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A 28-day subacute oral toxicity study of *Apis cerana* (Fabricius) honey in Wistar rats

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Abstract

The use of honey as food and medicine is widespread, but insufficient data support that it is safe, especially when consumed in high doses. As a result, the present work aimed to investigate the potential toxicity using a repeated dose oral toxicity study. In the toxicity study, Wistar rats were divided into five groups, and orally administered with distilled water (control), 3, 6, 12, and 24 g/kg body weight (BW)/day of honey for 28 days in a row. Body weight, food consumption, clinical pathology, and histopathology were then examined. Significant suppression of body weight, food consumption, and body weight gain was observed at the dose of 24 g/kg BW in both sexes. Honey administration had no statistically significant effect on any of the haematological parameters. The clinical observations, blood coagulation and biochemical parameters, target organs, or histopathology did not reveal any additional nor other treatment-related adverse effects. Mild pathological changes in hepatic tissues were observed in the control, 12, or 24 g/kg BW dose groups, which were common spontaneous lesions unrelated to honey treatment. In the 24 g/kg BW group, one male rat showed non-specific reactions such as focal basophilic change of renal tubule cells, which were also regarded as spontaneous lesions. Based on these results, the no-observed-adverse-effect level (NOAEL) of honey in this repeat dose oral toxicity study was determined to be 12 g/kg BW in both sexes of Wistar rats.

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Introduction

Honey is a natural product made by honeybees collecting nectar from various flowering plants. Honey has been a common food and therapeutic material in most countries around the world due to its high nutritional and medicinal values (Rao *et al*., 2016).

Honey is primarily composed of water (about 17%), carbohydrates such as fructose (about 40%), glucose (about 30%), sucrose (about 0.1 - 5%), and disaccharides (about 9%) (Boateng and Diunase, 2015). It also contains trace amounts of proteins, amino acids, vitamins, minerals (Na, K, Ca, Mg, *etc.*), enzymes (glucose oxidase, invertase, catalase, *etc.*), polyphenols such as flavonoids (Yao *et al*., 2004) and phenolic acids, which may act as natural antioxidants (Blasa *et al*., 2006). Generally, honey is used orally to boost the immune system (Al-Waili and Haq, 2004), and fight ageing (Chepulis, 2007). Honey also contains antioxidant (Gheldof *et al*., 2003), antibacterial (French *et al*., 2005), and anti-inflammatory properties (Al-Waili and Boni, 2003) due to its high osmolarity, acidity, and phenolic content. Honey has been shown to be effective in accelerating wound healing (Cooper *et al*., 2001), and treating chronic gastric ulcers (Almasaudi *et al*., 2017), owing to its antibacterial and anti-inflammatory properties. Honey has also been shown to have cardiovascular protective effects (Dogan and Kolankaya, 2005), lower the risk of heart disease (Khalil and Sulaiman, 2010), and have anti-cancer properties (Badolato *et al*., 2017).

However, most people are unaware that honey contains toxic compounds that might be harmful to one's health. The composition of honey is determined

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by the plant species visited by the honeybees. In addition, the processing and storage conditions may influence its composition (Bertoncelj *et al*., 2007). A highly toxic compound of honey is 5-hydroxymethylfurfural (HMF), which occurs when honey is heated (Spano *et al*., 2006) or stored for an extended period of time (Khalil *et al*., 2010). In mice, HMF (0.08 - 500.00 mg/kg) was found to be nephrotoxic and hepatotoxic (Godfrey *et al*., 1999). According to the Codex Alimentarius Commission Food Standards, the HMF concentration in honey after processing and/or blending should be less than 80 mg/kg. However, the European Union recommends a lower limit of 40 mg/kg (EU, 2002). Natural honey derived from pollen and nectar of certain plants may contain a variety of plant toxins, such as pyrrolizidine alkaloids (PAs), grayanotoxanes (GTXs), hyoscyamine, strychnine, and tutin. GTXs may cause excessive salivation, dizziness, vomiting (Aliyev *et al*., 2009), and cardiac conduction abnormalities (Sayin *et al*., 2012). Tutin has been found to be toxic to the nervous system (MPI, 2010). Furthermore, residual antibiotics, pesticides (Al-Waili *et al*., 2012), and heavy metals, such as arsenic (As), lead (Pb), and mercury (Hg) (Loannidou *et al*., 2004) in honey may also pose a risk to human health.

Honey poisoning has been widely reported in many countries for a long time (Gunduz *et al*., 2009; Dubey *et al*., 2009). However, the causes of the poisoning remain unknown, and there are insufficient data to establish an adequate safety assessment for this widely consumed food. As a result, there is an urgent need to examine the potential toxicity and edible safety of honey. The present work was then carried out to investigate the potential toxicity of honey by performing a 28-day repeated oral dose toxicity in Wistar rats.

Materials and methods

Test material

The commercial honey (lot number D190701; white, faint yellow, or orange liquid) used as the test substrate was obtained from Chunyuan Food Co. Ltd., Fuzhou, China. It was brewed by *Apis cerana* (Fabricius). The quality inspection results of the honey after purchase were in accordance with the provisions of the Chinese Codex Alimentarius. The following criteria were used: water content must not exceed 24.0%, HMF must not exceed 0.004%, and sucrose and maltose must not exceed 5.0%. Honey was dissolved in distilled water, and administered once daily in doses of 0, 3, 6, 12, and 24 g/kg BW. These samples were prepared once a week, and kept in a refrigerator at 2 - 6°C.

Animals and ethics approval

A total of 100 4-week-old male and female Wistar rats were purchased from Si Beifu Biotechnology Inc., Beijing, China. The rats were kept in individual polycarbonate cages with a barrier system under a 12-h light-dark cycle. The room temperature was kept at 22 - 25°C, and the relative humidity was kept between 40 - 70% throughout the study. The rats were acclimated for one week prior to administration. Sterile filtered water and rodent standard diet (Si Beifu Biotechnology Inc., Beijing, China) were supplied to the rats *ad libitum*. All animal protocols were approved by the Committee for the Ethics of Animal Experiments of Beijing Center for Disease Prevention and Control (ref. no.: BJCDCDL20201110; dated 11 November 2020), Beijing, China. The experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 2011).

Study design

The experimental procedures for this 28-day toxicity study were carried out in accordance with OECD Guideline 407 (OECD, 2018). Based on the results of our preliminary acute oral toxicity test (unpublished) of honey, one female mouse died at a dose of 46.4 g/kg BW, and the LD_{50} for female or male mice was 92.6 g/kg BW. In this subacute study, the rats (aged five weeks) were then given five different doses (ten males and females per dose group): distilled water (control), 3, 6, 12, and 24 g/kg BW/day of honey. These samples were administered to rats daily *via* oral gavage for a total of 28 days. The sample gavage volume of rats was set at 10 mL/kg BW. The 24 g/kg BW dose group was administered twice within 24 h at 4 h intervals. Throughout the 28-day experiment, general symptoms and clinical signs of toxicity were observed and recorded once daily. The body weight of each rat was measured once a week, just before administration on day 1, and the mean body weights were calculated. The amount of added and residual diet in each cage was weighed weekly, and the average consumption was calculated. To assess relative organ weight, the fasting (approximately 16 h) body weight of all surviving rats

was measured just prior to terminal sacrifice. All rats were anaesthetised with an intraperitoneal injection of 150 mg/kg sodium pentobarbital on autopsy day, and blood samples were collected from the abdominal aorta during necropsy for further analysis.

Haematological and blood biochemical analyses

For detection of haematology parameters, approximately 0.5 mL of blood per rat was put into tubes containing EDTA-K2 for blood anticoagulation. Haematological parameters such as white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB) concentration, haematocrit (HCT) value, platelet (PLT) count, lymphocyte (LYMPH) count, and differential leukocytes count (neutrophils, lymphocytes, eosinophils, and monocytes) were analysed immediately using an automated analyser (XT 2000i; Sysmex Corporation., Hyogo, Japan). For biochemical analysis, approximately 1.5 mL of blood per rat was put into the tubes, and the partially clotted blood samples were centrifuged at 3,000 *g* for 10 min at 4°C to obtain the serum. Serum samples were separated and analysed within 24 h using a Hitachi 7600 automatic analyser (Hitachi Ltd., Tokyo, Japan). The biochemical parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (GLU), urea (URE), creatinine (CRE), cholesterol (CHOL), triglyceride (TG), albumin (ALB), and total protein (TP) were measured using the corresponding commercial test kits (FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Blood coagulation analysis

For blood coagulation activity analysis, approximately 1.0 mL of blood per rat was put in tubes containing 3.2% (109 mmol/L) trisodium citrate for blood anticoagulation. The anticoagulant to blood ratio was 1:9. These blood samples were thoroughly mixed before being centrifuged at 3,000 *g* for 10 min at 25°C. Their supernatants (platelet-poor plasma) were obtained and detected within 2 h using a Sysmex CA-1500 (Sysmex Co., Kobe, Japan) automated blood coagulation analyser. The values of prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT) were measured using commercial assay kits (Siemens Healthcare Diagnostics Inc., Marburg, Germany) following the manufacturer instructions and guidelines of US CLSI (Kamal *et al*., 2007; CLSI, 2008).

Gross necropsy and histopathology

Following blood collection, all rats were sacrificed, and gross necropsies were performed. During necropsy, the liver, kidneys, spleen, thymus, adrenal glands, salivary glands, lungs, brain, heart, pituitary gland, prostate gland, testes, epididymis, and ovaries of each rat were grossly examined for any lesions. The weight of the liver, kidneys, spleen, brain, heart, lung, testes, thymus, and adrenal glands was determined. The relative organ-to-body weight ratios were calculated after weighing paired organs together. During necropsy, all of these organs and tissues from each rat were fixed with 10% neutral-buffered formalin solution. The paraffin-embedded sections of all organs and tissues from the control, 12 and 24 g/kg BW dose groups were sliced into approximately 5 μm thickness, and stained with haematoxylin and eosin (H&E). Treatment-related histological changes were examined under a light microscope at a magnification of 400× (Olympus, Tokyo, Japan).

Statistical analysis

All data were presented as mean ± standard deviation (SD). The data analysed included body weight, food consumption, haematological parameters, biochemical parameters, blood coagulation parameters, and organ weight/organ-to-body weight ratios. The One-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test was used to analyse the differences between control and treated groups. The difference was considered significant when $p < 0.05$ (one-tailed test). Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc., IBM, USA).

Results

Body weight and food consumption

Following honey oral administration, body weight in the 24 g/kg BW group significantly decreased on day 7, and had a trend of decreasing from day 14 to day 28 as compared to the control group ($p < 0.05$ or $p < 0.01$) for both sexes of rats (Figure 1). Food consumption data are shown in Figure 2. Female rats in the 24 g/kg BW group had significant decreases on day 7 ($p < 0.01$). Food

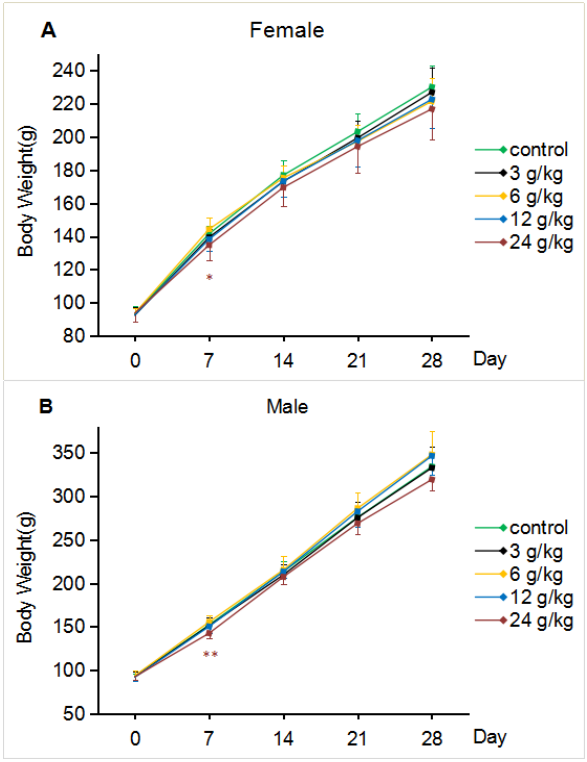

Figure 1. Changes in body weight of female **(A)** and male **(B)** Wistar rats following oral exposure to honey for 28 days. Values are mean ± SD (n = 10/group). *p < 0.05 and **p < 0.01 *versus* control group.

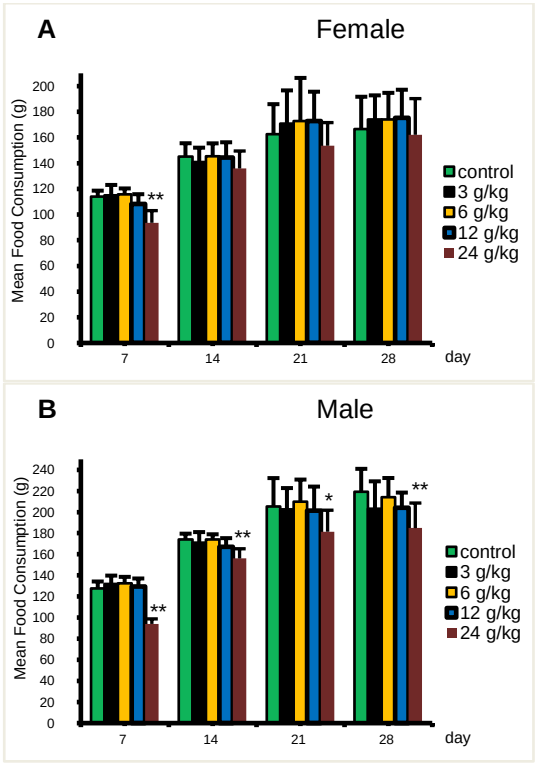

Figure 2. Weekly food consumption of female **(A)** and male **(B)** Wistar rats following oral exposure to DHA-S for 28 days. Values are mean ± SD (n = 10/group). *p < 0.05 and **p < 0.01 *versus* control group.

consumption in male rats decreased significantly from day 7 to day 28 in the 24 g/kg BW group (p < 0.05 or p < 0.01). As shown in Figure 3, the total body weight gain in female rats was significantly lower in the 24 g/kg BW group (p < 0.05). Total food consumption also decreased significantly in male rats in the 24 g/kg BW group (p < 0.01).

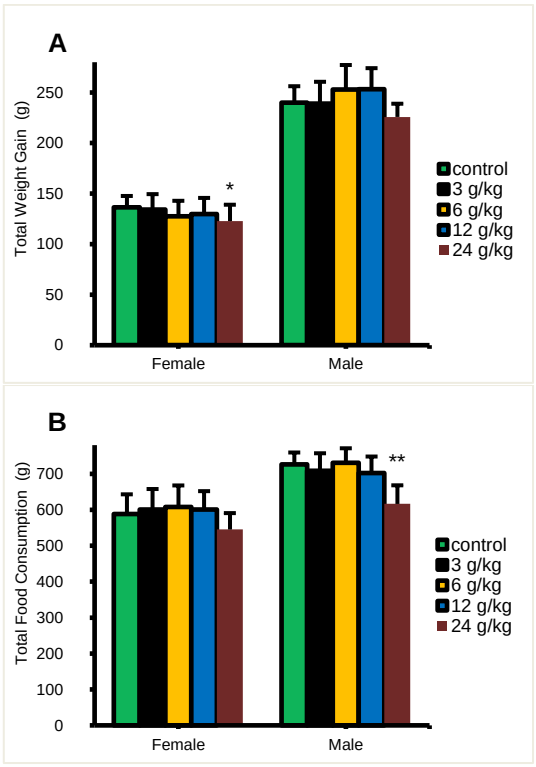

Figure 3. Total body weight gain **(A)** and total food consumption **(B)** of female and male Wistar rats following oral exposure to DHA-S for 28 days. Values are mean ± SD (n = 10/group). *p < 0.05 and **p < 0.01 *versus* control group.

Haematological parameters

The data of haematological parameters are shown in Table 1. For both sexes of rats, no statistically significant differences were observed between the control and any of the treatment groups.

Blood coagulation parameters

As shown in Table 2, the values of PT, APTT, and TT did not differ significantly between the control and honey-treated groups in female rats. For male rats, the values of TT significantly decreased in the 12 and 24 g/kg BW dose groups as compared to the control group.

Biochemical parameters

The results of biochemical parameters are shown in Table 3. Serum levels of AST, CRE, and

GLU showed similar trends in both sexes of rats. AST and CRE levels were significantly lower in the 12 and 24 g/kg BW dose groups in female rats, and in the 24 g/kg BW dose group in male rats ($p < 0.05$ or $p < 0.01$). Serum GLU levels increased significantly in both sexes of rats in the 24 g/kg BW group ($p < 0.05$ or $p < 0.01$). In addition, a significant increase in ALB was observed in female rats in the 6 and 12 g/kg BW dose groups ($p < 0.05$). Significant increases in Na were observed in the 12 and 24 g/kg BW groups ($p < 0.05$ or $p < 0.01$), in K in all the 3 - 24 g/kg BW groups ($p < 0.05$ or $p < 0.01$), and in Cl in the 24 g/kg BW group ($p < 0.01$). For male rats, significant increases were observed in Na in all of the 3 - 24 g/kg BW groups ($p < 0.05$ or $p < 0.01$), and in Cl in the 24 g/kg BW group ($p < 0.01$).

Table 1. Haematological data of Wistar rats following oral exposure to honey for 28 days.

Sex	Endpoint	Control	3 g/kg BW	6 g/kg BW	12 g/kg BW	24 g/kg BW
Female	WBC ($\times 10^3$/μL)	1.32 ± 0.26	1.33 ± 0.38	1.05 ± 0.19	1.51 ± 0.46	1.67 ± 0.82
	RBC ($\times 10^6$/μL)	6.16 ± 0.24	5.88 ± 0.41	5.96 ± 0.26	6.10 ± 0.40	6.00 ± 0.30
	HGB (g/L)	125.20 ± 4.66	121.90 ± 8.17	122.10 ± 4.68	122.90 ± 5.86	123.20 ± 7.04
	HCT (%)	39.08 ± 1.84	37.87 ± 2.50	37.86 ± 1.30	38.24 ± 1.78	38.49 ± 2.17
	PLT ($\times 10^4$/μL)	110.36 ± 14.23	104.49 ± 14.26	99.29 ± 11.76	107.81 ± 12.69	110.78 ± 10.17
	NEUT (%)	12.08 ± 4.81	9.43 ± 3.65	10.98 ± 6.61	17.56 ± 15.66	21.20 ± 13.61
	LYMPH (%)	81.82 ± 6.00	84.79 ± 5.70	81.38 ± 10.45	78.29 ± 15.31	72.71 ± 11.78
	MONO (%)	3.25 ± 2.85	2.59 ± 2.10	4.74 ± 3.51	2.55 ± 1.26	3.03 ± 1.84
	EO (%)	2.85 ± 1.81	3.19 ± 2.34	2.65 ± 2.70	1.60 ± 0.63	3.06 ± 2.74
	BAS (%)	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.79	0.00 ± 0.00	0.00 ± 0.00
Male	WBC ($\times 10^3$/μL)	1.71 ± 0.87	1.68 ± 1.35	1.01 ± 0.65	1.43 ± 1.48	1.14 ± 0.72
	RBC ($\times 10^6$/μL)	6.33 ± 0.59	6.60 ± 0.21	6.58 ± 0.41	6.50 ± 0.40	6.6 0± 0.30
	HGB (g/L)	127.00 ± 10.33	131.70 ± 4.60	132.50 ± 7.68	128.40 ± 5.74	130.40 ± 5.25
	HCT (%)	38.69 ± 3.07	40.70 ± 1.29	40.70 ± 2.52	39.94 ± 1.80	40.99 ± 1.55
	PLT ($\times 10^4$/μL)	86.24 ± 12.25	91.24 ± 11.98	83.89 ± 6.02	86.26 ± 17.21	85.81 ± 13.27
	NEUT (%)	15.27 ± 4.48	15.98 ± 12.44	14.27 ± 4.81	19.26 ± 4.65	19.67 ± 5.14
	LYMPH (%)	80.31 ± 4.54	78.89 ± 12.84	79.71 ± 4.11	75.58 ± 4.98	75.66 ± 4.67
	MONO (%)	2.62 ± 1.00	2.89 ± 1.30	3.24 ± 1.55	2.56 ± 1.04	3.02 ± 1.34
	EO (%)	1.80 ± 0.67	2.22 ± 0.93	2.78 ± 1.79	2.54 ± 0.96	1.65 ± 0.80
	BAS (%)	0.00 ± 0.00	0.02 ± 0.06	0.0 0± 0.00	0.06 ± 0.19	0.00 ± 0.00

Values are mean ± SD (n = 10/group). There was no significant difference.

Table 2. Blood coagulation data of Wistar rats following oral exposure to honey for 28 days.

Sex	Group (g/kg BW)	PT (s)	ATPP (s)	TT (s)
Female	Control	9.31 ± 0.63	15.83 ± 1.21	46.35 ± 6.01
	3	9.67 ± 1.09	16.39 ± 1.17	47.16 ± 2.21
	6	9.80 ± 1.72	17.48 ± 3.08	44.54 ± 2.22
	12	10.09 ± 1.14	16.88 ± 1.48	46.37 ± 2.37
	24	9.26 ± 1.01	16.46 ± 1.54	45.44 ± 5.25
Male	Control	10.45 ± 1.48	16.64 ± 2.35	53.43 ± 1.74
	3	10.91 ± 1.68	17.31 ± 1.90	52.64 ± 3.93
	6	11.56 ± 2.58	18.22 ± 1.26	50.78 ± 2.29
	12	10.73 ± 1.55	17.93 ± 2.27	50.68 ± 1.67*
	24	10.90 ± 0.93	17.74 ± 0.67	49.81 ± 1.45**

Values are mean ± SD (n = 10/group). *$p < 0.05$ and **$p < 0.01$ *versus* control group.

Table 3. Serum biochemical data of Wistar rats following oral exposure to honey for 28 days.

Sex	Endpoint	Control	3 g/kg BW	6 g/kg BW	12 g/kg BW	24 g/kg BW
Female	ALT (IU/L)	32.34 ± 14.01	27.37 ± 5.23	24.51 ± 4.82	27.09 ± 4.82	22.47 ± 4.41
	AST (IU/L)	141.10 ± 27.04	112.74 ± 11.50	117.65 ± 20.50	104.65 ± 9.25*	85.51 ± 10.49**
	GLU (mmol/L)	5.84 ± 0.84	6.07 ± 0.56	6.44 ± 0.80	6.34 ± 0.54	7.21 ± 0.64**
	URE (mmol/L)	7.25 ± 0.95	7.47 ± 0.78	7.73 ± 1.02	7.43 ± 1.32	8.12 ± 0.80
	CRE (μmol/L)	35.60 ± 7.57	32.40 ± 2.59	34.60 ± 6.45	30.70 ± 3.53*	30.90 ± 2.96*
	CHOL (mmol/L)	2.01 ± 0.39	1.93 ± 0.32	1.88 ± 0.41	2.13 ± 0.44	1.82 ± 0.29
	TG (mmol/L)	0.21 ± 0.12	0.26 ± 0.07	0.23 ± 0.09	0.29 ± 0.08	0.23 ± 0.05
	ALB (g/L)	37.65 ± 1.84	37.67 ± 1.34	39.53 ± 2.48*	39.79 ± 1.64*	39.23 ± 1.64
	TP (g/L)	51.03 ± 2.14	50.32 ± 1.73	52.85 ± 3.49	52.76 ± 1.78	52.58 ± 2.28
	GGT (IU/L)	0.37 ± 0.39	0.20 ± 0.23	0.39 ± 0.31	0.40 ± 0.25	0.59 ± 0.22
	ALP (IU/L)	105.40 ± 33.89	138.90 ± 23.68	131.00 ± 40.07	117.70 ± 39.67	126.20 ± 28.17
	Ig E (IU/ml)	9.30 ± 7.10	5.50 ± 1.58	8.00 ± 3.77	5.90 ± 2.42	7.00 ± 4.62
	CRP (mg/L)	0.03 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
	Na (mmol/L)	155.50 ± 1.51	155.00 ± 1.49	155.50 ± 1.65	156.90 ± 1.29*	159.10 ± 1.66**
	K (mmol/L)	4.31 ± 0.29	4.56 ± 0.18*	4.71 ± 0.19**	5.01 ± 0.26**	4.96 ± 0.23**
	Cl (mmol/L)	102.90 ± 2.08	103.80 ± 1.14	104.10 ± 0.74	104.60 ± 1.84	106.10 ± 0.99**
Male	ALT (IU/L)	31.01 ± 4.75	35.33 ± 12.70	36.88 ± 7.39	40.34 ± 13.68	30.48 ± 6.52
	AST (IU/L)	139.45 ± 21.58	151.71 ± 34.95	149.14 ± 28.17	138.25 ± 35.50	101.94 ± 18.01**
	GLU (mmol/L)	5.59 ± 0.84	6.08 ± 1.27	5.61 ± 0.60	6.28 ± 0.76	6.56 ± 0.87*
	URE (mmol/L)	6.32 ± 0.85	6.22 ± 1.11	6.55 ± 0.99	6.47 ± 0.97	5.97 ± 1.27
	CRE (μmol/L)	30.70 ± 3.83	31.00 ± 4.92	28.00 ± 2.16	29.30 ± 3.86	26.20 ± 4.80*
	CHOL (mmol/L)	1.47 ± 0.31	1.47 ± 0.32	1.37 ± 0.31	1.43 ± 0.25	1.43 ± 0.26
	TG (mmol/L)	0.44 ± 0.21	0.31 ± 0.10	0.31 ± 0.11	0.28 ± 0.05	0.31 ± 0.16
	ALB (g/dL)	37.71 ± 2.29	36.82 ± 1.17	37.87 ± 1.52	37.21 ± 0.85	37.21 ± 1.60
	TP (g/dL)	51.54 ± 2.69	51.34 ± 1.50	51.36 ± 1.78	50.71 ± 1.76	50.46 ± 2.21
	GGT (IU/L)	0.08 ± 0.11	0.21 ± 0.24	0.13 ± 0.13	0.10 ± 0.12	0.10 ± 0.17
	ALP (IU/L)	220.80 ± 41.04	224.90 ± 52.32	216.80 ± 39.21	213.90 ± 37.39	206.10 ± 53.17
	Ig E (IU/ml)	5.50 ± 2.95	7.20 ± 4.87	6.60 ± 3.81	6.80 ± 4.08	6.40 ± 4.84
	CRP (mg/L)	0.05 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.04
	Na (mmol/L)	154.00 ± 1.63	155.80 ± 0.79**	155.60 ± 1.51*	157.50 ± 1.27**	159.50 ± 1.72**
	K (mmol/L)	4.74 ± 0.18	4.69 ± 0.24	4.86 ± 0.35	4.93 ± 0.33	4.98 ± 0.49
	Cl (mmol/L)	102.30 ± 1.64	101.50 ± 1.78	102.20 ± 1.32	102.80 ± 1.23	104.70 ± 1.64**

Values are mean ± SD (n = 10/group). *$p < 0.05$ and **$p < 0.01$ *versus* control group.

Terminal body and organ weights

The terminal body, wet organ, and organ-to-body weight ratio for female and male rats are shown in Tables 4 and 5, respectively. In female rats, the terminal body weight significantly decreased in the 24 g/kg BW dose group as compared to the control group ($p < 0.05$). Significant increases in organ-to-body weight ratios of liver and brain were discovered in the 12 or 24 g/kg BW dose group in female rats. No significant differences were observed in these parameters in male rats.

Histopathological results

Table 6 shows the number of different pathological findings in the liver and kidney of rats in the control, 12, or 24 g/kg BW dose groups. In both the control and dose groups, there was mild inflammatory cell infiltration in the hepatic lobule. Mild hepatocyte steatosis was observed in five rats in the control group, five rats in 12 g/kg BW group, and six rats in 24 g/kg BW group. One rat in the control group showed focal haemorrhage and hepatocyte necrosis. Focal basophilic change of renal tubule cells

Table 4. Body and organ weight data of female Wistar rats following oral exposure to honey for 28 days.

Weight	Control	3 g/kg BW	6 g/kg BW	12 g/kg BW	24 g/kg BW
Body weight (g)	204.55 ± 9.94	200.23 ± 12.65	198.41 ± 11.13	198.84 ± 15.03	191.50 ± 17.68*
Liver wet weight (g)	7.25 ± 0.51	7.24 ± 0.63	6.94 ± 0.64	7.13 ± 0.55	7.13 ± 0.81
Organ/body weight (%)	3.55 ± 0.16	3.61 ± 0.18	3.50 ± 0.24	3.59 ± 0.22	3.72 ± 0.13*
Brain Wet weight (g)	1.74 ± 0.17	1.71 ± 0.24	1.82 ± 0.11	1.84 ± 0.06	1.85 ± 0.08
Organ/body weight (%)	0.85 ± 0.09	0.85 ± 0.11	0.92 ± 0.07	0.93 ± 0.06*	0.97 ± 0.08**
Kidney wet weight (g)	2.06 ± 0.16	2.03 ± 0.20	1.95 ± 0.17	2.02 ± 0.19	2.05 ± 0.22
Organ/body weight (%)	1.01 ± 0.07	1.02 ± 0.09	0.98 ± 0.08	1.01 ± 0.05	1.08 ± 0.12
Adrenal gland wet weight (g)	0.11 ± 0.02	0.10 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.10 ± 0.02
Organ/body weight (%)	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.01
Spleen wet weight (g)	0.59 ± 0.11	0.56 ± 0.10	0.54 ± 0.09	0.52 ± 0.08	0.52 ± 0.07
Organ/body weight (%)	0.29 ± 0.05	0.28 ± 0.04	0.28 ± 0.05	0.26 ± 0.04	0.27 ± 0.03
Heart wet weight (g)	0.87 ± 0.06	0.90 ± 0.11	0.84 ± 0.09	0.83 ± 0.09	0.83 ± 0.09
Organ/body weight (%)	0.42 ± 0.03	0.45 ± 0.04	0.42 ± 0.04	0.42 ± 0.04	0.44 ± 0.05
Thymus wet weight (g)	0.62 ± 0.08	0.61 ± 0.09	0.60 ± 0.11	0.67 ± 0.06	0.57 ± 0.05
Organ/body weight (%)	0.30 ± 0.04	0.31 ± 0.05	0.30 ± 0.05	0.33 ± 0.03	0.30 ± 0.02
Lung wet weight (g)	1.35 ± 0.32	1.35 ± 0.35	1.37 ± 0.41	1.44 ± 0.42	1.40 ± 0.35
Organ/body weight (%)	0.66 ± 0.16	0.67 ± 0.16	0.70 ± 0.22	0.73 ± 0.23	0.74 ± 0.19

Values are mean ± SD (n = 10/group). *$p < 0.05$ and **$p < 0.01$ *versus* control group.

Table 5. Body and organ weight data of male Wistar rats following oral exposure to honey for 28 days.

Weight	Control	3 g/kg BW	6 g/kg BW	12 g/kg BW	24 g/kg BW
Body weight (g)	299.31 ± 14.00	301.49 ± 24.65	315.34 ± 25.81	314.77 ± 20.97	295.75 ± 17.80
Liver wet weight (g)	10.24 ± 0.89	10.29 ± 1.25	10.65 ± 1.18	10.43 ± 0.96	10.12 ± 0.90
Organ/body weight (%)	3.42 ± 0.25	3.42 ± 0.32	3.37 ± 0.15	3.31 ± 0.23	3.43 ± 0.27
Brain wet weight (g)	1.99 ± 0.20	1.95 ± 0.20	1.98 ± 0.22	1.96 ± 0.19	1.88 ± 0.29
Organ/body weight (%)	0.66 ± 0.05	0.65 ± 0.09	0.63 ± 0.07	0.63 ± 0.07	0.64 ± 0.11
Kidney wet weight (g)	2.79 ± 0.24	2.81 ± 0.42	2.88 ± 0.30	2.91 ± 0.22	2.98 ± 0.41
Organ/body weight (%)	0.93 ± 0.09	0.93 ± 0.10	0.91 ± 0.07	0.93 ± 0.04	1.07 ± 0.32
Adrenal gland wet weight (g)	0.12 ± 0.04	0.10 ± 0.04	0.13 ± 0.04	0.12 ± 0.03	0.13 ± 0.06
Organ/body weight (%)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
Spleen wet weight (g)	0.73 ± 0.11	0.75 ± 0.10	0.75 ± 0.12	0.75 ± 0.11	0.74 ± 0.10
Organ/body weight (%)	0.24 ± 0.04	0.25 ± 0.03	0.24 ± 0.03	0.24 ± 0.04	0.25 ± 0.03
Heart wet weight (g)	1.26 ± 0.09	1.38 ± 0.17	1.39 ± 0.33	1.30 ± 0.13	1.34 ± 0.15
Organ/body weight (%)	0.42 ± 0.03	0.46 ± 0.04	0.44 ± 0.07	0.41 ± 0.03	0.45 ± 0.04
Thymus wet weight (g)	0.73 ± 0.14	0.76 ± 0.11	0.83 ± 0.16	0.70 ± 0.16	0.76 ± 0.13
Organ/body weight (%)	0.24 ± 0.05	0.26 ± 0.05	0.26 ± 0.05	0.22 ± 0.05	0.26 ± 0.03
Lung wet weight (g)	1.80 ± 0.49	1.80 ± 0.57	1.94 ± 0.67	1.96 ± 0.57	1.78 ± 0.43
Organ/body weight (%)	0.60 ± 0.17	0.60 ± 0.18	0.62 ± 0.21	0.62 ± 0.16	0.61 ± 0.16
Testis wet weight (g)	3.07 ± 0.57	2.92 ± 0.51	3.12 ± 0.19	3.14 ± 0.24	3.09 ± 0.21
Organ/body weight (%)	1.02 ± 0.17	0.97 ± 0.13	0.99 ± 0.07	1.00 ± 0.07	1.04 ± 0.03

Values are mean ± SD (n = 10/group). There was no significant difference.

Table 6. Histopathological findings of liver and kidney in female and male Wistar rats (n = 10) following oral exposure to honey for 28 days.

Pathological findings	No. of rat					
	Control		12 g/kg BW		24 g/kg BW	
	Female	Male	Female	Male	Female	Male
Liver						
Inflammatory cell infiltration of hepatic lobule	8	6	7	7	8	7
Mild steatosis of hepatocytes	3	2	3	2	4	2
Focal haemorrhage and necrosis of hepatocytes	0	1	0	0	0	0
Kidney						
Focal basophilic change of renal tubule cells	0	0	0	0	0	1

was found in one rat in the 24 g/kg BW group. Figures 4 and 5 are representative histopathology images. However, there were no obvious dose-response relationships or correlations with honey administration for any of these mild and common pathological changes. As a result, they were regarded as spontaneous lesions in the test rats. There were also no significant pathological abnormalities in any organs or tissues in the 12 or 24 g/kg BW dose group as compared to the control group.

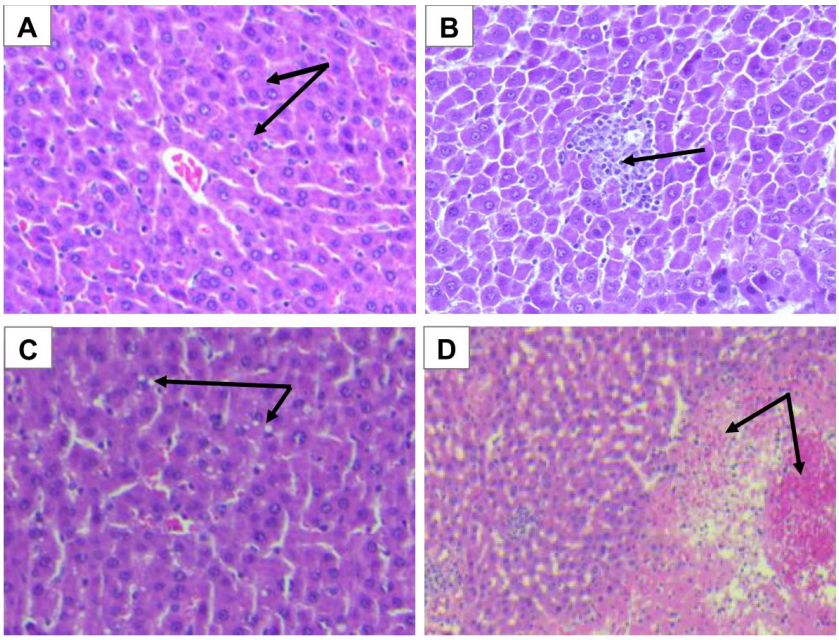

Figure 4. Hepatic histopathology of Wistar rats stained with haematoxylin and eosin (H&E), and observed at 100× magnification using a light microscope: **(A)** normal hepatocytes of rats fed with 24 g/kg honey; **(B)** inflammatory cell infiltration of hepatic lobule of rats fed with water (control group); **(C)** mild steatosis of hepatocytes of rats fed with 24 g/kg honey; and **(D)** focal haemorrhage and necrosis of hepatocytes of rats fed with water (control group).

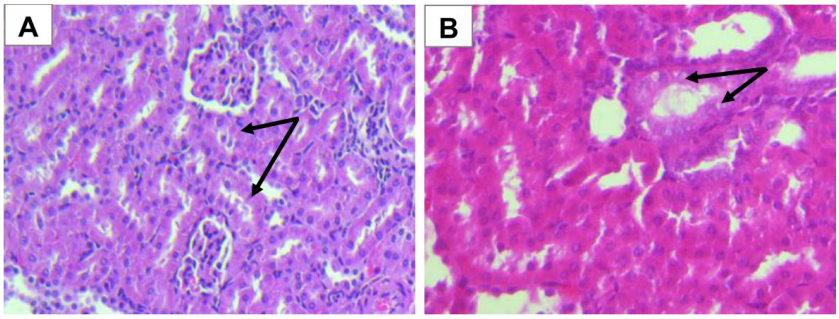

Figure 5. Renal histopathology of Wistar rats stained with haematoxylin and eosin (H&E), and observed at 100× magnification using a light microscope: **(A)** normal renal tubule of rats fed with water (control group); and **(B)** focal basophilic change of renal tubule cells of rats fed with 24 g/kg honey.

Discussion

Honey is a nutrient-rich compound produced by honeybees. The global honey industry is very large at the moment, with China having both the largest honey market and being the top producer, accounting for nearly 30% of global production (García, 2018). Poisoning incidents caused by consuming honey have been reported frequently in China and other countries, however the causes of the poisoning have not been determined until now, and data on the toxicity of honey are insufficient, thus urgently needed. As a result, the present work sought to investigate the safety and potential toxicity of honey.

During the experiment, there was no mortality among any of the test rats. Therefore, oral administration of honey at doses ranging from 3 - 24 g/kg BW for 28 days had no effect on the survival of Wistar rats. As compared to the control group, body weight in the highest dose group (24 g/kg BW) decreased significantly on day 7, and had a trend of decreasing from day 14 to day 28 in both sexes. This significant decrease in body weight in the 24 g/kg BW group could be attributed to a decrease in food consumption. Results showed that food consumption in the 24 g/kg BW group decreased significantly on day 7 in female rats, and from day 7 to day 28 in male rats. The obvious suppression of food consumption in the highest dose group of honey could be attributed to its high nutritional and calorie content, which makes it more likely to promote satiety in the gastro-intestinal tract (Blundell *et al*., 2010). Another sensitive toxicity index following the administration of test substances is a decrease in body weight gain (Hayelom *et al*., 2012). Results showed that the total body weight gain in the 24 g/kg BW group was lower in male rats, and significantly lower in female rats as compared to the control group. Total food consumption decreased significantly in male rats, and decreased with no statistically significant differences in female rats in the 24 g/kg BW group. Another study also found that honey may decrease rat weight gain due to decreased food intake after a 33-day diet (Nemoseck *et al*., 2011).

Haematological parameters are critical for assessing the toxic effects of toxic substances (Yohannan *et al*., 1994; Sonnenberg and Hepworth, 2019). In the present work, the WBC, RBC, HGB, HCT, PLT, and differential leukocyte count in blood were measured following 28-day honey treatment. Results showed that different honey doses had no significant effects on haematological parameters in both sexes of rats as compared to the control group.

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are the most commonly used screening test indexes to reflect the internal and external coagulation systems, respectively, and have very important clinical significance for the diagnosis and treatment of coagulation-related diseases (Kamal *et al*., 2007). Thrombin time (TT) determines whether the quantity and quality of fibrinogen in the blood are abnormal, as well as the time it takes for fibrinogen to be converted to fibrin. In this coagulation analysis, there were no statistically significant differences in PT or APTT values between each dose group and the control group. In male rats, TT significantly decreased in the 12 and 24 g/kg BW groups, but no significant differences were observed between the control and treatment groups in female rats. Mildly lowering TT levels had no obvious clinical implications. Based on the results of haematological and coagulation parameters, we believe that administering honey for 28 days had no discernible negative effects on coagulation indexes.

Some important serum liver enzymes, such as AST, ALT, and ALP are commonly used transaminase indexes associated with liver injury (Tice and Parry, 2002). Generally, elevated levels of those enzymes in serum may indicate hepatotoxicity (Ekam and Ebong, 2007). In the present work, repeated administration of honey had no significant effects on serum levels of ALT and ALP in both sexes of rats. The levels of AST in female rats significantly decreased in the 12 and 24 g/kg BW groups, with dose-dependent effects. In male rats, AST levels significantly decreased only in the highest dose group (24 g/kg BW). Several studies have shown that honey, at doses of 2.5, 5, and 20 g/kg BW, can alleviate the liver toxicity caused by melamine (El Rabey *et al*., 2013) or CCl_4 (El Denshary *et al*., 2012), lower serum liver enzyme markers, and decrease oxidative stress and inflammatory cytokines. Given the normal pathological results of the liver in the 12 or 24 g/kg BW groups as compared to the control group in the present work, the significant decrease in AST levels could be attributed to the significant protective effect of honey on the liver, which means that repeated administration of honey significantly ameliorated AST levels in rats (El-Haskoury *et al*., 2018).

Serum URE and CRE concentrations are measured to assess renal function and diagnose renal diseases (Khan and Alden, 2002; Arosalo *et al*., 2007). Serum URE and CRE levels would be higher than normal when the renal filtration rate is significantly decreased due to various renal diseases. CRE levels were lower in the 12 and 24 g/kg BW female groups and the 24 g/kg BW male group as compared to the control group. In both sexes, there were no significant differences in URE levels between the treatment and control groups. Oral administration of honey (0.4 and 0.8 g/kg BW) to rats has been reported to significantly decrease serum CRE, ALT, and AST values, as well as alleviated the harmful effects of lead on the liver and kidney (Elmenoufy, 2012). The probable reason for the decrease in serum CRE in the present work could be attributed to the protective effect of honey on the kidney by promoting the filtration and elimination of CRE from the blood, which was also consistent with previous study results (Hamad *et al*., 2015; Al-Waili *et al*., 2016).

The measurement of blood glucose (GLU) levels is an important tool for monitoring the progression of diabetes in rats (Elena and Ottavop, 2008). Food consumption, organ and tissue absorption, hepatic release and storage, and other factors all contribute to normal *in vivo* blood GLU levels (Smith *et al*., 2002). In the present work, GLU levels increased significantly in the 24 g/kg BW groups in both sexes with certain dose-dependent effects as compared to the control group. Previous research reported that blood GLU levels in STZ-induced diabetic rats were significantly decreased after four weeks of oral administration of 1.0 g/kg BW honey (Erejuwa *et al*., 2009). Adult male Wistar rats given daily carob honey (2 g/kg BW) for 12 days showed no obvious changes in GLU levels (El-Haskoury *et al*., 2018). The elevated blood GLU levels in the present work could be attributed to the higher dose and longer duration of honey administration to rats. There is currently no unified standard for diagnosing diabetes in rats. Based on published literature, the normal ranges of blood GLU values in rats were about 82 - 187 mg/dL (approximately 4.6 - 10.4 mmol/L) (Car *et al*., 2006). Although the blood GLU levels in the present work were within the normal range despite an increase post-treatment, the potential impact on human health of increased blood GLU caused by high-dose honey consumption should be considered.

Serum sodium, potassium, and chloride levels increased in both sexes after consecutively administering 3 - 24 g/kg BW doses of honey as compared to the control group, thus indicating the effects of honey treatment on electrolyte regulation within the body. This could be due to a high intake of mineral components contained in honey. However, the degrees of these variations were slight, and should not affect the body. Serum ALB levels in female rats increased significantly in the 6 and 12 g/kg BW groups, but no dose-dependent effects were observed, and no obvious changes were observed in male rats as compared to the control group. Therefore, the changes in ALB values were considered to have no obvious treatment-related clinical significance. There were also no discernible changes in other biochemical parameters such as CHOL, TG, TP, GGT, Ig E, and CRP.

The organ weight is another index to assess the toxicity of chemical substances. Generally, organotoxicity is associated with an increase or decrease in organ weight (Bailey *et al*., 2004; Michael *et al*., 2007). In the present work, there were no significant differences in terminal body, wet organ, or organ-to-body weight ratio between male dose groups and controls. In female dose groups, the terminal body weight decreased slightly in the 24 g/kg BW group, while the organ-to-body weight ratio of the liver and brain increased slightly in the 12 or 24 g/kg BW group. No obvious treatment-related lesions of the liver, brain, or other organs in test rats were found during the examination of gross necropsy. Results of the histological analysis showed no obvious pathological changes in the brain, and the mild and common pathological changes in the liver were classified as spontaneous lesions with no significant correlations to honey administration. Therefore, in the present work, minor increases in organ-to-body weight ratio in the liver or brain had no obvious practical clinical implications.

Conclusion

Repeated oral administration of 24 g/kg BW honey to Wistar rats for 28 days resulted in significant suppression of body weight, food consumption, and body weight gain. Aside from that, no obvious toxic effects of honey were observed in haematological or biochemical parameters. Necropsy and histological examinations revealed no evidence of systemic or target organ toxicity associated with honey

administration. Based on these findings, the NOAEL of honey in Wistar rats was determined to be 12 g/kg BW.

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References

Aliyev, F., Türkoğlu, C., Çeliker, C., Firatli, I., Alici, G. and Uzunhasan, I. 2009. Chronic mad honey intoxication syndrome: A new form of an old disease? EuropAce 11(7): 954-956.

Almasaudi S. B., Abbas A. T., Al-Hindi, R. R., El-Shitany, N. A., Abdel-Dayem, U. A., Ali, S. S., ... and Harakeh, S. M. 2017. Manuka honey exerts antioxidant and anti-inflammatory activities that promote healing of acetic acid-induced gastric ulcer in rats. Evidence-Based Complementary and Alternative Medicine 2017: 5413917.

Al-Waili, N. S. and Boni, N. S. 2003. Natural honey lowers plasma prostaglandin concentrations in normal individuals. Journal of Medicinal Food 6: 129-133.

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: 491-494.

Al-Waili, N., Salom, K., Al-Ghamdi, A. and Ansari, M. J. 2012. Antibiotic, pesticide, and microbial contaminants of honey: Human health hazards. The Scientific World Journal 2012: 930849.

Al-Waili, N. S., El-Haskoury, R., Bakour, M., Amarti, A. and Lyoussi, B. 2016. Protective effect of Morocco carob honey against lead-induced anemia and hepato-renal toxicity. Cellular Physiology and Biochemistry 39(1): 115-122

Arosalo, B. M., Raekallio, M., Raekallio, M., Holopainen, E., Kastevaara, T., Salonen, H. and Sankari, S. 2007. Detecting early kidney damage in horses with colic by measuring matrix metalloproteinase -9 and -2, other enzymes, urinary glucose and total proteins. Acta Veterinaria Scandinavica 49(1): 4.

Badolato, M., Carullo, G., Cione, E., Aiello, F. and Caroleo, M. C. 2017. From the hive: Honey, a novel weapon against cancer. European Journal of Medicinal Chemistry 142: 290-299.

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

Bertoncelj, J., Dobersek, U., Jamnik, M. and Golob, T. 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chemistry 105(2): 822-828.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P., Albertini, M. C. and Piatti, E. 2006. Raw Millefiori honey is packed full of antioxidants. Food Chemistry 97(2): 217-222.

Blundell, J., De Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., ... and Van Der Knaap, H. 2010. Appetite control: Methodological aspects of the evaluation of foods. Obesity Reviews 11(3): 251-270.

Boateng, J. and Diunase, K. N. 2015. Comparing the antibacterial and functional properties of Cameroonian and Manuka honeys for potential wound healing-have we come full cycle in dealing with antibiotic resistance? Molecules 20(9): 16068-16084.

Car, B. D., Eng, V. M., Everds, N. E. and Bounous, D. I. 2006. Clinical pathology of rat. In Suckow, M. A., Weisbroth, S. H. and Franklin, C. L. (eds). The Laboratory Rat, p. 127-145. United States: Elsevier.

Chepulis, L. M. 2007. The effect of honey compared to sucrose, mixed sugars, and a sugar-free diet on weight gain in young rats. Journal of Food Science 72: S224-S229.

Clinical and Laboratory Standards Institute (CLSI). 2008. Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays. United States: CLSI.

Cooper, R. A., Molan, P. C., Krishnamoorthy, L. and Harding, K. G. 2001. Manuka honey used to heal a recalcitrant surgical wound. European Journal of Clinical Microbiology and Infectious Diseases 20: 758-759.

Dogan, A. and Kolankaya, D. 2005. Protective effect of Anzer honey against ethanol-induced increased vascular permeability in the rat stomach. Experimental and Toxicologic Pathology 57(2): 173-178.

Dubey, L., Maskey, A. and Regmi, S. 2009. Bradycardia and severe hypotension caused by wild honey poisoning. Hellenic Journal of Cardiology 50: 426-428.

Ekam, V. S. and Ebong, P. E. 2007. Serum protein and enzyme levels in rats following administration of antioxidant vitamins during caffeinated and non-caffeinated paracetamol induced hepatotoxicity. Nigerian Journal of Physiological Sciences 22(1-2): 65-68.

El Denshary, E. S., Al-Gahazali, M. A., Mannaa, F. A., Salem, H. A., Hassan, N. S. and Abdel-Wahhab, M. A. 2012. Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats. Experimental and Toxicologic Pathology 64(7-8): 753-760.

El Rabey, H. A., Al-Seeni, M. N. and Al-Solamy, S. M. 2013. Bees' honey protects the liver of male rats against melamine toxicity. BioMed Research International 2013: 786051.

Elena, M. and Ottavop, G. 2008. Proposal open for discussion: Defining agreed diagnostic procedures in experimental diabetes research. Journal of Ethnopharmacology 115(2): 163-172.

El-Haskoury, R., Al-Waili, N., Kamoun, Z., Makni, M., Al-Waili, H. and Lyoussi, B. 2018. Antioxidant activity and protective effect of carob honey in CCl_4-induced kidney and liver injury. Archives of Medical Research 49(5): 306-313.

Elmenoufy, G. A. M. 2012. Bee honey dose-dependently ameliorates lead acetate-mediated hepatorenal toxicity in rats. Life Science Journal 9(4): 780-788.

Erejuwa, O. O., Sulaiman, S. A., Wahab, M. S. A., Sirajudeen, K. N. S. and Salzihan, M. S. 2009. Effects of Malaysian Tualang honey supplementation on glycemia, free radical scavenging enzymes and markers of oxidative stress in kidneys of normal and streptozotocin-induced diabetic rats. International Journal of Cardiology 137(1): S45.

European Union (EU). 2002. Council Directive 2001/110/EC of December 2001 relating to honey. Official Journal of the European Communities L10: 47-52.

French, V. M., Cooper, R. A. and Molan, P. C. 2005. The antibacterial activity of honey against coagulase-negative staphylococci. Journal of Antimicrobial Chemotherapy 56: 228-231.

García, N. L. 2018. The current situation on the international honey market. Bee World 95: 89-94.

Gheldof, N., Wang, X. H. and Engeseth, N. J. 2003. Buckwheat honey increases serum antioxidant capacity in humans. Journal of Agricultural and Food Chemistry 51: 1500-1505.

Godfrey, V. B., Chen, L. J., Griffin, R. J., Lebetkin, E. H. and Burka, L. T. 1999. Distribution and metabolism of (5-hydroxymethyl)furfural in male F344 rats and B6C3F1 mice after oral administration. Journal of Toxicology and Environmental Health - Part A 57: 199-210.

Gunduz, A., Merice, E. S., Baydin, A., Topbas, M., Uzun, H., Turedi, S. and Kalkan, A. 2009. Does mad honey poisoning require hospital admission? American Journal of Emergency Medicine 27(4): 424-427.

Hamad, R., Jayakumar, C., Ranganathan, P., Mohamed, R., El-Hamamy, M. M. I., Dessouki, A. A., ... and Ramesh, G. 2015. Honey feeding protects kidney against cisplatin nephrotoxicity through suppression of inflammation. Clinical and Experimental Pharmacology and Physiology 42(8): 843-848.

Hayelom, K., Mekbeb, A., Eyasu, M., Wondwossen, E. and Kelbesa, U. 2012. Methanolic effect of *Clerodendrum myricoides* root extract on blood, liver and kidney tissues of mice. African Health Sciences 12(4): 489-497.

Kamal, A. H., Tefferi, A. and Pruthi, R. K. 2007. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clinic Proceedings 82(7): 864-873.

Khalil, M. I. and Sulaiman, S. A. 2010. The potential role of honey and its polyphenols in preventing heart diseases: A review. African Journal of Traditional, Complementary and Alternative Medicines 7: 315-321.

Khalil, M. I., Sulaiman, S. A. and Gan, S. H. 2010. High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored

for more than one year. Food and Chemical Toxicology 48(8-9): 2388-2392.

Khan, K. N. M. and Alden, C. L. 2002. Kidney. In Haschek, W. M., Rousseaux, C. G. and Wallig, M. A. (eds). Handbook of Toxicologic Pathology, p. 255-336. United States: Academic Press.

Loannidou, M. D., Zachariadis, G. A., Anthemidis, A. N. and Stratis, J. A. 2004. Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. Talanta 65: 92-97.

Michael, B., Yano, B. L., Sellers, R. S., Perry, R., Morton, D., Roome, N., ... and Schafer, K. 2007. Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. Toxicologic Pathology 35(5): 742-750.

National Institute of Health (NIH). 2011. Guidelines for the care and use of laboratory animals. United States: NIH.

Nemoseck, T. M., Carmody, E. G., Furchner-Evanson, A., Gleason, M., Li, A., Potter, H., ... and Kern, M. 2011. Honey promotes lower weight gain, adiposity, and triglycerides than sucrose in rats. Nutrition Research 31: 55-60.

New Zealand Ministry for Primary Industries (MPI). 2010. Compliance guide to the food (tutin in honey) standard 2010. New Zealand: MPI.

Organization for Economic Co-operation and Development (OECD). 2018. OECD guidelines for the testing of chemicals (no. 407) - Repeated dose 28-day oral toxicity study in rodents. France: OECD.

Rao, P. V., Krishnan, K. T., Salleh, N. and Gan, S. H. 2016. Biological and therapeutic effects of honey produced by honey bees and stingless bees: A comparative review. Revista Brasileira de Farmacognosia 26: 657-664.

Sayin, M. R., Karabag, T., Dogan, S. M., Akpinar, I. and Aydin, M. 2012. Transient ST segment elevation and left bundle branch block caused by mad-honey poisoning. Wiener Klinische Wochenschrift 124(7-8): 278-281.

Smith, G. J., Rhodes, E. C. and Langill, R. H. 2002. The effect of pre-exercise glucose ingestion on performance during prolonged swimming. International Journal of Sport Nutrition and Exercise Metabolism 12(2): 136-144.

Sonnenberg, G. F. and Hepworth, M. R. 2019. Functional interactions between innate lymphoid cells and adaptive immunity. Nature Reviews Immunology 19(10): 599-613.

Spano, N., Casula, L., Panzanelli. A., Pilo, M. I., Piu, P. C., Scanu, R., ... and Sanna, G. 2006. An RP-HPLC determination of 5-hydroxymethylfurfural in honey: The case of strawberry tree honey. Talanta 68: 1390-1395.

Tice, S. A. and Parry, D. 2002. Medications that need hepatic monitoring. Hospital Pharmacy 37(2): 1-9.

Yao, L., Jiang, Y., D'Arcy, B., Singanusong, R., Datta, N., Caffin, N. and Raymont, K. 2004. Quantitative high-performance liquid chromatography analyses of flavonoids in Australian Eucalyptus honeys. Journal of Agricultural and Food Chemistry 52: 210-214.

Yohannan, M. D., Higgy, K. E., Al-Mashhadani, S. A. and Santhosh-Kumar, C. R. 1994. Thrombocytosis: Etiologic analysis of 663 patients. Clinical Pediatrics 33(6): 340-343.