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In vivo study of subacute oral toxicity of kelulut honey

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Abstract

Kelulut honey or also known as stingless bee honey is favoured for its tremendous nutritional benefits. However, the lack of systematic safety studies leads to it having no quality control or safety guarantee for the consumers. Consequently, the present work was designed to assess the effect of daily kelulut honey consumption. Subacute oral toxicity study was conducted following the Organization for Economic Co-operation and Development (OECD) test guideline 407. Sprague Dawley rats were administered with kelulut honey at the concentrations of 500, 1,000, and 2,000 mg/kg for four weeks, and observed for any changes or toxicity signs following daily consumption. The rats were physically and biochemically analysed, and the serum of highest honey concentration (2,000 mg/kg) consumption underwent metabolite analysis. Histopathology observations on the kidney and liver were also performed. The highest concentration of kelulut honey did not show any mortality or toxicity. Overall, there were no significant differences in all parameters, physically and biochemically, as compared to the control (distilled water), thus indicating the absence of toxicity of kelulut honey daily consumption. It was found that kelulut honey consumption demonstrated generally good health effects, such as in controlling food intake, weight gain, and increasing immune function. The honey's lethal medium dose surpassed 2,000 mg/kg, thus classified in category 5 according to the Globally Harmonized System of Classification and Labelling of Chemicals, which means that it is safe to consume at a high dose.

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Introduction

Stingless bee honey, also known as kelulut honey in Malaysia, is well-known for its high medicinal properties (Abu Bakar *et al.*, 2017). Generally, honey such as *Apis* sp. honey, contains sugars, amino acids, organic acids, vitamins, and minerals (Abu Bakar *et al.*, 2017). However, the sugars in kelulut honey are lower than that in common honey from *Apis* sp., whereas moisture and free acidity are higher (de Almeida-Muradian, 2013; Monggudal *et al*., 2018). Kelulut honey is less sticky, and has a tangy smell and distinct sour taste. Kelulut honey is produced by different stingless bee species such as *Heterotrigona itama*, *Geniotrigona thoracica*, and *Tetragonula laeviceps*. As the name implies, they have a reduced sting which makes them accessible among the beekeepers and in the honey production business which is called meliponiculture (Jaapar *et al.*, 2016).

Kelulut honey is usually stored in a propolis pot-shaped storage container known for its high

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antioxidant and anti-inflammatory properties (Abdullah *et al.*, 2019). The propolis composition of fibres, proteins, and minerals indirectly diffuse and increase the nutritional value of kelulut honey (Abdullah *et al.*, 2019). Unique preferences of stingless bees which forage on polyfloral sources in the lowest shrub contribute to this honey's high medicinal value (Jaapar *et al.*, 2016). Tiny body of stingless bees also allow them to sip nectar from the inner part of a flower, thus allowing for more nutrient content (Ab Hamid *et al.*, 2016). This condition increases phenolic acids and flavonoids in it, thus leading to high antioxidant content (Ab Hamid *et al.*, 2016). Furthermore, the watery feature enables the fermentation of osmophilic yeast carried by the bees, thus leading to the sour taste and antibacterial properties of kelulut honey (Abdullah *et al.*, 2019). The fermentation of glucose in kelulut honey produces glycolic acid and hydrogen peroxide which kills the bacteria (Yaacob *et al.*, 2018). Kelulut honey is also a good wound dresser due to these properties. The moisture in honey also helps prevent dehydration, and improves angiogenesis and collagen synthesis which further enhance wound healing (Zulkhairi Amin *et al.*, 2018), while giving aesthetic effect and reducing wound pain (Yaacob *et al.*, 2018). In honey, antibacterial properties fight bacterial infection, and antioxidant controls oxidative stress, thus accelerating wound healing (Zulkhairi Amin *et al.*, 2018).

Apart from all the benefits of kelulut honey, it is not recorded in the Codex Alimentarius Standard due to its lack of information, especially in the systematic safety study. Differences in physicochemical properties and chemical compositions as compared to *Apis* sp. honey cause it to have different quality standards, thus stressing the demand for its individual standards. Thus far, there is no proper study regarding the toxicity of kelulut honey; thus, there is no safety guarantee on its consumption. Therefore, a daily systematic safety study is crucial, and was conducted in the present work to provide information regarding consumer health. The present work also provides baseline data for further medicinal and clinical studies in the future.

Materials and methods

Chemicals and reagents

Abscisic acid (ABA), diethyl ether, sodium chloride, sodium hydroxide, Harris haematoxylin and eosin (H&E), deuterium oxide (D_2O), and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) were purchased from Sigma-Aldrich (UK). Xylene, formalin, and ethanol were purchased from Merck (Germany). Paraffin plasticised pellets were purchased from R&M (UK).

Sample collection

Malaysian kelulut honey from *H. itama* was obtained from the Kelulut Park of Malaysian Agricultural Research and Development Institute (MARDI) Centre, MAEPS, Serdang, Selangor, Malaysia. The honey was then refrigerated at 4°C away from direct sunlight in amber glass bottles.

Preparation of kelulut honey

The kelulut honey was administered orally using oral wire gavage which entered directly into the rats' stomach. Three different treatments at the doses of 500 (T1), 1,000 (T2), and 2,000 mg/kg (T3) were given based on the rats' body weight (BW). Distilled water served as the negative control (C1), whereas 0.0056 mg/kg of ABA (C2) served as the positive control. Identification and quantification of the marker compound abscisic acid in kelulut honey was performed using ultra performance liquid chromatography (UPLC) according to Fu *et al.* (2012) with modifications.

Experimental animal husbandry

The study protocol was approved by the Universiti Sultan Zainal Abidin (UniSZA) Animal and Plant Research Ethics Committee (ref. no.: UAPREC/04/041; dated 04/01/2019). The rats were obtained from the Animal Laboratory, Faculty of Bioresources and Food Industry, UniSZA. Both male and female Sprague Dawley (SD) rats were used as the test model. Both genders were used due to differences in toxicity tolerance towards substances. Thirty male rats and 30 female rats were divided into five groups with six rats ($n = 6$) in each (following the resource equation calculation) for each gender. The rats used were eight weeks old (age of young adult), and weighed from 190 - 230 g (Samat *et al.*, 2014; 2018). The rats were individually caged in standard environmental conditions under ambient temperature of 21 ± 3°C and 40 - 65% relative humidity, with a 12-h light/dark cycle, and fed with certified rodent food (Rodent Diet Specialty Feeds, Glen Forrest,

Australia) and water *ad libitum*. The animals were acclimatised a week before the beginning of the study, and individually labelled.

Subacute administration test and physical observation

The subacute oral test was performed based on the guidelines of the Organization for Economic Co-operation and Development (OECD) for the testing of chemicals, TG 407 (OECD, 2008) with modifications. This method measures the adverse effects after daily oral administration of a substance on several parameters such as general behaviour, reflexes, motor activities, respiratory patterns, and macroscopic changes on the skin and fur. From this experiment, the median lethal dose (LD_{50}) was obtained, and the value was expressed with respect to the weight of the test substance per unit weight of the test animal (mg/kg).

Initially, rats were deprived of food every 3 h before dose consumption, and water was excluded to avoid any food scrap disturbance. For the subacute toxicity study, 30 healthy male and female SD rats were used. The rats were divided into five groups of six animals (following the resource equation calculation) for each gender. The dose selected was based on a previous study which revealed the safety of the highest dose of 2,000 mg/kg in an acute study (Samat *et al.*, 2014). However, the effect of kelulut honey using this highest dose for daily consumption was still unknown. The next dose was reduced by half. Mortality and clinical toxicity signs were systematically recorded. Observations for severe effects including convulsions, tremors, diarrhoea, salivation, lethargy, sleep, and coma were also done at 0.5, 1, 2, and 4 h daily for four weeks following kelulut honey administration. Food was given after the 4 h observation period (OECD, 2008). The movement of the rats were also recorded upon dose consumption.

Body weight (BW) and meal pattern analysis

The BW of each rat was recorded weekly, and the changes in BW were recorded. The BW was calculated as the percentage of BW increment using Eq. 1 (Al-Afifi *et al.*, 2018):

$$\text{BW \%} = \frac{(\text{Body weight at the end of each week} - \text{Initial body weight})}{\text{Initial body weight}} \times 100\% \quad \text{(Eq. 1)}$$

In meal pattern analysis, the amount of food consumed was measured weekly by subtracting from the initial food supply (Samat *et al.*, 2014).

Anaesthesia of rats

The rats were anesthetised using diethyl ether on day 29 following the method by Tuffery (1995). For the rat's anaesthesia, 20 mL of 20% diethyl ether was soaked in a pad, and placed in the euthanasia chamber. The euthanasia agent was allowed to vaporise for 5 min. The rats were then individually placed in the chamber for 10 min until unconscious, as evidenced by no tickle on their whiskers, and no pain reaction when pressed on their leg palms. Then, blood was immediately drawn from the rats.

Blood collection

The rats were deprived of food overnight beforehand. The cardiac puncture was done on day 29 after the rats were confirmed to be anesthetised, blood samples of approximately 4 - 5 mL each were collected using a 5-mL syringe and 18 G needles for complete blood count (haematological test) and serum biochemical analyses.

Serum analyses by proton nuclear magnetic resonance

Rats' serum from subacute toxicity treatment 3, T3 (2,000 mg/kg kelulut honey) for both genders was analysed using a nuclear magnetic resonance (NMR) spectrometer (Bruker Avance 400 MHz, Germany) at the Institute of Marine Biotechnology, Universiti Malaysia Terengganu following the method by Lin *et al.* (2016) with modifications. Rats' serum (200 µL) was transferred into a clean vial and added with 400 µL of D_2O containing 0.1% TSP, and the mixture was vortexed until completely dissolved. Then, the mixture was transferred into an NMR glass tube until filling 3 - 4 cm of the tube, capped, and labelled. Next, the tube was inserted into the NMR spinner. A pre-saturation and the Carr-Purcell-Meiboom-Gill pulse sequence were applied to suppress water signal at 4.70 - 4.90 ppm, and broad protein resonances of the serum samples. The processed NMR spectrum was analysed using MestReNova 8.1 software (Mestrelab Research S.L, Spain) and Chenomx NMR Suite Profiler (Chenomx Inc., Canada).

Relative organ weight and histopathological observation

The five vital organs, namely heart, lung, kidney, liver, and spleen were collected from each gender to measure their percentage of relative organ weight (ROW). A gross observation was conducted on the internal organs to check for any signs of abnormality and the presence of lesions due to any effects following kelulut honey administration. The organs were carefully dissected, removed of any fats, and weighed. The ROW of each organ was obtained using Eq. 2 (Samat *et al.*, 2014):

$$\text{ROW} = \left[\frac{\text{absolute organ weight (g)}}{\text{BW of rat on sacrifice day (g)}}\right] \times 100\% \quad \text{(Eq. 2)}$$

The liver and kidney specimens were then subjected to histopathology since they play a crucial role in filtering the body wastes/metabolites. The organs were fixed 24 h in 10% neutral buffered formalin for histopathological processes. Then, the organ samples were cut and followed by dehydration using a tissue processor. The hardened specimens were embedded into moulds with paraffin wax, sectioned into 5 µm using a microtome, and stained with H&E staining. The histological procedure was adapted from Junqueira and Carneiro (2002).

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using Statistical Package for Social Sciences (SPSS) version 22.0 and student's *t*-test One-way ANOVA, followed by Tukey's *post-hoc* test. Values with a confidence level of $p < 0.05$ were considered significant.

Results

Physical analysis

Observation on the daily consumption of kelulut honey on rats showed no mortality throughout four weeks of the study period. Rats also displayed no toxicity signs such as abnormal urine or stool, changes in skin or eye colour, excessive shedding of fur, or abnormality in their behaviours for example vomiting, convulsion, and tremor. Subacute study of kelulut honey up to 2,000 mg/kg showed no mortality; thus, we could not determine the LD_{50}, which is greater than the used highest dose. Therefore, kelulut honey could be classified in category 5 or unclassified, which is the safest category in subacute oral toxicity according to the Globally Harmonized System of Classification and Labelling of Chemicals.

Body weight and total food intake

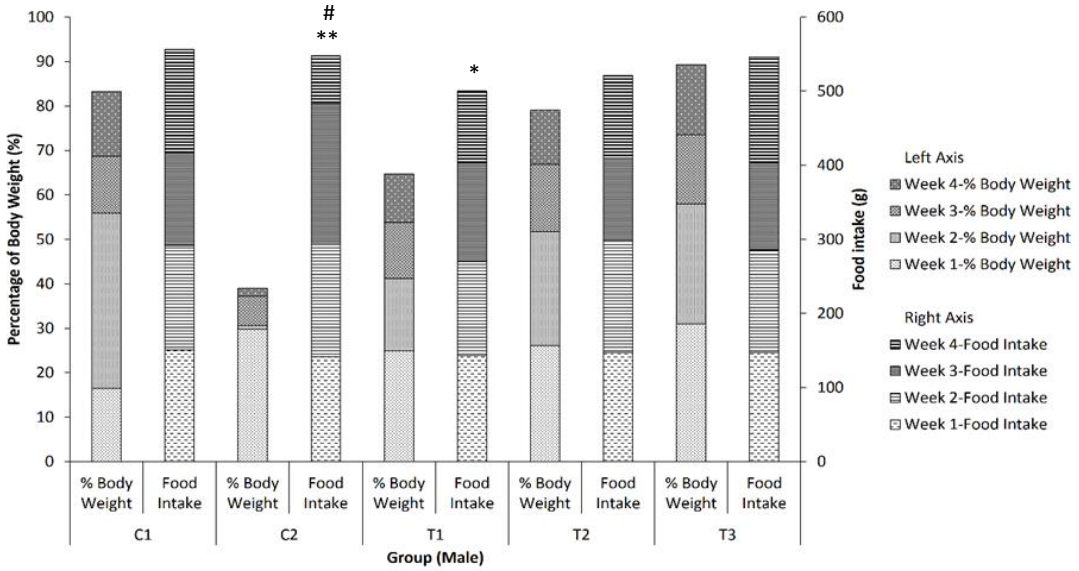
After four weeks of the study period, male rats' (Figure 1) showed a significant difference between C2 and C1 for food intake in Week 3, C2 and T1 also had a significant difference as compared to C1 in Week 4. T1 yielded the lowest total food intake (517.07 ± 13.24 g) whereas C1 yielded the highest (556.48 ± 9.69 g). Interestingly, C2 (547.72 ± 25.12 g) had relatively similar food intake with T3 (546.09 ± 5.66 g). For male percentage BW increment, C2 yielded the lowest of only 38.97 ± 14.67% for the four weeks of ABA consumption, whereas T3 the highest (89.33 ± 15.37%). We may assume that ABA consumption caused high food intake in male rats but lower BW increment. Female rats showed the lowest percentage of BW for C1 (9.74 ± 1.11%), whereas the highest was for T1 (22.00 ± 4.53%) (Figure 1). For total food intake, C2 yielded the lowest (360.17 ± 17.47 g), whereas C1 the highest (440.72 ± 24.02 g).

Haematological analysis

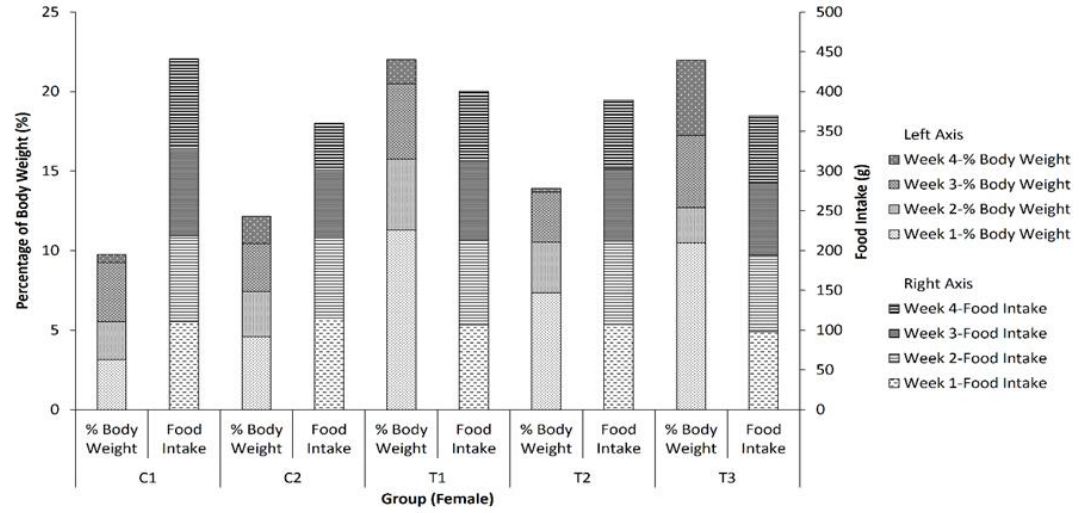
In male rats, analysis of erythrocyte (normal range = 6390 - 8010 × 10^9/L for both genders) (He *et al.*, 2017; Figure 2) showed a significant decrease in T2 (6880 ± 220 × 10^9/L) as compared to C1 (7520 ± 70 × 10^9/L), whereas in female, T1 (6800 ± 110 × 10^9/L) and T3 (6780 ± 210 × 10^9/L) significantly decreased as compared to C1 (7320 ± 310 × 10^9/L). Meanwhile, thrombocyte count (normal range, male = 638 - 1177 × 10^9/L; female = 674 - 1373 × 10^9/L) (Han *et al.*, 2010) showed a significant increase in male, T3 (1093.60 ± 83.24 × 10^9/L) as compared to C1 (848.40 ± 70.35 × 10^9/L), whereas thrombocyte count of female T1 (906.60 ± 108.21 × 10^9/L) and T2 (881.60 ± 44.74 × 10^9/L) also had a significant decrease as compared to C1 (1289.40 ± 70.19 × 10^9/L). Our data also recorded the increment of leukocytes with kelulut honey consumption (Figure 2). For instance, lymphocytes (a type of leukocytes) of group T2 (83.00 ± 1.05%) recorded the highest count in male, and T3 (80.20 ± 0.20%) in female rats.

Serum biochemical analyses

Serum urea (normal range, male = 4.32 - 8.97

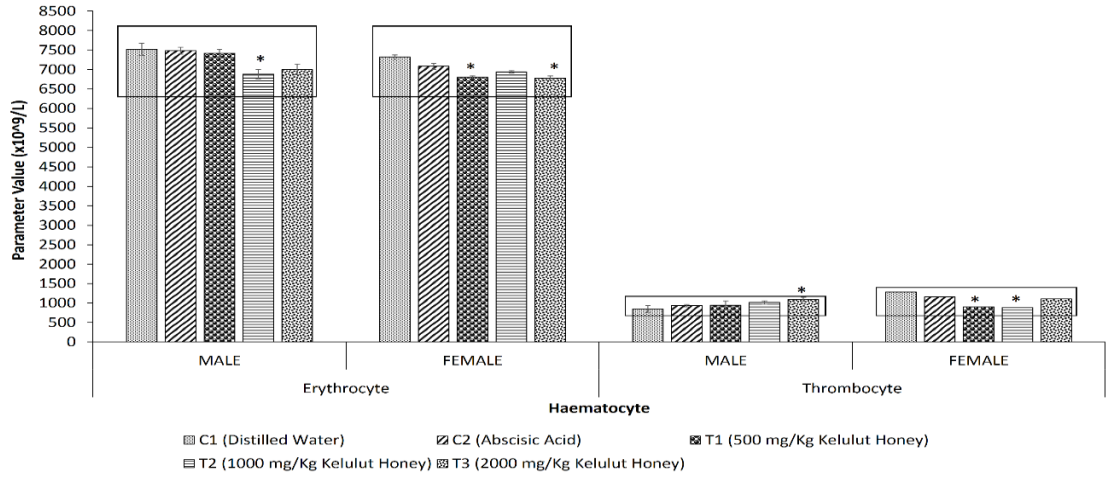


(A)

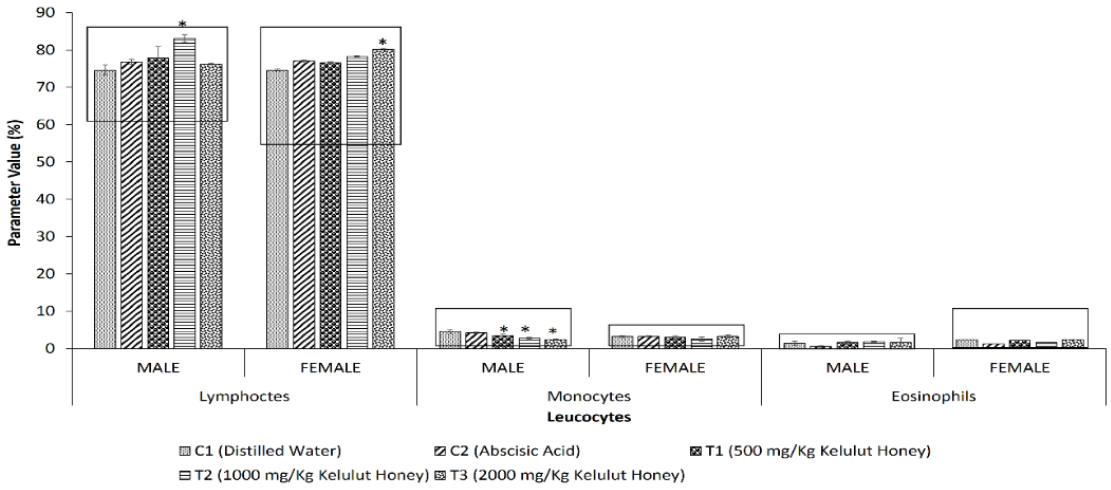


(B)

Figure 1. Percentage of body weight and total food intake for male rats **(A)** and female rats **(B)** (C1 = distilled water; C2 = abscisic acid; T1 = 500 mg/kg; T2 = 1,000 mg/kg; and T3 = 2,000 mg/kg) for the subacute study following daily administration of kelulut honey for four weeks. Values are mean of each week. $^{\#}p < 0.05$ for week 3, $^{*}p < 0.05$ and $^{**}p < 0.01$ for week 4 in male rats indicate significant difference as compared to negative control (C1).



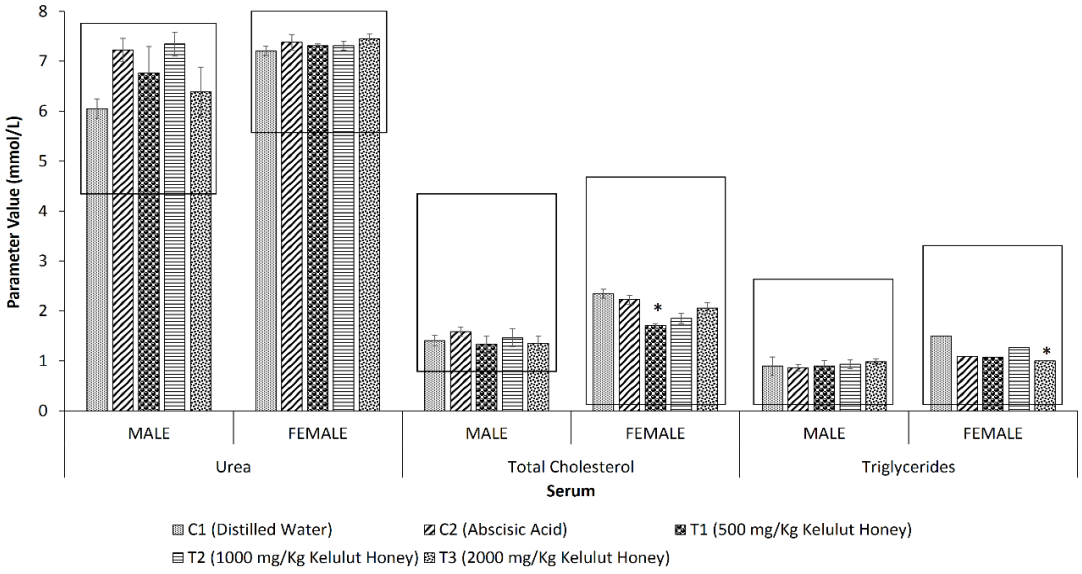
(A)



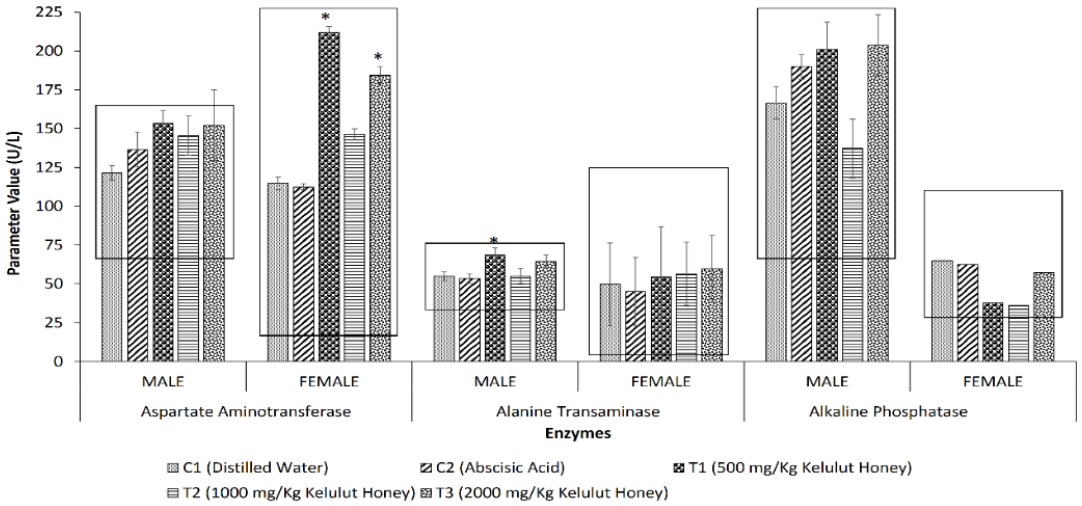
(B)

Figure 2. Haematology parameters of erythrocyte and thrombocyte **(A)** and some differential white blood count **(B)** (lymphocyte, monocyte, and eosinophil) of male and female rats for the subacute study following daily administration of kelulut honey for four weeks. Boxes indicate the normal range for each parameter in both genders. Values are mean ± SEM (n = 5) *significant difference ($p < 0.05$) as compared to negative control (C1).

mmol/L; female = 5.56 - 12.67 mmol/L) was analysed for kidney function test, total cholesterol (normal range, male = 0.80 - 4.53 mmol/L; female = 1.13 - 4.86 mmol/L), and triglyceride (normal range, male = 0.15 - 2.65 mmol/L; female = 0.48 - 3.37 mmol/L) to analyse blood parameters and liver enzymes to monitor the kelulut honey effect toward the liver function (Han *et al.*, 2010; He *et al.*, 2017; Delwatta *et al.*, 2018). Figure 3 shows the higher value of urea with kelulut honey consumption than the control. C1 (male = 6.05 ± 0.18 mmol/L; female = 7.21 ± 0.20 mmol/L) and T2 (7.34 ± 0.27 mmol/L) yielded the highest value in male, and T3 (7.45 ± 0.49 mmol/L) in female rats, but not significant. The total cholesterol in both male and female rats showed that T1 (1.34 ± 0.04 and 1.71 ± 0.17 mmol/L, respectively) as having the lowest value, but not significant in male rats (Figure 3).



(A)



(B)

Figure 3. Biochemical parameters of urea, total cholesterol, and triglyceride **(A)**, and some parameters of liver function tests **(B)** of male and female rats for the subacute study following daily administration of kelulut honey for four weeks. Boxes indicate the normal range for each parameter in both genders, respectively. Values are mean ± SEM (n = 5) *significant difference ($p < 0.05$) as compared to negative control (C1).

Serum analyses of enzymes alkaline phosphatase (ALP, normal range, male = 62.0 - 230.0 U/L; female = 30.1 - 116.0 U/L), alanine transaminase (ALT, normal range, male = 30.8 - 73.4 U/L; female = 2.1 - 426.9 U/L), and aspartate transaminase (AST, normal range, male = 64.1 - 168.1 U/L; female = 20.8 - 470.2 U/L) (De Biasio *et al.*, 2006) somehow revealed the condition of the liver following kelulut honey consumption. Figure 3 shows that T1 male and female rats (153.40 ± 12.87 and 212.2 ± 8.21 U/L, respectively) having the highest value in AST but not significant in male rats as compared to C1 (male = 121.60 ± 7.23 U/L; female = 114.80 ± 4.71 U/L). ALT in male rats also showed that T1 as having the significantly highest value (68.80 ± 3.87 U/L) as compared to C1 (55.00 ± 4.00 U/L), whereas in female rats, T3 (59.80 ± 4.10 U/L) had the highest value but not significant.

Detection of biomarkers in rats' serum after four weeks of kelulut honey consumption

Analysis of proton nuclear magnetic resonance (^{1}H NMR) for rats' serum of the highest concentration of kelulut honey daily consumption (2,000 mg/kg) allowed for the detection of any defect or abnormal metabolites in the rats' body (Figure 4). Analysis of the rats' serum grouped the compounds such as amino acids (betaine and glycine), protein (creatinine), organic acids (acetate, lactate, succinate, and glycolate), and organic compounds (acetone, glucose, trimethylamine-N-oxide (TMAO), and imidazole). There were no visible unfamiliar compounds following kelulut honey daily administration.

Relative organ weight (ROW)

Figure 5 shows a significant increase in the ROW of male rats' lungs for C2 (0.87%) and T3 (0.89%) as compared to C1 (0.70%). A significant decrease for female liver ROW of T2 (2.08%) and T3 (3.07%) as compared to C1 (3.81%) was also observed. Meanwhile, C2 female kidney ROW (0.43%) showed a significant increase as compared to C1 (0.40%).

Organ histopathological analyses

Histology results of the liver and kidney following kelulut honey daily administration showed normal tissue structure with no visible abnormality as compared to C1 (Figure 6). However, in female rats, T2 showed the presence of mononuclear cell infiltration but was absent in T1 and T3. This condition is questionable since both doses lower and higher than T2 displayed normal and healthy liver tissue. In addition, there were no visible toxicity signs on the kidney structure for kidney organs such as necrosis of proximal and distal tubular or sloughing of tubular epithelial cells. The kidney structure was also free from any injury such as the formation of interstitial inflammatory cells, glomerular atrophy, or capillaries congestion (De Biasio *et al.*, 2006).

Discussion

Kelulut honey has demonstrated various medicinal properties such as antioxidant, anti-obesity, antibacterial, wound-healing, and anticancer (Saiful Yazan *et al.*, 2016; Mohd Rafie *et al.*, 2018; Biluca *et al.*, 2020). These benefits come from its polyfloral sources, thus contributing to its antioxidant properties (Jaapar *et al.*, 2016). Plants naturally produce antioxidants as protection under abiotic and biotic stresses. A plant hormone that explicitly controls antioxidant production is ABA. Sah *et al.* (2016) found ABA to be a phytohormone that acts as a central regulator that signals the emergence of reactive oxygen species, and activates the genetic makeup for antioxidant production to discard it in plants (Sah *et al.*, 2016). Therefore, ABA is a prominent natural occurrence in plants. ABA has also been found to be present in all kelulut honey from different continents, thus serving as its biomarker (de Almeida-Muradian, 2013; Abu Bakar *et al.*, 2017).

According to the OECD 407 for oral subacute toxicity study, a highly expected dose to cause death based on the previous study should be considered as a starting dose. Samat *et al.* (2014) reported no toxicity signs on rats for the highest dose of 2,000 mg/kg for gelam and acacia honey. Therefore, the highest dose of 2,000 mg/kg of kelulut honey was selected as the starting dose, and reduced by half to evaluate its daily consumption effectiveness.

Based on the observation, no abnormal signs were observed on the movement and physical of each rat from the first day until the sacrifice day (day 29). The outcome agrees with the study from Samat *et al.* (2014), which showed no physical and behaviour abnormalities throughout the study period for the common *Apis* sp. honey. In contrast to BW and total

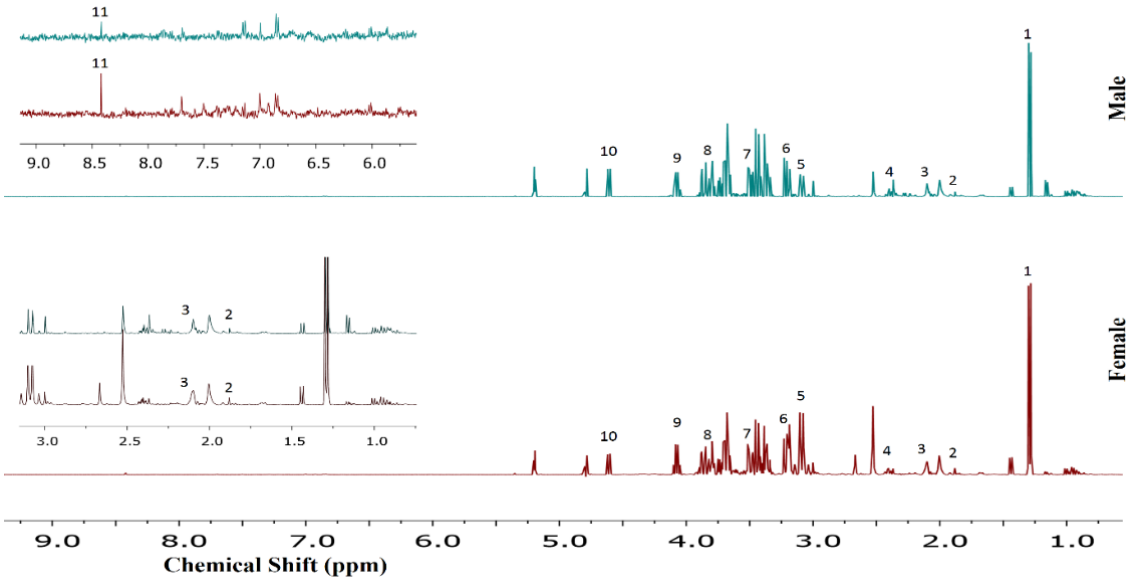


Figure 4. [1]H NMR spectra of male and female rat's serum fed with 2,000 mg/kg kelulut honey for four weeks. Identified signals: 1 = lactate; 2 = acetate; 3 = acetone; 4 = succinate; 5 = trimethylamine-N-oxide; 6 = creatinine; 7 = glycine; 8 = glycolate; 9 = betaine; 10 = glucose; and 11 = imidazole.

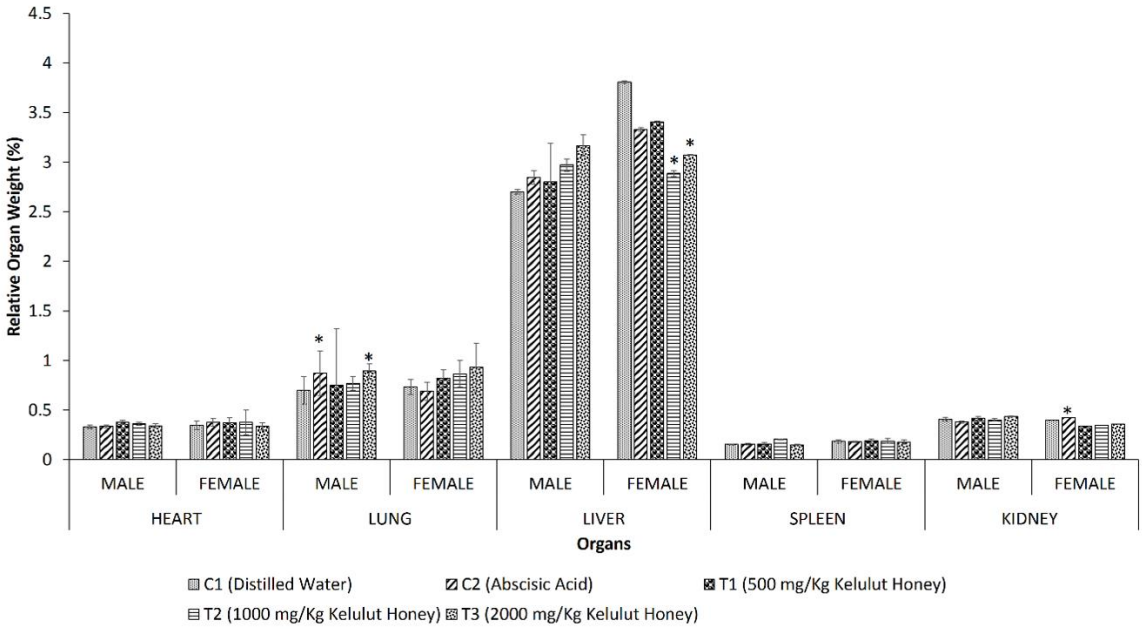
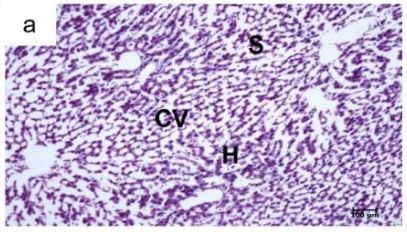
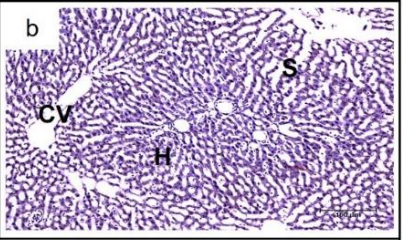
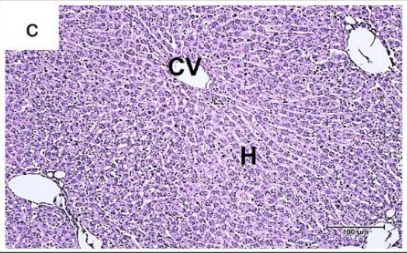
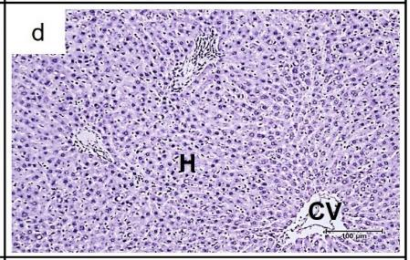
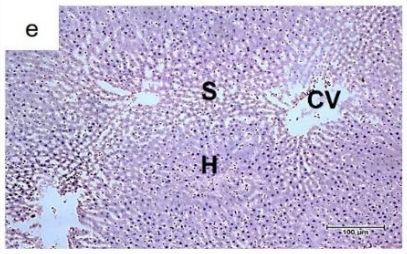
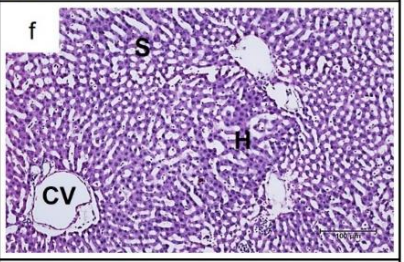
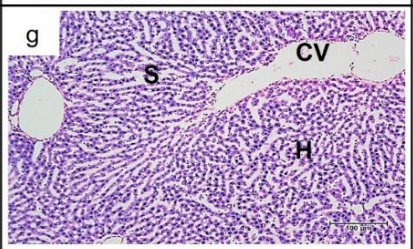
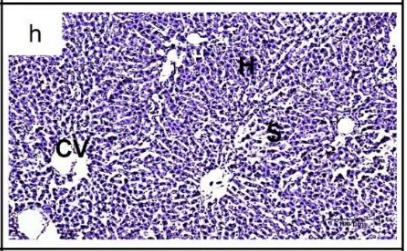
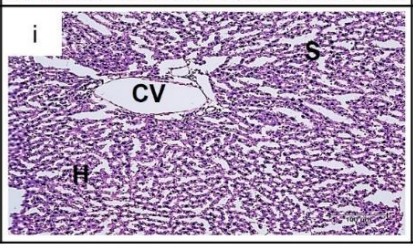
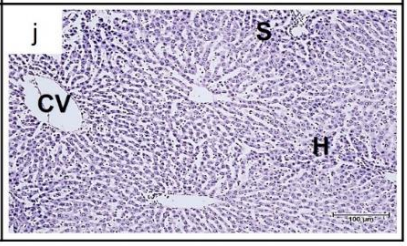
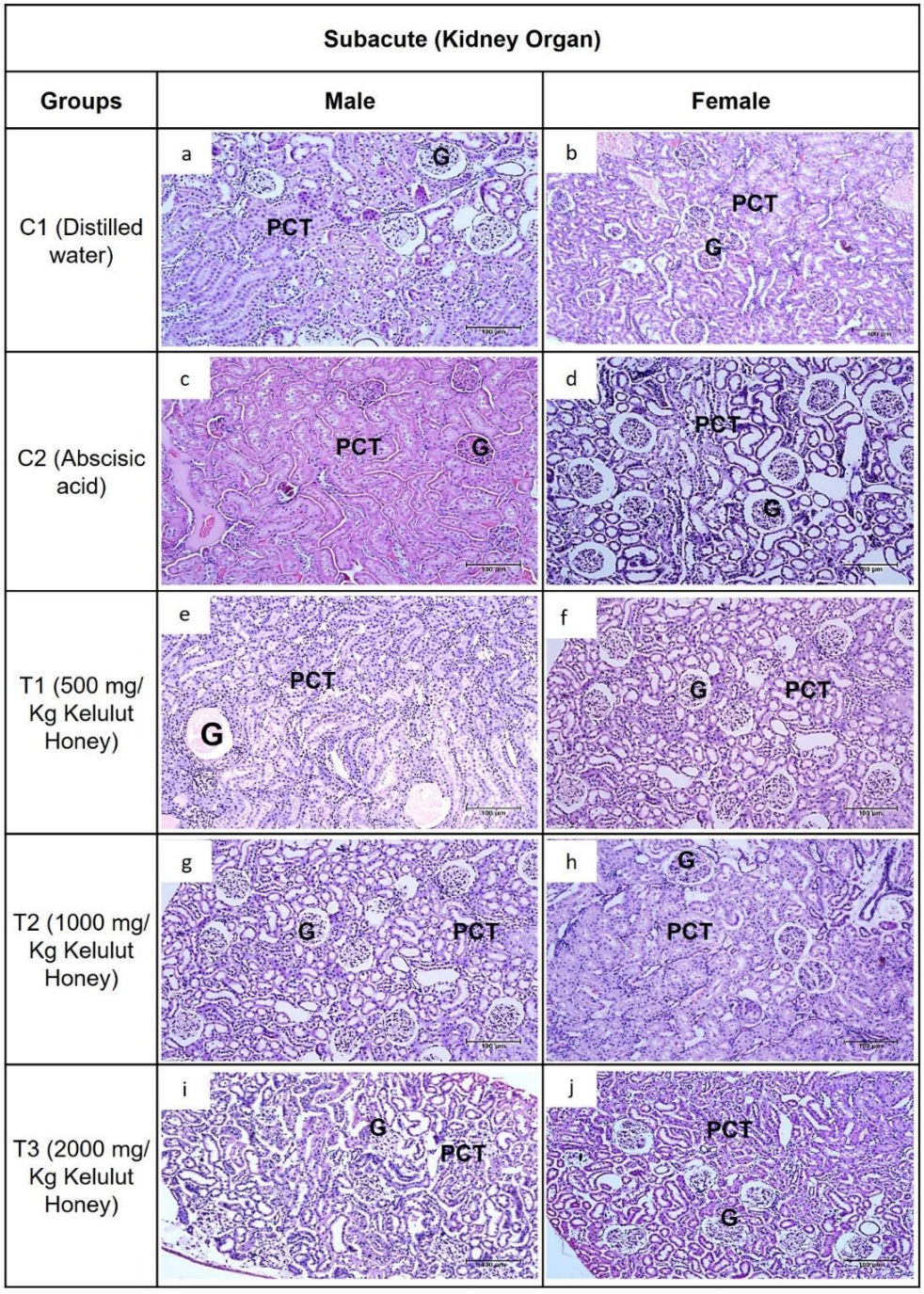


Figure 5. Relative organ weights of male and female rats following daily administration of kelulut honey for four weeks. Values are mean ± SEM (n = 5) *significant difference (p < 0.05) as compared to negative control (C1).

Subacute (Liver Organ)		
Groups	**Male**	**Female**
C1 (Distilled water)		
C2 (Abscisic acid)		
T1 (500 mg/ Kg Kelulut Honey)		
T2 (1000 mg/ Kg Kelulut Honey)		
T3 (2000 mg/ Kg Kelulut Honey)		

(A)



(B)

Figure 6. Subacute toxicity of haematoxylin and eosin (H&E) staining of liver **(A)** and kidney **(B)** after four weeks of kelulut honey administration. Left panel is representative of male rats, while right panel is representative of female rats: **a** and **b** = kidney and liver sections from rats fed with distilled water (negative control, C1) which show normal histological structure with hepatocytes (H), sinusoids (S), and central vein (CV) for liver, while glomerulus (G) and proximal convoluted tubules (PCT) for kidney; **c** and **d** = consumption of abscisic acid (C2); **e** and **f** = consumption of 500 mg/kg kelulut honey (T1); **g** and **h** = consumption of 1,000 mg/kg kelulut honey (T2); **i** and **j** = consumption of 2,000 mg/kg kelulut honey; both liver and kidney show the normal structure of the organs (H&E, 100× magnification).

food intake, different concentrations of honey showed distinctive effects on the rats. This might have been caused by the individual variation in the rats' metabolism (Hsu *et al.*, 2015). BW is a crucial indicator in analysing toxicity with losses of ≥ 20% of the original BW is regarded as severe damage, and recorded as a humane endpoint in some international guidelines (OECD, 2008). Food intake is associated with BW. However, the percentage of BW increment for different individuals might not be directly proportional to food intake because other factors such as energy expenditure or body metabolism also affect BW (Nordin *et al.*, 2018). This condition might be due to the increase in sugar intake by the rats while the energy expenditure is less due to the limited space of the rats. Meanwhile, in female rats, the percentage of BW increment varied in groups, probably due to hormonal effects. Deibert *et al.* (2010) also pointed out that the complicated effect of BW on honey consumption may be due to different metabolisms and body hormones in male and female rats.

ABA as the biomarker for kelulut honey may play an important role in the medicinal properties since it is constantly found in the honey. Soti *et al.* (2019) found that male rats treated with ABA have significantly decreased BW and increased appetite, which also correlates with our result on male rats. The finding was due to ABA's significant impact on insulin secretion, which decreases the concentration of fasting blood glucose while improving glucose tolerance in mice (Guri *et al.*, 2007). The condition results in low glycaemia that induced gluconeogenesis and increased food intake (Soti *et al.* 2019). However, BW change is still mainly affected by the caloric intake and energy expenditure of the organisms (Hsu *et al.*, 2015). Meanwhile, female rats did not exhibit the same effect as male rats. This could have been due to the variation in the modulation of hypothalamic regulatory on the basal metabolic rate that could cause changes in energy expenditure without food restriction controlling the different psychological and physiological factors, including the change in mood, emotional state, and activity (Saiful Yazan *et al.*, 2016; Nordin *et al.*, 2018). Since ABA is a hormone that may participate in body metabolism and food appetite, disturbance and change in hormone regulation may also be affected by the effect of ABA since both genders had contrast body conditions, and the substance may interact with the body differently (Deibert *et al.*, 2010).

Blood parameter data play a significant role in determining the possible adverse effect since blood serves as the primary medium for substance transfer in the body (Abotsi *et al.*, 2011). Robust changes in blood components are inimical to normal bodily function (Abotsi *et al.*, 2011). Low erythrocyte counts are usually associated with anaemia or erythropoietin deficiency resulting from chronic kidney diseases and deprivation of the body's oxygen. In contrast, high erythrocyte counts indicate the increase in oxygen needs of body cells due to some conditions that need oxygen. However, the erythrocyte count in the present work was still in the normal range (He *et al.*, 2017), thus indicating that daily kelulut honey administration did not alter the erythrocyte counts. Meanwhile, thrombocyte counts were different in male and female rats due to daily kelulut honey administration. Saiful Yazan *et al.* (2016) also reported a decrease in thrombocyte count but in male rats after being treated with kelulut honey. The decrease in the body's thrombocyte needs might be due to the antioxidant and wound healing effect of kelulut honey that improves body cell function (Mohd Rafie *et al.*, 2018; Nordin *et al.*, 2018; Biluca *et al.*, 2020). The effect was different for different doses and genders, thus suggesting different probabilities of the optimum dose of kelulut honey. Differential leukocytes also play a vital role in protecting the body. Any adverse effect of a substance will affect the prognostic value of leukocytes. Apart from being rich in antioxidants, kelulut honey is also found to improve the immune system by increasing the number of leukocytes. Saiful Yazan *et al.* (2016) again supported our finding with the high leukocyte count for kelulut honey treatment ($10.12 \pm 0.95 \times 10^9$/L) as compared to rats fed with distilled water ($7.45 \pm 0.88 \times 10^9$/L). Al-Waili and Haq (2004) also found that natural honey stimulates the antibody production for the primary and secondary immune responses against thymus-dependent and thymus-independent antigens, thus suggesting that honey has a significant effect on the body immune responses. However, high leukocyte count can also induce anxiety since it may indicate body infections or diseases (such as leukaemia, immune system disorder, or stress), which is in contrast with our data since all the blood parameters were in the normal range (Han *et al.*, 2010; Delwatta *et al.*, 2018).

Analysis of blood serum can also be one of the specific and essential precursors for the adverse effect

of substances (Samat *et al.*, 2014). Nitrogen waste product results from the breaks of protein by the liver, combined with other elements such as carbon, hydrogen, and oxygen, thus forming urea. This molecule is then transferred to the kidney to be filtered and flushed out from the body (Stepanova *et al.*, 2013). Therefore, high blood urea may indicate the ineffectiveness of kidney function. Apart from that, an increase in urination elaborates the diuretic properties of kelulut honey which mimicks furosemide. Furosemide increases the urinary excretion of certain ions such as potassium and chloride in the thick ascending limb of the Henle loop, thus causing hypokalaemia, a contrast effect as compared to honey (El-Guendouz *et al.*, 2017). An increase in urea value may denote an increase in urine volume, which removes toxins and reduces water retention problems. A previous study had also reported an increase in urea value upon kelulut honey consumption (Saiful Yazan *et al.*, 2016). Other than that, lipid is one of the critical elements in the body that functions as cushions for internal organs and prominent energy storage. However, hyperlipidaemia is toxic to the body by promoting many severe diseases such as atherosclerosis, hypothyroidism, and liver and kidney failure (Stepanova *et al.*, 2013). Lipid acts as a crucial toxicity indicator in honey quality assessment since honey is generally speculated as an obesity agent. Conversely, our data found that kelulut honey consumption reduced the total cholesterol and triglyceride values, which showed the anti-obesity effect. Many preceding studies also reported the anti-obesity effect of natural honey such as in our data. Our results coincide with Mohd Rafie *et al.* (2018) who found a significant decrease in low-density lipoprotein cholesterol and triglyceride in rats following kelulut honey consumption. The anti-obesity properties of kelulut honey were found due to the insulin-mimetic effect of hydrogen peroxide in the honey, which increased the rats' metabolism (Mohd Rafie *et al.* 2018).

Liver is a vital organ that functions to filter blood, detoxify chemicals, and metabolise drugs in the body. Therefore, abnormality of liver performance also marks the adverse effect of a substance. Elevation of these enzymes might occur due to the enzyme leakage from the damaged cellular membrane. Currently, the increment in ALT and AST levels is commonly associated with drugs, chronic viral infection, chronic alcohol consumption, and non-alcoholic steatohepatitis (NASH) (Stepanova *et al.*, 2013). NASH can occur with the abnormality in the bodies' metabolic performance due to diseases such as obesity, type II diabetes, or hyperlipidaemia (Bailey *et al.*, 2004; Stepanova *et al.*, 2013; Craig *et al.*, 2015; El-Guendouz *et al.*, 2017). NASH can be severe and lead to further complications, for instance, cirrhosis and hepatocellular carcinoma. This happens because of high toxicity in the blood, increased liver burden to remove the toxins, thus leading to damage and reduced liver function. Mohd Rafie *et al.* (2018) also recorded a high value of ALP for high-fat diet male rats (obese-induced) treated with 500 mg/kg kelulut honey (140.5 ± 9.0 U/L) as compared to control (89.8 ± 4.8 U/L). Other than that, Samat *et al.* (2014) reported a high AST value after consumption of 2,000 mg/kg acacia honey (154.00 ± 5.48 U/L) as compared to control (112.00 ± 4.47 U/L) for female rats. Although our data found high value of the enzymes following kelulut honey administration, the value was still within the normal range (Figure 3) (Han *et al.*, 2010). Therefore, the rats' liver was subjected to ^{1}H NMR and histopathology to confirm any abnormality of the metabolites and organs.

Apart from serum analyses, the rats' serum also underwent ^{1}H NMR to detect any abnormal serum metabolites following kelulut honey adminsitration. The ^{1}H NMR analysis is vastly used for structure determination since it can elaborate complex structure in a complicated system, has a non-invasive nature, has rapid data elimination, presents a broad range of metabolites in one analysis, and above all, requires only a small amount of sample (Lin *et al.*, 2016). One of the metabolites found in our analysis was glycine. Glycine usually represents 11.5% of the total amino acids in the human body, which functions in protein synthesis and collagen formation (Razak *et al.*, 2017). Collagen is essential to sustain the normal structure, strength of connective tissue, and elasticity of the skin, and also a precursor for several key metabolites of low molecular weight (Li and Wu, 2018). Supplementation of glycine is also able to enhance the quality of sleep and neurological functions, and prevent disease including cancers (Razak *et al.*, 2017). Succinate serves as a critical metabolite intermediate in the tricarboxylic acid cycle, and connects directly with the electron transport chain of the mitochondria, thus allowing the direct production of adenosine triphosphate (ATP) via oxidative metabolism (Giorgi-Coll *et al.*, 2017). A reduced level of succinate might decrease the production of ATP and increase the oxidative stress

of body cells. Next, oxidation of serum glycolate by glycolate oxidase to glyoxylate, and then to oxalate, produces hydroxyl acids, a carbohydrate precursor, and serves as an energy source (Kubáň *et al.*, 2014). Other than that, acetone (product of fat breakdown) and glucose (product of carbohydrate breakdown) are also energy providers (Abu Bakar *et al.*, 2017), bearing a pivotal role to kick-start oxidative metabolism. Interestingly, TMAO peak was higher in female rats and lower in male rats. Elevation in TMAO level is usually associated with cardiovascular disease. Wang *et al.* (2011) reported that a diet containing choline, TMAO, or betaine can induce the activeness of multiple macrophage scavenger receptors that are vulnerable to atherosclerosis. Nevertheless, no harm was observed in the female rats. Last but not the least, the imidazole ring is ubiquitous and significant as a build-up for histamine and histidine in humans and many organisms. Histidine can be found in many proteins, and is essential as the iron binder for haemoglobin in red blood cells (Krishna Deepak and Sankararamakrishnan, 2016). Decarboxylation of histidine produced histamine, an allergic agent alleviating inflammatory responses in immunity (Krishna Deepak and Sankararamakrishnan, 2016). All these metabolites are essential in maintaining optimum bodily functions. All the metabolites found in the rat serum are needed by the body, and promoted their normal condition.

Other than that, analysis of organ weight is also critical in determining xenobiotics' effect on the animals' body performance. ROW can be the more sensitive detector for a substance effect since ROW significance may occur without any morphological changes (Bailey *et al.*, 2004). Interestingly, data showed a significant increment of male rats' lung ROW with ABA intake, and T3 indicated that ABA might have an effect in increasing lung ROW. However, Hontecillas *et al.* (2013) reported the effectiveness of ABA as an immune-modulatory for respiratory infection without any severe side effects. Apart from that, the result also showed an increment in male liver ROW, an intriguing concern since hepatomegaly is usually associated with fat accumulation. Despite that, the increment in lung and liver ROW might also be due to the different individual organ sizes (Samat *et al.*, 2014). The decrease in female ROW can suggest the liver's water loss or reduction of original liver fats (Mohd Rafie *et al.*, 2018). Coincidently, our results were supported by Mohd Rafie *et al.* (2018) who found a significant reduction in adiposity index and liver ROW after being treated with kelulut honey. The resulted condition suggested that kelulut honey highly possesses the anti-obesity effect, which was found to be affected by the ABA that ameliorates glucose tolerance in rats (Soti *et al.*, 2019). Other than that, the significant increase of ROW does not necessarily cause defects in histopathology as compared to the absolute organ weight of the organs (Craig *et al.*, 2015).

Observation of histology has a deep relation with xenobiotics' effect on the body (Samat *et al.*, 2014). Moreover, liver and kidney serve as the crucial organs in maintaining body homeostasis by eliminating unnecessary and waste compounds. Therefore, histological analyses of these organs may help explain the exact body condition upon consumption of substances (Craig *et al.*, 2015). Hepatocytes are the basic unit of the liver with a specific function to synthesise protein, detoxify metabolites, and channel biochemicals for body growth and digestion (De Biasio *et al.*, 2006). Meanwhile, kidney is another filtration organ, and will also be damaged by severe toxins. As expected, all groups display normal morphology of the kidney and liver cells based on the histology analysis. However, the abnormality of female rats' liver for T1 and T3 tissues somehow might be associated with specific autoantibodies inducing mononuclear cell infiltration and immunoglobulin G (De Biasio *et al.*, 2006). Saiful Yazan *et al.* (2016) also reported the visibility of cancerous cells in their negative control (treatment with distilled water, C1) that was assumed due to cell mutation. In addition, other studies also reported the effectiveness of kelulut honey consumption in eliminating cancer cells in the colon (De Biasio *et al.*, 2006; Saiful Yazan *et al.*, 2016; Mohd Rafie *et al.*, 2018; Nordin *et al.*, 2018; Biluca *et al.*, 2020). According to Abdel-Moneim and Ghafeer (2007), the treatment of rats with honey on renal tubular toxicity from cadmium chloride reduces lipid peroxidation, and improves tissue activity for enzyme glutathione and glutathione peroxidase.

Conclusion

The medium lethal dose for daily consumption of kelulut honey was higher than 2,000 mg/kg rats' BW. Therefore, it is classified in category 5 or unclassified according to the Globally Harmonized

System of Classification and Labelling of Chemicals, which is the safest in subacute oral toxicity. Generally, there was no abnormality in rats' behaviour and individual performance. In the present work, we found that kelulut honey had a prognostic value in decreasing BW (anti-obesity) while increasing food intake (increase appetite). Haematological analyses showed an increment in lymphocytes necessary for antibody formation in the immune system to fight pathogens, and blood serum analyses showed normal value for each serum and enzyme analysed. Results also indicated general good health effects of kelulut honey consumption. ^{1}H NMR analysis revealed the metabolites in male and female rats treated with kelulut honey, and those metabolites in the rats' serum are essential for bodily functions. Kelulut honey consumption also significantly decreased female liver ROW. At the same time, histopathology observation showed normal tissue structure for both the liver and kidney, the prominent organs in filtering toxins, thus further indicating the safety of kelulut honey. Nevertheless, the optimum doses for males and females might be different due to hormone regulation in the body. The effect on individuals may also vary based on the current condition of each individual. Overall, kelulut honey at a concentration up to 2,000 mg/kg (which is equal to 518.912 mg/m^2 for human adults and 384.000 mg/m^2 for children) is safe for daily consumption, and does not cause any adverse effect while improving healthy bodily function on individuals (Reagan-Shaw *et al.*, 2007). However, the effect of honey might vary with individuals, and the dose needed for specific treatment might also be different. The long-term effects for 90 days also need to be investigated. Therefore, in the future, toxicity safety study can be pursued with a clinical study, especially in highlighting the medicinal effects of kelulut honey.

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References

Ab Hamid, S., Salleh, M. S., Krishnan, K. T. and Hashim, N. A. 2016. Distribution and morphometrical variations of stingless bees (Apidae: Meliponini) in urban and forest areas of Penang Island, Malaysia. Journal of Tropical Resources and Sustainable Science 4(1): 1-5.

Abdel-Moneim, W. M. and Ghafeer, H. H. 2007. The potential protective effect of natural honey against cadmium-induced hepatotoxicity and nephrotoxicity. Mansoura Journal of Forensic Medicine and Clinical Toxicology 15(2): 75-98.

Abdullah, N. A., Ja'afar, F., Yasin, H. M., Taha, H., Petalcorin, M. I., Mamit, M. H., ... and Usman, A. 2019. Physicochemical analyses, antioxidant, antibacterial, and toxicity of propolis particles produced by stingless bee *Heterotrigona itama* found in Brunei Darussalam. Heliyon 5(9): 1-8.

Abotsi, W. K., Ainooson, G. K., Gyasi, E. B. and Abotsi, W. K. 2011. Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae) in rodents. West African Journal of Pharmacy 22(1): 27-35.

Abu Bakar, M. F., Sanusi, S. B., Abu Bakar, F. I., Cong, O. J. and Mian, Z. 2017. Physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. Pakistan Journal of Nutrition 16(11): 888-894.

Al-Afifi, N. A., Alabsi, A. M., Bakri, M. M. and Ramanathan, A. 2018. Acute and sub-acute oral toxicity of *Dracaena cinnabari* resin methanol extract in rats. BMC Complementary and Alternative Medicine 18(1): 50.

Al-Waili, N. S. and Haq, A. 2004. Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. Journal of Medicinal Food 7(4): 491-494.

Bailey, S. A., Zidell, R. H. and Perry, R. W. 2004. Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? Toxicologic Pathology 32(4): 448-466.

Biluca, F. C., da Silva, B., Caon, T., Mohr, E. T., Vieira, G. N., Gonzaga, L. V., ... and Costa, A. C. O. 2020. Investigation of phenolic compounds, antioxidant, and anti-inflammatory activities in stingless bee honey (Meliponinae). Food Research International 129(7): 108756.

Craig, E. A., Yan, Z. and Zhao, Q. J. 2015. The relationship between chemical-induced kidney

weight increases and kidney histopathology in rats. Journal of Applied Toxicology 35(7): 729-736.

De Almeida-Muradian, L. B. 2013. *Tetragonisca angustula* pot-honey compared to *Apis mellifera* honey from Brazil. In Vit, P., Pedro, S. R. M. and Roubik, D. (eds). Pot-Honey, p. 375-382. New York: Springer.

De Biasio, M. B., Periolo, N., Avagnina, A., de Dávila, M. G., Ciocca, M., Goñi, J., … and Munoz, A. E. 2006. Liver infiltrating mononuclear cells in children with type 1 autoimmune hepatitis. Journal of Clinical Pathology 59(4): 417-423.

Deibert, P., König, D., Kloock, B., Groenefeld, M. and Berg, A. 2010. Glycaemic and insulinaemic properties of some German honey varieties. European Journal of Clinical Nutrition 64(7): 762-764.

Delwatta, S. L., Gunatilake, M., Baumans, V., Seneviratne, M. D., Dissanayaka, M. L., Batagoda, S. S., ... and Walpola, P. B. 2018. Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. Animal Models and Experimental Medicine 1(4): 250-254.

El-Guendouz, S., Al-Waili, N., Aazza, S., Elamine, Y., Zizi, S., Al-Waili, T., ... and Lyoussi, B. 2017. Antioxidant and diuretic activity of co-administration of *Capparis spinosa* honey and propolis in comparison to furosemide. Asian Pacific Journal of Tropical Medicine 10(10): 974-980.

Fu, J., Chu, J., Sun, X., Wang, J. and Yan, C. 2012. Simple, rapid, and simultaneous assay of multiple carboxyl containing phytohormones in wounded tomatoes by UPLC-MS/MS using single SPE purification and isotope dilution. Analytical Sciences 28(11): 1081-1087.

Giorgi-Coll, S., Amaral, A. I., Hutchinson, P. J., Kotter, M. R. and Carpenter, K. L. 2017. Succinate supplementation improves metabolic performance of mixed glial cell cultures with mitochondrial dysfunction. Scientific Reports 7(1): 1003.

Guri, A. J., Hontecillas, R., Si, H., Liu, D. and Bassaganya-Riera, J. 2007. Dietary abscisic acid ameliorates glucose tolerance and obesity-related inflammation in db/db mice fed high-fat diets. Clinical Nutrient 26(1): 107-116.

Han, Z. Z., Xu, H. D., Kim, K. H., Ahn, T. H., Bae, J. S., Lee, J. Y., ... and Lee, H. K. 2010. Reference data of the main physiological parameters in control Sprague-Dawley rats from pre-clinical toxicity studies. Journal of Laboratory Animal Research 26(2): 153-164.

He, Q., Su, G., Liu, K., Zhang, F., Jiang, Y., Gao, J., ... and Xie, H. 2017. Sex-specific reference intervals of hematologic and biochemical analytes in Sprague-Dawley rats using the nonparametric rank percentile method. PLoS One 12(12): e0189837.

Hontecillas, R., Roberts, P. C., Carbo, A., Vives, C., Horne, W. T., ... and Bassaganya-Riera, J. 2013. Dietary abscisic acid ameliorates influenza-virus-associated disease and pulmonary immunopathology through a PPARγ-dependent mechanism. Journal of Nutritional Biochemistry 24(6): 1019-1027.

Hsu, T. M., Hahn, J. D., Konanur, V. R., Lam, A. and Kanoski, S. E. 2015. Hippocampal GLP-1 receptors influence food intake, meal size, and effort-based responding for food through volume transmission. Neuropsychopharmacology 40(2): 327-337.

Jaapar, M. F., Halim, M., Mispan, M. R., Jajuli, R., Saranum, M. M., Zainuddin, M. Y., ... and Abd Ghani, I. 2016. The diversity and abundance of stingless bee (Hymenoptera: Meliponini) in Peninsular Malaysia. Advances in Environmental Biology 10(9): 1-7.

Junqueira, L. C. and Carneiro, J. 2002. Basic histology - text and atlas. 10th ed. New York: McGraw-Hill Professional.

Krishna Deepak, R. N. and Sankararamakrishnan, R. 2016. N–H···N hydrogen bonds involving histidine imidazole nitrogen atoms: A new structural role for histidine residues in proteins. Biochemistry 55(27): 3774-3783.

Kubáň, P., Ďurč, P., Bittová, M. and Foret, F. 2014. Separation of oxalate, formate, and glycolate in human body fluid samples by capillary electrophoresis with contactless conductometric detection. Journal of Chromatography A 1325: 241-246.

Li, P. and Wu, G. 2018. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino Acids 50(1): 29-38.

Lin, T., Gu, J., Huang, C., Zheng, S., Lin, X., Xie, L. and Lin, D. 2016. ^{1}H NMR-based analysis of serum metabolites in monocrotaline-induced pulmonary arterial hypertensive rats. Disease Markers 2016: 5803031.

Mohd Rafie, A. Z., Syahir, A., Wan Ahmad, W. A., Mustafa, M. Z. and Mariatulqabtiah, A. R. 2018. Supplementation of stingless bee honey from *Heterotrigona itama* improves anti-obesity parameters in high-fat diet induced obese rat model. Evidence-Based Complementary and Alternative Medicine 2018: 6371582.

Monggudal, M. B., Mohd Radzi, M. N. F., Ismail, M. M. and Ismail, W. I. W. 2018. Effect of six month storage on physicochemical analysis and antioxidant activity of several types of honey. IOP Conference Series - Materials Science and Engineering 440: 012047

Nordin, A., Omar, N., Sainik, N. Q., Chowdhury, S. R., Omar, E., Saim, A. B. and Idrus, R. B. H. 2018. Low dose stingless bee honey increases viability of human dermal fibroblasts that could potentially promote wound healing. Wound Medicine 23: 22-27.

Organisation for Economic Co-operation and Development (OECD). 2018. OECD guideline for the testing of chemicals 407 - sub-acute oral toxicity-acute toxic class method. France: OECD.

Razak, M. A., Begum, P. S., Viswanath, B. and Rajagopal, S. 2017. Multifarious beneficial effect of nonessential amino acid, glycine: A review. Oxidative Medicine and Cellular Longevity 2017: 1716701.

Reagan-Shaw, S., Nihal, M. and Ahmad, N. 2007 Dose translation from animal to human studies revisited. The FASEB Journal 22(3): 659-661.

Sah, S. K., Reddy, K. R. and Li, J. 2016. Abscisic acid and abiotic stress tolerance in crop plants. Frontier in Plant Science 7: 571- 596.

Saiful Yazan, L., Muhamad Zali, M. F., Mohd Ali, R., Zainal, N. A., Esa, N., Sapuan, S., … and Syed Alwi, S. S. 2016. Chemopreventive properties and toxicity of Kelulut honey in Sprague-Dawley rats induced with azoxymethane. BioMed Research International 2016: 4036926.

Samat, S., Enchang, F. K., Abd Razak, A., Hussein, F. N. and Wan Ismail, W. I. 2018. Adulterated honey consumption can induce obesity, increase blood glucose level and demonstrate toxicity effects. Sains Malaysiana 45(5): 363-377.

Samat, S., Nor, N. A., Hussein, F. N. and Wan Ismail, W. I. 2014. Effects of Gelam and Acacia honey acute administration on some biochemical parameters of Sprague-Dawley rats. BMC Complementary and Alternative Medicine 14(1): 146.

Soti, M., Abbasnejad, M., Kooshki, R. and Esmaeili-Mahani, S. 2019. Central microinjection of phytohormone abscisic acid changes feeding behavior, decreases body weight and reduces brain oxidative stress in rats. Nutritional Neuroscience 22(10): 678-687.

Stepanova, M., Rafiq, N., Makhlouf, H., Agrawal, R., Kaur, I., Younoszai, Z., ... and Younossi, Z. M. 2013. Predictors of all-cause mortality and liver-related mortality in patients with non-alcoholic fatty liver disease (NAFLD). Digestive Diseases and Sciences 58(10): 3017-3023.

Tuffery, A. A. 1995. Laboratory animals: An introduction for experimenters. 2nd ed. New York: John Wiley & Sons.

Wang, Z., Klipfell, E., Bennett, B. J., Koeth, R., Levison, B. S., DuGar, B., ... and Wu, Y. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472: 57-63.

Yaacob, M., Rajab, N. F., Shahar, S. and Sharif, R. 2018. Stingless bee honey and its potential value: A systematic review. Food Research 2(2): 124-133.

Zulkhairi Amin, F. A., Sabri, S., Mohammad, S. M., Ismail, M., Chan, K. W., Ismail, N., ... and Zawawi, N. 2018. Therapeutic properties of stingless bee honey in comparison with European bee honey. Advances in Pharmacological and Pharmaceutical Sciences 2018: 6179596.