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Adulterated Honey Consumption can Induce Obesity, Increase Blood Glucose Level and Demonstrate Toxicity Effects

(Pengambilan Madu Tidak Asli boleh Menyebabkan Keobesan, Meningkatkan Kadar Paras Gula dan Menunjukkan Kesan Toksik)

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ABSTRACT

The effects of adulterated honey consumption towards human health is not widely known; mainly due to lack of systematic and scientific studies and low public awareness. In this study, short-term and long-term effects of consumption on two brands of commercially honey are available in Malaysian market was investigated and compared to normal control (NC) rats and rats fed with natural pineapple honey (PH) using male Sprague dawley rats. Adulteration of honey used in the study was measured using physicochemical and antioxidant analyses and identified as adulterated honey A (FHA) and B (FHB). No toxicity effect was found for short-term consumption (14 days with one honey consumption). However, visible effects were observed after 16 weeks of study. Both FHA and FHB showed a significant increase ($p>0.05$) in cholesterol (48.6 ± 4.8 mmol/L, 46.5 ± 3.6 mmol/L), triglycerides (26 ± 1.2 mmol/L, 24.4 ± 1.8 mmol/L) and glucose (28.4 ± 2.5 mmol/L, 25 ± 2.6 mmol/L) level respectively. In contrast, rats from NC and PH groups have lower cholesterol (26.5 ± 4.4 mmol/L, 18.94 ± 3.6 mmol/L), triglycerides (17.5 ± 1.2 mmol/L, 13.5 ± 1.5 mmol/L) and glucose (6.4 ± 1.4 mmol/L, 8.0 ± 1.5 mmol/L) level, respectively. The most critical finding was in total five rats from both fake honey groups showed early mortality. This intensive study indicates long-term adulterated honey may harm to human health and required prompt actions from various authorities locally and internationally to avoid other consequences in the future.

Keywords: Adulterated honey consumption; health effects; honey analysis; honey authenticity; long-term effects; obesity; short-term effects

ABSTRAK

Kesan pengambilan madu yang tidak asli terhadap kesihatan manusia masih lagi banyak yang belum diketahui; berikutan kekurangan penyelidikan secara sistematik dan saintifik, serta kesedaran masyarakat awam tentang kewujudan madu tidak asli. Kajian yang dijalankan ini menumpukan pada kesan jangka masa pendek dan panjang penggunaan dua madu komersial dijual di pasaran Malaysia dan dibandingkan dengan tikus kawalan (NC) dan tikus yang diberi makan madu asli nenas (PH) menggunakan tikus jantan strain Sprague Dawley. Ketulenan madu tersebut dikaji menggunakan analisis fizikokimia dan antioksida sebelum ia dilabel sebagai madu tidak asli A (FHA) dan B (FHB). Tiada kesan keracunan dilaporkan untuk pengambilan madu tersebut dalam masa yang singkat (satu pengambilan madu dalam masa 14 hari). Walau bagaimanapun, kesan yang ketara dapat diperhatikan apabila kedua-dua FHA dan FHB diambil selama 16 minggu. Kedua-dua FHA dan FHB telah meningkatkan secara signifikan ($p>0.05$) aras kolesterol (48.6 ± 4.8 mmol/L, 46.5 ± 3.6 mmol/L), trigliserida (26 ± 1.2 mmol/L, 24.4 ± 1.8 mmol/L) dan glukosa (28.4 ± 2.5 mmol/L, 25 ± 2.6 mmol/L). Ini berbeza dengan tikus daripada kumpulan NC dan PH yang menunjukkan aras kolesterol (26.5 ± 4.4 mmol/L, 18.94 ± 3.6 mmol/L), trigliserida (17.5 ± 1.2 mmol/L, 13.5 ± 1.5 mmol/L) dan glukosa (6.4 ± 1.4 mmol/L, 8.0 ± 1.5 mmol/L) yang lebih rendah. Pemerhatian yang paling kritikal di dalam kajian ini ialah lima ekor tikus yang diberi makan dengan kedua madu FHA dan FHB secara berasingan telah menyebabkan kematian awal. Kajian secara intensif ini menunjukkan bahawa pengambilan madu yang tidak asli dalam jangka masa yang panjang boleh mengancam kesihatan manusia. Ini amat memerlukan perhatian dan tindakan serius oleh pihak berkuasa tempatan dan antarabangsa untuk mengambil tindakan ke atas pengeluaran dan penjualan madu tidak asli di dalam pasaran untuk mengelak sebarang mudharat pada masa hadapan.

Kata kunci: Analisis madu; kesan jangka masa pendek dan panjang; kesan terhadap kesihatan; ketulenan madu; keobesan; pengambilan madu tidak asli

Introduction

Honey adulteration is one of the main issues in honey fraud. It is emerging as a threat to beekeeping industry worldwide including Malaysia. The main reasons contribute to the issue are small production of pure (unadulterated) honey compared to the honey demand, and its commercial value

(Strayer et al. 2014). Various actions have been taken to solve the problem both locally and internationally, but until to date no effective result was perceived in controlling the adulterated honey production.

Pure honey, also known as natural honey, is a pre-digested food and it is the best alternative sweetener for many people who could not digest plain cane sugar. It may be originated from either nectar in flowers or plant saps which is processed in the bee's stomach (Codex 2001; Rahaman et al. 2013). It contains 80% of simple sugars such as fructose and glucose, which are readily to be broken down by the digestive system. Therefore, it is directly absorbed into our bloodstream and is converted directly into energy. Besides simple sugar, approximately 200 substances were recorded from pure honey such as antioxidant properties, water, vitamins, minerals, phenolic acids, proteins and enzymes (Bogdanov et al. 2008). However, the exact composition of honey may vary according to the plant species on which the bee forages, but the main constituents are almost similar in all honeys (Almeida-Muradian et al. 2013). Previous researches proved that all compositions in the honey showed synergistic action and may contribute several health benefits to our health instead of the individual component (Chavan et al. 2014; Zainol & Mohd 2013).

On the other hand, adulterated honey is made from table sugar, sucrose syrup or high fructose corn syrup which is not originated from the honey bee stomach (Weihrauch & Diehl 2004). Due to profit motive and scarcity of pure honey in the market, irresponsible traders started to produce and selling adulterated honey which is far cheaper and easier to produce compared to the pure honey.

Identification of adulterated honey is challenging particularly for consumers with lack of knowledge on characteristics of pure honey. With current advanced technology, doctored or fraudulent adulterated honey becomes more sophisticated leading to misidentification even for experts.

Besides these challenges, not much knowledge is known on the effect of adulterated honey on health. The data is crucial to support enforcement on preventing adulterated honey production and vending throughout the regions. Thus, the present study was conducted to measure health effects of adulterated honey available in the local market using Sprague Dawley rats for short- and long-term consumptions and compared to rats fed with natural honey.

Materials and Methods

Adulterated honey A (FHA) and B (FHB) were purchased randomly from groceries in Shah Alam, Selangor, Malaysia. Pineapple honey harvested from the pineapple farm in Johor, Malaysia was used as a pure honey control. All the samples were stored at 4°C, away from direct sunlight, in amber bottles throughout the study. Chemicals and reagents were purchased either from Sigma-Aldrich (St. Louis, Mo., USA.) or from Merck (Darmstadt, Germany). All chemicals used were at the analytical grade.

PHYSIOCHEMICAL ANALYSIS

Physicochemical analysis of honey was conducted to identify authenticity of honey according to the International Honey Commission (IHC), 2009 such as moisture content, pH, acidity, hydroxymethylfurfural (HMF) and sugar profile.

Moisture content Moisture content of each honey was determined using a refractometric method based on the refractive index increases with solids content. Each series of measurement was determined in triplicate using a digital refractometer at ambient temperature (Bogdanov et al. 1997; Codex 2001).

pH and Acidity pH of all honeys were determined according to the method described by the IHC. Five grams of the honey was diluted with 50 ml distilled water to make a 10% solution. The pH was measured using a digital pH meter (HI 98127, Hanna instruments, Mauritius), which was calibrated at room temperature using buffer solutions at pH4 and 7. Meanwhile, the acidity was determined by the titrimetric method (with 0.1M sodium hydroxide solution) (IHC 2009).

Hydroxymethylfurfural (HMF) Five grams of honey were dissolved in 25 mL of distilled water. The absorbance was then measured at 284 and 336 nm using a spectrophotometer in triplicate. Level of HMF was determined according to the equation, where D = dilution factor and W = sample weight in grams:

$$\text{HMF (mg/kg of honey)} = (\text{Abs}_{284} - \text{Abs}_{336}) \times 149.7 \times 5 \times \text{D/W}.$$

Sugar Analysis Honey samples were weight and placed in the glass vials prior to added 0.45 mL of pyridine. After that, the vials were immersed in the waterbath at 70°C for 10 min. Next, 0.5 mL of hexamethyldisalizane (HMDS) was added and mixed well. Then, 0.05 mL of triflouroacetic (TFA) was added slowly, mixed gently for 30 s and allowed to stand for 15 min until a homogenous clear solution was obtained. For injection, 1.0 µL solution was required. The sugar contents in honey were compared to the standard; fructose, glucose, maltose and sucrose. Gas chromatography mass spectrophotometry (GCMS) Agilent Technologies Model 7890A couple with inert MSD with Triple-Axis detector model 5975C was used in the study.

Determination of Total Phenolic and Flavanoid Compounds Antioxidant levels of honey can be measured with total phenolic and flavonoid compounds. The concentration of the phenolic compounds in honey and adulterated honey samples were estimated using Folin-Ciocalteu method with a slight modification (Singleton et al. 1999). Gallic acid was used to calculate a standard curve (20, 40, 60, 80 and 100 µg/mL; $r^2 = 0.9336$). The concentration of the phenolic compounds was measured in triplicate. The results were reported as milligram (mg) of gallic acid equivalents (GAEs) per kilogram (kg) of honey.

The total flavonoid content in samples was measured using the colorimetric assay as developed by Jia et al. (1999). A calibration curve was created using a standard solution of catechin (20, 40, 60, 80 and 100 μg/mL; r^2 = 0.998). The results were expressed as mg catechin equivalents (CE) per kg of honey.

ANIMAL STUDY

The study was approved under the Ethical Use of Animals (UiTM Care), Reference No. 05/2012. Forty eight healthy Sprague Dawley male rats were obtained from Laboratory Facilities of Animal Management (LAFAM), Universiti Teknologi MARA (UiTM), Puncak Alam, Selangor, Malaysia. Eight weeks old rats with weight between 180 and 220 g were housed and managed according to Samat et al. (2014). Two types of animal study were conducted to observe the honey consumption for short- and long-term effects.

Short-term Study for Adulterated Honey Consumption Short-term or acute study via a single administration test was performed according to the guidelines of the Organisation for Economic Co-operation and Development (OECD) for the testing of chemicals, TG 423 with slight modifications (OECD 2001). The rats were divided into four groups: normal control and rats fed with 2000 mg/kg body weight honey; either with pineapple honey, adulterated honey A (FHA) or adulterated honey B (FHB), with one single oral dose of the substance. Mortality and general behaviour of the animals were observed over a 24 h period and thereafter once a day for the next 14 days. Any clinical signs of toxicity were recorded. On day 15, the overnight-fasted animals (water allowed) were euthanized using diethyl ether and subjected to gross pathological examination of all the major internal organs, such as liver, kidneys, heart, lung, spleen and brain.

Long-term Study for Adulterated Honey Consumption Long-term or chronic effects of adulterated honey consumption with a repeat-dose oral administration was carried out according to OECD guideline 408 with a slightly modification. Four groups of eight male rats each were kept individually in separate cages. Each rat was fed with respective honey once daily for sixteen weeks via oral administration. Group 1 was kept as control and received normal clean drinking water *ad libitum*. Groups 2, 3 and 4 were treated with 2500 mg/kg of pineapple honey, FHA and FHB, respectively. Animals in each group were weighed on day zero (baseline) and weekly thereafter. They were observed for general behavioral and signs of abnormalities during the experiment duration. Relative organ weight of the organs was also calculated.

Body Weight and Meal Pattern Analysis Body weight, amount of food and water consumed and food efficiency were calculated as mentioned in Samat et al. (2014).

Biochemical Analysis Clear serum obtained from blood samples were separated and collected for analysis of serum levels for the hepatic function tests, including aspartate aminotransferase (AST), alanine transaminase (ALT), total bilirubin and total protein, a renal function test (urea and creatinine) and serum lipid profile (glucose, triglycerides and total cholesterol) (Samat et al. 2014).

Anthropometrical Determinations In this study, the body weight and body length were determined for anthropometrical parameters. Body mass index (BMI) was determined by body weight (g) divided by root of length (cm^2). Meanwhile for adiposity index was determined by the sum of epididymal, visceral and retroperitoneal fat weights divided by body weight × 100 and expressed as adiposity percentage (% AI) (Abu et al. 2014).

Relative Organ Weights Liver and kidneys were then carefully dissected, cleaned of any fat and weighed. The relative organ weight (ROW) of each organ was then calculated according to the following:

$$\text{ROW} = \frac{(\text{absolute organ weight (g)} \times 100)}{\text{body weight of rat on sacrifice day (g)}}.$$

Histological Evaluation Liver and kidneys were processed for histological evaluation (Samat et al. 2014). Any signs of abnormality and toxicity owing to any effects of the administration of pineapple honey and adulterated honeys were observed and recorded.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard error mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA). Values with a confidence level of $p \leq 0.05$ were considered significant.

Results and Discussion

PHYSIOCHEMICAL ANALYSIS

Measurement of physicochemical properties such as moisture content, pH, acidity, hydroxymethylfurfural (HMF) and sugar profile are criteria set by Codex to prove the authentication of honey.

Moisture content of pineapple honey (pure honey), adulterated honey A (FHA) and adulterated honey B (FHB) used in the study was varied with pineapple honey showed the highest moisture content (Table 1). Meanwhile, pH of both FHA and FHB showed the highest pH value (Table 1). Free acidity and HMF value for both adulterated honeys was significantly higher compared to the pineapple honey (Table 1).

Moisture content determines the amount of water present in honey. According to Codex, acceptable moisture content in the honey is less than 20% of honey (Codex 2001). However, moisture content of pure honey from the tropical regions is higher than the temperate countries due to high humidity in tropical region. Moreover, honey is hygroscopic which can readily absorb water from its surrounding. Moisture content in the pure honey

TABLE 1. Physiochemical analysis of moisture content, pH, acidity, and HMF from pineapple honey (PH), adulterated honey A (FHA) and adulterated honey B (FHB)

Parameter	IHC Value	Types of honey		
		PH	FHA	FHB
Moisture content (%)	<20	25.10 ± 0.08	18.70 ± 0.02*	18.10 ± 0.08*
pH	-	3.63 ± 0.07	3.79 ± 0.03	4.10 ± 0.02*
Free Acidity	-	96.00 ± 0.05	24.00 ± 0.05*	8.00 ± 0.02*
HMF (mg/kg)	<80	14.31 ± 1.50	195.4 ± 3.50*	151.5 ± 3.45*

The results are presented as means ± SEM (standard error of the mean) for triplicate reading. Statistically significant data are given as *p<0.05 compared to pineapple honey

was slightly higher but is not significant compared to adulterated honeys. The results reflected to the seasonal climate in Malaysia whereby the sampling period was at the end of the year (rainy season, between September and December) (Moniruzzaman et al. 2013). Meanwhile, moisture content of adulterated honeys can be engineered according to the Codex and stable because of lacking hygroscopic behavior even at the room temperature.

pH value for both pure honey and adulterated honeys were insignificantly different. However, value of acidity was evidently low in the adulterated honey. In contrast, data of pH and acidity of adulterated honeys contradicted and questionable. Acidity in honey is associated with same factors, such as floral sources, amount of minerals, time of harvesting and also the amount of gluconic acid resulting from enzymatic action on glucose (Fredijs et al. 2006; Moniruzzaman et al. 2013). This factor clearly observed in pure honey but not in adulterated honeys. Therefore, free acidity in adulterated honeys was low compared to pure honey.

HMF value for both adulterated honeys was significantly higher compared to the pineapple honey and beyond accepted level set by the Codex. Production HMF is a natural process in all sugary natural products particularly fructose. The value is varying from 0 mg/kg in the fresh harvested honey to 40 mg/kg of temperate honey or 80 mg/kg of tropical honey set by the Codex. However, process of HMF can be accelerated by the addition of commercial sugar and heating process, which is a common practice in the adulterated honey production. The process can be observed in both FHA and FHB samples used in the study (Khalil et al. 2010; LeBlanc et al. 2009). Effects of excessive HMF in the adulterated honey are subjected for investigations. A few studies revealed the formation of HMF solely was toxic to rats and mice because it induces severe toxicity into the liver, mutagenesis and carcinogenic effects (Abraham et al. 2011).

High level of antioxidant, in terms of total phenol and total flavonoid contents, can be measured in pure honey. Meanwhile, adulterated honey will either shows none or small amount of antioxidant properties. In this study, pineapple honey exhibited significantly high value of both phenolic and flavonoid contents than both adulterated honey (Figure 1). High value of antioxidants in pineapple honey associated to a correlation between floral, geographical and botanical origin (Alvarez-Suarez et al. 2013; Lee et al. 2013). In contrast, antioxidant value showed by the adulterated honeys solely contributed from high fructose corn syrup or sucrose and commonly it is in low level.

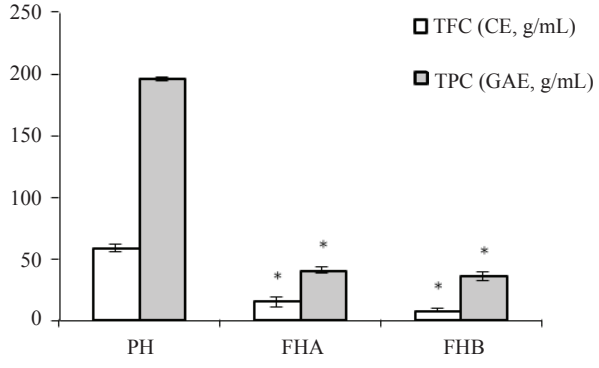

FIGURE 1. Antioxidant properties (total flavonoid and total phenolic contents) of pineapple honey (PH), adulterated honey A (FHA) and adulterated honey B (FHB). TFC: Total flavanoid content, TPC: Total phenolic content

The results are presented as means ± SEM (standard error of the mean) for triplicate reading. Statistically significant data are given as *p<0.05 compared to pineapple honey

Confirmation of the honey status can be obtained from the measurement of sugar profile either by using GCMS or high performance liquid chromatography (Wang et al. 2015). In this study, total reducing sugar and fructose over glucose ratio in both adulterated honey samples detected by GCMS were significantly lower compared to pineapple honey (Table 2). The value was below range the highest limit of total sugar content (≥ 60%) determined by the International Honey Commission (IHC) (2009). Moreover, the percentage of sucrose content in FHA and FHB was significantly higher compared to pineapple honey and more than acceptable limit approved by Codex (2001) and IHC (2009). Based on the physicochemical results, both FHA and FHB in this study were identified as adulterated honey with sucrose.

ANIMAL STUDY

Short-term or acute effect of adulterated honey consumption was observed using a single dose administration test. All rats fed once with 2000 mg/kg body weight of each honey for 14 days showed no sign of toxicities either apparent

TABLE 2. Sugar contents of Pineapple honey (PH), adulterated honey A (FHA) and adulterated honey B (FHB). The results are presented as means ± SEM (standard error of the mean) for triplicate reading. Statistically significant data are given as *p<0.05 compared to pineapple honey

Parameter	IHC Value	Types of honey		
		Pineapple Honey	FHA	FHB
Total reducing sugar (%)	>60	60.69 ± 2.07	49.29 ± 1.05*	44.99 ± 1.70*
Fructose/Glucose (F/G) ratio	1	1.21 ± 0.20	0.75 ± 0.06	0.87 ± 0.07
Sucrose (%)	<5	0.97 ± 0.08	5.75 ± 0.43*	5.58 ± 0.62*

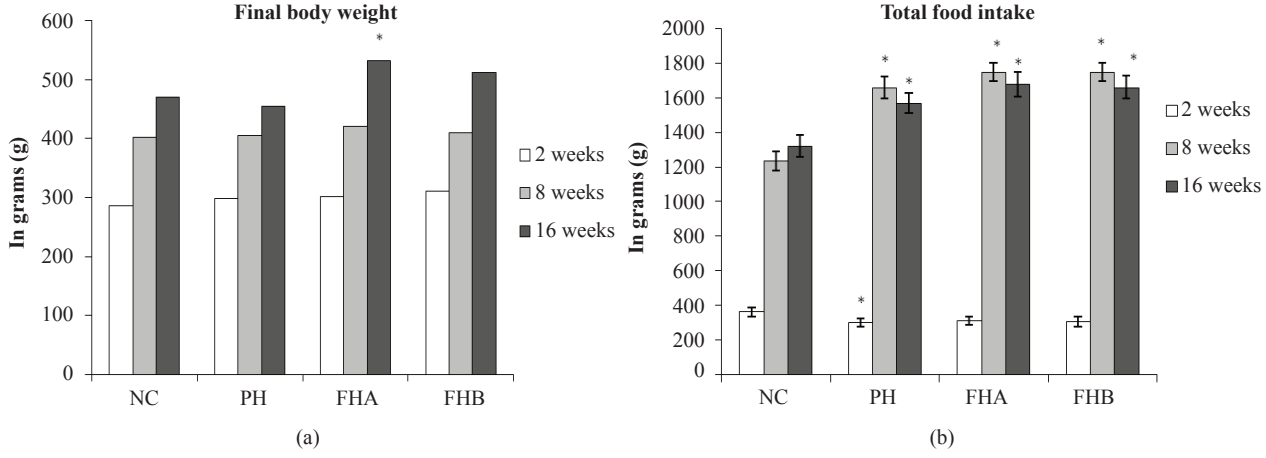

FIGURE 2. Body weight gains (a) and total food intake (b) after 2, 8 and 16 weeks of study

The results are presented as means ± SEM (standard error of the mean) for triplicate reading. Statistically significant data are given as *p<0.05 compared to NC. NC: normal control, PH: pineapple honey, FHA: adulterated honey A, FHB: adulterated honey B

differences in physical activity or other behaviours, no significant changes in the nature of stool, urine and eye colour of any rat, no diarrhoea, salivation, convulsion, sleep or coma and no symptoms of toxicity and mortality were observed. The reason is because the rats were fed with honeys only once a day for total period of 14 days. The study was short and require longer period of study to observe the honey's effects.

In contrast, chronic effects of the adulterated honey consumption were investigated using a long-term study i.e. 16 weeks with daily consumption of honey. There were obviously treatment-related deaths observed and many abnormal signs developed in both groups fed with the adulterated honeys during and after the study. On week seven, three rats fed with FHA and two rats fed with FHB were dead. Throughout the study, the rest rats from both groups fed with the adulterated honeys showed abnormal behavioural activities like aggressive, excessive drinking intake compare to control rats and rats fed with pineapple honey. On the last few weeks of study, some of the rats fed with both adulterated honeys showed grooming tachycardia, thinning hair, heavy breathing and lethargy (shown by reduced activities). Meanwhile, rat fed with pineapple honey did not showed any abnormalities in behavioral activities but showed healthy, physically active, consuming food and water in regular way.

The remaining rats in both adulterated honey groups showed a significant increment in the body weight, accompanied by an increase in fat pads and BMI even though their total food intake was slightly less compared to eight weeks (Figure 2). The results reflect on the tendency and appearance of signs indicating obesity, hyperglycemia and also diabetes; and based on the rat's final body weight and anthropometrical observation; it clearly indicates the obese condition (Figure 3).

Serum lipid profile including triglycerides and cholesterol and glucose levels were observed and it showed drastically and significantly increased in rats fed with adulterated honeys as early as after two weeks of the study (Figure 4).

Based on the findings, rats fed with both adulterated honeys fulfilled all the criteria of obesity and hyperglycemia such as increased body weight gain, fat pads, BMI, triglyceride, cholesterol and glucose levels, which indicates adulterated honey consumption leads to obesity and potentially diabetes (Belobrajdic et al. 2012; Bocarsly et al. 2010). These findings are in concurrent to previous studies that long-term consumption of golden syrup, sucrose or/and fructose diet or fructose cane syrup alone demonstrated a high mean total percent body fat, increased visceral fat pads which then leads to hypercholesterolemia, hypertriglyceridemia and hyperinsulinemia (Ajibola et al. 2013; Bocarsly et al. 2010; Cao et al. 2012). As a result, lipogenesis was enhanced and gene expressions of several enzymes including acetyl coA carboxylase and fatty acid synthase that responsible for the synthesis of triacylglycerides in the liver was increased (Larson-Meyer et al. 2010).

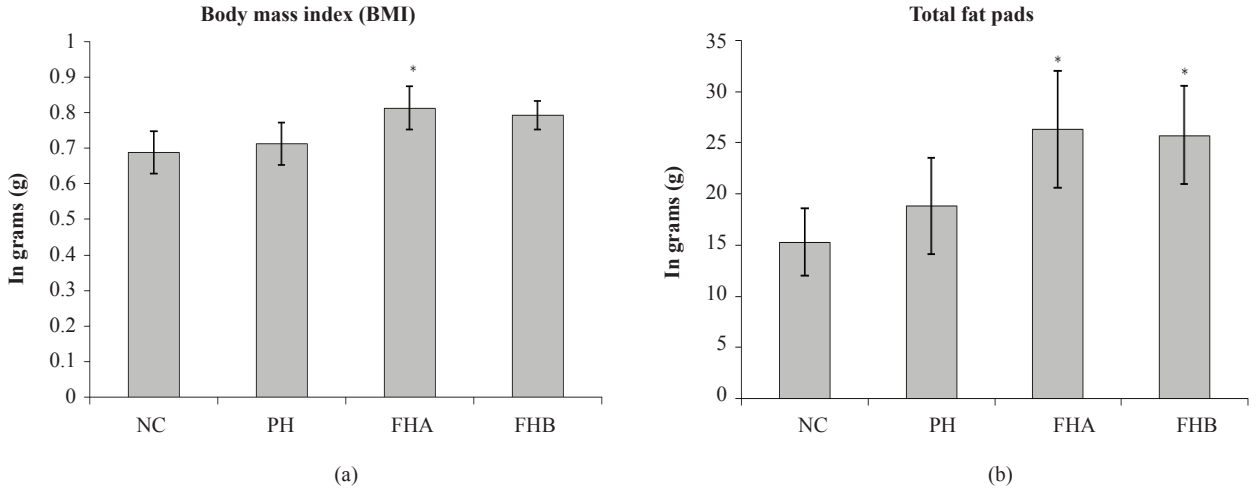

FIGURE 3. Effects of nomal diet in control rats (NC), rat fed with either pineapple honey (PH), adulterated honey A (FHA) or adulterated honey B (FHB) on (a) body mass index (BMI) and (b) total fat pads

Values are expressed as mean ± SEM (standard error of the mean) (n=6). Statistically significant data are given as *p<0.05 compared to NC

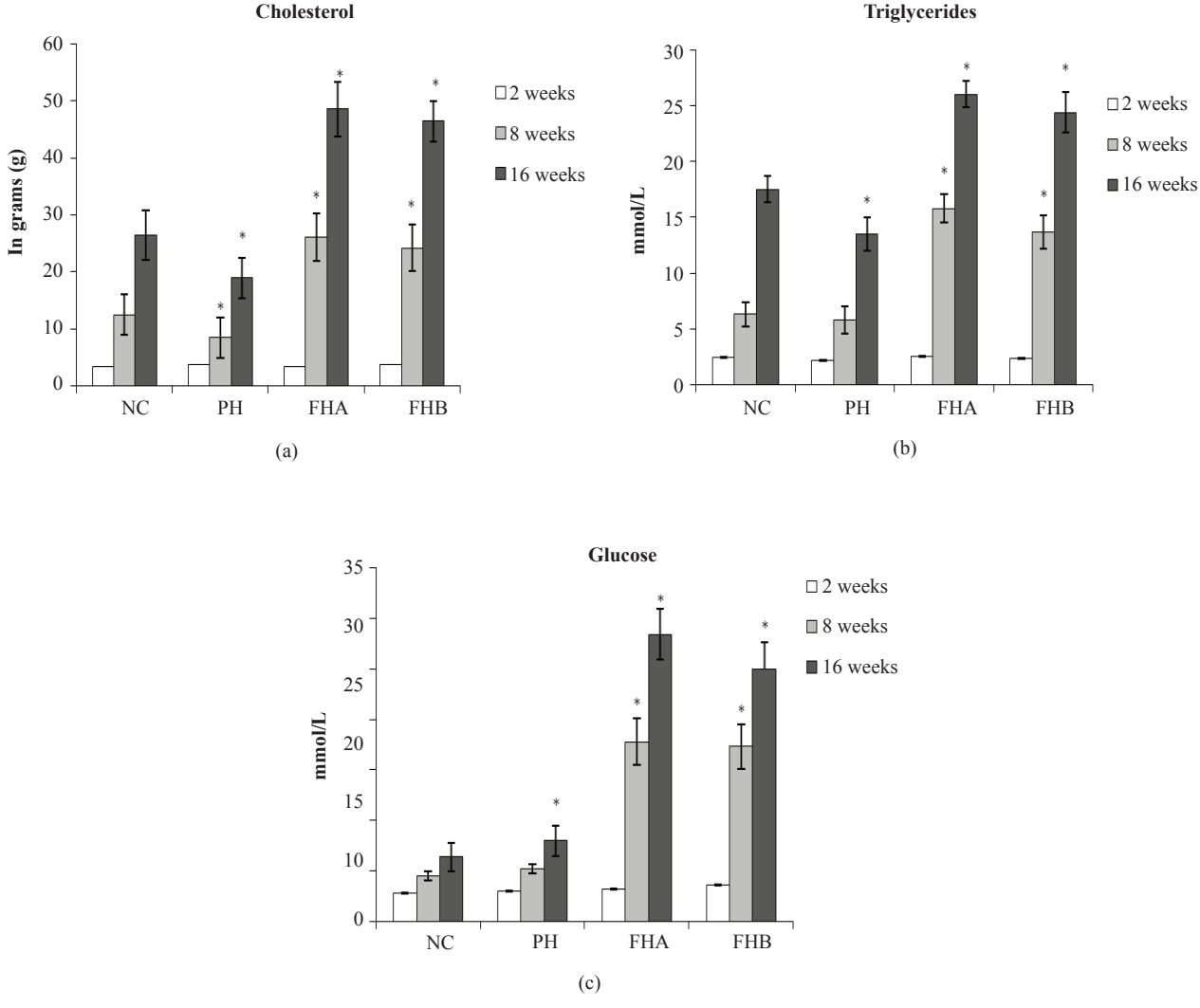

FIGURE 4. Effects of nomal diet in control rats (NC), rat fed with either pineapple honey (PH), adulterated honey A (FHA) or adulterated honey B (FHB) on (a) cholesterol, (b) triglycerides and (c) glucose on rats

Values are expressed as mean ± SEM (standard error of the mean) (n=6). Statistically significant data are given as *p<0.05 compared to NC

Besides, rats fed with pineapple honey, a natural and pure honey used in this study, showed a slight reduction in the body weight gain, no significant change in total fat pads and BMI compared to control rats, even though the rats had a high food intake. The honey significantly reduced the level of triglycerides, cholesterol and glucose compared to rats fed with both adulterated honeys. These were similar observations as reported by the other studies either in animal or human study (Abdulrhman et al. 2013; Al-Waili, 2004; Mushtaq et al. 2011; Nazir et al. 2014; Yaghoobi et al. 2008). The effects appeared because of existence of simpler sugars and high levels of antioxidants in the pure honey compared to the adulterated honeys (Ajibola et al. 2013; Alvarez-Suarez et al. 2013). Simple sugar such as glucose and fructose supply instant energy to human's cells and have low glycemic index compared to more complex sugars such as sucrose. It reduces blood glucose level and supply energy efficiently (Abdulrhman et al. 2013; Al-Waili, 2004; Mushtaq et al. 2011; Nazir et al. 2014; Yaghoobi et al. 2008). Similar mechanism may occur in the rat's body.

In addition, antioxidant properties such as flavonoids and phenolic in green tea were reported to be able to reduce excess body weight gain, lipid profile and blood glucose levels (Forester & Lambert 2011). In addition, each antioxidant properties have ability to show the similar results, either the properties in honey or green tea, in reducing weight and lipid levels. It was confirmed in a study conducted by Zainol and Mohd (2013). In addition, antioxidant found in honey did not worked individually. They found that each component in a pure honey including simple sugars and antioxidant properties work synergistically to show its antibacterial effects (Zainol & Mohd 2013). However, once each of the components was extracted and tested individually in the study, less effect was observed. Similar notion was suggested to the pineapple honey whereby the simple sugars, antioxidant properties and other components in the honey may interact together to reduce signs of obesity and diabetes in the rats. Even though the major ingredient of adulterated honey (i.e. high fructose corn syrup, table sugar and/or sucrose syrup) is similar to the pure honey, different effects were recorded in the present study. It is because of lacking the other components in the adulterated honey as found in the pure honey including the antioxidant properties.

After 16 weeks, serum levels of total bilirubin, ALT and AST showed a significant increase in rats fed with both FHA and FHB (Figure 5). Whereas, rats fed with pineapple honey showed significant decrease in total bilirubin and ALT level compared to the control groups.

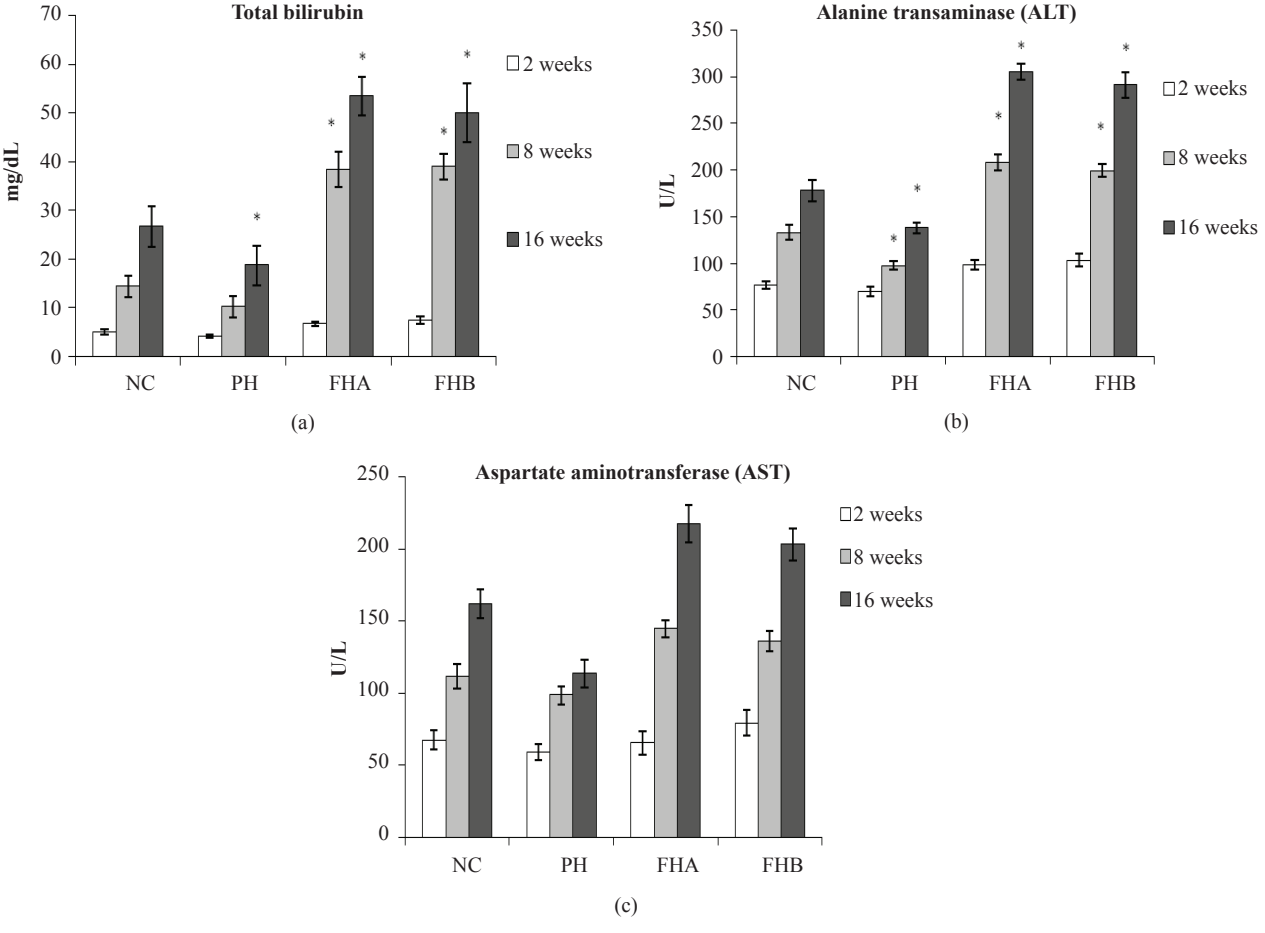

FIGURE 5. Effects of nomal diet in control rats (NC), rat fed with either pineapple honey (PH), adulterated honey A (FHA) or adulterated honey B (FHB) on (a) total bilirubin, (b) alanine transaminase (ALT) and (c) aspartate aminotransferase on rats

Values are expressed as mean ± SEM (standard error of the mean) (n=6). Statistically significant data are given as *p<0.05 compared to NC

Besides obesity and diabetes, long-term consumption of adulterated honey also demonstrated severe toxicity which shows significant increase of liver and kidney marker enzymes such as AST, ALT, total bilirubin and total protein, urea and creatinine and induced abnormalities in organs weight. Levels of AST, ALT and total bilirubin indicate hepatocellular damage effectively by providing a quantitative evaluation on the degree of damage to the liver. High values of liver marker enzymes obtained from the rats fed with both adulterated honeys showed that the livers were severely damaged. The increment levels of lipid profile as previously mentioned supported the results.

A long-term consumption of adulterated honey resulted is a significant decrease of urea and creatinine levels (Figure 6). In contrast, rats fed with pineapple honey showed significant decrease of urea and creatinine levels compared with control group.

High levels of markers such as serum urea and creatinine when evaluating kidneys function are commonly recorded due to acute and chronic intrinsic renal diseases and also when there is decreased effective circulating blood volume with decreased renal perfusion. This resulted to kidneys of rats fed FHA and FHB for 16 weeks lose their ability to excrete serum urea and creatinine, leading to kidney damage. Our findings are parallel to a previous research of Li et al. (2015), in which the increment of both urea and creatinine were caused by the long-term consumption of high sucrose diet. It was also aligned with another research conducted by Arise and Malomo (2009), as they reported that malfunction in the glomerular filtration of rats were due to long-term consumption of high fructose corn syrup.

Liver from control rats exhibited normal reddish color, regular smooth and glossy surfaces with a soft texture and appeared healthy (Table 3). The similar observation was reported for livers from rats fed with pineapple honey with brighter reddish color. In contrast, livers from rats fed with adulterated honeys showed abnormal differences appearances, with a slight discoloration from reddish to brown, particularly at the middle of the liver surface. In details, some of the livers (FHA group) showed roughly surface with the color pale brown (Table 3), their sizes were smaller with whitish micronodule on the entire liver surface. In FHB group, livers from the rats showed more severe macroscopic conditions with their surface were rough, scattered patches and pale yellowish fibrotic with few nodules on the liver surface (Table 3).

Kidneys from control rats and rats fed with pineapple honey showed normal reddish color with a regular size for both left and right kidneys (Table 3). Surprisingly, rats fed with adulterated honeys exhibited divergent results i.e. rats fed with FHA had pale reddish color, abnormal right kidney size and presence of nodules outside surface of the kidneys. Similar results were observed from kidneys of rats fed with FHB but with more apparent nodules on the surface of kidneys.

Anatomopathological observation showed major changes (abnormal) in the liver and kidney appearances and surfaces of rats fed with FHA and FHB. The results were confirmed by the changes in relative organs weight of these groups compared to control rats and rats fed with pineapple honey. Relative liver weight of rats fed with FHA showed significant increased, whereas the relative weight from rats fed with FHB groups showed significantly decreased compared to the rats' livers in control and pineapple honey groups (Table 4). Moreover, relative weight for the kidneys and lung from rats fed with adulterated honeys showed significant increase compared to control rats. Meanwhile, heart and brain from the rats fed with adulterated honey exhibited significant decreased. No changes were recorded for the relative spleen weight except for the rats fed with pineapple honey.

Frequency and severity of histopathological alterations in liver and kidneys were observed and showed increase in rats administered with FHA and FHB, compared to the control rats and rats fed with pineapple honey (Table 5).

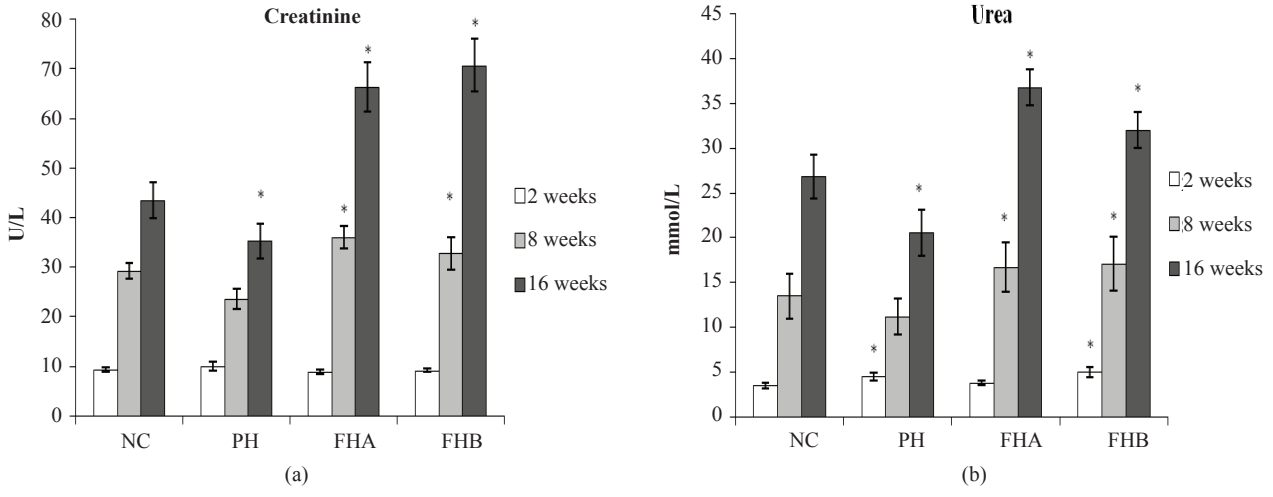

FIGURE 6. Effects of nomal diet in control rats (NC), rat fed with either pineapple honey (PH), adulterated honey A (FHA) or adulterated honey B (FHB) on (a) creatinine and (b) urea on rats

Values are expressed as mean ± SEM (standard error of the mean) (n=6). Statistically significant data are given as *p<0.05 compared to NC

TABLE 3. Frequency and severity of anatomopathological observations in liver and kidney of rats that administered with normal diet as control (NC), pineapple honey (PH), adulterated honey A (FHA) and adulterated honey B (FHB)

Anatomopathological observation	Group			
	NC	PH	FHA	FHB
Liver				
Discoloration in the middle	0	0	4	4
Roughly surface	0	0	3	1
Color pale brown	0	0	4	1
Whitish micronodula	0	0	2	3
Scattered patches	0	0	4	1
Kidney				
Abnormal in size	0	0	4	4
Color (pale reddish)	0	0	4	4
Nodules on the surface	0	0	3	4

Scale for the severity of liver and kidney histological changes; 0 = a change that was either absent or sporadic in all animals of a group; 1= a change that was found in a few animals of a group; 2 = a change that was rare in all animals of a group; 3 = a change that was relatively common in all animals of a group; 4 = a change that was very often found in all animals of a group

TABLE 4. Effects of control rats (NC), rat fed with either pineapple honey (PH), adulterated honey A (FHA) or adulterated honey B (FHB) on relative organs weight on male rats

Organ	Group			
	NC	PH	FHA	FHB
Liver	2.733 ± 0.076	2.730 ± 0.073	2.866 ± 0.038*	2.647 ± 0.095*
Kidney	0.710 ± 0.022	0.716 ± 0.023	0.756 ± 0.047*	0.794 ± 0.073*
Spleen	0.132 ± 0.002	0.121 ± 0.013*	0.132 ± 0.020	0.148 ± 0.080
Heart	0.364 ± 0.007	0.352 ± 0.010*	0.312 ± 0.030*	0.323 ± 0.030*
Lung	0.551 ± 0.016	0.567 ± 0.018*	0.595 ± 0.030*	0.601 ± 0.080*
Brain	0.464 ± 0.013	0.444 ± 0.013	0.395 ± 0.030*	0.408 ± 0.030*

Values are expressed as mean ± SEM (standard error of the mean) (n=6). Statistically significant data are given as *p<0.05 compared to NC

In addition, congestion and degeneration was relatively common in rats fed with adulterated honeys.

Macroscopic observations on the liver and kidneys were conducted to observe effect of the adulterated honey consumption. Microscopic examination on the rat's liver from control group showed normal histology with sinusoidal cards of hepatocytes with central vein and portal tracts (Figure 7(a)). Similar observation was recorded on

TABLE 5. Frequency and severity of histopathological alterations in liver and kidney of rats that administered with control (NC), pineapple honey adulterated honey A (FHA) and adulterated honey B (FHB)

Histological changes	Group			
	NC	PH	FHA	FHB
Liver				
Necrosis	0	0	2	2
Cellular infiltration	0	0	3	2
Congestion	0	0	3	2
Degeneration	0	0	3	2
Vacuolation	0	0	3	2
Kidney				
Congestion	0	0	2	2
Slightly Necrosis	0	0	1	1
Slightly Haemorrhage	0	0	1	1

Scale for the severity of liver and kidney histological changes; 0 = a change that was either absent or sporadic in all animals of a group; 1 = a change that was found in a few animals of a group; 2 = a change that was rare in all animals of a group; 3 = a change that was relatively common in all animals of a group; 4 = a change that was very often found in all animals of a group

the rat's liver fed with pineapple honey, which is normal lobular architecture with a central vein and radiating hepatic cords and no pathological changes. However, major pathological changes and severe sign of damage were observed in the livers of rats in adulterated honey groups after 16 weeks of study (Figure 7(c) and (d)). The livers showed distortion in the arrangement of cells and also development of fibrosis with necrosis of hepatocytes with slight congestion of liver particularly from rats that died earlier in this study. Liver of rats in FHA group showed distortion in the arrangement of cells around the central vein (⇢), thickening of the walls of veins and capillaries, development of fibrosis with necrosis of hepatocytes (⇢), a slight congestion of the liver and periportal fatty infiltration. Meanwhile, rat's liver from FHB group (Figure 7(d)) shows moderate to severe macrovesicular steatosis and hepatocyte ballooning (⇢) with mild to moderate intrahepatic hemorrhage and few erythrocytes in central vein.

Similar to previous research done by Cheplius and Starkey (2008), they documented a higher visceral adiposity in their sucrose-fed rats' livers than the honey-fed rats' livers. The elevated visceral adiposity coupled with hepatomegaly could cause hepatic steatosis, with eventual progression to nonalcoholic fatty liver disease (NALFD). Furthermore, rats fed with a golden syrup for long period also caused hepatic inflammation, which manifested as Kupffer cells accumulation in the liver (Chepulis & Starkey 2008; Figlewicz et al. 2009). The same findings were observed in the study (Figure 7(c) and 7(d)). Contrary to the livers from rats fed with pineapple honey, there were no differences and abnormalities observed compared to the livers from control group, in terms of relative liver weight and structure of the organ at the end of study. Histological evaluation of kidneys from adulterated honey groups showed notable distortion, shrunken glomerulus and thickening of vesicles (Figure 8(c) and 8(d)) especially in rats that died earlier in this study. The severe damages might be due to the high sugar levels in adulterated honey. Consequently, high sugar intake has been reported to cause kidney damage in a human and may ultimately lead to nephropathy in rats (Yudkin et al. 1980).

Rat's kidneys from control group showed normal histology with well-developed proximal and distal convoluted tubules and normal glomeruli and Bowman's capsule. Similarly, kidneys from rats fed with pineapple honey showed well arrangement structure of the renal corpuscles and renal tubules i.e. the renal corpuscle consisted of tuft of blood capillaries surrounded by the Bowmann's capsule (→). Besides, rats in FHA and FHB groups showed shrunken glomerulus (→) and thickening of vesicles (Figure 8 (a) and 8 (b)). Furthermore, glomerulus in kidneys of rat fed with FHA showed some cellular proliferation with fibrosis and some congestion, marked tubular damage, hemorrhage in the Bowman's space.

In contrast, histological evaluation of liver and kidneys and the relative organs weight of rats fed with

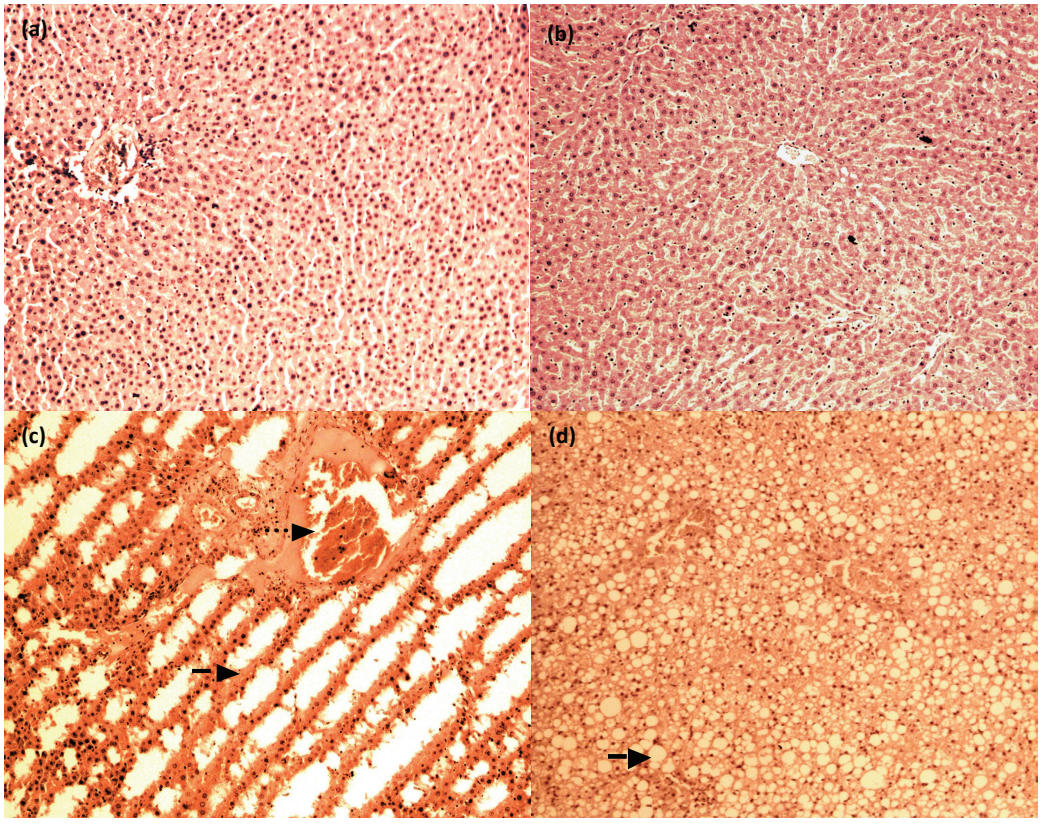

FIGURE 7. Photomicrograph of rat's liver either orally administered with (a) normal diet, (b) pineapple honey, (c) adulterated honey A (FHA) or (d) adulterated honey B (FHB) at a dose of 2500 mg/kg body weight for 16 weeks (HE staining ×10)

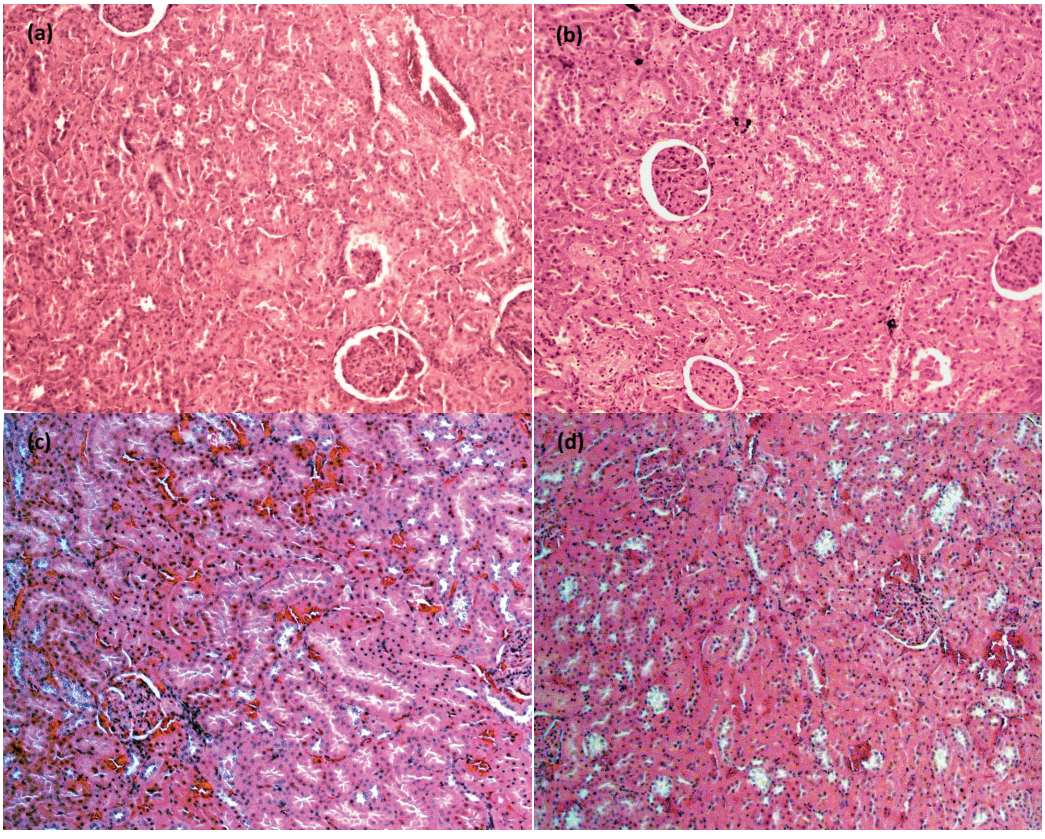

FIGURE 8. Photomicrograph of rat's kidneys either orally administered with (a) normal diet, (b) pineapple honey, (c) adulterated honey A (FHA) or (d) adulterated honey B (FHB) at a dose of 2500 mg/kg body weight for 16 weeks (HE staining ×10)

pineapple honey showed insignificant different compared to the control rats. The observation showed that pure honey is not toxic, but instead exhibited extraordinary medicinal effects by reducing excess body weight gain and other obesity and diabetes parameters. Meanwhile, adulterated honeys display contrasting effects that are harmful to body and could induce several diseases (Cao et al. 2012; Jurgens et al. 2005; Light et al. 2009; Lindqvist et al. 2008). The reason is because pure honey contains not only simple sugars (fructose and glucose) but also other nutrients such as proteins, antioxidants and minerals which are essential to our health (Bogdanov et al. 2012). As such, it is commonly used as a natural sweetener and nutritional food and its benefit to our health has been known for thousands of years (Alvarez-Suarez et al. 2010).

Study on the direct effects of adulterated honey consumption on human is difficult to be designed and related to ethical approval. Moreover, data on the honey's effects at the animal model is still lacking. Thus, to the best of our knowledge, this is the first study conducted to observe the effects of adulterated honey consumption intensively at *in vivo* level. Moreover, the honey was purchased randomly from the local market and was confirmed as adulterated honey using the established physicochemical and antioxidant methods. Most of parameters related to obesity, diabetes and toxicity have been investigated including histological observations on the major organs such as liver, kidney, spleen, heart, lung and brain. However, effects of the honey at the molecular level were not covered in this study.

Intriguingly, data obtained from *in vivo* study can be used as guidelines or notion for human study (Hau & Hoosier 2003). For example, from the study we showed that adulterated honey consumption could induce obesity, increase blood glucose level and demonstrate toxicity effects in rats. The similar effects may observe when human consumes the adulterated honey for long period. However, this hypothesis is projected for further study.

Conclusion

In summary, adulteration status of FHA and FHB is confirmed using physicochemical analysis. No toxicity observed in adulterated honey consumption for short period (14 days with one single consumption). However, apparent effects of adulterated honey consumption are recorded from the long-term study, which the rats showed weight increased, abnormal renal and hepatic function parameters, increased circulating triglycerides, cholesterol and glucose level and augmented fat deposition. Shocking findings are shown from the long-term consumption of adulterated honey, which could induce obesity, diabetes, causes severe organ toxicities in rats and could lead to early mortality. Even though the issue of the adulterated honey production is well-known across the globe, not

much research on its effect towards human health has been made a priority. Findings from this study will accelerate more prompt actions to be taken by authorities to prevent production, trading and marketing of the adulterated honey. In addition, more plans will be designed to improve the production of pure honey, which will obviously contribute to human health in general.

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