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

Somin Lee,†, Kyu Sup Ahn,‡, Hyeon Yeol Ryu, Hye Jin Kim, Jin Kyu Lee, Myung-Haing Cho, Mi Young Ahn and Kyung Seuk Song

†Toxicity Evaluation Center, Korea conformity laboratories (KCL) 406-840, Incheon, South Korea
‡Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

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.

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...
resource is ongoing (Belluco et al., 2015). In 2015, cricket has been newly approved as a temporal food ingredient with mealworm beetle, and protaetia brevitaris 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.