.7±0.2 | 2.7±0.2 |
| Na[20] (mmol/L) | 141±3 | 141±3 | 140±2 | 141±2 |
| K[21] (mmol/L) | 5.7±1.0 | 5.8±0.8 | 6.0±1.0 | 6.3±0.6 |
| Cl[22] (mmol/L) | 103±3 | 103±3 | 104±3 | 104±2 |

Mean±SD (n=10 per each groups). 1: Aspartate aminotransferase, 2: Alanine aminotransferase, 3: Alkaline phosphatase, 4: Gamma(γ)-glutamyl transferase, 5: Total bilirubin, 6: Blood urea nitrogen, 7: Creatinine, 8: Uric acid, 9: Glucose, 10: Total cholesterol, 11: Triglycerides, 12: Total protein, 13: Albumin, 14: Albumin/ Globulin ratio, 15: Lactate dehydrogenase, 16: Creatine phosphokinase, 17: Calcium, 18: Inorganic phosphorus, 19: Magnesium, 20: Sodium, 21: Potassium. , 22: Chloride

inner and outer strip of kidney, scar with focal inflammation and tubular regeneration in kidney, ectopic thymus, ultimobranchial cyst, focal granulomatous inflammation in lung, spermatogranuloma in epididymis, mononuclear cell infiltration in prostate stroma were detected in both vehicle and control and 1,000 mg/kg test groups. However, it is considered not related to cricket ethanol extract administration as their severities were minimal to mild and they were present in vehicle control group as well. There was no difference in the incidence rate compared to the vehicle control group. Other than these findings, there were no abnormalities detected in the other organs.

In conclusion, under the study condition, there were not found target organs and systemic toxicological effects related with cricket ethanol extract in 4 wk oral repeated dose toxicity study. NOAEL of cricket ethanol extract administrated through oral gavage for 28 d in SD rats was found to be greater than 1,000 mg/kg body weight and acceptable daily intake is up to 10 mg per kg of body weight.

**Table 6-1.** Absolute organ weights of the male and female rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF SERUM BIOCHEMICAL TESTS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SEX : MALE | | | | SEX : FEMALE | | | |
| TEST ITEM | GROUP(mg/kg/d) | | | | GROUP(mg/kg/d) | | | |
| (Unit) | 0 | 250 | 500 | 1,000 | 0 | 250 | 500 | 1,000 |
| Body weight | 364.70±19.22 | 358.01±24.86 | 345.89±18.76 | 358.30±19.64 | 221.51±17.27 | 223.98±20.10 | 219.95±19.14 | 220.67±25.19 |
| Testis/Ovary (Lt.) | 1.6670±0.1425 | 1.7172±0.1165 | 1.6974±0.1994 | 1.6750±0.1180 | 0.0410±0.0090 | 0.0439±0.0109 | 0.0383±0.0058 | 0.0431±0.0115 |
| Testis/Ovary (Rt.) | 1.6789±0.1250 | 1.7306±0.1174 | 1.6765±0.1817 | 1.6893±0.1130 | 0.0406±0.0081 | 0.0419±0.0111 | 0.0422±0.0091 | 0.0413±0.0071 |
| Prostate/Uterus | 0.5917±0.0907 | 0.6322±0.1077 | 0.5535±0.0730 | 0.4808** ±0.0713 | 0.4555±0.1581 | 0.4388±0.0541 | 0.4776±0.1899 | 0.4622±0.1415 |
| Spleen | 0.8457±0.1285 | 0.7803±0.0774 | 0.7583±0.1307 | 0.7572±0.1125 | 0.5100±0.0482 | 0.4985±0.0658 | 0.6225±0.1958 | 0.5129±0.0690 |
| Liver | 11.1450±1.2861 | 10.5031±1.3177 | 9.9194±0.6281 | 10.7571±0.7982 | 6.5266±0.6266 | 6.6122±0.8633 | 6.4402±0.7147 | 6.3762±0.9835 |
| Adrenal gland (Lt.) | 0.0277±0.0034 | 0.0295±0.0066 | 0.0303±0.0043 | 0.0303±0.0066 | 0.0360±0.0051 | 0.0379±0.0064 | 0.0347±0.0038 | 0.0347±0.0068 |
| Adrenal gland (Rt.) | 0.0271±0.0042 | 0.0280±0.0071 | 0.0271±0.0043 | 0.0296±0.0068 | 0.0340±0.0043 | 0.0346±0.0068 | 0.0323±0.0034 | 0.0347±0.0045 |
| Kidney (Lt.) | 1.3224±0.1381 | 1.3629±0.0630 | 1.2735±0.1607 | 1.3197±0.0903 | 0.7988±0.0742 | 0.8342±0.1137 | 0.8286±0.1092 | 0.8479±0.0855 |
| Kidney (Rt.) | 1.3327±0.1375 | 1.3729±0.0782 | 1.2970±0.1316 | 1.3602±0.0791 | 0.8140±0.0816 | 0.8830±0.0990 | 0.8469±0.0900 | 0.8637±0.0992 |
| Heart | 1.3507±0.0584 | 1.3055±0.1666 | 1.3044±0.1638 | 1.2973±0.1149 | 0.8464±0.0871 | 0.8482±0.0743 | 0.8325±0.0976 | 0.8244±0.1008 |
| Lung | 1.5951±0.1743 | 1.5659±0.1249 | 1.5968±0.2277 | 1.5764±0.2138 | 1.1736±0.1355 | 1.1729±0.1070 | 1.1910±0.0941 | 1.2375±0.1787 |
| Brain | 2.0347±0.0667 | 2.0125±0.0552 | 2.0047±0.0521 | 2.0913±0.1135 | 1.9110±0.0804 | 1.9307±0.0753 | 1.9189±0.0585 | 1.9166±0.0931 |
| Pituitary | 0.0106±0.0024 | 0.0108±0.0016 | 0.0101±0.0012 | 0.0103±0.0031 | 0.0138±0.0021 | 0.0129±0.0031 | 0.0126±0.0022 | 0.0156±0.0066 |
| Thymus | 0.4462±0.0991 | 0.4503±0.0823 | 0.4214±0.0733 | 0.4774±0.1348 | 0.4260±0.1122 | 0.4292±0.0868 | 0.4750±0.0919 | 0.4125±0.1018 |

Mean±SD (n=10 per each groups). Mean±S.D. Lt: Left, Rt: Right.
** : Significant difference compared to the control group, $p<0.01$

**Table 6-2.** Relative organ weights of the male and female rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF RELATIVE ORGAN WEIGHTS (% Body weight) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | SEX : MALE | | | | SEX : FEMALE | | | |
| TEST ITEM | GROUP(mg/kg/d) | | | | GROUP(mg/kg/d) | | | |
| (Unit) | 0 | 250 | 500 | 1,000 | 0 | 250 | 500 | 1,000 |
| Testis/Ovary (Lt.) | 0.4577±0.0387 | 0.4830±0.0593 | 0.4932±0.0756 | 0.4687±0.0412 | 0.0184±0.0035 | 0.0194±0.0039 | 0.0175±0.0024 | 0.0194±0.0039 |
| Testis/Ovary (Rt.) | 0.4612±0.0360 | 0.4866±0.0584 | 0.4869±0.0696 | 0.4726±0.0380 | 0.0184±0.0041 | 0.0186±0.0042 | 0.0191±0.0035 | 0.0188±0.0030 |
| Prostate/Uterus | 0.1623±0.0234 | 0.1773±0.0320 | 0.1602±0.0207 | 0.1345**±0.0210 | 0.2079±0.0790 | 0.1966±0.0238 | 0.2194±0.0905 | 0.2102±0.0632 |
| Spleen | 0.2324±0.0369 | 0.2182±0.0182 | 0.2195±0.0369 | 0.2110±0.0256 | 0.2304±0.0166 | 0.2228±0.0244 | 0.2820* ±0.0790 | 0.2332±0.0271 |
| Liver | 3.0542±0.2788 | 2.9264±0.2063 | 2.8681±0.1036 | 3.0015±0.1328 | 2.9456±0.1563 | 2.9451±0.1628 | 2.9246±0.1301 | 2.8800±0.1503 |
| Adrenal gland (Lt.) | 0.0076±0.0010 | 0.0082±0.0015 | 0.0088±0.0012 | 0.0085±0.0019 | 0.0163±0.0022 | 0.0169±0.0021 | 0.0159±0.0024 | 0.0157±0.0021 |
| Adrenal gland (Rt.) | 0.0074±0.0011 | 0.0078±0.0015 | 0.0078±0.0012 | 0.0083±0.0019 | 0.0154±0.0019 | 0.0154±0.0021 | 0.0148±0.0017 | 0.0158±0.0021 |
| Kidney (Lt.) | 0.3631±0.0379 | 0.3819±0.0253 | 0.3691±0.0508 | 0.3686±0.0225 | 0.3609±0.0264 | 0.3720±0.0332 | 0.3760±0.0272 | 0.3854±0.0230 |
| Kidney (Rt.) | 0.3660±0.0384 | 0.3846±0.0281 | 0.3756±0.0402 | 0.3799±0.0170 | 0.3673±0.0214 | 0.3943±0.0285 | 0.3849±0.0249 | 0.3922±0.0290 |
| Heart | 0.3711±0.0229 | 0.3656±0.0486 | 0.3789±0.0569 | 0.3624±0.0292 | 0.3820±0.0249 | 0.3792±0.0182 | 0.3778±0.0189 | 0.3737±0.0215 |
| Lung | 0.4377±0.0442 | 0.4382±0.0336 | 0.4617±0.0612 | 0.4397±0.0529 | 0.5313±0.0646 | 0.5256±0.0481 | 0.5435±0.0448 | 0.5641±0.0840 |
| Brain | 0.5597±0.0424 | 0.5645±0.0402 | 0.5814±0.0392 | 0.5850±0.0424 | 0.8656±0.0510 | 0.8663±0.0602 | 0.8784±0.0808 | 0.8777±0.0989 |
| Pituitary | 0.0029±0.0006 | 0.0030±0.0003 | 0.0029±0.0003 | 0.0029±0.0008 | 0.0063±0.0009 | 0.0057±0.0011 | 0.0057±0.0007 | 0.0071±0.0030 |
| Thymus | 0.1221±0.0247 | 0.1252±0.0181 | 0.1220±0.0219 | 0.1327±0.0347 | 0.1913±0.0424 | 0.1912±0.0311 | 0.2177±0.0484 | 0.1866±0.0401 |

Mean±SD (n=10 per each groups). Mean±S.D. Lt: Left, Rt: Right.
*: Significant difference compared to the control group, $p<0.05$, **: Significant difference compared to the control group, $p<0.01$



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**Table 7-1**. Histopathological findings in the male rats in the 4 wk repeated oral dose toxicity study

| SUMMARY OF SERUM BIOCHEMICAL TESTS | | | | | |
|---|---|---|---|---|---|
| | | | | | SEX : MALE |
| Organs | Sings | GROUP( mg/kg/d ) | | | |
| | | 0 | | 1,000 | |
| | | N | % | N | % |
| Liver | No remarkable lesions | 10/10 | 100 | 9/10 | 90 |
| | Remarkable lesions | 0/10 | 0 | 1/10 | 10 |
| | -Cell infiltration, mononuclear, focal | ±0/10 | 0 | 1/10 | 10 |
| Kidney | No remarkable lesions | 9/10 | 90 | 7/10 | 30 |
| | Remarkable lesions | 1/10 | 10 | 3/10 | 30 |
| | -Cyst, inner stripe | +1/10 | 10 | 0/10 | 0 |
| | -Dilatation, tubule, inner/outer stripe | +1/10 | 10 | 1/10 | 10 |
| | -Scar, with focal inflammation, cortex | ±0/10 | 0 | 1/10 | 10 |
| | -Regeneration, tubule, with focal inflammation, cortex | ±0/10 | 0 | 1/10 | 10 |
| Lung | No remarkable lesions | 10/10 | 100 | 9/10 | 90 |
| | Remarkable lesions | 0/10 | 0 | 1/10 | 10 |
| | -Focal inflammation, granulomatous | ±0/10 | 0 | 1/10 | 10 |
| Epididymis | No remarkable lesions | 9/10 | 90 | 10/10 | 100 |
| | Remarkable lesions | 1/10 | 10 | 0/10 | 0 |
| | -Spermatogranuloma | +1/10 | 10 | 0/10 | 0 |
| Prostate | No remarkable lesions | 9/10 | 90 | 10/10 | 100 |
| | Remarkable lesions | 1/10 | 10 | 0/10 | 0 |
| | -Cell infiltration, mononuclear, interstitial | ±1/10 | 10 | 0/10 | 0 |

N: Number of animals with the signs / Number of examined animals, +: Mild, ±: Minimal

## Discussion

People worldwide mostly in Africa, Asia, and Latin America have been traditionally consuming about 2,000 species of insects (Premalatha *et al*., 2011). In current, edible insect products have been produced in North American or European countries (Hamerman, 2015). In 2015, KMFDS approved crickets as temporary food ingredient (no. 2015-09). Also, it is the one of seven eatable insect species including beetles, mealworms and protaetia brevitarsis larvae. Especially, crickets were widely consumed as not only a feeding a pet or zoo animals, but also a human food (Ahn *et al*., 2000). With respect to health, the environment, and livelihood, the cricket farming is likely to have advantages over the livestock business in the future (Oonincx *et al*., 2010). Cricket rearing requires fewer rearing coast than cattle rearing, with fewer animal welfare issues, and also poses a low risk of transmitting zoonotic infections (van Huis *et al*., 2013). Due to this resource use efficiency, cricket rearing for entomophagy has the potential to become a modern and sustainable food production system.

General contents of crickets have a very different trend compared to existing traditional food (Adebowale *et al*., 2005). Although fat and protein content showed the characteristics of animal products which are relatively large portion of cricket contents as well, it is also high in polyunsaturated fatty acid, mineral, and fiber at the same time. When assessing the value in food science, this implies that cricket has a high development potential as a functional food. Chitin is an insoluble carbohydrate which is a main component constituting the outer shell of crickets. Because chitin has an effect of enhancing immunity, crickets can be used as a dietary supplement (Wang *et al*., 2004). Therefore, the cricket is expected higher utilization of chitin

**Table 7-2.** Histopathological findings in the female rats in the 13 wk repeated oral dose toxicity study.

| SUMMARY OF SERUM BIOCHEMICAL TESTS | | | | | | | |
|---|---|---|---|---|---|---|---|
| SEX : FEMALE | | | | | | | |
| Organs | Sings | GROUP(mg/kg/d) | | | | | |
| | | 0 | | 500 | | 1,000 | |
| | | N | % | N | % | N | % |
| Liver | No remarkable lesions | 9/10 | 90 | | | 10/10 | 100 |
| | Remarkable lesions | 1/10 | 10 | | | 0/10 | 0 |
| | -Cell infiltration, mononuclear, focal | +1/10 | 10 | | | 0/10 | 0 |
| Kidney | No remarkable lesions | 8/10 | 80 | | | 8/10 | 80 |
| | Remarkable lesions | 2/10 | 20 | | | 2/10 | 20 |
| | -Mineralization, outer stripe | ±2/10 | 20 | | | 2/10 | 20 |
| Spleen | No remarkable lesions | 10/10 | 100 | 0/1 | 0 | 10/10 | 100 |
| | Remarkable lesions | 0/10 | 0 | 1/1 | 100 | 0/10 | 0 |
| | -Congestion | +0/10 | 0 | 1/1 | 100 | 0/10 | 0 |
| Thyroid | No remarkable lesions | 9/10 | 90 | | | 9/10 | 90 |
| | Remarkable lesions | 1/10 | 10 | | | 1/10 | 10 |
| | - Ectopic thymus | √1/10 | 10 | | | 1/10 | 10 |
| | -Ultimobranchial cyst | √1/10 | 10 | | | 0/10 | 0 |
| Lung | No remarkable lesions | 10/10 | 100 | | | 9/10 | 90 |
| | Remarkable lesions | 0/10 | 0 | | | 1/10 | 10 |
| | - Focal inflammation, granulomatous | +0/10 | 0 | | | 1/10 | 10 |

N: Number of animals with the signs / Number of examined animals, ±: minimal, +: mild, v: present

derivatives in health supplement research in present. Cricket is high in lysine, leucine, valine, and isoleucine. These are essential amino acids which is consumed with food because it does not synthesize enough or not synthesized in human body (Belluco *et al*., 2013). In addition, it seems to be used for strengthening and development muscle of athletes, because the level of branched chain amino acids such as leucine, valine, and isoleucine is high in cricket (Ismasyahir *et al*., 2012). Crickets also contain high concentrations of fatty acids especially unsaturated fatty acids (68.6 %) such as linoleic and oleic acid (Wang *et al*., 2004; Kim and Jung, 2013). Unsaturated fatty acid is known as functioning of lowering blood cholesterol level (Sampath and Ntambi, 2005). Since there is relationship between a high fat content of crickets, crickets in particular contain high content of vitamin D and E (Finke, 2002). Vitamin D is recommended to consume with high Ca content food due to absorption of calcium from the stomach and functioning of calcium in the body (Ross, 2001). Vitamin E plays a key role in antioxidant (Jiang, 2014). In this aspect, it is highly advantageous to use cricket as a food source.

Recent studies have supported that crickets can be used for medicinal food and health supplement. Ahn *et al.* reported that *G. bimaculatus* extract containing glycosaminoglycan had a therapeutic potential for inflammatory disease, chronic arthritis in rats (Ahn *et al*., 2014). In 2015, Ahn *et al.* reported that cricket extract was potentially effective in anti-aging while reducing creatinine phosphokinase level in blood serum of aged rats (Ahn *et al*., 2015a). In addition, cricket ethanol extract inhibited adipose tissue accumulation in high phosphate dieted Wister rats. When high fat dieted rats were administered cricket ethanol extract for 1 month, fasin-related fatty acid synthesis and adipose differentiation related protein were upregulated. It is indicated that cricket can be used as an anti-atherosclerosis or inflammation medicine (Ahn *et al*., 2015b).

To enhance the utilization of cricket as a food material, quality improvement is being made in accordance with the various processing methods (FAO, 2011). In recent study, Ahn *et al*. reported the quality characteristics such as the microbial and nutrient content of crickets under different processing conditions

(Kim *et al*., 2015). They aimed development of functional food or functional product by extracting a specific component out and increasing food quality through optimizing processing of cricket. Previously, safety evaluation of cricket powder in phosphate-buffered saline was conducted. NOAEL of cricket powder dissolved in PBS was higher than 5,000 mg/kg/d in rats of 13 wk oral dose toxicity study and there was no mutagenic effect in genotoxic evaluation (Ahn *et al*., 2005; Ahn *et al*., 2011). According to the increased interest in the edible insect, quality change in processing method of insects was emphasized. Safety evaluation of the insects under different processing method should be followed to tighten the food safety regulation. There is a need to evaluate the bio-accessibility of the nutrients and the safety of bioactive compounds with regard to human consumption.

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