EVALUATION OF LONG TERM ORAL ADMINISTRATION OF GAMMA IRRADIATED TUALANG HONEY IN SPECIFIC PATHOGEN FREE SPRAGUE-DAWLEY RATS

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by

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

%	Percentage	BW	Body weight
<	Less than	BWG	Body weight gain
>	Greater than	C	Cortex
≈	Approximate	Ca	Calcium
°C	Degree Celsius	CAM	Complementary and alternative medicine
♀	Female	cc	cubic centimetre
♂	Male	Cd	Cadmium
ABS_{450}	Absorbance: 450	CHO	Carbohydrates
AEC	Animal Ethics Committee	Cl	Chlorine
AIDS	Acquired immune deficiency syndrome	cm	Centimetres
Al	Aluminium	CRP	C- reactive protein
Alb	Albumin	CVD	Cardiovascular disease
ALP	Alkaline phosphatase	D0	Day zero
ALT	Alanine transaminae	D183	Day 183
am	ante meridiem	DM	Diabetes mellitus
ANOVA	Analysis of variance	Group III (GIII)	Group 3 (Medium dose, 1.0g/kg body weight)
ARASC	Animal Research and Service Centre	Group IV (GIV)	Group 4 (High dose, 2.0g/kg body weight)
As	Arsenic	H&E	Haematoxylin and eosin
AST	Aspartate Transaminase	Hb	Haemoglobin
DNA	Deoxyribonucleic acid	HbA1c	Glycated haemoglobin
DW	Distilled water	HDL	High density Lipoprotein
EDTA	Ethlylene diamine tetraacetic acid	FAO	Food and Agricultural Organizations of United Nations
EU	European Union	Fe	Iron
F	Flouride	fl	femtoliters
FAMA	Federal Agricultural Marketing Authority Malaysia	FRAP	Fluorescence Recovery After Photobleaching
BC	Bowman's capsule	g	Grams
BCS	Body Condition Scoring	G	Gauge
BIL (D)	Bilirubin direct	g/cm	Grams per centimetre
BIL (I)	Bilirubin indirect	g/dl	Gram per deciliter
BM	Basement membrane	g/kg	Gram per kilogram
BSA	Bowman's space Area	g/L	Grams per litre
BUN	Blood Urea Nitrogen	GA	Glomerular area
GGT	Gamma-glutamyl transpeptidase	IQR	Interquartile range
GIT	Gastrointestinal tract	IU	International Unit

GITH	Gamma Irradiated Tualang honey	IVC	Individual Ventilated cages
GLM	General Linear Model (GLM) Repeated Measures	K	Potassium
GN	Glomerular number	kg	Kilogram
GP	Glomerular perimeter	kGy	kilo gray
GR	Glomerular radius	LD_{50}	Median lethal dose (50%)
Group I (GI)	Group 1 (Control)	LDL	Low density Lipoprotein
Group II (GII)	Group 2 (Low dose, 0.2g/kg body weight)	LFT	Liver Functions tests
LOQ	Limit of Quantification	Li	Lithium
Lt	Left	LOD	Limit of detection
M	Medulla	mg/100g	Milligram per hundred gram
m	meter	mg/dl	Milligrams per decilitre
MCH	Mean corpuscular haemoglobin	mg/kg	milligrams per kilgram
MCHC	Mean corpuscular haemoglobin concentration	ml	mililitres
MCV	Mean corpuscular volume	ml/kg	militre per kilogram
meq/kg	Miliequivalent per kilogram	mmol/l	milimol per liter
Mg	Magnesium	Mn	Manganese
mg	miligram	Mo	Molybdenum
mg TE/100g honey	milligrames of Trolox equivalents (TE) per 100g of honey	MOH	Ministry of Health Malaysia
HMF	Hydoroxymethylfurfuraldehyde	MT	Masson's Trichrome
HRT	Hormone Replacement Treatment	n	Sample size
HUSM	Hospital Universiti Sains Malaysia	Na	Sodium
I	Iodide	NACAD	North American Control Animal Database
IACUC	Institutional Animal Care and Use Committee	NCCIH	The National Centre for Complementary and Integrative Health
IAEA	International Atomic Energy Agency	Ni	Nickel
ICH	International Conference on Harmonization	NIH	National Institute of Health
IM	Intramuscular	no.	Number
In	Indium		
NOAEL	No Observable Adverse Effects	Si	Silicon
NOEL	No Observable Effects Level	SPF	Specific Pathogen Free
OECD	Organization for Economic Co-operation and Development	Sr	Strontium
OW	Organ weight	TBIL	Total bilirubin
P	Phosphorus	TBW	Terminal body weight

Pb	Lead	TH	Tualang honey
PCV	Packed cell volume	U/L	Units per litre
pg	picogram	USA	United States of America
pH	Potential of hydrogen	USM	Universiti Sains Malaysia
PL	glomerulus parietal layer	UVB	Ultaviolet B
PTA	Phosphotungistic acid-Phosphomolybdic acid (PTA) working solution	V	Vanadium
r	radius	VL	Visceral layer
RBC	Red blood cell	WBC	White blood cells
RC	Renal corpuscle	WHO	World Health Organization
RCA	Renal corpuscle area	WI	Weigert's iron working solution
RCP	Renal corpuscle perimeter	ZF	Zona fasciculata
RDW	Red Cell distribution width	ZG	Zona Glomerulosa
RM	Ringgit Malaysia	Zn	Zinc
ROS	Reactive Oxygen Species	ZR	Zona reticularis
ROW	Relative organ weight	β	Beta
Rt	Right	μm	micrometers
Sdn. Bhd.	Sendirian Berhad	μm^2	micrometer squares
Se	Selenium		
SEM	Standard error of mean		

PENILAIAN PENGAMBILAN ORAL JANGKA PANJANG MADU TUALANG DIIRIDIASI GAMMA DALAM TIKUS '*SPRAGUE-DAWLEY*' BEBAS PATOGEN KHUSUS

ABSTRAK

Madu Tualang diiridiasi gamma (GITH) dilaporkan mempunyai aktiviti antioksidan, keupayaan menggarut radikal bebas yang tinggi, aktiviti anti kanser, anti-bakteria, anti-radang dan juga penyembuhan luka. Oleh yang demikian, kajian terperinci bagi menilai kesan pengambilan oral jangka panjang diperlukan. Kajian dilaksanakan menurut Garis Panduan '*The Organisation for Economic Cooperation and Development'* (OECD) no. 452, dengan sedikit pengubahsuaian. Tikus '*Sprague-Dawley'* bebas patogen khusus (SPF) berjumlah 120 ekor (berumur 6 ± 1 minggu) jantan dan betina dibahagi secara rawak kepada empat kumpulan (n = 10-20 setiap satu) dan GITH diberi secara oral pada dos [0 (Kumpulan I), 0.2 (Kumpulan II), 1.0 (Kumpulan III) dan 2.0 g / kg berat badan (Kumpulan IV)] setiap hari selama enam bulan. Kadar mortaliti, morbiditi, penilaian fizikal, tingkah, berat badan direkod. Selepas enam bulan, sampel darah tikus yang dipuasakan semalaman dikumpulkan untuk pemeriksaan parameter hematologi dan biokimia klinikal. Bedahsiasat penuh dijalankan. Berat organ mutlak (AOW) dan relatif (ROW) telah diukur. Kajian histologi ke atas kelenjar adrenal, ginjal dan hepar dijalankan. Tiada mortaliti, morbiditi, perubahan signifikan pada rekod berat badan diantara semua kumpulan dicerap. Kumpulan II (jantan dan betina) menunjukkan peningkatan dalam *'mean corpuscular haemoglobin concentrations'* (MCHC) berbanding Kumpulan IV (jantan) dan semua kumpulan lain

(betina). Nilai '*mean corpuscular haemoglobin'* (MCH) yang tinggi diperhatikan bagi tikus betina (Kumpulan II) berbanding kumpulan lain. Kumpulan IV (jantan) mempunyai nilai *'blood urea nitrogen'* (BUN) yang rendah berbanding Kumpulan I dan II. Nilai rendah yang signifikan bagi '*alanine transaminase'* diperhatikan pada semua kumpulan GITH (jantan) berbanding Kumpulan I. Tiada perubahan ketara pada organ dalaman tikus dan tiada perbezaan AOW dan ROW. Penilaian histologi menunjukkan morfologi normal pada kelenjar adrenal, ginjal dan hepar untuk semua kumpulan tikus. Analisis histomorfometri kelenjar adrenal menunjukkan kelebaran kawasan zona fasciculata yang menurun pada semua kumpulan penerima rawatan GITH (jantan) dan Kumpulan II (betina) berbanding Kumpulan I. Pembesaran kawasan '*Bowman's space'* diperhatikan pada Kumpulan IV (jantan) berbanding Kumpulan I dan III. Perbezaan signifikan pada saiz sel hepar dwinukleus dalam Kumpulan II dan III berbanding Kumpulan I (jantan); dan Kumpuan IV berbanding Kumpulan II dan III (betina) diperhatikan. Kesimpulannya, pengambilan oral jangka panjang GITH pada tikus SPF tidak memberi kesan negatif dalam parameter yang dikaji. Secara keseluruhan, kajian menunjukkan GITH memelihara fungsi normal kelenjar adrenal, ginjal dan hepar tikus dan mungkin ada kesan positif yang memerlukan kajian lanjut.

EVALUATION OF LONG TERM ORAL ADMINISTRATION OF GAMMA IRRADIATED TUALANG HONEY IN SPECIFIC PATHOGEN FREE SPRAGUE-DAWLEY RATS

ABSTRACT

Gamma irradiated Tualang honey (GITH) is reported to have high antioxidant and free radical scavenging activities along with its anticancer, antibacterial, anti-inflammatory and wound healing properties. A detailed study to evaluate its long term oral administration is therefore, necessary. The study design was adapted from The Organisation for Economic Cooperation and Development (OECD) Guideline no. 452 with slight modifications. A total of 120 healthy male and female specific pathogen free (SPF) Sprague-Dawley rats (6±1 weeks old) were randomly divided into four groups (*n*=10-20 each) fed with different GITH doses [0 (Group I), 0.2 (Group II), 1.0 (Group III) and 2.0 g/kg body weight] (Group IV) daily for six months. Weekly body weight, mortality and morbidity, physical appearance and behavioural characteristics were recorded. After six months, blood sample of overnight fasted rats were analysed for haematology and clinical biochemistry parameters. Full gross necropsies were conducted followed by the determination of absolute and relative organ weights (ROW) were determined. Adrenal glands, kidneys and liver were then subjected to histological assessments. The study revealed that no morbidity and mortality were observed and no significant changes in body weight recorded between all groups. Group II (male and female) possessed significantly higher mean corpuscular haemoglobin concentrations (MCHC) when compared to Group IV (male) and other groups (female). Higher mean

corpuscular haemoglobin (MCH) values were observed in female rats (Group II) when compared with other groups. Group IV (male) have lower blood urea nitrogen (BUN) when compared to Group I and II. Significantly lower alanine transaminase were observed in all GITH treated groups (male) in comparison with control group. No noticeable changes in gross appearance of rats internal organs were seen with no marked changes in absolute and ROW. Histological examinations revealed normal morphology of adrenal glands, kidneys and livers for all the rats. Histomorphometry analysis of adrenal glands revealed lower width of zona fasciculata regions in all GITH treated groups (male) and Group III (female) when compared to Group I. Dilatation of Bowman's space were observed in Group IV (male) in comparison to Group I and III. Significant variations of binucleated hepatocytes size in Group II and III as compared to Group I (male); and Group IV when compared to Group II and III (female) was observed. In conclusion, long term oral administration of GITH does not produce any detrimental effects in the body weight, physical and behavioural characteristics, as well as clinical laboratory analysis and histopathological parameters. Hence, overall findings suggested that GITH preserved the normal functions of adrenal glands, kidney and liver of SPF rats and may contribute to positive effects that warrant further evaluations.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Complementary medicines, Apitherapy and natural medicines

Complementary and alternative medicines (CAM) are defined as a group of diverse medical and health care organizations that are not considered as a part of modern conventional medicine. Complementary medicines are usually practiced along with conventional methods. While, Integrative medicine combines mainstream medical therapies and CAM based on its scientific evidence of the safety and effectiveness (NCCIH, 2008). Normally in some countries, the terms complementary or alternative medicines are utilized interchangeably with traditional medicines (WHO, 2000).

An alternative medicinal branch called Apitherapy has been developed recently utilizing honey and other bee products such as bee venom, propolis and royal jelly in the treatments of many illnesses (Bogdanov *et al.*, 2008; Bogdanov, 2010). Some of the ailments treated are multiple sclerosis, shingles, gout, burns and wounds, tendonitis, pain, and infections.

The National Centre for Complementary and Integrative Health (NCCIH) (2008) estimated that approximately 40% Americans resorts to alternative medicine for their general well being. Moreover, in a 2007 interview surveys done by the National Institute of Health (NIH) concluded that approximately 17.7% adults in America had used natural products in the past such as fish oil or omega- 3.

On the other hand, based on the survey conducted by Malaysian Ministry of Health (MOH) in 2004, approximately 69% of Malaysians have utilized traditional and

complementary medicine in their lifetime and 88.9% among them use a biologically-based therapy such as herbal medicines or vitamins supplements either for their general health and boosting immune system, improving quality of life or combat symptoms of illnesses (Dhanoa *et al.*, 2014; Globinmed, 2015). Additionally, Aziz and Tey (2008) reviewed that the estimated annual sales of traditional medicine in Malaysia have increased from RM 1billion to RM 4.5 billion within the duration of five years (2000 to 2005).

Moreover interview-based studies done by a team of researchers showed high prevalence in CAM utilization amongst general populations and cancer patients as well (Aziz and Tey, 2008; Siti *et al.*, 2009; Dhanoa *et al.*, 2014). Mostly their choice of treatment alongside with conventional modern medicine is by consumptions of traditional herbs and vitamins and mind-body techniques such as prayers, yoga and meditations (Siti *et al.*, 2009; Dhanoa *et al.*, 2014).

In line with the marketing upsurge and easily available dietary supplements such as herbs, vitamins and minerals, it raised a special concern on the occurrence of adverse effects which are likely to arise from overdoses or herbal-drugs interaction that can put an additional risk to the consumer. Consequently, the need to learn about the effects of these products to the human body and their safety profile becomes of great importance.

1.2 Honey

Honey is a complex substance which composed mainly of water, different types of carbohydrates, proteins, small quantities of vitamins, minerals and trace elements along with aromatic compounds and polyphenols. Nevertheless, the exact contents and

composition of honey are in accordance to its floral origin, plant species as well as geographical and climates in which the honeybees forages.

This sweet, viscous substance is made by means of several processes of regurgitation by bees. The flower nectars (mono/multi-floral) comprise almost 80% of water and complex sugars, were carried by bees through their honey stomach back to the hive and passed to other worker bees where it will undergo the chewing process and converted into simple sugars. Evaporation of excess water from the nectars was allowed in the honeycombs. The bees will then chew the nectars once more after the nectar is thicken and honey is produced (Al-Waili, 2003b).

Well known to be flavourful with high nutritional and therapeutic values. It has been widely marketed as a natural sweetener and dietary supplements. As some called it a 'functional food' (Bogdanov, 2010) that is capable of exhibiting positive health effects (Nantel, 1999).

1.2.1 Honey and its nutritional components

1.2.1.1 Carbohydrates

Ninety-five percent of honey dry weight comprises of carbohydrates predominantly fructose (38.5%) and glucose (31.0%). Additionally, about 25 different sugars were detected with oligosaccharides (sucrose, maltose, turanose, erlose, melezitose and raffinose) while trace amounts of tetra and pentasaccharides have been identified. Generally, honeys with high fructose are sweeter compared with the ones with high glucose content (Bogdanov *et al.*, 2008; Bogdanov, 2009a; Bogdanov, 2009b). Since fructose and glucose were readily transported in the blood stream, honey can be an

excellent source of instant energy. Twenty grams of honey taken orally will cover about three percent of human required daily energy (Bogdanov *et al.*, 2008; Bogdanov, 2009b). Oligosaccharides is a product of honey enzyme invertase activities which can serve as prebiotic agent (White, 1975; Chow, 2002; Bogdanov, 2009a).

1.2.1.2 Proteins

Proteins, primarily enzymes and amino acids made up about 0.5%-0.7% of honey contents. Virtually all physiologically important amino acids were present in honey. However, since the amount is relatively small, it has very little nutritive effects to the human body (Bogdanov *et al.*, 2008; Bogdanov, 2009b; Bogdanov, 2009a)

Besides amino acids, several enzymes were present in honey. These enzymes namely were diastase, invertase, glucose oxidase, catalase, acid phosphatase, protease, esterase and β-glucosidase (Bogdanov *et al.*, 2008; Bogdanov, 2009b; Bogdanov, 2009a) and were responsible in honey functional properties which make it a unique sweetener.

Major enzymes in honey are diastase (amylase) which is capable of converting polysaccharides (starch) or oligosaccharides (glycogen) to simpler sugar by breaking the glycosidic bonds. Next is invertase which catalyses conversion of sucrose to fructose and glucose and glucose oxidase which are responsible for conversion of glucose to gluconic acid and hydrogen peroxide (contributes to antibacterial properties of honey) (Bogdanov *et al.*, 2008; Bogdanov, 2009a; Bogdanov, 2009b; Kowalski *et al.*, 2012).

Glucose oxidase is generally inactive in honey which is merely activated by dilution of pure honey (Al-Mamary *et al.*, 2002). In earlier years, invertase and diastase were used to evaluate honey freshness upon storage or heating (Bogdanov *et al.*, 2008; Bogdanov, 2009a)

1.2.1.3 Vitamins, mineral and trace elements

Fairly small amounts of vitamins, minerals and trace elements were found in honey (Table 1.1) which has very little influence in accordance with the recommended daily intake for humans. The different values were mainly due to the botanical origin of honeys (Bogdanov *et al.*, 2008; Bogdanov, 2009a; Bogdanov, 2009b)

1.2.1.4 Aromatic and volatile compounds of honey

Volatile compounds which originate mostly from botanical origin of honey are responsible for its tastes and aroma. Widespread research on honey aromatic compounds had identified about 600 compounds (Bogdanov, 2009a). One of it is polyphenols groups, mainly flavonoids for example quercetin, luteolin, kaempferol, apigenin, galangin, phenolic acids and phenolic acid derivatives (Bogdanov, 2009b), which are plant-derived secondary metabolites. These compounds are mainly accountable for the honey anti-oxidative capacity. Darker coloured honeys reportedly have more phenolic acid derivatives but lesser flavonoids (Bogdanov, 2009a). It was proven by several studies that darker coloured honey has more antioxidant content than honey with lighter colour (Taormina *et al.*, 2001; Gheldof *et al.*, 2002; Schramm *et al.*, 2003; Kinoo *et al.*, 2012).

Table 1.1 Honey vitamins and minerals values as well as trace elements adapted from Bogdanov *et al*, (2008) and Bogdanov, (2009b)

Vitamins (mg)	**Amount in 100g**	**Minerals (mg)**	**Amount in 100g**	**Element**	**mg/100 g**	**Element**	**mg/100 g**
Phyllochinon (K)	Ca. 0.025	Sodium (Na)	1.6-1.7	Aluminium (Al)	0.01-2.4	Lead (Pb) *	0.001-0.03
Thiamin (B_1)	0.00-0.001	Calcium (Ca)	3- 3.31	Arsenic (As)	0.014-0.026	Lithium (Li)	0.225-1.56
Riboflavin (B_2)	0.01-0.02	Potassium (K)	40-3500	Barium (Ba)	0.01-0.08	Molybdenum (Mo)	0-0.004
Pyridoxine (B_6)	0.01-0.32	Magnesium (Mg)	0.7-1.3	Boron (B)	0.05-0.3	Nickel (Ni)	0-0.051
Niacin	0.1-0.2	Phosphorus (P)	2-15	Bromine (Br)	0.4-1.3	Rubidium (Rb)	0.040-3.5
Panthothenic acid	0.02-0.11	Zinc (Zn)	0.05-2	Cadmium (Cd) *	0-0.001	Silicon (Si)	0.05-24
Ascorbic Acid (C)	2.2-2.5	Copper (Cu)	0.02-0.6	Chlorine (Cl)	0.4-56	Strontium (Sr)	0.04-0.35
		Iron (Fe)	0.03-4	Cobalt (Co)	0.1-0.35	Sulphur (S)	0.7-26
		Manganese (Mn)	0.02-2	Floride (F)	0.4-1.34	Vanadium (V)	0-0.013
		Chromium (Cr)	0.01-0.3	Iodide (I)	10-100	Zirconium	0.05-0.08
		Selenium (Se)	0.002-0.01				

(*) Toxic elements

1.3 Tualang honey

Tualang honey is named after one of the tallest trees (>85m) in Malaysia, Tualang tree (*Koompasia excelsa*), which is indigenous, mostly to peninsular Malaysia, southern Thailand, Borneo and Palawan region. It is produced by Asian rock bees (*Apis dorsata*) deemed to be the world's largest and most ferocious bees (mySinchew, 2011; Shah *et al.*, 2013).

1.3.1 Physicochemical characteristics and nutritional values of Tualang honey

Due to its multifloral botanical origin, it has dark brown (Tumin *et al.*, 2005) to amber (Moniruzzaman *et al.*, 2013a) in appearance. Because of its tropical origin, Tualang honey has somewhat higher water content (23.6%) compared to honeys originating from temperate regions (Jeffrey and Echazarreta, 1996). This high moisture content can be reduced by the process of evaporation to decrease the probability of honey spoilage by fermentation. It is acidic with an average pH value of 3.4 and total acid content of 35.12 meq/kg. The other criteria such as specific gravity, HMF value, soluble solid were within the limit set up by international regulations (Codex Alimentarious Commission, 2000). Table 1.2 summarized the physicochemical properties of Tualang honey as described by extensive research carried out by previous researchers.

Trace elements (Table 1.3) that are commonly found in different types of honeys were present in Tualang honeys with sodium (Na), potassium (K), iron (Fe) and calcium (Ca) being the most abundant (Chua *et al.*, 2012; Moniruzzaman *et al.*, 2014). The level of trace elements found in honey is within permissible limits set by a joint committee of FAO/WHO (2007).

Over 35 volatile compounds has been identified in Tualang honey which consist of 59% of hydrocarbons, 11% ketones, 10% aldehydes, 8% furans, 7% acid/alcohol, 1% organic acids (Shah *et al.*, 2013).

Tualang honey possessed relatively high antioxidants contents mainly phenolics and flavonoids compounds (Table 1.4) which in turn gives high antioxidant capacities (Mohamed *et al.*, 2010; Khalil *et al.*, 2011a; Khalil *et al.*, 2011b; Kishore *et al.*, 2011; Khalil *et al.*, 2012; Chua *et al.*, 2013; Moniruzzaman *et al.*, 2013a).

Table 1.2 Physicochemical properties and nutritional profile of Tualang honey

Parameters	**Amount**	**Parameters**	**Amount**
Colour appearance	Dark brown to amber	Glycaemic Index	Intermediate 65
Intensity (ABS_{450})	489.5-544.33	Total CHO (%)	72.94
Moisture content (%)	22.32-26.51	Total Sugar content (g/100g honey)	63.60
pH	3.2-3.8	Reducing sugar (g/100g honey)	61.94-67.50
Specific gravity	1.335	Apparent sucrose (g/100g honey)	0.60- 1.66
Water insoluble solid (%)	0.8	Fructose/glucose ratio (%)	0.885
Ash	0.19-0.2	Sucrose/ maltose ratio (%)	0.226
Electrical conductivity	0.5-1.37	Glucose/water (%)	1.574
Diastase activity (Number)	2.0	Fructose (%)	29.60 -41.732
HMF (mg/kg)	1.7	Glucose (%)	30- 47.13
Free acid (Meq/kg)	32.9 – 37.33	Maltose	4.49 -7.90
		Total Lipid content (mg/100g)	100
		Protein (%)	0.36- 0.483
		Proline (mg/kg)	248.53
		Thiamine (B1)	<LOQ
		Riboflavin (B2)	<LOD
		Nicotinic acid (B3)	170.38
		Folic acid (B9)	<LOQ
		Cyanocobalamin (B12)	<LOD
		Ascorbic acid (C)	52.20

LOQ: Limit of Quantification; LOD: Limit of Detection

Range of values in the Table 1.2 is as described in various studies by different researchers all over Malaysia (Tumin *et al.*, 2005; Robert and Ismail, 2009; Khalil *et al.*, 2010; Mohamed *et al.*, 2010; Zakaria *et al.*, 2011; Jaafar *et al.*, 2012; Ahmed and Othman, 2013; Chua *et al.*, 2013; Moniruzzaman *et al.*, 2013a; Chua and Adnan, 2014).

Table 1.3 Minerals and trace elements identified in Tualang honey

Elements	Amount (ppm) as determine by Chua *et al* (2012)	Amount (mg/kg) as determined by Moniruzzaman (2014)	Amount (%) as summarized by Ahmed and Othman (2013)
Aluminium (Al)	1.120	-	-
Arsenic (As)	nd	0.062	-
Barium (Ba)	nd	-	-
Boron (B)	-	-	-
Bromine (Br)	-	-	-
Cadmium (Cd)*	nd	nd	-
Calcium	-	165.10	0.18
Chlorine (Cl)	-	-	-
Chromium (Cr)	1.845	-	-
Cobalt (Co)	nd	0.033	-
Copper (Cu)	0.093	1.25	-
Fluoride (F)	-	-	-
Indium (In)*	0.226	-	-
Iodide (I)	-	-	-
Iron (Fe)	11.097	128.13	-
Lead (Pb)*	nd	0.183	-
Lithium (Li)	nd	-	-
Magnesium	5.209	35.03	0.11
Manganese	1.992	-	-
Molybdenum (Mo)	-	-	-
Nickel (Ni)	nd	-	-
Potassium	1199.65	1576.40	0.51
Rubidium (Rb)	5.188	-	-
Selenium	17.202	-	-
Silicon (Si)	-	-	-
Sodium (Na)	370	268.23	0.26
Uranium*	0.040	-	-
Strontium (Sr)*	0.113	-	-
Sulfur (S)	-	-	-
Vanadium (V)	nd	-	-
Zinc	3.316	13.20	-
Zirconium	-	-	-

(*) Toxic elements that could be due to man-made origin, (nd) non detected, (-) not considered in the particular study

Table 1.4 Antioxidant activities and antioxidant compounds found in Tualang honey

Parameters	**Values**
Total phenolics (mg/GAE/100g honey)	83.96-110.39
Total flavonoids (mg catechin/kg)	50.45
FRAP values (mg TE/100g honey)	52.39
DPPH (% inhibition)	9.65
Phenolic Acids	*p*-coumaric acid
	Caffeic acid
	Ferulic acid
	Trans-cinnamic acid
	Chlorogenic acid
	Gallic acid
	Syringic acid
	Benzoic acid
Flavonoids	Pinobanksin-3-O-butyrate
	Quercetin
	Catechin
	Naringenin
	Kaempferol
Organic acids	Fumaric acid
	Gluconic acid

As determined by Mohamed *et al*., (2010); Khalil *et al*., (2011a; 2011b); Kishore *et al*., (2011) Chua *et al*., (2013) and Moniruzzaman *et al*., (2013a).

1.4 Contaminants in honey

Like any other food source, honey could be exposed to different kinds of contaminants (microorganisms, heavy metal, pesticide, antibiotic and other toxic materials) from the environment either by bees or in the processing and packaging of the honey (Bogdanov, 2006; Bogdanov, 2008; Bogdanov, 2009a; Bogdanov, 2009b).

1.4.1 Heavy metals, pesticides and antibiotics

There have been incidences in contamination of honey with heavy metals such as arsenic (As), lead (Pb) and cadmium (Cd), radioactive materials (Bogdanov, 2006; Chua *et al.*, 2012; Islam *et al.*, 2013) as well as organic contaminants such as polychlorinated biphenyls. These contaminants are mainly introduced into the honey through the environment, for instance from soil and man-made products which include motor oil, during packaging as well as indirectly through the honeybees (Bogdanov, 2006; Islam *et al.*, 2013).

The residual amount of pesticides that were used in agricultural industries had been detected in European honeys (Bogdanov, 2006), moreover there have been growing concern over the contaminations by antibiotics that was used against bee brood diseases (Bogdanov, 2006; Bogdanov *et al.*, 2008; Bogdanov, 2009a).

1.4.2 Microorganisms

Due to its high osmotic pressure, acidity and low pH value as well as hydrogen peroxide content, the microorganism found in honeys is very limited since the condition is not favourable for microorganism growth (Al-Mamary *et al.*, 2002; Bogdanov, 2010). However, studies have reported finding colonies of osmotolerant yeasts, moulds,

Bacillus species as well as *Clostridium botulinum* spores (Snowdon and Cliver, 1996; Iurlina and Fritz, 2005).

Most honeys contain small amounts of yeasts and moulds (Snowdon and Cliver, 1996; Iurlina and Fritz, 2005). In a study done by Iurlina and colleague (2005), they found out that yeasts and were found in 57% out of 70 unpasteurized Argentineans honeys. These yeasts that were present in honeys can cause unwanted fermentation and it was made possible by honey with high moisture content. A moisture content above 20% raised the chances of yeast fermentation in honeys (Bogdanov, 2009a).

Paenibacilus larvae (spore forming bacteria), major pathogens for honey bees were also identified alongside *Bacillus sp* such as *B. cereus*, *B. pumilus* and *B. Laterosporus* spores (Snowdon and Cliver, 1996; Iurlina and Fritz, 2005).

Usually, the primary source of microbial contaminations is likely introduced by pollen, digestive tract of honey bees, dusty air, earth and nectar. Secondary sources of contamination were due to post harvesting of the honeys such as packaging, food handlers, cross-contamination, equipment and building (Bogdanov, 2006).

1.4.3 Hydoroxymethylfurfuraldehyde (HMF)

HMF normally presents in trace amounts in fresh honeys which are a product of fructose decomposition. Concentration of HMF is normally increased, with storage and prolonged heating (Bogdanov, 2009a). Codex Alimentarious Commissions and European Union (EU) (2000) have standardized the limit of HMF content in honey to be not exceeding 40mg/kg and for honey originating from tropical countries to be not exceeding 80mg/kg.

1.5 Remedial use of honey

Copious health benefits of honey made it an important aspect of complementary and traditional medicines especially in Apitherapy. Growing interest has been based on multi faceted medicinal properties of honey namely antibacterial; wound healing, antioxidant, anticancer and anti-inflammatory activities. Honey has gained great work momentum by researchers both *in vitro* and *in vivo* round the globe to spark the unknown benefit of the infinite attributes as well as its applications to modern medicine.

1.5.1 Antimicrobial activity

The most studied medicinal attributes of honey were its anti-microbial activities. Generally honey possess highly effective broad spectrum antibacterial activities against infections caused by both gram positive and gram negative bacteria (Molan and Allen, 1996; Taormina *et al.*, 2001; Aljadi and Yusoff, 2003; Lusby *et al.*, 2005; Estevinho *et al.*, 2008; Tan *et al.*, 2009; Khoo *et al.*, 2010; Nasir *et al.*, 2010; Saxena *et al.*, 2010; Kirnpal-Kaur *et al.*, 2011; Kinoo *et al.*, 2012; Zainol *et al.*, 2013)

Besides its natural physical attributes such as high osmolarity, low pH value and low water content that are unfavourable for bacterial growth, hydrogen peroxide activation is primarily the contributing factors in bacteriostatic and bactericidal effects of honey. Some phytochemicals compounds (Al-Mamary *et al.*, 2002; Kinoo *et al.*, 2012) that are present in honey also contribute to its antibacterial effects. Even though the chemical constituents of honey might be a little different from one another, they all still exhibit good antimicrobial activities. Table 1.5 summarizes the antibacterial properties of Tualang honey against gram positive and gram negative bacteria.

Table 1.5 List of bacteria that are known to be sensitive to Tualang honey

Gram positive bacteria	**Gram negative bacteria**
Streptococcus pyogenes	*Escherichia coli*
Coagulase-negative Staphylococci	*Salmonella enterica* serovar typhi
Staphylococcus aureus	*Stenotrophomonas maltophilia*
Methicillin- resistant Staphylococcus aureus	*Acinetobacter baumanii*
Streptococcus agalacticae	*Pseudomonas aeruginosa*
Bacillus cereus	*Proteus mirabilis*
	Shigella flexneri
	Enterobacter cloacae

(Tumin *et al.*, 2005; Tan *et al.*, 2009; Kirnpal-Kaur *et al.*, 2011; Ahmed and Othman, 2013; Zainol *et al.*, 2013)

1.5.2 Wound healing

It was revealed that honey can support wound healing either burn and infected wounds or ulcers by accelerating the re-epithelisation and promoting granulation and tissue formation (Nawfar *et al.*, 2011), eliminating bacterial infections (Nasir *et al.*, 2010; Sukur *et al.*, 2011) and providing moist environment and nutrient essential for wound healing.

Different therapeutics properties of honey exhibits synergistic effect in combination with conventional antibiotics or wound dressing biomaterial such as hydrogel and hydrofiber® and showed comparable results when compared with commercial dressing materials such as silver hydrogel and so on (Imran *et al.*, 2011; Rodzaian *et al.*, 2011; Lazim *et al.*, 2013).

1.5.3 Source of antioxidants and protective effects of honey against oxidative stress

Antioxidants compounds are nutritive and non-nutritive agents that can impede biologically detrimental chemical reaction of free radicals and reactive oxygen species (ROS). Free radicals and ROS compounds are responsible in increasing oxidative damage in many molecules for examples lipids, proteins and nucleic acids (Erguder *et al.*, 2008) which contributes to cellular dysfunction and accountable for pathogenesis of diseases, such as cancer, cardiovascular diseases and neurodegenerative diseases (Busserolles *et al.*, 2002; Gheldof *et al.*, 2003; Schramm *et al.*, 2003).

Separate studies done by different researchers have reported that consumption of honey can increase serum antioxidant capacity, plasma antioxidant content and consequently will increase the protectiveness of human body against damaging effects of free radicals species (Al-Waili, 2003a; Gheldof *et al.*, 2003; Schramm *et al.*, 2003).

Number of studies performed by different researchers concluded that Tualang honey has high antioxidant content which has significant correlation between high total phenolic content and free radical scavenging activities of honey (Hussein *et al.*, 2011; Kishore *et al.*, 2011; A-Rahaman *et al.*, 2013). This is also substantiated via *in vitro* studies that showed protective effects in cellular resistance to oxidative stress (Tan *et al.*, 2014) and Ultraviolet B (UVB) radiation (Ahmad *et al.*, 2012).

1.5.4 Anti-proliferative, anti-cancer and anti-inflammatory effects of honey

Tualang honey has been shown to exhibit anti-proliferative and apoptotic effects in human keloid fibroblast (Syazana *et al.*, 2011), oral squamous and osteosarcoma cell lines (Ghashm *et al.*, 2010), as well as human breast and cervical cell lines without

harming normal healthy cells (Fauzi *et al.*, 2011). Similar study done using Greek honey extracts revealed a significant effect on human cancer cells mainly breast, endometrial and prostate (Tsiapara *et al.*, 2009) owing to its phenolic contents. Additionally, *in vivo* study conducted by Kadir *et al* (2013) showed that long term oral ingestion of Tualang honey can reduce aggressiveness of DMBA-induced breast cancer development in female rats. It is also observed in this study that with increasing doses of Tualang honey (0.2, 1.0 and 2.0g/kg); there was an increasing trend of apoptotic index (AI) of the cancer cells.

Adequate inflammatory response is needed in order to eliminate harmful irritants whether it is a microbial or non-microbial origin. However, excessive inflammatory responses can be detrimental to human health. Honey exhibits potent anti-inflammatory responses. Othman (2012) reviewed that topical application of a mixture containing honey, olive oil and beeswax can reduce the occurrence of diaper dermatitis in infants and topical application of honey can improve dermatitis and *Psoriasis vulgaris* pathogenesis. Furthermore, it is proven that oral supplementation of Tualang honey reduces inflammatory response of upper respiratory tracts (Sulaiman *et al.*, 2011; Asha'ari *et al.*, 2013) among affected individuals. Similarly, inhalation of Tualang honey reduces inflammatory cells response and airway inflammation in rabbits (Kamaruzaman *et al.*, 2014).

1.5.5 Protective effects of honey against metabolic diseases

Several animal studies and clinical trials had concluded that honey intake either short term or long term can ameliorates the risk factors for metabolic and cardiovascular diseases in animal models or in individuals at risk and patients of cardiovascular diseases

(Busserolles *et al.*, 2002; Chepulis, 2007a; Chepulis and Starkey, 2008; Fasanmade and Alabi, 2008; Yaghoobi *et al.*, 2008; Najafi *et al.*, 2011).

Earlier researchers further established that honey intake had metabolic and cardiovascular significance by evaluating the routine health profiles. There were reductions in indices that are known as risk factors for cardiovascular disease such as total cholesterol, triglycerides, low density lipoprotein (LDL) and C-reactive protein with the increment of high-density lipoprotein (HDL) level, higher plasma vitamin E and C (Busserolles *et al.*, 2002; Al-Waili, 2003a; Chepulis and Starkey, 2008; Yaghoobi *et al.*, 2008; Alagwu *et al.*, 2009; Mundstedt *et al.*, 2009; Alagwu *et al.*, 2011; Nemoseck *et al.*, 2011; Nasrolahi *et al.*, 2012; Majid *et al.*, 2013).

Honey has been proven to decrease blood glucose (Erejuwa *et al.*, 2011a; Nazir *et al.*, 2014), glycated haemoglobin (HbA1c) level (Chepulis, 2007a) and increase blood insulin level (Erejuwa *et al.*, 2011a) in both healthy and diabetic patients as well as animal models. Moreover in separate studies it is revealed that honey consumption by patients with type I (Abdulrhman *et al.*, 2013) and type II (Nazir *et al.*, 2014) diabetes mellitus (DM) had lower glycaemic response in comparison with glucose consumption. These indicate that honey is suitable as a sugar alternative in diabetic management and prevention.

1.5.6 Kidney functions

Reduced level of blood urea nitrogen (BUN) (Al-Waili, 2003c; Al-Waili *et al.*, 2006b; Abdel-Moneim and Ghafeer, 2007), creatinine (Al-Waili, 2003c; Abdel-Moneim and Ghafeer, 2007; Erejuwa *et al.*, 2011a) were observed in honey fed rats either healthy or diseased.

1.5.7 Hepatoprotective effects

Both clinical trial and *in vivo* studies showed that when honey was supplied via inhalation (Al-Waili, 2003c; Mostafa *et al.*, 2012) or oral consumption (Al-Waili *et al.*, 2006a; Al-Waili *et al.*, 2006b), it can exhibit hepatoprotective attributes. Reduction of liver enzyme activity such as aspartate aminotransferase (AST) ,alanine transaminase (ALT) and alkaline phosphatase (ALP) (Erejuwa *et al.*, 2012) was observed after repeated inhalation or oral consumption of honey (Al-Waili, 2003c; Al-Waili *et al.*, 2006a; Al-Waili *et al.*, 2006b; Abdel-Moneim and Ghafeer, 2007; Erejuwa *et al.*, 2012; Mostafa *et al.*, 2012). Significant decrease of total bilirubin was also seen after repeated exposures to honey (Mostafa *et al.*, 2012).

1.5.8 Haematology and immunity

Honey has been found to be beneficial to reduce anaemic incidence in individuals and enhance the immunity parameters. Chepulis (2007b) documented that dietary intake of honey in young rats increases the percentages of neutrophil and lymphocytes. This aligned with earlier studies by Al-Waili (2003c) that disclosed honey intravenous infusion elevates the level of haemoglobin, white blood cells and eosinophil percentage in healthy sheep. Same researcher also examined the effects of honey ingestion after acute blood loss in animal models. This study revealed that there is an increment in the level of haemoglobin before and after acute haemorrhage (Al-Waili *et al.*, 2006b). Nutritional constituents in honey such as minerals and trace elements might serve to stimulate haemoglobin synthesis. In separate clinical trial, Al-Waili (2006a) also documented an enhanced immunological indices such as lymphocytes percentage, platelet count in AIDS patient given 3 weeks of supplementation of honey.

1.5.9 Reproductive and developmental

Honey (Tualang honey) has been shown to have hormonal regulations effects either in male or females subjects. Daily oral supplementation of Tualang honey for two weeks prevents uterine atrophy and excessive weight gain as well as, increased bone mass in ovariectomised rats (Zaid *et al.*, 2010). Two other studies showed that Tualang honey enhances the memory of postmenopausal women compared to women receiving hormone replacement treatment (HRT) therapy (Othman *et al.*, 2011; Shafin *et al.*, 2014). Consequently Tualang honey can be suggested as an alternative to HRT therapy in postmenopausal women. In a different study, daily supplementation of Tualang honey for four weeks at the dose of 1.2g/kg significantly enhanced spermiogenesis in rats by increasing epididymal sperm count (Mohamed *et al.*, 2011).

1.6 Review on other Malaysian honey

There are several types of local Malaysian honey (Table 1.6) that has been studied apart from Tualang honey which includes Malaysian Gelam honey, Acacia honey, Nenas honey and recently Borneo honey namely for their physicochemical and antioxidant properties (Aljadi and Kamaruddin, 2004; Khalil *et al.*, 2011b; Moniruzzaman *et al.*, 2013a). These honeys are called tropical honey since it is originated from tropical countries (Khalil *et al.*, 2011b) and were collected from several states across Malaysia.

Among these types of honeys, Tualang and Gelam honey were the most widely studied followed by Acacia due to their therapeutic properties and availabilities (Khalil *et al.*, 2011b). However recently, Trigona honey has gained recognition amongst local population.

Gelam honey is a wild honey produced by two species of bees which are *Apis dorsata* and *Apis mellifera*. Normally the bees' colonies collect nectar from the same floral source which is Gelam tree (*Melaleuca cajuputi* powell) (Khalil *et al.,* 2011b; Mohd Zohdi *et al.*, 2011; Moniruzzaman *et al.*, 2013b; Aziz *et al.*, 2014)

Acacia honey is produced by *Apis mellifera*, cultured bees, derived from a plant widely used in forest plantation known as tropical acacia species (*Acacia mangium*) (Moniruzzaman *et al.*, 2013a; Zainol *et al.*, 2013). Acacia honeys are famous for its pleasant and mild taste and have low pollen content, which can reduce the risks of allergies. It is also said that Acacia honey showed slower crystallization process (bees-products.com).

Pineapple honey, which is produced by bees species *Apis mellifera* normally collects nectars form pineapple plants hence the name Pineapple honey (Moniruzzaman *et al.*, 2013a). Borneo honeys that were normally used in the studies were from Apiary in Kudat Sabah (Khalil *et al.*, 2011b; Moniruzzaman *et al.*, 2013a). The nectar in which the smaller size bee (*Apis cerana*) forages came from *Acacia mangium* trees and flowers.

While Trigona (Kelulut) honey derived from species of stingless bees (Tribe Meliponini) normally from genus *Trigona* (Oddo *et al.*, 2008; Zainol *et al.*, 2013; Mail, 2014; Tualanghoney.com, 2015). These bees stored their honey in pots rather than in combs as other honeybees (Oddo *et al.*, 2008) inside hollow tree. Physicochemical characterization that was done for Trigona carbonara honey from Australia were shown in Table 1.7, and they concluded that several physicochemical properties of Trigona honey (moisture content, electrical conductivity and free acidity) are somewhat different from honey produced by *Apis mellifera* bees.

Recently in Malaysia there has been increasing effort in rearing these stingless bees inside manmade boxes attached to a tree stump which normally contained the queen bee that was found in the wild or near village area (Mail, 2014). So far, there are still no literature published on Malaysian Trigona honey and its composition maybe somewhat different from Australian Trigona honey due to dissimilarities in floral origin and geographical region. However according to Malaysian Agricultural Research and Development institute (MARDI), Trigona honey possess twice as nutritious as ordinary honey (Mail, 2014). It is also believed by the Malays that Trigona (Kelulut) possesses anti-ageing and could possibly treat tumour cells when combine with the honey comb (Sirisinghe *et al.*, 2007). Recent study on Indonesian methanolic crude extract of Trigona honey (three different types) showed a promising result in cytotoxic effect towards human cancer cell lines (Kustiawan *et al.*, 2014)

Table 1.6 Honey type and honeybees

Honey type	Floral type	Bee species	Tree name
Tualang	Multifloral	*Apis dorsata*	Tualang tree (*Koompassia excelsa*)
Gelam	Monofloral	*Apis dorsata, Apis mellifera*	Gelam tree (*Melaleuca cajuputi* powell)
Acacia	Monofloral	*Apis mellifera*	Forest Mangrove (*Acacia mangium*)
Pineapple	Monofloral	*Apis mellifera*	Pineapple plant (*Ananas cornosus*)
Borneo	Monofloral	*Apis cerana*	Forest Mangrove (*Acacia mangium*)
Kelulut (Trigona)	Multifloral	*Trigona spp.*	Multiforal foraging activities of bees, Manmade boxes/treestump

Summarized from different studies all over Malaysia (Aljadi and Yusoff, 2003; Aljadi and Kamaruddin, 2004; Hussein *et al.*, 2011; Khalil *et al.*, 2011b; Mohd Zohdi *et al.*, 2011; Moniruzzaman *et al.*, 2013a; Zainol *et al.*, 2013; Mail, 2014; Samat *et al.*, 2014; Tualanghoney.com, 2015).

Table 1.7 Characterization of Australian Trigona carbonara honey

Physicochemical properties	Mean ±SD
Colour (Pfund units)	84.6 10.3
Moisture (g/100 g of honey)	26.5 ± 0.8
Electrical conductivity (mS/cm)	1.64 ±0.12
Ash (g/100 g of honey)	0.48 ± 0.06
HMF (mg/kg of honey	1.2 ±0.6
pH	4.0 ± 0.1
Total acidity (mEq/kg of honey)	128.9 ± 23.3
Diastase (Diastase number)	0.4 ±0.5
Invertase number	5.7 ±1.5
Sugars (g/100 g of honey)	
Fructose	24.5 ± 1.9
Glucose	17.5 ± 2.8
Maltose	20.3 ±2.9
Sucrose	1.8 ±0.4
Fructose + Glucose	42.0 ± 4.5
Total sugars	64.1 ±1.9
Water activity (A_w)	0.74 ±0.01
Flavonoids (mg EQ/100g of honey)	10.02 ± 1.59
Polyphenols (mg EGA/100g of honey)	55.74 ± 6.11

Adapted from Oddo *et al.*, 2008

Even though the honey is produced by different bees' species and originating from different floral source, its basic composition and also its properties is almost similar. However, its biochemical and pharmacological activities vary depending on its origin and processing.

Based on the studies done by honey research team of Universiti Sains Malaysia (USM) (Khalil *et al.*, 2011b; Moniruzzaman *et al.*, 2013a; Moniruzzaman *et al.*, 2013b) on several local Malaysian honeys (Acacia, Pineapple, Borneo, and Tualang, Gelam, Longan, Rubbertree, and Sourwood) revealed that their physicochemical properties such as pH, moisture content, electrical conductivity, colour intensity as well as HMF content were within the acceptable range approved by international guideline (Codex Alimentarious). Malaysian honeys were found to be more acidic when compared to Manuka honey. Additionally, Malaysia is currently in the process of developing Malaysian honey standards (personal communication).

All local honeys studied by USM honey research team exhibits antioxidant activities with TH have the highest antioxidant properties. Acacia honey is the most acidic with highest total sugar and reducing sugar. Pineapple honey had the highest concentration of proline and with the lowest moisture content, which means it has the highest resistant towards microbial growth and fermentation (Moniruzzaman *et al.*, 2013a). Studies revealed that the antioxidant capacities and antimicrobial activities of local honey is comparable or more superior to Manuka honey (Tan *et al.*, 2009; Ahmed and Othman, 2013; Moniruzzaman *et al.*, 2013b).

Studies done by teams of researchers from Universiti Kebangsaan Malaysia (UKM) and Universiti Malaya (UM), showed that Gelam honey exhibit high antioxidant properties mainly due to its flavonoids and phenolic content (Hussein *et al.*, 2011). Due