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Safety assessment of freeze-dried powdered *Tenebrio molitor* larvae (yellow mealworm) as novel food source: Evaluation of 90-day toxicity in Sprague-Dawley rats

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ABSTRACT

Worldwide demand for novel food source has grown and edible insects are a promising food sources for humans. *Tenebrio molitor*, as known as yellow mealworm, has advantages of being rich in protein, and easy to raise as a novel food source. The objective of this study was to evaluate subchronic toxicity, including potential hypersensitivity, of freeze-dried powdered *T. molitor* larvae (fdTML) in male and female Sprague-Dawley rats. The fdTML was administered orally once daily at dose levels of 0, 300, 1000 and 3000 mg/kg/day for 90 days. A toxicological assessment was performed, which included mortality, clinical signs, body and organ weights, food consumption, ophthalmology, urinalysis, hematology, serum chemistry, gross findings, histopathologic examination and allergic reaction. There were no fdTML-related findings in clinical signs, urinalysis, hematology and serum chemistry, gross examination, histopathologic examination or allergic reaction. In conclusion, the No Observed Adverse Effect Level (NOAEL) for fdTML was determined to be in excess of 3000 mg/kg/day in both sexes of rats under the experimental conditions of this study.

1. Introduction

Insects are a traditional food for humans and animals, especially in Asia, Africa, the Americas and Australia (Siemianowska et al., 2013). Demand for novel sources of food, in particular protein, is growing with the increase in the world population. From efficiency and environmental perspectives, there are many benefits to using insects as food. They are a more sustainable food source and alternative to animal protein (Oonincx et al., 2010; Yen, 2009). Insects have higher feed conversion efficiencies than homeothermic animals, because they are poikilotherms (Oonincx et al., 2010). Furthermore, insects are rich in protein and beneficial fatty acids (Heinrich and Prieto, 2008). The issue of edible insects has been discussed actively by the Food and Agriculture Organization of the United Nations (FAO) since 2003(Huis et al., 2013).

Tenebrio molitor larvae (yellow mealworm) are used widely as animal feed for reptiles, birds and monkeys (Huis et al., 2013; Jones et al., 1972; Ng et al., 2001). There have been many attempts to investigate *T. molitor* as food, as it is a commonly bred species worldwide (Bednářová et al., 2013; Mlcek et al., 2014), has a short life cycle and is easy to handle and raise (Ghaly and Alkoaik, 2009). Furthermore, several studies have shown that the nutritional content and composition of *T. molitor* is adequate for use as food (Ghaly and Alkoaik, 2009; Ravzanaadii et al., 2012; Yi et al., 2013; Yoo et al., 2013).

As a novel food, the nutritional value of *T. molitor* and its larva has been established (Ghaly and Alkoaik, 2009; Ravzanaadii et al., 2012; Yi et al., 2013; Yoo et al., 2013) and the genotoxicity and subchronic toxicity for 28 days has been assessed (Han et al., 2014); however, its long-term safety, including potential hypersensitivity for human consumption, has not been evaluated. The potential detrimental effects from eating *T. molitor* include microbial and chemical hazards, allergy and toxicity (Belluco et al., 2013; Mlcek et al., 2014). Moreover, it is possible to develop allergic sensitivity through long-term exposure in people with a history of atopy

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(allergic hypersensitivity) (Huis et al., 2013). This study examined potential hypersensitivity and subchronic toxicities in Sprague-Dawley (SD) rats administered freeze-dried powdered *T. molitor* larvae (fdTML) for 90 days. The study was performed in compliance with the Good Laboratory Practice (GLP) guidelines of the Organization for Economic Cooperation and Development (OECD, 1997).

2. Materials and methods

2.1. Preparation of freeze-dried powdered Tenebrio molitor larvae

T. molitor larvae were purchased from Sworm Farm (Cheonan, Chungcheongnam-do, Republic of Korea) and ground into a powder after a freeze-drying and sterilized using autoclave at 115 °C for 10 min by Worldway (Yeongi, Republic of Korea). The fdTML were examined for food poisoning pathogen contamination by assessing *Escherichia coli* O157:H7 and *Salmonella* spp. and for heavy metal content including Pb, Hg, As and Cd. They were found to be safe from food poisoning pathogens and Pb, As and Cd were undetectable (Yoo et al., 2013). Furthermore, the Hg concentration was 0.03 mg/kg, a concentration lower than the standard index for food (Yoo et al., 2013).

The general components were measured using the official analytical methods of the Association of Official Analytical Chemists and the marker compound was measured using gas chromatography (GC) (GC-2010 Plus, Shimadzu, Japan). Oleic acid comprised the highest proportion of fatty acids (51.4%) (Yoo et al., 2013); therefore, oleic acid was selected as the representative marker for the fdTML analysis. The nutritional components of *T. molitor* larvae are shown in Table 1.

2.2. Formulation and analysis of fdTML

For oral administration via gavage, fdTML was measured and suspended in distilled water at the concentration for the highest dose group. This suspension was diluted to prepare the lower doses.

Before administration, the fdTML analysis using GC was validated by the Korea Institute of Toxicology (KIT) (not published). The validation study included specificity, system suitability, linearity, calibration curve reproducibility, accuracy, precision, homogeneity and stability. The formulated fdTML was analyzed at the start (Day 1) and end (Week 13) of dosing for homogeneity and content.

Table 1
The nutritional components of *Tenebrio molitor* larvae.

General components	Contents (g/100 g)
Total carbohydrate	10.28
Protein	48.26
Fat	35.81
Moisture	2.47
Ash	3.17
Trans fat	0.11
Cholesterol	97.81
Saturated fat	8.80
Dietary fiber	5.89
Vitamin	**Contents (mg/100 g)**
Vitamin A	–[a]
Vitamin C	–
Vitamin D	–
Vitamin E (Tocopherol)	–
Vitamin B6 (Pyridoxine)	–
Vitamin B3 (Niacin)	7.83
Vitamin B5 (Pantothenic acid)	2.56
Mineral	**Contents (mg/kg or mg/100 g)**
Copper (Cu)	7.83/kg
Magnesium (Mg)	2388.00/kg
Manganese (Mn)	9.16/kg
Phosphorus (P)	680.80/100 g
Zinc (Zn)	106.70/kg
Iron (Fe)	4.65/100 g
Calcium (Ca)	37.02/100 g
Potassium (K)	656.90/100 g

[a] Not detected.

2.3. Experimental animals

This study was performed using male and female specific pathogen-free SD rats obtained from Orient Bio Co. (Seongnam-si, Republic of Korea) at 5 weeks of age. The animals were acclimatized for 7 days and healthy animals were selected for the study. Then, 50 male and 50 female rats were assigned randomly to four groups, one vehicle control and three treatment groups, using the Path/Tox system (ver. 4.2.2. Xybion Medical Systems Corp., Cedar Knolls, NJ, USA). A total of 10 male and 10 female rats were assigned to controls and the highest dose group served as recovery animals. Each group consisted of 15 (including recovery animals) or 10 rats of each sex. At the start of dosing, body weight ranged from 177.6 to 212.3 g [coefficient variation; CV (%) = 5.04] for males and 142.5 to 167.2 g [CV (%) = 4.30] for females.

Two or three animals were housed per stainless steel cage throughout the study period. Sterilized tap water and pellet food for rodents (PMI Nutrition International, Richmond, IN, USA) were provided *ad libitum*. The animal room was maintained at a temperature of 22 ± 3 °C, a relative humidity of ~30–70%, air ventilation of 10–20 times/h and light intensity of 150–300 lux with 12-h light–dark cycles. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the KIT and conducted in compliance with the Testing Guidelines for Safety Evaluation of Drugs (Notification No. 2012-86 issued by Korea Food and Drug Administration on August 24, 2012) and OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No. 408, Repeated Dose 90-Day Oral Toxicity Study in Rodents (September 21, 1998).

2.4. Treatment and toxicity assessments

In a previous oral single dose and 28-day repeated dose study doses up to 3000 mg/kg/day were well tolerated in both sexes of SD rats (Han et al., 2014). Therefore, doses of 0 (vehicle control), 300, 1000 and 3000 mg/kg/day were selected for this 90-day repeated dose study, and general and pathology observations were performed similar to the previous study (Han et al., 2014). The dosing volume, 10 mL/kg, was based on the most recent body weight and all measurements and examination records were calculated or collected using the Path/Tox system. Animal condition and behavior were checked once daily throughout the acclimation and recovery periods. Clinical observations were recorded twice daily, before and after dosing, during the treatment period and once during the recovery period and on the day of necropsy.

Animals were weighed on arrival, before the randomization, before dosing on the first day of dosing and once weekly thereafter. Final body weight was measured on the day of necropsy. Cage food consumption was recorded once during the acclimation period and once weekly during the treatment and recovery periods. Individual food consumption was calculated as g/rat/day. The amount of food consumed by each rat was determined by weighing each feeder at the beginning and end of the week and dividing by the number of animals in the cage. External eye examinations were performed on all animals before dosing began. External and fundus examination of animals in the vehicle control and highest dose (3000 mg/kg/day) groups were performed with an indirect ophthalmoscope (Vantage Plus Digital, Keeler Ltd., London, UK) in the last week of