



***EVALUATION OF STINGLESS BEE HONEY FROM *Heterotrigona itama*
COCKERELL AS ANTI-OBESITY AGENT IN HIGH FAT DIET-INDUCED
OBESITY SPRAGUE-DAWLEY RAT MODEL***

AHMAD ZULKIFLI BIN MOHD RAFIE

FBSB 2017 48



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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science**

December 2017

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EVALUATION OF STINGLESS BEE HONEY FROM *Heterotrigona itama* COCKERELL AS ANTI-OBESITY AGENT IN HIGH FAT DIET-INDUCED OBESITY SPRAGUE-DAWLEY RAT MODEL

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December 2017

Chairman : Mariatulqabtiah Bte Abdul Razak, PhD
Faculty : Biotechnology and Biomolecular Sciences

Heterotrigona itama is a common stingless bee species of Malaysia. However, studies on the health benefits of its honey are relatively limited compared to other stingless bee species of the world. In this study, the anti-obesity properties of stingless bee honey from *H. itama* were evaluated based on the weight changes, biochemistry parameters and morphological structures in diet-induced obese rat model. 56 male Sprague Dawley (SD) rats were induced with formulated high fat diet (HFD) to become obese, indicated by the value of 0.68 g/cm^2 , for 6 weeks. Then, treatment phase was carried out for the next 6 weeks. At the end of the experiment, rats were euthanized and their blood and organs (liver, aorta and adipose tissue) were sampled. Results showed significant reduction in percentages of body weight gain and adiposity index in groups treated with all the three dosages of stingless bee honey with percentages of body weight gain of 12.0 ± 3.1 , -2.3 ± 2.9 and 11.1 ± 4.6 and adiposity index of 5.6 ± 0.3 , 4.6 ± 0.5 and 6.8 ± 0.8 , for 1000 mg/kg, 750 mg/kg and 500 mg/kg supplementations, respectively, compared to the control obese group with excess of percentage of body weight gain of 33.7 ± 2.8 and adiposity index of 10.1 ± 0.5 . For the biochemistry analysis based on blood serum, levels of liver enzymes (ALT, AST and alkaline phosphatase) were significantly lower in all treated groups with 1000 mg/kg showing 51.0 ± 4.9 , 154.5 ± 19.0 and 104.2 ± 25.5 , 750 mg/kg showing 56.3 ± 5.6 , 183.3 ± 18.0 and 122.0 ± 9.3 , and 500 mg/kg showing 64.8 ± 8.6 , 206.3 ± 18.9 and 140.5 ± 9.0 , respectively. Lipid profiles of (triglycerides and LDL-cholesterol) were also significantly lower in all treated groups with 1000 mg/kg showing 0.73 ± 0.08 and 0.17 ± 0.061 , 750 mg/kg showing 0.83 ± 0.08 and 0.12 ± 0.031 , and 500 mg/kg showing 0.78 ± 0.08 and 0.17 ± 0.021 , respectively, compared to control obese group which showed 1.33 ± 0.22 and 0.70 ± 0.132 , respectively. Level of HDL-cholesterol in treated groups was significantly higher with 1000 mg/kg showing 1.33 ± 0.10 , 750 mg/kg showing 1.35 ± 0.09 , and

500 mg/kg showing 1.30 ± 0.09 , compared to control obese group which showed 0.93 ± 0.11 . Based on morphological structures, adipocyte size was smaller and hepatocytes were less ruptured in treated groups compared to untreated groups at 40 \times magnification. Histology results of aorta in treated groups directly mimic to the normal-like structure, compared to untreated groups. As a conclusion, the intervention study suggested that administration of stingless bee honey was successful to reduce the complication risks related to obesity such as liver disease. Our study also suggests that stingless bee honey possesses hepatoprotective action that could be used to control obesity by regulating lipid metabolism.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENILAIAN MADU KELULUT DARIPADA SPESIS *Heterotrigona itama* SEBAGAI AGEN ANTI-OBESITI TERHADAP MODEL TIKUS SPRAGUE-DAWLEY OBES YANG DIARUH DENGAN MAKANAN TINGGI LEMAK

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Heterotrigona itama merupakan salah satu spesies lebah kelulut di Malaysia. Setakat ini, penyelidikan tentang kebaikan madu itu terhadap kesihatan masih lagi kurang jika dibandingkan dengan spesies lebah kelulut yang lain di dunia. Ciri-ciri anti-obesiti madu kelulut spesies *H. itama* terhadap model tikus yang obesiti dinilai berdasarkan perubahan berat, parameter-parameter biokimia dan struktur morfologi. Sebanyak 56 tikus jantan jenis 'Sprague Dawley' diberi makan setiap hari dengan makanan diet berlemak tinggi yang dihasilkan sendiri sehingga tikus-tikus itu mencapai tahap obesiti iaitu pada kadar melebihi 0.68g/cm^2 sehingga enam minggu. Selepas itu, fasa rawatan dijalankan selama enam minggu. Pada akhir minggu keenam, haiwan dikorbankan dan darah serta organ (hati, aorta, dan tisu adipos) diambil. Keputusan menunjukkan terdapat pengurangan dalam peratusan pertambahan berat dan indeks adipositi dalam kumpulan yang menerima rawatan tiga dos madu kelulut dengan peratusan pertambahan berat adalah 12.0 ± 3.1 , -2.3 ± 2.9 dan 11.1 ± 4.6 dan indeks adipositi adalah 5.6 ± 0.3 , 4.6 ± 0.5 dan 6.8 ± 0.8 untuk 1000 mg/kg, 750 mg/kg dan 500 mg/kg suplemen, masing-masing, dibandingkan dengan kumpulan kawalan obes yang mempunyai lebih peratusan pertambahan berat sebanyak 33.7 ± 2.8 dan indeks adipositi sebanyak 10.1 ± 0.5 . Bagi analisis biokimia serum darah, kadar bacaan enzim hati (ALT, AST dan phosphatase alkali) adalah ketara lebih rendah dalam semua kumpulan yang menerima rawatan dengan 1000 mg/kg menunjukkan 51.0 ± 4.9 , 154.5 ± 19.0 dan 104.2 ± 25.5 , 750 mg/kg menunjukkan 56.3 ± 5.6 , 183.3 ± 18.0 dan 122.0 ± 9.3 , dan 500 mg/kg menunjukkan 64.8 ± 8.6 , 206.3 ± 18.9 dan 140.5 ± 9.0 , masing-masing. Profil lipid (trigliserida dan kolesterol lipoprotein ketumpatan rendah) adalah ketara lebih rendah di semua kumpulan tikus yang menerima rawatan dengan 1000 mg/kg menunjukkan 0.73 ± 0.08 dan 0.17 ± 0.061 , 750 mg/kg menunjukkan 0.83 ± 0.08 dan 0.12 ± 0.031 , dan 500 mg/kg menunjukkan 0.78 ± 0.08 dan 0.17 ± 0.021 , masing-

masing, berbanding kumpulan kawalan obes yang menunjukkan 1.33 ± 0.22 dan 0.70 ± 0.132 , masing-masing. Kadar bacaan kolesterol lipoprotein ketumpatan tinggi di kumpulan tikus yang menerima rawatan adalah ketara lebih tinggi dengan 1000 mg/kg menunjukkan 1.33 ± 0.10 , 750 mg/kg menunjukkan 1.35 ± 0.09 , dan 500 mg/kg menunjukkan 1.30 ± 0.09 , masing-masing, berbanding kumpulan kawalan obes yang menunjukkan 0.93 ± 0.11 . Struktur morfologi menunjukkan saiz adipos lebih kecil dan struktur hepatosit kurang rosak di kumpulan tikus yang menerima rawatan madu berbanding yang tidak mendapat rawatan madu di bawah pembesaran 40 \times . Keputusan histologi aorta menunjukkan struktur yang sama di antara kumpulan tikus yang mendapat rawatan madu dan kumpulan tikus normal, berbanding kumpulan tikus yang tidak menerima rawatan madu. Kesimpulannya, rawatan menggunakan madu kelulut berjaya menurunkan risiko komplikasi obesiti seperti penyakit hati. Hasil penyiasatan ini mencadangkann madu kelulut mempunyai tindakan protektif-hepa yang mampu mengawal obesiti dengan kawalan metabolisme lipid.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

FA	Fatty Acids
HDL	High-Density Lipoprotein
H&E	Hematoxylin & Eosin
HFD	High Fat Diet
HPE	Histopathological Examination
IP	Intraperitoneal
LDL	Low-Density Lipoprotein
LPL	Lipoprotein Lipase
MOA	Mechanism of Action
NAFLD	Non-Alcoholic Fatty Liver Disease
ND	Normal Diet
OS	Oxidative Stress
ROS	Reactive Oxygen Species
ROW	Relative Organ Weight
SD	Sprague-Dawley
SBH	Stingless Bee Honey
SEM	Scanning Electron Microscope
TG	Triglyceride

CHAPTER 1

INTRODUCTION

1.1 Obesity

Obesity is one type of disease that increase among people globally and spread by increasing urbanization. Haslam and James (2005) have stated that rapidly growing epidemic worldwide of this problem may lead to the negative effect on health, which risk to reduce life expectancy and /or increase health problem. Approximately 2.8 million deaths happended per year due to the uncontrolled obesity according to the World Health Organization (WHO) 2013. Due to this, it has been listed as the fifth risk factor for mortality and morbidity around the world (WHO, 2013). Statistically, in year of 2014, it was reported that 1.9 billion adults (18 years and above) worldwide were overweight. More than 600 million from this range or about 13% of the world's adult population s (11% of men and 15% of women) were facing obesity problem (WHO, 2015). Fatimah *et al* (2005) have mentioned that in Malaysia, 23% of the adult population was found to be overweight and 14% was obese. Based form this data, it can be highlighted that Malaysia has been rated as the highest among Asian countries for obesity (Bernama, 2014).

Obesity has been defined as excessive or abnormal fat accumulation that is detrimental to human health, whereby there is an increase in adipose tissue mass as a result of an enlargement and increase number of fat cells. Obesity measurement can be calculated by Body Mass Index (BMI), calculated as body weight in kilogram divided by the square of height in meters. Range of BMI between 25-29.9 kgm^{-2} categorised as overweight and BMI exceeding 30 kgm^{-2} is considered as obese (WHO, 2015).

Individuals who have BMI greater or equal to 30 are more likely to have health complication problems which include hyperlipidemia, cardiovascular disease, high blood pressure, diabetes, stroke, arthritis, cancer, breathing problems and metabolic syndrome (Nguyen *et al.*, 2009). However, the most relevant disease which affect from obesity complication are high blood pressure, diabetes mellitus and cardiovascular diseases (Malnick and Knobler, 2006; Pagotto *et al.*, 2008; Kurukulasuriya *et al.*, 2011).

1.2 Stingless Bee Honey (*Heterotrigona itama* species)

Generally, stingless bees can be found in most of tropical and subtropical regions around the world such as Africa, Southeast Asia, Australia and South America. There are more than 500 species described including 32 genera worldwide with perhaps more than 100 new species to be characterized (Chuttong *et al.*, 2014). Eighty nine species in 15 genera from the Indo/Australian region has been listed

(Rasmussen, 2008) meanwhile in Thailand, 32 species of stingless bees in 10 genera have been identified (Chuttong *et al.*, 2014). Before the production of honey, floral nectars were collected, stored and chemically modified by social bees. Unlike western honey bee *Apis mellifera*, they produce and store much more honey compared to stingless bees and it become world leader in honey production. In Thailand, it was estimated that total national production of stingless bee honey to be 2.5-3 metric tons which is lower compared to honey from *A. mellifera* (Chuttong *et al.*, 2014). Hence, this limited production results in lack of quality standard of stingless bee honey and insufficient knowledge and unregulation by food control authorities leads to the exclusion from international standards (Chuttong *et al.*, 2014).

To expand the knowledge with scientific research related to stingless bee honey, the aim of this study was to evaluate the effect of stingless bee honey from *H. itama* species to obese rat model. The work includes evaluating the effects of different dosages of stingless bee honey on weight, lipid profile, liver function test, and histopathology of liver and aorta in each respected groups of treated and untreated rats. This finding would create the path for further studies, in order to produce medicinal product from this natural medicine with acceptable levels of safety, efficacy and tolerability especially in the management of obesity and also as preventive medicine against several degenerative diseases such as liver disease, blood pressure etc.

1.3 Objectives

1.3.1 General Objective

- To investigate the therapeutic effect of stingless bee honey in obesity and complication risks associated with obesity in rat model of obesity.

1.3.2 Specific Objectives

- To determine the body weight, body mass index (BMI), percentages of body weight gain (%), and food intake of all treated and untreated obese rats.
- To evaluate the biochemistry parameters including lipid profile and liver function test between treated and untreated obese rats.
- To evaluate the histopathological changes of organ structures (liver, aorta and adipose tissue) in treated and untreated obese rats.

1.4 Hypothesis

- Stingless bee honey is able to reduce the weight and other complication risks associated with obesity in diet-induced rats.

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