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Evaluation of acute toxicity of *Ducetia japonica* on mice model

Pura Yashung*, Jharna Chakravorty

Biochemical Nutritional Laboratory, Department of Zoology, Rajiv Gandhi University, Doimukh- 791112, Arunachal Pradesh, India.

Keywords

Ducetia japonica
Acute toxicity
Entomophagy
Mice

Abstract

Ducetia japonica, also known as bush cricket, is an edible insect that is valued as a food source by various tribal communities in Arunachal Pradesh. Its nutrient content makes it a novel source for both human food and animal feed. However, no research has been done on its food safety or possible toxicity. The toxicological evaluation was carried out on mice for 2-weeks at four distinct dose levels of 2000, 3000, 4000, and 5000 mg/kg bodyweight, according to the criteria of the Organisation for Economic Co-operation and Development (OECD). Bodyweight and clinical indicators showed no substantial toxicological-related alterations. Furthermore, no toxicological changes in haematology were observed. When compared to a vehicle control group, serum alanine aminotransferase and creatinine levels did not differ between the experimental groups. In the liver and kidney tissues of mice, no histological and gross abnormalities were found. The relative organ weight of the treatment groups did not differ significantly from that of the vehicle control group. As a result, the LD_{50} value for *Ducetia japonica* is considered to be greater than 5000 mg/kg body weight and no evidence of toxic changes were observed in the present study.

1 Introduction

According to numerous sources, feeding the entire population, which is expected to reach 9 billion by 2050, will be extremely tough [1]. Therefore, researchers are trying to explore other food sources for alternative food supplies, which are nutritious, eco-friendly, and take less space to farm. As a result, insects get the most enthusiastic response [2]. Entomophagy is the consumption of insects as food [3,4,5] and it has been mentioned in Christian, Islamic, and Jewish literature for quite some time [6]. Entomophagy can also be proven through archeological findings [7], which reveal that it has been practiced for millions of years [8]. They are consumed for a variety of reasons, including cultural significance, taste, and seasonal availability. They have also been shown to be high in energy, protein, mono and polyunsaturated fatty acids, as well as trace elements such as biotin, folic acid, pantothenic acid, and riboflavin [9,10]. Furthermore, studies have shown that edible insects have the potential antioxidant property to combat the effects of oxidative stress [11,12,13,14] and are also proven to have antimicrobial properties [15]. *Ducetia japonica*, commonly known as bush cricket, is a member of the Tettigoniidae family that can be found in East Asia, South Asia, and Southeast Asia [16]. Arunachal Pradesh is one of the Indian states that fall under the South Asian countries where *Ducetia japonica* can be found eaten whenever it is seasonally available. According to studies, *Ducetia japonica* is nutritionally dense, containing 56% protein, 14.99% fat, 11.84% fiber, 4.59% ash, and 11.84% carbohydrates. It can also deliver the daily recommended dose of amino acids, including essential amino acids while also containing 22.77% MUFA and 18.93% PUFA [17]. According to the studies on the nutrient content of *Ducetia japonica*, it can be considered a novel food source. Hence, the current research is being carried out to determine whether it is harmful at a specific level.

2 Materials and Methods

2.1 Sample Collection

From December to January, the adult of *Ducetia japonica* was handpicked from various locations on and around the campus, including the botanical garden, rice fields, trees, and shrubs, in Doimukh, Papum pare District, Arunachal Pradesh, India.

2.2 Sample Preparation

The insect sample was brought to the laboratory for further processing after it was collected. The insects were thoroughly cleaned with distilled water and then pat dried with a paper towel. After that, the samples were dried for

*Corresponding Author: Pura Yashung 0000-0001-8505-6203
E-mail: purayashung78@gmail.com
Phone No.: +918825304721

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72 hours at 50-60°C in an oven. After drying, the sample was milled into powder and stored in a deep freezer at −20°C for subsequent examination.

2.3 Animal and Maintenance

Three-week-old healthy female albino mice (n = 25) were housed in a polypropylene cage under light (12hr light: 12hr dark) and temperature (25°C) control conditions. The animals were given regular laboratory food and free access to water. The animals were acquired from the Department of Zoology, Rajiv Gandhi University, Animal facility. The experiment followed the National Institutes of Health's (NIH) guideline for the care and use of animal models (NIH, 1985). The institutional animal ethical committee (No. RGU/IAEC/20/03) reviewed and approved all of the experimental procedures.

2.4 Experimental Design

Healthy 12-weeks old female mice were randomly assigned to five groups (n = 5 mice per group). The method complies with the OECD Test Guideline 423 for acute dose toxicity [18]. The study was conducted in accordance with Good Laboratory Practice (GLP) guidelines. Mice were given distilled water as a vehicle and graded dosages of *Ducetia japonica* aqueous extract (2000, 3000, 4000, 5000 mg/kg body weight) via oral gavage once a day for two weeks until the completion of the experiment, the mice were monitored daily for clinical symptoms such as general appearance, mortality, and behavioural abnormality. Throughout the study, the body weight was recorded every week.

2.5 Blood Analysis

Before dissection, all of the mice have fasted overnight and 1-2ml of blood was obtained by cardiac puncture under diethyl ether anaesthesia. An automatic haematology analyzer, Mythic^{TM} 18 Vet (*Orphee*), Germany, was used to examine haematological parameters such as Red Blood Cell Count (RBC), White Blood Count (WBC), haemoglobin, different leukocyte count, and platelets. The biochemical parameters such as ALT and creatinine levels were determined by using commercial kits Reitman and Frankel, 1975 and Coral system, Goa, India respectively.

2.6 Histopathology and Organ Weight

At the end of the experiment, the mice were anaesthetized with diethyl ether and sacrificed, a complete necropsy was performed on all animals, followed by a gross inspection. The brain, liver, lung, kidney, and spleen were immediately removed and weighed. The tissue of the liver and kidney was fixed overnight in Bouin's solution. The organs were then transferred to 70% alcohol and dehydrated by passing through ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax after that sectioned at 5µ using a Leica RM2125 RTS rotator microtome. The section was stained with haematoxylin and eosin, then photographed and examined under Leica DM500 B (Leica Microsystems, Germany) microscope.

2.7 Statistical Analysis

The mean ± standard deviation was used to express all of the data. One-way analysis of variance (ANOVA) is used in the statistical analysis. The statistical analysis was performed using IBM SPSS Statistics v20. The statistically significant result was denoted by $p < 0.05$.

3 Result and Discussion

People have been eating insects since the dawn of time, not only to overcome hunger but also for their nutritional and medicinal benefits [19,20]. Insects are being considered as an alternative food source among all the other non-conventional food resources due to factors such as global warming and population growth [21]. Insects are sold and consumed as delicacies on the streets and at edible insects markets in various Southeast Asian countries. As a result, concerns about food safety and the potential toxicity of edible insects have arisen. Thus, toxicological analysis of the edible insect *Ducetia japonica* was conducted to assess its food safety. Throughout the 14-days oral dose toxicity study, there were no signs of clinical harm (Table 1). Also, no mortality was recorded during the study period (Table 2). The animals were euthanized after the 14-days experiment period, and their organs were inspected for gross lesions such as a change in colour, shape, and swelling. No gross lesions were found throughout the observation (Table 3). During the first week of the experiment, all the treatment groups, including the control group, lost weight. This could be because the animals in the study were not accustomed to the oral mode of dosing, resulting in stress-related bodyweight loss. However, the following week, all of the treatment groups gained weight, resulting in significant ($p < 0.05$) bodyweight change among the treatment groups compared to the vehicle control group (Fig. 1). Comparing the haematological test result of the dosed group with the vehicle control group showed no significant difference between them (Table 4).

Table 1: Clinical signs in female mice within 2-weeks of oral dosing of *Ducetia jopanica* extract.

Days of oral treatment	Groups (mg/kg/day)				
	0	2000	3000	4000	5000
1	0/5	0/5	0/5	0/5	0/5
7	0/5	0/5	0/5	0/5	0/5
14	0/5	0/5	0/5	0/5	0/5

*Number of deceased mice/Total mice.

Table 2: Case of mortality in female mice within 2-weeks of oral dosing of *Ducetia jopanica* extract.

Days of oral treatment	Group (mg/kg/day)				
	0	2000	3000	4000	5000
1	0/5	0/5	0/5	0/5	0/5
7	0/5	0/5	0/5	0/5	0/5
14	0/5	0/5	0/5	0/5	0/5

*Number of deceased mice/Total mice.

Table 3: Gross Finding in female mice after 2-weeks of oral dosing of *Ducetia japonica* extract.

Group (mg/kg/day)	Gross finding	Frequency
0	No gross findings	5/5
2000	No gross findings	5/5
3000	No gross findings	5/5
4000	No gross findings	5/5
5000	No gross findings	5/5

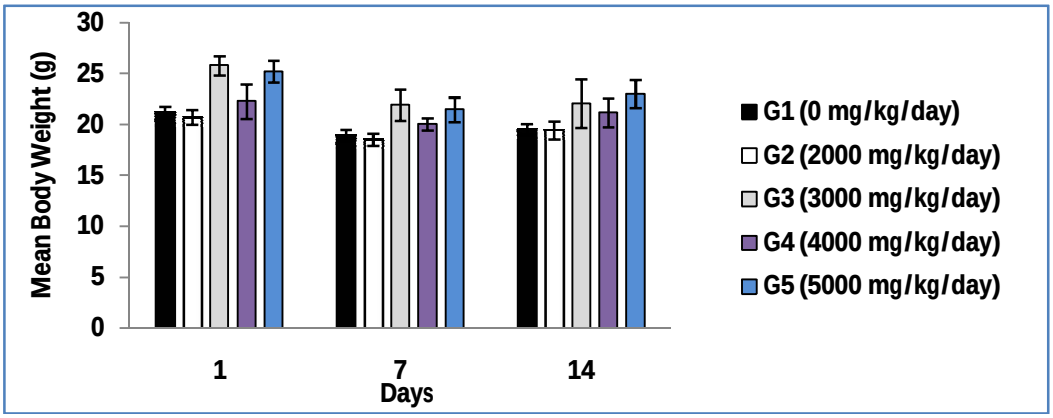

Fig. 1: Mean body weight of mice administered with *Ducetia japonica* extract for 2-weeks.

Table 4: Haematological data of mice orally administered with *Ducetia japonica* extract for 2-weeks.

Parameters	0mg/kg/day	2000mg/kg/day	3000mg/kg/day	4000mg/kg/day	5000mg/kg/day
RBC (10^6/µl)	7.56 ± 0.28	7.39 ± 0.15	7.32 ± 0.29	7.33 ± 0.15	7.44 ± 0.24
HGB (g/dl)	14.05 ± 0.3	13.9 ± 0.3	14.2 ± 0.3	13.7 ± 0.2	14.2 ± 0.3
HCT (%)	39.5 ± 0.3	39.7 ± 0.08	39.75 ± 0.3	39.5 ± 0.1	39.5 ± 0.2
MCH (pg)	17.5 ± 0.31	17.1 ± 0.29	17.3 ± 0.54	17.0 ± 0.32	17.4 ± 0.38
PLT (10^3/µl)	720.5 ± 3.5	713.8 ± 2. 1	718.3 ± 7.4	715 ± 6.2	717.6 ± 1.3
WBC (10^3/µl)	3.07 ± 0.36	3.03 ± 0.37	3.26 ± 0.22	3.17 ± 0.35	2.98 ± 0.29
LYM (%)	77.5 ± 0.4	76.4 ± 1.1	77.3 ± 0.1	77.4 ± 0.1	77.7 ± 0.1
NEU (%)	13.2 ± 0.3	13.6 ± 0.8	13.0 ± 0.5	14.0 ± 0.3	14.5 ± 0.5
MON (%)	2.30 ± 0.1	2.16 ± 0.08	2.28 ± 0.2	2.72 ± 0.1	2.69 ± 0.1

Each value represent the mean ± standard deviation (n = 5). RBC - Red blood cell; HGB - Hemoglobin; HCT - Hematocrit; MCH - Mean corpuscular hemoglobin; PLT - Platelet; WBC - White blood cell; LYM - Lymphocyte; NEU - Neutrophil; MON - Monocyte.

To assess the liver and renal toxicity of *Ducetia japonica* aqueous extract, serum ALT and creatinine levels was measured. The serum ALT and creatinine levels did not differ significantly between the study groups (Table 5). The activity of the blood toxicity marker enzymes ALT and creatinine in the liver and kidney respectively was not aided by the administration of *Ducetia japonica* aqueous extract. Hence, it was confirmed that the liver and kidney of all the animals in the dosed groups were functioning normally. When the weight of organs such as the brain, lung, liver, spleen, and kidney of dosed groups was compared to the weight of the vehicle control group, no significant ($p > 0.05$) variations in organ weight were found between the vehicle control and treatment groups (Table 6). Furthermore, microscopic observations of liver and kidney tissues in all the treatment groups were compared to the vehicle control group, no significant differences were observed (Fig. 2 & 3).

No significant toxicological alterations were seen in the 2-weeks oral dosage study, based on the findings of the parameters used in the investigation. As a result, the aqueous extract of *Ducetia japonica* caused no toxicity in mice, and the lethal dose was higher than 5000 mg/kg body weight.

Table 5. Serum ALT and creatinine value of mice orally administered with *Ducetia japonica* extract for 2-weeks.

Parameters	0mg/kg/day	2000mg/kg/day	3000mg/kg/day	4000mg/kg/day	5000mg/kg/day
ALT (IU/L)	11.4 ± 0.39	11.3 ± 0.38	11.5 ± 0.24	11.4 ± 0.21	11.56 ± 0.10
CREA (mg/dL)	0.72 ± 0.1	0.7 ± 0.14	0.78± 0.08	7.2 ± 0.13	0.8 ± 0.08

Each value represents the mean ± standard deviation (n = 5). ALT - Alanine aminotransferase; CREA - Creatinine.

Table 6. Organ weight of mice orally administered with *Ducetia japonica* extract for 2-weeks.

Parameters	Organ Weight (g)				
	0mg/kg/day	2000mg/kg/day	3000mg/kg/day	4000mg/kg/day	5000mg/kg/day
Liver (g)	0.9531 ± 0.0152	0.9246 ± 0.0195	0.9381 ± 0.0237	0.9278 ± 0.0224	0.9516 ± 0.0304
Kidney (g)	0.2038 ± 0.0210	0.1937 ± 0.0032	0.2074 ± 0.0097	0.2062 ± 0.0050	0.2116 ± 0.0199
Lung (g)	0.1532 ± 0.0033	0.1539 ± 0.0043	1.6315 ± 0.0433	0.1525 ± 0.0037	1.5856 ± 0.0287
Spleen (g)	0.0221 ± 0.0013	0.0231 ± 0.0021	0.0300 ± 0.0017	0.0313 ± 0.0033	0.0297 ± 0.0035
Brain (g)	0.4036 ± 0.0076	0.4177 ± 0.0198	0.409 ± 0.0340	0.3999 ± 0.0311	0.4554 ± 0.0246

Each value represents the mean ± standard deviation (n = 5).

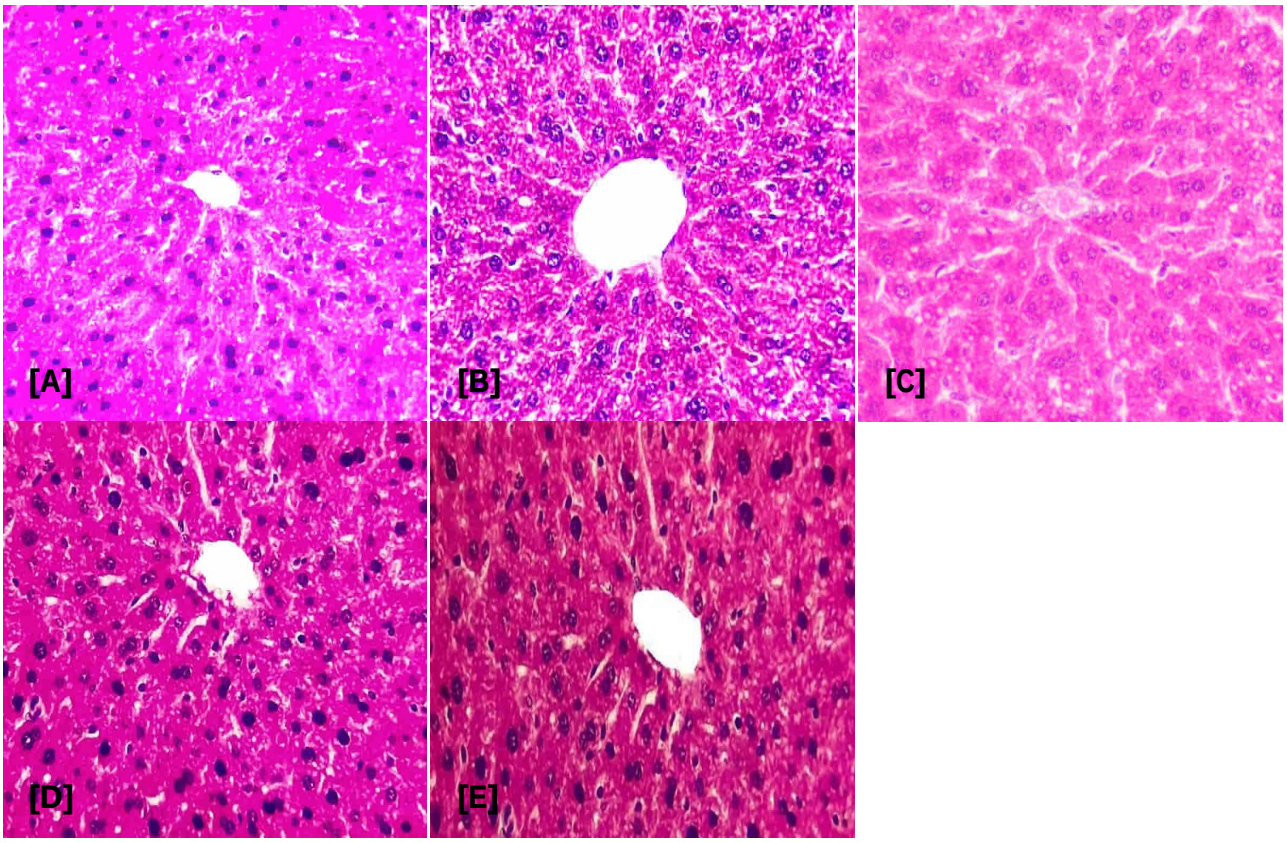

Fig. 2: Selected micrographs of liver from mice administered with *Ducetia japonica* extract for 2-weeks. (A) Vehicle Control liver, (B) Liver of 2000 mg/kg body weight, (C) Liver of 3000 mg/kg body weight, (D) Liver of 4000 mg/kg body weight, and (E) Liver of 5000 mg/kg body weight. Hematoxylin and Eosin stain; magnification 40X; Leica DM5000 B.

4 Conclusion

Based on the results of a 2-weeks oral dose acute toxicity study of *Ducetia japonica* aqueous extract, we found that no-observed-adverse-effect level (NOAEL) of aqueous extract of *Ducetia japonica* in female mice after treatment with four distinct dose levels of 2000, 3000, 4000, and 5000 mg/kg bodyweight. The highest dose administered was 5000 mg/kg/day. In conclusion, based on the current trial setting and conditions, *Ducetia japonica* is considered safe as human food and animal feed.

Conflicts of interest

The author declares that there is no conflict of interest.

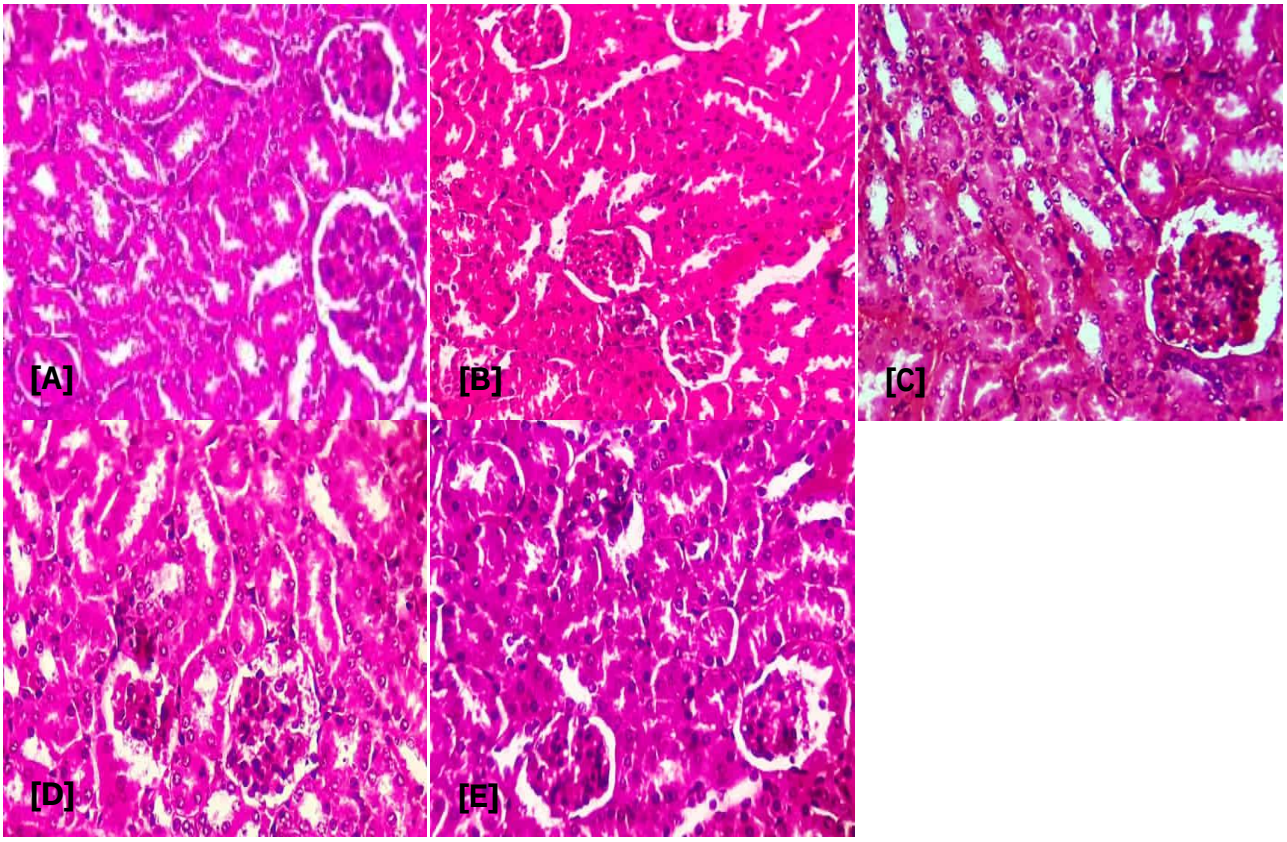

Fig. 3: Selected microphotographs of kidney from mice administered with *Ducetia japonica* extract for 2- weeks. (A) Vehicle control kidney, (B) kidney of 2000 mg/kg body weight, (C) kidney of 3000 mg/kg body weight, (D) kidney of 4000 mg/kg body weight (E), and kidney of 5000 mg/kg body weight, Hematoxylin and Eosin stain; magnification 40X; Leica DM5000 B.

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