

## EFFECTS OF PURE AND FAKE HONEY ON GLUCOSE AND LIPID PROFILE *in vitro* AND *in vivo* STUDY

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### ABSTRACT

Honey authenticity is one of the most critical honey trading issues worldwide. Fake honey (FH) represents almost 80% of the honey market in Malaysia. Apart from violating consumer's rights, FH consists of none of the beneficial nutritional value other than pure honey (PH). Moreover, prolonged consumption of FH may cause harm to human health. However, data regarding these issues is limited. In this study, both PH and FH were tested using 3T3-L1 adipocytes and in high-fat diet (HFD)-induced obese Sprague Dawley rats. Initially, a physicochemical analysis was performed on both the honey samples according to the Codex Alimentarius Commission and International Honey Commission procedures. After 72 hr, PH significantly reduced lipid accumulation and triglyceride levels in the adipocytes. In the rats, low glucose, cholesterol, and triglyceride levels were significantly detected after orally administered for 16 weeks. Conversely, FH significantly induced higher lipid accumulation and triglyceride levels in adipocytes and excess high blood glucose, cholesterol, and triglyceride levels in rats. The results demonstrated that FH has negative effects on lipid metabolism, and prolonged consumption may cause health complications. Furthermore, prompt actions are required to address this problem.

**Key words:** Fake honey, health complications, natural honey, pure honey, obesity

### INTRODUCTION

Honey is a natural sweetener that is widely used globally across cultures, religions, and socio-economic backgrounds. In 2015, approximately 520,336 tons of honey was produced, valuing EUR 1.6 million (UN Comtrade, 2018). According to the Food and Agriculture Organization (FAO), honey production has been consistently increasing from 1980 to 2015 (UN Comtrade, 2018). However, even with a production rate of 0.23–0.24 kg/annum, the production is insufficient as in some countries of central Europe, for example, Austria, Germany, and Switzerland, honey consumption can reach up to 1 kg/annum (Bradbear *et al.*, 2009). High demand for honey but a shortage in supply has resulted in

adulteration and the production of fake honey (FH). Fraudulent practices about honey production are a major problem within this industry, and currently, there is no effective solution for tackling this issue.

One of the problems that may occur from the consumption of FH is adverse effects on health. The honey consists of high fructose corn syrup or caramelized sucrose and gives little nutritional and medicinal value compare to pure honey (PH) or natural honey (Damto *et al.*, 2019). The consumption of these types of sugar are known to increase body weight and body fats, and long term effect may increase the risk of obesity, hypertension, heart disease and other health complications (Ko *et al.*, 2017). However, the number of studies analyzing these effects of fake honey is scarce. In this study, we investigated the effects of one type of fake honey (FH) and pure honey (PH), which are available in

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our local market using 3T3-L1 adipocytes and a high-fat diet (HFD)-induced obese rats.

## MATERIALS AND METHODS

### Honey sample preparation

PH used in this study was raw Acacia honey, a unifloral honey that comes to the nectar from acacia tree (*Acacia mangium*) and collected by the *Apis mellifera* bee species. The honey was collected in 2012 from an Acacia apiary farm in Ulu Sedili, Johor, Malaysia. FH was purchased in the same year from a local grocery market in Selangor, Malaysia.

### Physicochemical analysis and antioxidant properties

Physicochemical analysis, including moisture content, pH, acidity, ash content, hydroxymethylfurfural (HMF) level, the presence of glucose oxidase, and sugar composition were assessed according to the procedures listed in the Codex Alimentarius Commission (CAC) (2001) and International Honey Commission (IHC) (2009). Antioxidant properties, particularly total phenolic content (TPC) and total flavonoid content (TFC) were determined using the method developed by Salleh *et al.* (2017).

### Cell culture study

3T3-L1 cell lines (ATC.CL-173) were purchased from the American Type Culture Collection (ATCC, USA). The pre-adipocyte cells were cultured and induced to differentiate into adipocytes according to the method reported by Ismail *et al.* (2014). Lipid accumulation and triglyceride levels in the adipocytes were measured after treatment with PH and FH at concentrations of 0–100 v/v, according to the method reported by Samat *et al.* (2018).

### Animal study

Seven weeks old male Sprague-Dawley rats, with bodyweight ranging from 200 to 220 g, obtained from Laboratory Facilities of Animal Management (LAFAM), Universiti Teknologi MARA (UiTM), Puncak Alam, Selangor. The rats were housed at one rat per cage and maintained under an ambient room temperature and 40–65% relative humidity, with a 12-hr cycle of light and dark according to Samat *et al.* (2014). Twenty-four rats were divided into four groups (n=6): Control, HFD, HFD + PH, and HFD + FH. The control rats were orally administered with distilled water and rodent food ad libitum. Obesity was induced in these rats by providing a HFD according to the procedure reported by Samat *et al.* (2017). Two groups of rats were orally fed using oral gavage with 2000 mg/kg body weight of either PH or FH daily

for 16 weeks. On the last day of the feeding period, a blood sample was collected and centrifuged at 4000 rpm for 15 min at 4°C. The clear serum obtained was separated and subjected to lipid profile test, consist of cholesterol, triglycerides, and glucose levels. The procedures involving animals were approved by the UiTM Ethics Committee (05/2012).

### Statistical analysis

All data are presented as the mean  $\pm$  standard deviation. Statistical analysis for cell culture studies was performed using the Sigma Plot Version 12 (Systat Software Inc., CA, USA) followed by Dunnett's posthoc tests. Data from the animal study were analyzed using one-way analysis of variance followed by the posthoc Tukey's test (SPSS version 18). The data thus obtained were then compared with those obtained for the controls. Differences with *p*-values of *p*<0.05 were considered to be statistically significant.

## RESULTS AND DISCUSSION

Acacia honey was selected in the study as the pure honey sample. The honey is commercially available throughout the year compared to other honey, for instance, Tualang honey (Samat *et al.*, 2014). Tualang honey is seasonal honey, which is only available between June-September every year. Moreover, Acacia honey is a unifloral honey, making it more reliable to standardize the honey quality and to measure its authenticity honey using physicochemical analysis (Samat *et al.*, 2014). The health benefits of the local honey are also less reported compared to other honey samples, yet to be disclosed in this study.

The physicochemical data of PH were considerably different from those of FH, particularly in terms of free acidity, ash content, HMF level, and the presence of glucose oxidase (Table 1). PH showed high free acidity value, low HMF value, and the presence of glucose oxidase and ash content. On the contrary, FH displayed the characteristic of adulterated honey with a high value of HMF and the absence of glucose oxidase. In freshly harvested honey, HMF is absent or occurs at a very low concentration, however, heat treatment or long storage conditions can increase the level of HMF (Shapla *et al.*, 2018). In the case of fake honey production, high sugar (sugarcane syrup) content, and heat treatment caused a high level of HMF (Shapla *et al.*, 2018). Glucose oxidase is an enzyme found in honey that catalyzes glucose to form gluconic acid and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which are the agent responsible for antibacterial activity in honey (Hermanns *et al.*, 2020). The authenticity

**Table 1.** Physicochemical data of pure and fake honey

Parameter	PH	FH
Moisture Content (%)	22.30 ± 0.10	18.70 ± 0.02
pH	3.50 ± 0.03	3.79 ± 0.03
Free Acidity	85.20 ± 0.40*	24.00 ± 0.05
Ash Content (g)	0.18 ± 0.04	ND
HMF (mg/kg)	0.68 ± 0.03*	195.4 ± 3.50
Glucose oxidase	Active	Not Active

Values are expressed as means ± SD (n=3). PH (pure honey), FH (fake honey), ND (not determined). \*significantly different,  $p < 0.05$ .

**Table 2.** Sugar composition of pure and fake honey

Parameter	Codex Standard	PH	FH
Reducing Sugar (%)	>60	70.43 ± 3.51*	49.29 ± 1.05
Fructose/Glucose ratio	1	1.55 ± 0.43*	0.75 ± 0.06
Sucrose (%)	<5	2.63 ± 0.27*	5.75 ± 0.43

Values are expressed as means ± SD (n=3). PH (pure honey), FH (fake honey). \*significantly different,  $p < 0.05$ .

status of FH was confirmed by its sugar composition (Table 2). FH did not comply with the standards set by Codex for these components. The reducing sugar ( $49.29 \pm 1.05$ ), fructose/glucose ratio ( $0.75 \pm 0.06$ ), and sucrose composition ( $5.75 \pm 0.43$ ) of FH was not within the range.

The value of TPC and TFC of Malaysian honey varied greatly depending on the type of honey. As shown in Table 3, PH contained the highest level of TPC ( $186.56 \pm 4.03$  mg/kg) and TFC ( $36.66 \pm 1.50$  mg/kg) compared to FH. The value of TPC and TFC is due to the botanical sources of honey. However, the value is considered low if compared to other pure honey since our honey sample is unifloral honey. It has been reported that multifloral honey contains higher TPC and TFC values compared to unifloral honey (Machado De-Melo *et al.*, 2017). However, FH, which is not produced naturally by a honeybee, showed a significantly small amount of TPC and TFC value compared to the PH.

Based on the *in vitro* study, PH significantly reduced 3T3-L1 adipocytes lipid accumulation and triglycerides level in a dose-dependent manner (Figure 1). This result suggested that PH inhibited adipogenesis of the adipocytes by reducing lipid accumulation, thus reducing its triglyceride content. In contrast, FH significantly increased lipid accumulation and triglyceride level in the adipocytes, which indicates that the FH may induce adipogenesis in the cells.

Similar findings were observed *in vivo* study, where FH exacerbated the obesity in rats from group HFD + FH. The rats have displayed a significant increment in the glucose level, which was up to

two times ( $28.4 \pm 2.5$ ) and cholesterol level increased up to four times ( $48.6 \pm 4.8$ ) than rats in the HFD group. The high levels of glucose, cholesterol, and triglyceride lead to hyperglycemia and dyslipidemia, which increase the risk of obesity and its complication such as heart disease, diabetes, and cancer (Kinlen *et al.*, 2018).

In contrast, the level of blood glucose, triglyceride, and cholesterol was significantly reduced in HFD rats fed with PH when compared to HFD rats fed with FH (Table 4). The results were similar to a study conducted by Samat *et al.* (2014), where acacia honey reduced the level of glucose, cholesterol, and triglycerides in normal diet-fed male rats. The positive effects of PH may be due to its high phenolic, and flavonoids content. The phenolic and flavonoid in honey mainly depend on its floral origin and can also be used as a tool for classification and authentication, especially in the case of unifloral varieties, including Acacia honey (Cianciosi *et al.*, 2018). According to Santos-Buelga and Gonzalez-Paramas (2017), the phenolic compound in pure honey is responsible for many beneficial health benefits such as antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic activity. Therefore, based on our results, it is suggested that PH can ameliorate obesity by the inhibition of adipogenesis *in vitro* and reduce levels of glucose and lipid profile *in vitro*. Subsequently, pure honey has the potential to be used as an alternative to reduce obesity in obese and diabetic patients. However, more studies are required to understand the mechanism and to identify the phenolic compounds, which are responsible for the effects.

**Table 3.** Antioxidant properties of pure and fake honey

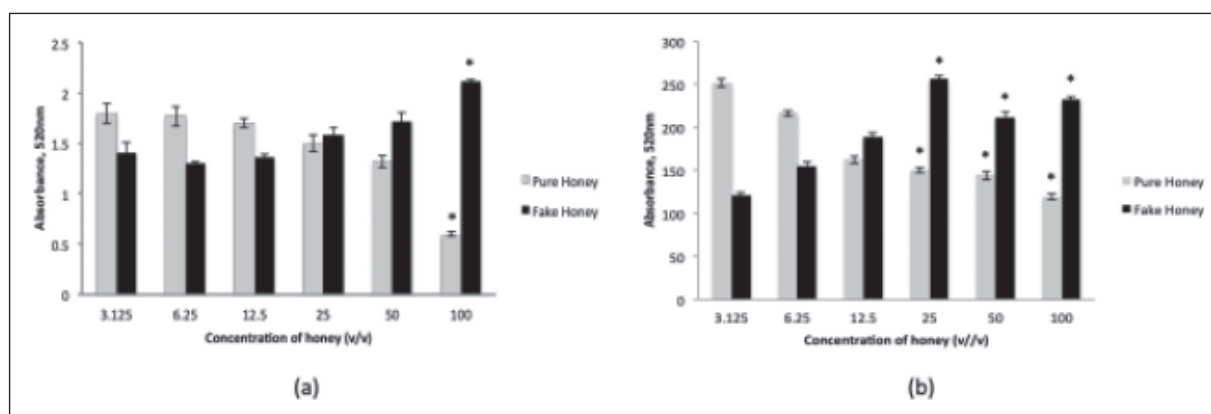
Parameter	PH	FH
TPC (mg gallic acid/kg)	186.56 ± 4.03*	40.93 ± 1.10
TFC (mg catechin/kg)	36.66 ± 1.50*	14.75 ± 1.11

Values are expressed as means ± SD (n=3). PH (pure honey), FH (fake honey).  
\*significantly different,  $p < 0.05$ .

**Table 4.** Glucose, triglyceride, and cholesterol levels in Sprague–Dawley rats with high-fat diet-induced obesity after being fed pure or fake honey for 16 weeks

Test	Control	HFD	HFD + PH	HFD + FH
Glucose level (mmol/L)	3.39 ± 0.01	12.55 ± 0.52*	7.13 ± 0.66	28.4 ± 2.5*
Triglycerides level (mmol/L)	2.55 ± 0.11	17.65 ± 0.72*	13.73 ± 0.61*	26 ± 1.2*
Cholesterol level (mmol/L)	5.63 ± 0.31	13.67 ± 0.50*	8.30 ± 0.45	48.6 ± 4.8*

HFD = high-fat diet, HFD + PH = rats on high-fat diet fed with pure honey, and HFD + FH = rats on high-fat diet fed with fake honey. Values are expressed as means ± SD (n=3). \*significantly different,  $p < 0.05$ .



**Fig. 1.** Lipid accumulation (a) and triglyceride levels (b) in differentiated 3T3-L1 adipocytes treated with either pure or fake honey. Lipid accumulation was spectrophotometrically assessed after treatment with several concentrations of honey (0–100% v/v). Results are presented as the mean ± standard deviation (n=4). \*Significantly different compared with control ( $p < 0.05$ , analysis of variance and Dunnett's test).

Results presented in this study are consistent with different types of pure honey reported by other studies (Chepulis *et al.*, 2009; Nemoseck *et al.*, 2011; Ajibola *et al.*, 2013; Samat *et al.*, 2018). Samat *et al.* (2018) reported that pineapple honey reduced the lipid droplet size and lipid accumulation of 3T3-L1 adipocytes. Another example, clover honey, another mono-floral honey was found to improve weight regulation and reduce triglycerides level in rats (Nemoseck *et al.*, 2011). Moreover, a diet comprising of low-dose golden syrup and sucrose (some FH types are invented with sugars) increased visceral adiposity in rats (Chepulis *et al.*, 2009; Ajibola *et al.*, 2013). Our study used commercial FH available in a local market. The fake honey has demonstrated the negative compared to the positive effects of consuming PH on glucose

and lipid metabolism using *in vitro* and *in vivo* models.

## CONCLUSION

The study highlights differences in physico-chemical analysis among PH and FH based on the established international standards set by the CAC and IHC. The analysis can identify the authenticity status of the honey. The study also shows PH can significantly reduce levels of lipid profiles in both *in vitro* and *in vivo* models. However, more studies are warranted to understand the positive effects of PH consumption and to confirm the adverse health effects of FH utilization. The data are crucial in providing additional justification for

ceasing fraudulent activities in commercial honey production.

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