EFFECTS OF TUALANG HONEY ON REPRODUCTIVE SYSTEM AND BONE PROPERTIES IN OVARIECTOMISED RATS

by

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SHAM	Sham operated group
OVX	Ovariectomised control group
THL	Tualang honey at low dose (0.2 g/kg/bw)
THM	Tualang honey at medium dose (1.0 g/kg/bw)
THH	Tualang honey at high dose (2.0 g/kg/bw)
bw	Body weight
D.P.X	Distyrene Plasticizer plus Xylene
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
ELISA	Enzyme-linked immunosorbent assay
TH	Tualang honey at 0.2 g/kg/bw
PC	Positive control (1% calcium)
IP	Intraperitoneum
BV/TV	Trabecular bone volume
Tb.Th	Trabecular thickness
Tb. N	Trabecular number
Tb. Sp	Trabecular separation (inter – trabecular space)

ABSTRAK

Madu adalah produk semulajadi bernutrisi tinggi yang digunakan secara meluas dalam bidang terapi perubatan sejak zaman dahulu lagi. Dalam kajian ini, keberkesanan madu lebah Tualang dalam mengurangkan komplikasi menopaus mengunakan tikus betina yang telah diovariektomi dua belah. Dalam kajian I (kajian pendek dua minggu), tikus dibahagikan secara rawak kepada lima kumpulan (n=7), terdiri daripada kumpulan dibedah sham (SHAM), kawalan ovariektomi (OVX), kumpulan ovariektomi THL (diberi 0.2 g/kg madu Tualang), kumpulan ovariektomi THM (diberi 1.0 g/kg madu Tualang) dan kumpulan ovariektomi THH (diberi 2.0 g/kg madu Tualang). Kedua-dua kumpulan SHAM dan OVX diberi rawatan 0.5 ml air suling. Rawatan diberi secara oral gavaj setiap hari selama dua minggu. Organ pembiakan (uterus dan vagina) dan tulang tibia diambil untuk pemeriksaan histopatologi manakala serum untuk analisis hormon. Rawatan madu Tualang selama dua minggu kepada tikus ovariektomi secara signifikan ($P<0.05$) meningkatkan berat uterus dan vagina ($P<0.05$), meningkatkan ketebalan lapisan epithelium dan otot vagina ($P<0.05$), meningkatkan ketebalan lapisan endometrium dan otot myometrium uterus ($P<0.05$), memulihkan ketebalan tulang trabekular tibia and menurunkan berat badan ($P<0.05$) berbanding kumpulan kawalan OVX. Tiada perbezaan signifikan ($P>0.05$) untuk profil hormon kecuali aras testosterone ($P<0.05$). Dalam kajian II (kajian dos ulangan enam minggu), tikus dibahagikan secara rawak kepada lima kumpulan (n=8) yang terdiri daripada kumpulan kawalan asas (BC; tidak diovariektomi atau dibedah sham), kumpulan dibedah sham (SHAM), kawalan ovariektomi (OVX), kumpulan rawatan madu Tualang (TH; diberi 0.2 g/kg madu Tualang) dan kumpulan kawalan positif (PC; diberi 1% kalsium dalam air minuman). Kedua-dua kumpulan SHAM dan OVX diberi rawatan 0.5 ml air

ternyahion. Rawatan selama enam minggu diberikan secara oral gavaj setiap hari iaitu bermula 14 hari selepas pembedahan dilakukan. Tulang femur diambil untuk pemeriksaan histopatologi manakala serum untuk analisis aras glukos. Rawatan madu Tualang selama enam minggu kepada tikus-tikus diovariektomi, analisis histomorphometri menunjukkan secara signifikan meningkatkan isipadu tulang per isipadu tisu (BV/TV) ($P<0.05$), ketebalan trabecular (Tb.Th) ($P<0.05$) dan bilangan trabecular (Tb.N) ($P<0.05$) termasuklah merendahkan jarak pemisahan trabecular (Tb.Sp) ($P<0.05$) berbanding dengan kumpulan OVX. Aras glukos didapati tidak berubah dengan signifikan ($P>0.05$) dalam semua kumpulan menunjukkan ovariektomi dan rawatan madu selama enam minggu tidak mempengaruhi sensitiviti insulin dalam sistem badan. Kesimpulan, madu Tualang berkesan dalam merawat gejala menopaus seperti mengurangkan keadaan atropi sistem reproduktif dan memelihara jisim tulang dalam model haiwan menopaus.

ABSTRACT

Honey is a highly nutritional natural product that has been widely used in the folk medicine for a number of therapeutic purposes. In this study, *Tualang honey* was evaluated for its effectiveness in reducing postmenopausal changes in bilaterally ovariectomised rats. In study I (short term two weeks study), rats were randomly divided into five groups (n=7), which were the of sham control group (SHAM), ovariectomised control group (OVX), THL ovariectomised group (received 0.2 g/kg of *Tualang honey*), THM ovariectomised group (received 1.0 g/kg of *Tualang honey*) and THH ovariectomised group (received 2.0 g/kg of *Tualang honey*). Both SHAM and OVX groups received 0.5 ml vehicle treatment of distilled water. The treatment was given orally by gavage daily for two weeks. The reproductive organs (uterus and vagina) and tibia bone were subjected to histopathological examination while the serum was for hormonal assays. Administration of *Tualang honey* for two weeks to ovariectomised rats significantly increased the weight of the uterus and vagina ($P<0.05$), increased the thickness of vaginal epithelium and muscular layers ($P<0.05$), increased the thickness of uterine endometrium and myometrium layers ($P<0.05$), restored the thickness of trabecular tibia bone and suppressed the body weight ($P<0.05$) compared to OVX group. There were no significant changes in hormonal profile except for free testosterone level ($P<0.05$). In study II (six weeks repeated dose study), rats were randomly divided into five groups according to body weight (n=8) consists of baseline control group (BC; not subjected to ovariectomy or sham-operation and killed without treatment), sham control group (SHAM), ovariectomised control group (OVX), *Tualang honey* treated group (TH; received 0.2 g/kg of *Tualang honey*) and positive control (PC; received 1% calcium in drinking water). Both SHAM and OVX groups received vehicle

treatment 0.5 ml of deionized water. The treatment was given by gavaging daily from day 14 post-operative for six weeks duration. The femur bone was taken for histopathological examination while serum for blood fasting glucose assay. After six weeks administration of *Tualang honey* to ovariectomised rats, histomorphometrical analysis of bone structures showed significant increase in the bone volume per tissue volume (BV/TV) ($P<0.05$), the trabecular bone thickness (Tb.Th) ($P<0.05$) and the trabecular bone number (Tb.N) ($P<0.05$), as well as significant lower in trabecular bone separation (Tb.Sp) ($P>0.05$) compared with OVX group. There were no significant changes in the fasting blood glucose levels ($P<0.05$) indicating that ovariectomy and six weeks treatment with honey did not influent the body sensitivity of towards insulin. In conclusion, *Tualang honey* improved the histology of atrophic reproductive organs and restored the bone mass in postmenopausal (bilateral ovariectomy) animal model.

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Menopause is the time of life in women when menstrual cycles and reproductive function permanently cease due to the loss of ovarian follicular activity (Amore *et al.*, 2007; Hunter and Rendall, 2007; Jahangar *et al.*, 2006; Stoppard, 1994). Clinically, natural menopause can be diagnosed in women in their 40s or 50s after 12 consecutive months of amenorrhea for which no other obvious pathological or physiological cause can be found (Wich and Carnes, 1995; Harlow and Signorello, 2000). However, it can also be induced by surgery, chemotherapy, or radiation (Harris *et al.*, 2002; Stoppard, 1994).

There are various early and late complications of menopausal period frequently referred to as postmenopausal syndrome such as vasomotor, psychogenic and sexual problems while other complications which could occur much later include increase in incidence of atherosclerosis, ischemic heart disease, stroke and osteoporosis (Mori-Okamoto *et al.*, 2004; Boreham *et al.*, 2002; Avis *et al.*, 2005; Kanis *et al.*, 1991; Stoppard, 1994). In order to reduce these complications, hormone replacement therapy (HRT) has been prescribed extensively over the last 25 years (Blumberg *et al.*, 1996). Findings from the Women's Health Initiative trial suggested that long-term HRT use increases the risk of breast cancer, endometrial cancer, thromboembolic events and vaginal bleeding (Termine and Wong, 1998). Such findings have resulted in a search for HRT alternatives and natural products therapies for postmenopausal complications. In fact, women need to approach the

transition into menopause in a holistic and integrative way by managing menopause in terms of the entire lifestyle, including eating correctly, using the correct supplements, dress management and healing herbs or natural products to assist gently through the menopause and perimenopause.

Honey is a natural product that has been widely used for its therapeutic benefits (Molan and Allen, 1996). It has been reported to contain about 200 substances such as mixture of sugars (fructose, glucose, maltose and sucrose) and also small amounts of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals (Aljadi and Kamaruddin, 2004; Al-Mamary *et al.*, 2002; Beretta *et al.*, 2007; Bergman *et al.*, 1983; Bertoncelj *et al.*, 2007; Blasa *et al.*, 2006; Blasa *et al.*, 2007; Chen *et al.*, 2000; Cooper *et al.*, 2001; Dustmann, 1979; Frankel *et al.*, 1998; Gheldof *et al.*, 2002; Ibrahim, 1981; McInerney, 1990). However, the nutritional profiles of honey are qualitatively and quantitatively variable depending on the regional plant ecology (Al-Mamary *et al.*, 2002). It is known to have some pharmacological functions in human such as anti inflammatory (Armon, 1980; Bergman *et al.*, 1983; Blomfield, 1973), antibacterial, immunomodulatory and antioxidant (Beretta *et al.*, 2007; Blasa *et al.*, 2007; Chen *et al.*, 2000). In Malaysia, honey has been used by local folk people during postpartum and postmenopausal period (usually with yolk egg) to improve health and to enhance vitality. Royal jelly and propolis are other beehive products that have been scientifically proven for their improvement of menopausal syndrome (Mishima *et al.*, 2005; Song *et al.*, 2002). *Tualang honey* is a wild honey in which the bees of the *Apis Dorsata* species form hives on the branch on tall trees named Tualang, found in the Malaysian Rain Forest in Malaysia.

Thus far, there are no data on the benefits of honey on postmenopausal syndrome. Hence a study to evaluate the effect of *Tualang honey* (AgroMas, Malaysia) on the reproductive system and bone using bilateral ovariectomised rats as postmenopausal animal model was conducted. According to previous researchers, ovariectomised female rats with or without supplementation of hormones are widely used as animal models to study the impact of hormones on physiological changes (Goss *et al.*, 2004; Mori-Okamoto *et al.*, 2004; Bradshaw and Berkley, 2002) and osteoporosis (Nazrun *et al.*, 2007; Kalu, 1991) that may occur during postmenopausal period. A short term two weeks study (Study I) and a six weeks repeated dose study (Study II) were conducted to evaluate the effects of honey supplementation on the reproductive system and bone properties in ovariectomised rats.

During the short term two weeks study (study I) honey supplementation was given immediately following ovariectomy and the reproductive organs (uterus and vagina) and hormonal profiles were studied. The undecalcified histology of the bone was reviewed under the microscope. More detailed study on the bone was done following six weeks repeated dose honey treatment (study II). In this study the animals were ovariectomised and two weeks later, honey was administered. Calcium supplementation was given to the positive control group to compare the effects of honey administration with calcium supplementation. Blood was also collected in this study to see the effect of honey supplementation on fasting blood glucose in ovariectomised rats.

1.2 OBJECTIVES OF THE STUDY

1.2.1 Study I - Short term two weeks study

1.2.1.1 General objectives

The objective of the study is to determine the effects of two weeks administration of *Tualang honey* (AgroMas, Malaysia) on the reproductive system and bone in ovariectomised rats (postmenopausal animal model).

1.2.1.2 Specific objectives

a) To determine the histomorphological effects of *Tualang honey* on the histomorphology the of uterus and vagina of ovariectomised rats.

b) To determine the effects of *Tualang honey* on reproductive hormones (17ß-estradiol, progesterone, free testosterone, FSH and LH) of ovariectomised rats.

c) To determine the effects of *Tualang honey* on the weight and morphology of tibia bone of ovariectomised rats.

1.2.2 Study II - Six weeks repeated dose study

1.2.2.1 General objectives

The general objective of the study is to determine the effects of longer period (six weeks) administration of *Tualang honey* (AgroMas, Malaysia) on bone properties and fasting blood glucose levels in ovariectomised rats (postmenopausal animal model).

1.2.2.2 The specific objectives

1) To determine the effects of *Tualang honey* on trabecular bone volume of ovariectomised rats.

2) To determine the effects of *Tualang honey* on trabecular bone thickness of ovariectomised rats.

3) To determine the effects of *Tualang honey* on trabecular bone number of ovariectomised rats.

4) To determine the effects of *Tualang honey* on trabecular bone separation of ovariectomised rats.

5) To determine the effects of *Tualang honey* on fasting blood glucose levels of ovariectomised rats.

1.3 HYPOTHESIS OF THE STUDY

1.3.1 Study I - Short term two weeks study

1.3.1.1) Ovariectomy causes regression of the morphology of reproductive organs (uterus and vagina) of rats due to estrogen deficiency and administration of *Tualang honey* improves the morphology of both organs in ovariectomised rats.

1.3.1.2) Ovariectomy disturbs the normal levels of reproductive hormones and administration of *Tualang honey* improves the hormonal levels of these hormones in ovariectomised rats.

1.3.1.3) Ovariectomy reduces the trabecular bone thickness due to estrogen deficiency and administration of *Tualang honey* restores this thickness in ovariectomised rats.

1.3.2 Study II- Six weeks repeated dose study

1.3.2.1) Ovariectomy causes regression structure of trabecular bone and administration of *Tualang honey* restores regression structure of trabecular bone in ovariectomised rats.

1.3.2.2) Administration of *Tualang honey* maintains the normal levels of fasting blood glucose in ovariectomised rats.

1.4 Significance of the study

Tualang honey (AgroMas, Malaysia) is a natural bioproduct that has not been extensively evaluated for its nutritional and medicinal properties. Thus, this study provides scientific information on the benefit of local *Tualang honey* as nutritional or nutraceutical products in preventing postmenopausal changes using ovariectomised rat as postmenopausal animal model.

CHAPTER TWO

LITERATURE REVIEW

2.1 HONEY

2.1.1 Sources and biochemical profile of honey

Honey is a sweet natural substance that contains a highly concentrated solution of a complex mixture of sugars produced by honeybees (of the *Apis genus*) in almost every country of the world and extracted generally from nectars (Blasa *et al.*, 2006; Gomez-Caravaca *et al.*, 2006). The main effect of nectar collecting by bees from various plants and flowers is pollination, which is crucial for flowering plants. Honey has been used extensively since ancient times and has been appreciated as the only concentrated form of sugar available worldwide (Nagai *et al.*, 2001).

Previous works have indicated that the composition of honey varies widely according to the floral source and geographical origin, climate, environmental condition and post-harvest processing condition such as processing, handling and storage (Cherchi *et al.*, 1994; Gheldof and Engeseth, 2002; Frankel *et al.*, 1998; Gheldof *et al.*, 2002; Turkmen *et al.*, 2006; Al-Mamary *et al.*, 2002; Beretta *et al.*, 2005; Chen *et al.*, 2000; Yao *et al.*, 2004; Kucuk *et al.*, 2007). A study has shown that the Italian honey collected from the nectar of wild blossoms on hill zones and mountain zones is contains higher organoleptic characteristics and nourishing values, low moisture content, high density and has a stability which guarantees long shelf live without the risk of fermentation or changes in its

properties (Blasa *et al.*, 2006). Multifloral honey differs from unifloral honey in that it is darker in color with solid crystallization and is thick while unifloral honey has light color, transparent appearance and is thin (Blasa *et al.*, 2006). The color of honey is affected by the content of minerals, pollens and phenolics (Baltrusaityte *et al.*, 2007; Lazaridou *et al.*, 2004). Darkening of honey during storage may occur due to Maillard reactions, fructose caramelization and reactions of polyphenols as well as temperature and/or time of storage (Bertoncelj *et al.*, 2007). These compositional differences can influence the value of a specific honey for medicinal or health-promoting purposes (Gomez-Caravaca *et al.*, 2006). However, the main constituents of all honey from all countries of the world are almost similar.

Honey, a supersaturated aqueous solution of inverted sugars mainly contains fructose (38%) and glucose (31%) as well as a small amount of very complex mixture of other saccharides such as disaccharides, trisaccharides and oligosaccharides. It also contains small amounts of enzymes, amino and organic acids, phytochemicals, carotenoid-like substances, Maillard reaction products, vitamins and minerals (Cherchi *et al.*, 1994; Antony *et al.*, 2000; Blasa *et al.*, 2006; Bertoncelj *et al.*, 2007; Vit *et al.*, 1997; Gomez-Caravaca *et al.*, 2006). The average composition of honey is given in Table 2.1. Honey is considered to be a suitable food for humans of every age due to its high nutritional value (304 kcal/100 g honey) and fast absorption of its carbohydrates. Honey is recommended as a supplement for children and sportsmen and used by itself or with appropriate therapies to help improve the efficiency of the elderly and invalids. It has a density of about 1.36 kg/liter (40% denser than water), with acidic environment ranging from 3.2 to 4.5 pH and high osmotic pressure and low water activity (Jeffrey and Echazareta, 1996). Raw honeys are usually produced by

small farms and left in their natural state without undergoing processing to thermal or pasteurization processes or any other operations to alter their natural composition. However, the extraneous matter that is contained in raw honey is removed in order to make it marketable on a large scale (Blasa *et al.*, 2006).

The main enzymes found in honey which are derived from the hypopharyngeal glands of worker honeybees are invertase, diastase (amylase), catalase, glucose oxidase and acid phosphatase (Jeffrey and Echazareta, 1996). Invertase is added to the nectar by the honeybees as either fructo-invertase or gluco-invertase that converts sucrose to fructose or glucose. However, this enzyme becomes inactive with high temperatures. Diastase originating from honeybees and pollen can breakdown or split the starch chains to randomly produce dextrins and maltose and can change to inactive forms after long periods of storage and exposure to high temperature. Catalase functions as a regulator of glucose oxidase activity and produces oxygen and water from hydrogen peroxide.The inverse relationship between catalase activity and hydrogen peroxide content has been used as an indicator for hydrogen peroxide level in honey, formerly known as 'inhibine number'. The glucose oxidase enzyme is of considerable interest because its activity causes the production of hydrogen peroxide which not only stablizes the ripening nectar against spoilage but has microbicidal action.

Table 2.1 Nutrient values of honey adopted from National Honey Board, 2002.

Nutrient	Average amount per 1 Tbsp serving (21g)	Average amount per 100g
Water	3.62g	17.10g
Calories	64	304
Total Carbohydrate	17.46g	82.40g
Fructose	8.16g	38.50g
Glucose	6.57g	31.00g
Maltose	1.53g	7.20g
Sucrose	0.32g	1.50g
Other carbohydrates	0.85g	4.00g
Dietary fiber	0.04g	0.20g
Total fat	0	0
Cholesterol	0	0
Total protein	0.06g	0.30g
Ash	0.04g	0.20g
Vitamins		
(data not available for biotin and vitamin B-12)		
Thiamin	0	0
Riboflavin	0.01mg	0.04mg
Niacin	0.03mg	0.12mg
Pantothenic acid	0.01mg	0.07mg
Vitamin B-6	0.01mg	0.02mg
Vitamin B-12	0	0
Folate	0.42mcg	2.00mcg
Vitamin C	0.11mg	0.50mg
Vitamin A	0	0
Vitamin D	0	0
Vitamin E	0	0
Vitamin K	0	0
Minerals		
Calcium	1.27g	6.00mg
Phosphorus	0.85g	4.00mg
Sodium	0.85mg	4.00mg
Potassium	11.02mg	52.00mg
Iron	0.09mg	0.42mg
Zinc	0.05mg	0.22m
Magnesium	0.42mg	2.00mg
Selenium	0.17mg	0.80mg
Copper	0.01mg	0.04mg
Manganese	0.02mg	0.08mg

Honey contains a number of amino acids such as proline, lysine, phenylalanine, tyrosine, glutamic and aspartic acids (Jeffrey and Echazareta, 1996) which are contributed by the pollens, nectar or by honeybees themselves. The predominant organic acid in honey is gluconic acid while the others are butyric, acetic, formic, lactic, succinic, malic, citric, maleic, oxalic and pyroglutamic. Gluconic acid in honey originates largely from the activity of enzymatic glucose oxidase reaction (Jeffrey and Echazareta, 1996). Indeed, it contributes a slight tartness to the flavor of honey and also adds antimicrobial properties as well as increase in calcium absorption**.**

In recent years, many studies have been conducted to identify the phytochemicals present in honey. Phytochemicals are non-nutrient compounds commonly found in plants such as fruits and vegetables (Kuhnle *et al.*, 2009) and have health-promoting activities by reducing the risk of oxidative tissue (Aljadi and Kamaruddin, 2004; Al-Mamary *et al.*, 2002). Honey is well known to be rich in both enzymatic and non-enzymatc antioxidants, including catalase, ascorbic acid, flavonoids, alkaloids**,** glucose oxidase, phenolics acid, carotenoid derivatives, Maillard reaction products, amino acid and proteins (Aljadi and Kamaruddin, 2004; Al-Mamary *et al.*, 2002; Gheldof and Engeseth, 2002; Gheldof *et al.*, 2002; 2003; Schramm *et al.*, 2003). Pinocembrin, a unique flavonoid is highly present in propolis and honey, while the others such as quercetin, chrysin, galangin, luteolin and kaemferol were also reported to be in honey (Gheldof *et al.*, 2002; 2003; Baltrusaityte *et al.*, 2007)**.** Previous works have indicated that the antioxidant activity of honey varies widely depending on the floral sources (Gheldof and Engeseth, 2002; Gheldof *et al.*, 2002; Al-Mamary *et al.*, 2002; Nagai *et al.*, 2001; Bertoncelj *et al.*, 2007; Chen *et al.*, 2000; Yao *et al.*, 2004; Frankel *et al.*, 1998). Several studies have shown that a strong correlation

between honey colour and antioxidant power, with darker and more crystallized honey having higher total phenolic content and hence a stronger antioxidant capacity than lighter and transparent honey (Blasa *et al.*, 2006; Nagai *et al.*, 2001; Bertoncelj *et al.*, 2007) as well as honey with higher content of water (Aljadi and Kamaruddin, 2004; Frankel *et al.*, 1998). Moreover, the color of honey depends on the potential alkalinity and ash content, as well as on the antioxidatively active pigments, including carotenoids and flavonoids (Frankel *et al.*, 1998).

2.1.2 Honey in modern medicinal uses

Nowadays, ancient remedies still survive together with modern medicine under the term 'modern folk medicine' since their effectiveness has not been scientifically proven through clinical trials. The ancient usage of honey into modern folk medicine has been continued in India as a traditional therapy for coughs and sore throats and eye diseases (using lotus honey), infected leg ulcers in Ghana, earaches in Nigeria, topical treatment for measles and eye measles to prevent corneal scarring, as well as for the treatment of gastric ulcers and constipation (Obi *et al.*, 1994; Zumla and Lulat, 1989; Imperato and Traore, 1969; Ankra-Badu, 1992; Kandil *et al.*, 1987)**.** In Germany, honey with cod liver oil was used for treatment of ulcerations, burns, fistulas and boils**.** During World War I, Russian soldiers used honey to prevent infections in wounds as well as to accelerate healing (Bergman *et al.*, 1983).

The mechanism of the effects of honey on human health has gradually became apparent in a number of scientific studies. The scientific support has been well documented

with a proliferation in publications on the successful therapeutic uses of honey in several general medical and surgical conditions (Somerfield, 1991; McInerney, 1990; Armon, 1980; Zumla and Lulat, 1989; Bergman *et al.*, 1983; Efem, 1988; Grange, 1990; Subrahmanyam, 1993; 1994; 1996; 1998; Efem *et al.*, 1992; Phuapradit and Saropala, 1992; Adesunkanmi and Oyelami, 1994; Hejase *et al.*, 1996; Wood *et al.*, 1997; Ndayisaba *et al.*, 1992; Ndayisaba *et al.*, 1993). The most active medical effect of honey is its antimicrobial property. The mechanism of this function was due to its high osmolarity (Willix *et al.*, 1992), acidity and content of inhibines, such as peroxide (Postmes *et al.*, 1993; Burdon, 1995; Cooper *et al.*, 1999; Bang *et al.*, 2003; Mani, 2006; Molan, 2001; 2002; Patton *et al.*, 2006), flavonoids and the phenolic acids (caffeic and ferulic acid) (Wahdan, 1998; Frankel *et al.*, 1998; Cao *et al.*, 1997; Baltrusaityte *et al.*, 2007). However, the antimicrobial ability of honey differs depending on its floral source (Gheldof and Engeseth, 2002; Al-Mamary *et al.*, 2002; Nagai *et al.*, 2001; Bertoncelj *et al.*, 2007; Chen *et al.*, 2000; Yao *et al.*, 2004; Frankel *et al.*, 1998) . In 1892, Van Ketel first recognized the antimicrobial activity of honey (Dustmann, 1979). Intensive study on the antimicrobial activity of honey had began in 1937 and found the antimicrobial name 'inhibine' was formed (Willix *et al.*, 1992). Later in 1965, other researchers identified 'inhibine' as hydrogen peroxide, which is formed by enzymatic glucose oxidase reaction. In other honeys, the antimicrobial agent is a non-peroxide compound derived directly from the flower (Patton *et al.*, 2006) . Therefore, not every type of honey has equally effective antimicrobial properties. The antimicrobial and antifungal properties of honey have been well documented through several studies. Honey at concentration of 40% found to be bactericidal to various gram-negative and gram-positive bacteria particularly *Salmonella shigella, E. coli* and *Bibrio cholera* (Jeddar *et al.*, 1985). Concentrations of between 30%

and 50% of honey were found to be as effective as cephaloridine, ampicilin, gentamycin, nitrofurantoin, nalidixic acid and cotrimoxazole in inhibiting growth of nine types of pathogenic organisms isolated from the urine samples of 149 patients with confirmed urinary tract infection (Ibrahim, 1981).

Honey has been proven to be effective in treating infected surgical wounds, burns and decubitus ulcer. A research found that local application of honey in the postoperative management of patients who had undergone radical vulvectomy for vulval carcinoma lead to succesfull healing (Cavanagh *et al.*, 1970). The rate of wound healing was accelerated and less bacterial colonization was observed. This observation has been proven in an animal model when pure commercially available honey applied on 12 mice significantly healed wounds faster than those of the control group (Bergman *et al.*, 1983). Subrahmanyam (1998) reported his findings in randomized controlled trial study in which honey was found to be more a efficient dressing for burns as compared to silver sulfadiazine. Treatment with silver sulfadiazine, the most widely used agent to prevent or clear infection in burns, resulted in 7% of the patients controlled from wound infection within seven days while treatment with honey resulted in 91% of the wounds to be sterile within the same period. Honey was observed to remove dead tissue and offensive smell from burns (Hutton, 1966; Efem, 1988). Healthy granulation tissue was observed to appear nearly twice as fast when using honey, and new skin cover developed faster also (Subrahmanyam, 1998; Hejase *et al.*, 1996). There was also better relief of pain, less exudation of lymph and less irritation when using honey. Honey also gave a lower incidence of raised scars and contractures. In the literature, honey was reported to successfully treat various types of wounds such as abrasions (Blomfield, 1973),

amputations (Hutton, 1966), abscesses (Farouk *et al.*, 1988), bed sores (pressure sores, decubitus ulcers) (Weheida *et al.*, 1991; Somerfield, 1991; Efem, 1988), burns (Ndayisaba *et al.*, 1993; Efem, 1988; Blomfield, 1973; Farouk *et al.*, 1988; Adesunkanmi and Oyelami, 1994; Subrahmanyam, 1993; 1994; 1996; 1998), burst abdominal wounds following caesarean delivery (Phuapradit and Saropala, 1992), cancrum (Efem, 1988), cervical ulcers, chilblains, cracked nipples (Seymour and West, 1951), cuts (Blomfield, 1973), diabetic foot ulcers and other diabetic ulcers (Tovey, 1991; Farouk *et al.*, 1988), a fistula , foot ulcers in lepers (Tovey, 1991), infected wounds arising from trauma (Efem, 1988; Ndayisaba *et al.*, 1993), large septic wounds, leg ulcers, malignant ulcers, sickle cell ulcers, tropical ulcers (Efem, 1988), skin ulcers (Tovey, 1991; Blomfield, 1973; Efem, 1988), surgical wounds (Bergman *et al.*, 1983; Farouk *et al.*, 1988; Ndayisaba *et al.*, 1993; Cavanagh *et al.*, 1970), wounds to the abdominal wall and perineum and varicose ulcers (McInerney, 1990). Of particular notice is the success of honey in treating Fournier's gangrene, a rapidly spreading infection that is usually managed by aggressive surgical removal of infected tissue (Efem 1988, 1993).

Honey has also been used for a long time in the treatment of various gastrointestinal diseases. The effect was proven when an oral rehydration therapy (ORT) solution containing 5% honey was given to infants and children suffering from bacterial gastroenteritis (*Salmonella, Shigella* and *E.coli)* (Haffejee and Moosa, 1985; Jeddar *et al.*, 1985). The convincing results showed that recovery time from bacterial diarrhea was significantly faster in patients given ORT solution containing equal concentration of glucose and concluded that honey can safely be used as a substitute for glucose in solution with electrolytes and in promoting sodium and water from the gut. Based on these data,

honey can provide an effective and cheap therapy for infected wounds and infectious gastrointestinal diseases. However, honey is a natural mixture of carbohydrates that may be contaminated with spores of *Clostridium botulinum* (Arnon, 1980,1979; Midura *et al.*, 1979) that can result in infant botulism and harmful effects to wound dressing. Sterilization of honey for these usages would eliminate this risk. However, the antibacterial activity of honey can be lost with standard sterilization procedures such as autoclaving, therefore gamma-radiation is the other alternative method since it does not affect its bactericidal activity (Molan and Allen, 1996). Hence, investigators who use honey must be aware of these potential complications associated with such treatments.

Nowadays, there are lots of research that has been conducted in Malaysia on honey, particularly by Universiti Sains Malaysia (USM) on the local wild honey known as the *Tualang honey*. The 1st International Conference on the Medicinal Uses of Honey in 2006 and 2nd International Conference on the Medicinal Uses of Honey in 2010 which were mainly organized by Universiti Sains Malaysia had successfully gathered the recent findings of clinical and laboratory studies from all over world. Some of the findings that were presented in these conferences were that *Tualang honey* has an important role in improvement of reproductive system in postmenopausal animal model (Siti Sarah *et al*., 2007a,b) and male rats exposed to cigarette smoke (Mahaneem *et al*., 2007a,b). It was also found that daily intake of *Tualang honey* at 20 mg/day for 4 months has similar effect on bone densitometry when compared with hormone replacement therapy (Lily Husniata *et al.*, 2010). Besides that, it was reported that *Tualang honey* has wound contraction and antimicrobial properties on full thickness burn wound in rats (Halima *et al.*, 2010). Combination of *Tualang honey* supplementation and jumping exercise may also elicit

beneficial effects on tibial bone mineral density (Kiew *et al.*, 2010). *Tualang honey* also has antiproliferative and apoptotic effect on oral squamous cell carcinoma and human osteosarcoma cell lines (Gashm *et al.*, 2010) and is also potentially used as therapeutic agent for diabetic foot wounds (Nawfar *et al.*, 2010), *Tualang honey* might reduce hyperglycemia, ameliorates oxidative stress and protects STZ-induced diabetic renal damage (Erejuwa *et al.*, 2010). Honey is an effective agent against yeast infections (Tuan Noorkorina and Mazatul Haizam, 2010) and can reduce apoptosis in leukemia cells (Rosline *et al.*, 2010), phenolic contents and antioxidant capacity (Khalil *et al.*, 2010). Last but not least, it was found that *Tualang honey* was significantly effective as a prophylactic measure in reducing acute respiratory symptoms among Hajj pilgrims (Siti Amrah *et al.*, 2010).

In recent years, attention has highly focused on natural food as nutraceuticals that have been used for the treatment of illnesses and diseases from ancient times. The mechanism of the effect of honey on human health has gradually become apparent in a number of studies. Many researchers demonstrated that honey contains a rich source of natural antioxidants and phytochemicals, which are effective in reducing the risk of heart disease, cancer, immune-system decline, cataracts, different inflammatory processes, as anti-allergic, anti-thrombotic, vasodilatory actions as well as its impact on gastrointestinal health and energy metabolism (Bertoncelj *et al.*, 2007)**.** Some suggested that oxidative and free radical-mediated reactions are implicated in degenerative processes related to aging with or without various diseases (Cerutti, 1994; Dean *et al.*, 1993) such as cancer, atherosclerosis, immune system decline, cataracts, diabetes etc (Bertoncelj *et al.*, 2007; Blasa *et al.*, 2006). According to Heim et al (2002), several polyphenols have been reported to quench reactive

oxygen species (ROS) and inhibit *in vitro* oxidation of low-density lipoproteins (LDL) and therefore reduce thrombotic tendency that is provided by endogenous antioxidants or by an array of different compounds contained in the diet (Manach *et al.*, 2004) while the others reported that diet rich in antioxidants would protect vital cell components from oxidative damage and slow down the aging process by neutralizing free radicals (Blasa *et al.*, 2007).

Honey also has been reported to prevent deteriorative oxidation reactions in foods such as enzymatic browning of fruit and vegetables (Chen *et al.*, 2000), lipid oxidation in meat (Nagai *et al.*, 2006; Gheldof and Engeseth, 2002; McKibben and Engeseth, 2002) and inhibit the growth of foodborne pathogens and spoilage organisms (Mundo *et al.*, 2004; Taormina *et al.*, 2001). A study has identified several key nutrients (namely, potassium, magnesium, fiber, β-carotene and vitamin C) which could be implicated in bone health (New *et al.*, 2000), while other researchers claimed that phytochemicals in several plants and fruits may contribute beneficial effects in postmenopausal animal model by showing an improvement in bone health, reproductive organs and exerting hormonal balancing effect (Devareddy *et al.*, 2006; Mori-Okamoto *et al.*, 2004; Puel *et al.*, 2006; Puel *et al.*, 2005; Shirwaikar *et al.*, 2003; Zhang *et al.*, 2007; Zhang *et al.*, 2006b). Royal jelly, which is one of the beehive products has been proven by a laboratory study to prevent osteoporosis in ovariectomised rats by enhancing intestinal calcium absorption (Nagai and Inoue, 2004). Royal jelly and propolis are beehive products that have been scientifically proven for their improvement of menopausal syndrome (Mishima *et al.*, 2005; Song *et al.*, 2002). Interestingly, honey contains all of these mentioned bioactive compounds such as polyphenols and flavonoids (Aljadi and Kamaruddin, 2004), the key nutrients for bone

health and phytochemicals which may be beneficial in relieving postmenopausal symptoms (Al-Mamary *et al.*, 2002).

To the researcher's knowledge, even though honey is traditionally well known to improve postmenopausal symptoms, there has been no research conducted to evaluate the effectiveness of honey in alleviating or improving postmenopausal symptoms. Therefore, this study was conducted to evaluate the efficacy of *Tualang honey* (AgroMas, Malaysia) in preventing some of the postmenopausal symptoms using ovariectomised rats as the postmenopausal animal model. The parameters assessed in this study include general health, body weight, food intake, reproductive organs (vagina and uterus), reproductive hormonal levels, bone properties and fasting blood glucose level.

2.1.3 *Tualang honey* (AgroMas, Malaysia)

The *Tualang honey* used in this study was supplied by Federal Agricultural Marketing Authority (FAMA), Kedah, Ministry of Agriculture and Agro-Based Industry. *Tualang honey* is produced by bees (*Apis Dorsata*) in which their hives are built hanging under the branch of Tualang tree at Sik, Kuala Nerang and Baling forest in Kedah. *Tualang honey* is collected from forests where the levels of dangerous chemicals in the atmosphere are insignificant and the environmental pollution is minimal. Furthermore, it is kept by the *Apis Dorsata* in fixed comb log and hives wall without the use of artificial comb foundations, sugar feeding and antibiotics in such hives. Therefore, *Tualang honey* is considered as Malaysian pure wild honey with dark color. Interestingly, honey that is produced by *Apis*