UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

COMPARISON OF THE EFFECTS OF TUALANG HONEY AND TIBESTAN IN SPERM PARAMETERS SEXUAL BEHAVIOUR AND HORMONAL PROFILE AMONG OLIGOSPERMIA MALES.

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2015

UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT FINAL REPORT
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A.	**PARTICULARS OF RESEARCH** / *MAKLUMAT PENYELIDIKAN:*
(i)	**Title of Research:** Comparison Of The Effects Of Tualang Honey And Tribestan In Sperm Parameters Sexual Behaviour And Hormonal Profile Among Oligospermia Males *Tajuk Penyelidikan:* (Perbandingan Kesan Tualang Honey dan Tribestan Ke Atas Parameter Sperma, Kesejahteraan Seksual dan Hormon di Kalangan Lelaki Kurang Bilangan Sperma)
(ii)	
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D.	**Duration of this research:** *Tempoh masa penyelidikan ini:* **Start Date** 01 / 08 / 2010 a) **Completion Date** 31/07/2012 b) **Duration** : 2 years c) **Revised Date** (*if any*) : 31 / 03 /2013 (*first revised*) 30 / 09 / 2013 (*second revised*)

E. ABSTRACT OF RESEARCH

(An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English**. This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**.
Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

ABSTRACT OF RESEARCH

Introduction: Honey is used as a medicine from the ancient to modern folks. In Malaysian population, honey was used as a supplement for men's health since many years although no clear scientific evidence of its benefit. This study aims to evaluate the effectiveness of Tualang honey on sperm parameters, erectile function, hormonal and safety profiles.

Methodology: A randomized control trial was done using Tualang honey (20 gram) and Tribestan (750 mg) over a period of 12 weeks. Sperm parameters including sperm concentration, motility and morphology were analyzed and erectile function was assessed using IIEF-5 questionnaire. Hormonal profile of testosterone, FSH and LH were studied. Safety profile was measured by hematology profile, renal and liver functions besides adverse event reporting. The volunteers were randomized into two groups and the outcomes were analyzed using SPSS version 18.

Results: A total of 66 participants were involved. A significant increment of mean sperm concentration ($p<0.001$), motility ($p=0.015$) and morphology ($p=0.008$) were seen in Tualang honey group. In Tribestan group, a significant increment of mean sperm concentration ($p=0.007$) and morphology ($p=0.009$) were seen. No significant different of sperm concentration, motility and morphology were seen in between Tualang honey and Tribestan group and similar results were also seen in erectile function and hormonal profile. All safety profiles were normal and no adverse event was reported.

Conclusion: Tualang honey effect among oligospermic males was comparable with Tribestan in improving sperm concentration, motility and morphology. The usage of Tualang honey was also safe with no reported adverse event.

ABSTRAK PENYELIDIKAN (BAHASA MALAYSIA)

Pengenalan: Madu digunakan sebagai ubat sejak zaman dahulu hingga ke zaman moden . Dalam populasi rakyat Malaysia, madu telah digunakan sebagai suplemen kesihatan lelaki sejak bertahun-tahun walaupun tiada bukti saintifik yang jelas tentang manfaat daripadanya. Kajian ini bertujuan untuk menilai keberkesanan madu Tualang pada parameter sperma, fungsi ereksi, hormon dan profil keselamatan.

Kaedah: Kajian kawalan secara rawak dilakukan dengan menggunakan madu Tualang (20 gram) dan Tribestan (750 mg) dalam tempoh 12 minggu. Parameter sperma termasuk kepekatan sperma, motiliti dan morfologi dianalisis dan fungsi ereksi dinilai menggunakan soal selidik IIEF-5. Profil hormon testosteron, FSH dan LH telah dikaji. Profil keselamatan diukur dengan profil hematologi, fungsi buah pinggang dan hati selain pelaporan kesan buruk. Sukarelawan di bahagi secara rawak kepada dua kumpulan dan keputusan telah dianalisis menggunakan SPSS versi 18.

Keputusan: Seramai 66 peserta terlibat. Kenaikan yang signifikan purata kepekatan sperma ($p <0.001$), motili ($p = 0.015$) dan morfologi ($p = 0.008$) diperolehi pada kumpulan madu Tualang. Di kumpulan Tribestan, kenaikan yang signifikan purata kepekatan sperma ($p = 0.007$) dan morfologi ($p = 0.009$) diperolehi. Tiada perbezaan yang signifikan daripada kepekatan sperma, motiliti dan morfologi diperolehi di antara kumpulan madu Tualang dan kumpulan Tribestan dan hasil yang sama juga dilihat dalam fungsi ereksi dan profil hormon. Semua profil keselamatan adalah normal dan tiada peristiwa buruk dilaporkan.

Kesimpulan: Kesan madu Tualang di kalangan lelaki oligospermia adalah setara dengan Tribestan dalam meningkatkan kepekatan sperma, motili dan morfologi. Penggunaan madu Tualang juga selamat dengan tiada pelaporan kesan buruk.

F. **SUMMARY OF RESEARCH FINDINGS**
Ringkasan dapatan Projek Penyelidikan

The effect of Tualang honey was comparable with Tribestan in improving the sperm parameters. Tualang honey and Tribestan showed significant difference in sperm parameters. Hormonal profile of testosterone, FSH and LH did not show any significant difference in both groups. The safety profile of Tualang honey and Tribestan showed no significant changes in hematological, renal or liver functions and no adverse effect was reported. Therefore, Tualang honey and Tribestan are comparable and safe to be consumed until 12 weeks in oligospermic males.

G. **COMPREHENSIVE TECHNICAL REPORT**
Laporan Teknikal Lengkap
Applicants are required to prepare a comprehensive technical report explaining the project.
(This report must be attached separately)
Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Laporan ini mesti dikepilkan]

List the key words that reflect our research:
Senaraikan kata kunci yang mencerminkan penyelidikan anda:

English	Bahasa Malaysia
Tualang honey	Madu Tualang
Tribestan	Tribestan
Sperm parameter	Parameter sperma
Erectile function	Fungsi ereksi
Hormonal profiles	Profile hormon
Oligosperima males	Lelaki oligospermia

H. a) **Results/Benefits of this research**
Hasil Penyelidikan

No. *Bil:*	Category/Number: *Kategori/ Bilangan:*	Promised	Achieved
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)*	1	1
2.	**Human Capital Development**		
	a. Ph. D Students		
	b. Masters Students	**1(MMED)**	**1 (MMED)**
	c. Undergraduates (Final Year Project)		
	d. Research Officers		
	e. Research Assisstants		
	f. Other: Please specify		
3.	**Patents** *Paten*		
4.	**Specific / Potential Applications** *Spesifik/Potensi aplikasin*		
5.	**Networking & Linkages** *Jaringan & Jalinan*	1	1
6.	**Possible External Research Grants to be Acquired** *Jangkaan Geran Penyelidikan Luar Diperoleh*		

- Kindly provide copies/evidence for Category 1 to 6.

b) Equipment used for this research.
Peralatan yang telah digunakan dalam penyelidikan ini.

Items *Perkara*	Approved Equipment	Approved Requested Equipment	Location
Specialized Equipment Peralatan khusus	Nil	Nil	Nil
Facility Kemudahan	Nil	Nil	Nil
Infrastructure Infrastruktur	Nil	Nil	Nil

- Please attach appendix if necessary.

COMMENTS OF PTJ'S RESEARCH COMMITTEE
KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ

General Comments:
Ulasan Umum:

Pencapaian yg baik.
Berpotensi untuk penyelidikan seterusnya.

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My sincere gratitude to FAMA, Malaysia for providing the honey, Pharmacology Department, USM staffs for helping in packaging the honey and also Laboratory USM staff for laboratory work.

Finally, my deepest gratitude goes to my lovely wife and children, for their understanding and support throughout my study period.

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ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine transaminase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AST	Aspartate transaminase
BMI	Body mass index
DBP	Diastolic blood pressure
FAMA	Federal Agriculture Marketing Authority
FBC	Full Blood Count
FSH	Follicular stimulating hormone
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
HUSM	Hospital Universiti Sains Malaysia
IIEF-5	International Index of Erectile Function 5
IIUM	International Islamic University of Malaysia
LFT	Liver Function Test
LH	Luteinizing hormone
RFT	Renal Function Test
SBP	Systolic blood pressure
SD	Standard deviation
SFA	Seminal fluid analysis
SPSS	Statistical Package Social Sciences
TWC	Total white cell count
WHO	World Health Organization

ABSTRAK

PERBANDINGAN KESAN DAN KESELAMATAN MADU TUALANG DAN TRIBESTAN PADA PARAMETER SPERMA, FUNGSI EREKSI DAN PROFIL HORMON DI KALANGAN LELAKI OLIGOSPERMIA.

Pengenalan: Madu digunakan sebagai ubat sejak dari zaman dahulu hingga ke zaman moden . Dalam populasi rakyat Malaysia, madu telah digunakan sebagai suplemen kesihatan lelaki sejak bertahun-tahun walaupun tiada bukti saintifik yang jelas tentang manfaat daripadanya. Kajian ini bertujuan untuk menilai keberkesanan madu Tualang pada parameter sperma, fungsi ereksi, hormon dan profil keselamatan.

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Kesimpulan: Kesan madu Tualang di kalangan lelaki oligospermia adalah setara dengan Tribestan dalam meningkatkan kepekatan sperma, motili dan morfologi. Penggunaan madu Tualang juga selamat dengan tiada pelaporan kesan buruk.

ABSTRACT

COMPARISON ON THE EFFECTS AND SAFETY OF TUALANG HONEY AND TRIBESTAN IN SPERM PARAMETERS, ERECTILE FUNCTION AND HORMONAL PROFILES AMONG OLIGOSPERMIC MALES.

Introduction: Honey is used as a medicine from the ancient to modern folks. In Malaysian population, honey was used as a supplement for men's health since many years although no clear scientific evidence of its benefit. This study aims to evaluate the effectiveness of Tualang honey on sperm parameters, erectile function, hormonal and safety profiles.

Methodology: A randomized control trial was done using Tualang honey (20 gram) and Tribestan (750 mg) over a period of 12 weeks. Sperm parameters including sperm concentration, motility and morphology were analyzed and erectile function was assessed using IIEF-5 questionnaire. Hormonal profile of testosterone, FSH and LH were studied. Safety profile was measured by hematology profile, renal and liver functions besides adverse event reporting. The volunteers were randomized into two groups and the outcomes were analyzed using SPSS version 18.

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Conclusion: Tualang honey effect among oligospermic males was comparable with Tribestan in improving sperm concentration, motility and morphology. The usage of Tualang honey was also safe with no reported adverse event.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Infertility

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Infertility is a global health problem affecting couples worldwide with estimation of about 8 to 12 % of them experiencing some form of infertility during their reproductive life [2]. The first epidemiological study on infertility started in 1866 when Matthews Duncan did a population survey among Scottish and the results was published in his famous book, Fecundity, Fertility, Sterility, and allied topics [3]. Since then, many population based study was done throughout the world to estimate infertility prevalence. However, there are differences of infertility prevalence among each region leading to difficulty in providing infertility services standardization. In view of lacking in magnitude and geographically distribution data of infertility, WHO did an initiative of infertility tabulation involve various part of the world in 1991 [2]. It was useful for researchers, health planners and policy makers to identify the information gaps, and suspected zones, illustrate and support the need to chart the problem and monitor the trends or to carry out follow up studies [2].

Data from the Department of Statistics Malaysia showed total fertility rate among Malaysia populations was declining from 4.9 in 1970 to 2.4 in 2010 [4]. Further, rising in the prevalence of infertility was also seen worldwide [5] [6]. Increasing the number of infertile couple will cause rising in public health care problem, affecting the health care

services, socio-environmental and economic burden [6]. Besides, infertility also affect the couple psychologically leading to distress, loss of control, stigmatization and a disruption in the developmental trajectory of adulthood [7].

From the literature survey by Kamel *et al.*, he elaborated causes of infertility include male, female, couple factors and unexplained causes. The male factors is usually related to sperm abnormality, while female factors was related to ovarian dysfunction and tubal pathology [8]. According to Niederberger *et al.*, about one third of infertile couple are due to the male solely, one-third due to the female and one- third due to both partners [9].

1.2 Male infertility

Male infertility involves a complex aetiology. There are many factors contributing to male infertility such as structural abnormality, hormonal imbalance, previous infection, environmental factor, immunological factor, genetic factor, systemic disease, erectile function, spermatogenic dysfunction and idiopathic. An evaluation related to spermatogenesis was done by many researchers using a variety of treatment models to treat infertility in male [10].

Spermatogenesis is a process of spermatozoa production. It requires a complex regulation of the endocrine hormone axis control release from hypothalamus, pituitary gland and testes. Many hypotheses were postulated for spermatogenesis, but the true pathophysiology process is still unknown. Basically, Gonadotrophin releasing hormone from the hypothalamus plays a role to stimulate the pituitary gland to produce Follicular stimulating hormone (FSH) and Luteinizing hormone (LH). LH will act on Leydig cell of the testes to produce testosterone hormone. This hormone was used for germinal cells

growth and division to form spermatozoa Meanwhile, FSH gives direct effect to Sertoli cells in the testes to produce nutrients, co-factors, and proteins. It will support spermatozoa transportation within seminiferous tubule lumen and progression of normal spermatozoa. [11].

A remarkable discovery of spermatozoa by Leeuwenhoek in 1677 [12] led development in the variety of sperm tests and semen parameters to clarify male infertility. Traditionally, the male infertility was diagnosed based on conventional seminal profiles constructed according to the recognized guideline [13] including measurement of specific of spermatozoa such as sperm concentration, sperm motility and sperm morphology of seminal. Normal values of semen parameters issued by the World Health Organisation (WHO) are generally used as the reference values.

Several versions of normal sperm parameter value manual were introduced by WHO starting from 1980 until the latest version in 2010. In the first WHO manual which was published in 1980, the description of normal sperm parameter was clearly stated beside sperm morphology [14]. Slight improvement was done in the second WHO manual in 1987 in term of sperm morphology but it was not sufficiently provided. In the third WHO manual 1992, spermatozoa morphology classification was described thoroughly at the side of sperm concentration and motility parameter. Unfortunately, the fourth WHO manual 1999 were not specifically indicate on sperm morphology, suggested to use from assisted reproductive technology programme. Latest version of WHO manual, the fifth WHO manual was recently published in 2010 controversially stated very low level of sperm morphology contributing a debate among researchers [14]. In view of their diagnostic value are not population based, suggestion was made to each laboratory to set

its own normal values, reflecting the specific population analyzed with standardized procedure [15]

Various researches were carried out treatment of oligospermic male using different types of medicines, hormones, vitamins, herbs or minerals. In relation to that, many experimental procedures were employed on animals with various type of materials to determine their effectiveness on spermatogenesis postulated for human used [10].

1.3 Tribestan (Tribulus terristris)

Usage of *Tribulus terrestis* (herbs) for enhancement of spermatogenesis has been reported since many years. Studies of *Tribulus terristris* in animals have showed intensification of spermatogenesis [16] and increased of testosterone level [17]. Besides, *Tribulus terristris* also have antioxidant activity [18], antimicrobial activity [19 20] and antihypertensive and vasodiator effect [15]. The first standardized preparation of *Tribulus territris* by Sopharma, Bulgaria; 'Tribestan' was well established three decades ago, and widely used in clinical trials. Tribestan has been recommended for the treatment of impotent and libido disorders in male [21 22], menopausal syndrome in women [22] and infertility [22 23 24].

1.4 Honey

Medically used of honey was documented in many ancient written records among Egyptians, Assyrians, Chinese, Greeks and Romans [25]. The Holy Quran and Bible also describe the importance of honey in human's life and one surah in Quran is named after the bees. Honey, naturally was produced from the belly of bees contain not less than 181 different compounds include simple and complex sugars, organic acids, minerals

and trace elements, vitamins, amino acids, proteins (mainly enzymes), lipids (simple, complex and wax), plant flavours and colouring materials, hydrocarbons, hormones, pollens and microorganisms (yeast) [25].

The honey has been used continuously as a modern folk medicine. In recent years, honey related research is expanded to include variety of the health aspects. Honey Scientific Report [26] showed use of honey in treatment for coughs and sore throats, infantile gastroenteritis, infected legs ulcers in Ghana, earaches in Nigeria, treatment of wound. In addition to that, a detail of study regarding effectiveness of honey on microbial activity [27] and treatment of wound healing [28] was also evaluated.

1.5 Tualang honey

Tualang honey is one of several types of honey found in Malaysia beside Gelam honey, Akasia honey and others. Tualang honey was name from Tualang tree (*Koompassia excelsa*) where *Apis dorsata* bees buid their nest to produce honey. The Tualang tree can be found in the lowland rainforest of southern Thailand, Northeastern Sumatra and also Malaysia [29]. About 14-18 compounds were identified in Tualang Honey. Indeed, the major chemical compound was furfural derivatives and others like formic and acetic acids and fatty acids such as palmitic acid, ethyl linoleate and ethyl oleate [30]. Besides, there are also hydrocarbon and volutile compound [31] and antioxidant properties [32] in Tualang honey.

Local research teams have evaluated the effect of Tualang honey in various studies. In animal studies, Tualang honey has been used in wound treatment [33] [34] [35] and as apoptosis inducer [36] [37] while in human, it has been used for post menopausal treatment

[38] [39], wound treatment [40], scar treatment [41] and acute respiratory symptoms [42]. Mahaneem *et al.* have used Tualang honey spermatogenesis studies in healthy rats and rats exposed to cigarette smoke [43]. The studies reported that Tualang honey had protective effect and enhanced spermatogenesis in both rat samples leading to increase sperm count and daily sperm production. The testosterone level also showed significant improvement although no changes were seen in Follicular Stimulating Hormone and Luteinizing Hormone level [43]. The quality of sperms too was improved with Tualang honey and the percentage of abnormal sperms reduced significantly [44] [45]. These results were probably due to the antioxidant protective effect of Tualang Honey as supported by Mohamed *et al.* which reported that Tualang honey has large amount of antioxidant properties derived from its phenolic constituents [32].

1.6 Justification of the study

There were numerous studies conducted in all over the world aiming to evaluate on the medicinal values of honey. However, there was no reported study on the effect of honey in improvement of sperm concentration, molitity and morphology in human as well as on erectile function and male hormone. Therefore, the main study objective is to determine the effectiveness of Tualang honey in improving sperm parameters, erectile function and hormonal profiles among oligospermic males. Data from this study is essential for future application on the use of honey for the treatment of male infertility.

CHAPTER 2

OBJECTIVES AND HYPOTHESIS

2.1 General objective

To determine the effectiveness of Tualang Honey versus Tribestan in improving sperm parameters, erectile function and hormonal profiles among oligospermic males.

2.2 Specific objectives

i. To determine the effect of Tualang Honey on sperm concentration, sperm motility and sperm morphology as compared to Tribestan.

ii. To determine the effect of Tualang Honey on erectile function as compared to Tribestan.

iii. To determine the effect of Tualang Honey on hormonal profiles (Luteinizing Hormone, Total Testosterone and Follicular Stimulating Hormone) as compared to Tribestan.

iv. To evaluate the safety profiles of Tualang Honey based on haematological and biochemical profile as compared to Tribestan.

2.3 Hypothesis

i. The effect of Tualang Honey on sperm concentration, sperm motility and sperm morphology is comparable with Tribestan.

ii. The effect of Tualang Honey on erectile function is comparable with Tribestan.

iii. The effect of Tualang Honey on hormonal profile is comparable with Tribestan.

iv. The safety profile of Tualang Honey on hematological and biochemical profile is comparable with Tribestan

2.4 Definition of operational term

i. Oligospermia- a condition of suboptimal concentration of spermatozoa in the ejaculated semen to ensure successful fertilization of ovum, defined as a sperm number in between 5 to 20 millions per mililiter semen in this study.

ii. Sperm concentration- a count of sperm in the ejaculum, expressed as number per mililiter.

iii. Sperm motility- the percentage of characteristics of spermatozoa in fresh specimen that productive flagellar motion such as rapid, linear and forward progression.

iv. Sperm morphology- the percentage of sperm with normal anatomical structure in seminal fluid.

v. Erectile function- the ability in the men to have a penile erection. It was graded according to the IIEF-5 score.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study design

Open-label randomized control trial.

3.2 Proposed sample

3.2.1 Source population

Male patients attended to infertility clinic at two centres which were Hospital Universiti Sains Malaysia (HUSM) Kubang Kerian Kelantan and International Islamic University of Malaysia (IIUM) Kuantan, Pahang.

3.2.2 Study population

Inclusion Criteria

i. Married

ii. Alive wife/wives

iii. Age > 20 and < 55 year old

iv. Sperm count in between 5 - 20 $x10^6$ /ml

Exclusion Criteria

i. Anatomical penile deformities

ii. History of undescended testes

iii. History of vasectomy or orchidectomy

iv. Major uncontrolled psychiatric disorders

v. History of alcohol or drug abuse

vi. History of major hematological, renal, hepatic or bleeding disorder

vii. Stroke or Myocardial Infarction within 6 months

viii. Systolic Blood Pressure < 90 or >170mmHg and /or Diastolic Blood Pressure < 50 or > 100 mmHg

ix. Abnormal Full Blood Count, Renal Profile or Liver Function test.

x. Uncontrolled Diabetes Mellitus (HbA1c > 7%)

xi. Use of herbal or drugs that could contain androgenic activity in the last three months

3.3 Sample size calculation

Study sample size was calculated using PS power and sample size calculation software version 3.

i. To determine the effects of Tualang honey on sperm concentration, sample size calculation for comparing two mean was done.

$\alpha = 0.05$ $\beta = 0.9$

σ = standard deviation of sperm concentration among infertile males was $42x10^6$/ml [46]

δ = detectable difference of sperm concentration was $35x10^6$/ml

m = ratio of Tribestan to Tualang honey group was 1

The minimum sample size calculated was 31. After considering non response rate of 10%, the calculated sample size for one group was 34.

ii. To determine the effects of Tualang honey on sperm motility, sample size calculation for comparing two mean was done.

$\alpha = 0.05 \quad \beta = 0.9$

σ = standard deviation of sperm motility among infertile males was 0.15 [46]

δ = detectable difference of sperm motility was 0.13

m = ratio of Tribestan to Tualang honey group was 1

The minimum sample size calculated was 29. After considering non response rate of 10%, the calculated sample size for one group was 32.

iii. To determine the effects of Tualang honey on sperm morphology, sample size calculation for comparing two mean was done.

$\alpha = 0.05 \quad \beta = 0.9$

σ = standard deviation of sperm morphology among infertile males was 0.06 [46]

δ = detectable difference of sperm morphology was 0.05 [47]

m = ratio of Tribestan to Tualang honey group was 1

The minimum sample size calculated was 31. After considering the non response rate of 10%, the calculated sample size for one group was 34

iv. To determine the effect of Tualang honey on erectile function using IIEF-5, sample size calculation for comparing two means should be done. However there was no available study done on IIEF-5 questionnaire to assess erectile function among oligospermic males.

v. To determine the effects of Tualang honey on Testosterone, sample size calculation for comparing two mean was done.

$\alpha = 0.05 \quad \beta = 0.9$

σ = standard deviation of Testosterone among oligospermia males was 0.82 [48]

δ = detectable difference of Testosterone hormone was 0.07

m = ratio of Tribestan to Tualang honey group was 1

The minimum sample size calculated was 30. After considering the non response rate of 10%, the calculated sample size for one group was 33.

vi. To determine the effects of Tualang honey on FSH, sample size calculation for comparing two mean was done.

$\alpha = 0.05 \quad \beta = 0.9$

σ = standard deviation of FSH among oligospermia males was 3.78 [48]

δ = detectable difference of FSH was 3.5

m = ratio of Tribestan to Tualang honey group was 1

The minimum sample size calculated was 24. After considering the non response rate of 10%, the calculated sample size for one group was 29.

vii. To determine the effects of Tualang honey on LH, sample size calculation for comparing two mean was done.

$\alpha = 0.05 \quad \beta = 0.9$

σ = standard deviation of LH among oligospermia males was 2.98 [48]

δ = detectable difference of LH was 2.5

m = ratio of Tribestan to Tualang Honey group was 1

The minimum sample size calculated was 31. After considering the non response

rate of 10%, the calculated sample size for one group was 34.

From the above calculation, the biggest sample size was 34 respondents for one group. Therefore, the number of respondents calculated for this study was 68.

3.4 Sampling method

Respondent was selected from infertility clinic in each centres using convenience sampling method.

3.5 Randomization

The selected respondents who fulfilled the inclusion and exclusion criteria were involved in this study. The equal numbers of respondents were divided into the Tualang Honey group and Tribestan group using random permuted block of four. A random sequence number of group selection either Tualang Honey or Tribestan was generated by using generator number from website www.randomization.com.

3.6 Study tools and interventions

3.6.1. Study tools

i. Seminal fluid analysis (SFA)

SFA was done according to the fourth WHO manual [49] sample collection and delivery. This investigation was sent and analysed either in IIUM or HUSM. The respondent underwent three days abstinence before semen collection. The seminal fluid was collected in a special sterile plastic container and it was sent to the respective lab within one hour after masturbation. SFA was done during second visit and it was

repeated during the fourth visit after completion of 12 weeks study. The sperm parameters used in this study are as showed below table;

Table 3.1 : Sperm parameters reference values according to the 4th WHO manual 1999

Semen parameters	Reference value
Volume (mL)	$\geq$ 2
pH	$\geq$ 7.2
Concentration (10^6/ mL)	$\geq$ 20
Motility (% motile)	$\geq$ 50
Morphology (% normal)	$\geq$ 15*

* From assisted reproductive technology programmes data.

ii. Blood investigations

The blood investigations done in this study were Full Blood Count (FBC), Renal Function Test (RFT), Liver Function Test (LFT), Total serum testosterone, Follicular Stimulating Hormone and Luteinizing Hormone. About 10 ml of the respondent's blood were taken from antecubital fossa and sent either to Gribbles Laboratory in Kuantan, Pahang or Kota Bharu, Kelantan for analysis. Blood sample were taken during the second and fourth visit.

iii. Questionnaire

The questionnaire used in this study was International Index of Erectile Function (IIEF-5). The IIEF-5 questionnaire contains five questions for patient self-assessment to detect and classify the severity of erectile dysfunction. Erectile dysfunction severity was classified into the following five categories based on IIEF-5 scores; no erectile dysfunction (22-25), mild erectile dysfunction (17-21), mild to moderate erectile dysfunction (12-16), moderate erectile dysfunction (8-11) and severe erectile dysfunction (5-7). IIEF-5 was given to the respondents in the second and fourth visit. A

validated IIEF-5 Malay version [50] was used in this study as all the respondents understand Malay language well.

iv. Physical examination

During physical examination, the respondents were examined for height, weight, blood pressure, pulse rate, testicular volume, cardiorespiratory and abdominal examination. Height and weight were measured using Seca scale with the height was recorded in meter (2 decimal) and the weight was recorded in kilogram (-0 decimal). Blood pressure was measured using calibrated manual sphygmomanometer and pulse rate was count manually within 60 seconds. Testicular size was measured comparing with orchidometer. These procedures were done during the second and fourth visit.

3.6.2 Study intervention.

i. Tualang honey

Tualang honey was collected from FAMA Kedah and sterilized by gamma radiation done before packing in a sachet form. Each respondent in Tualang honey group given 20 grams of Tualang honey per day. As estimated adult male body weight was 75 kg, hence, 90 gram of Tualang honey was calculated. However, based on traditional local human consumption of honey of 0.2 g/kg body weight [44] , an estimated around 15 grams of honey was used. Therefore, the amount of 20 grams of honey was a reasonable dose to be taken by the respondents in this study.

ii. Tribestan

Tribestan tablet was used in this study as a comparator. A dose of 250 mg per tablet taken three times per day (750 mg/day) was used based on the oligospesmic males research [23] .

3.7 Study procedure

This study was conducted in accordance with Good Clinical Practice guideline and Declaration of Helsinki. Approval from Human Research and Ethics Committee for Clinical Studies of Universiti Sains Malaysia (USM) (Reference Number: USMKK/PPP/JEPeM[223.3.911)]) was made and this study was funded by University research grant (1001/PPSP/812066). The study was done from June 2010 until December 2012 at Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan and International Islamic University of Malaysia, Kuantan, Pahang.

All men who involved in the study were voluntarily participated and written informed consent were taken after comprehensive explanation given regarding the proposed treatment involved, nature of the therapy, anticipated benefit and any known side-effects of the therapy during screening visit (first visit). A study information sheet was given and they were allowed to withdrawn from the study without penalty at any time.

During the second visit, socio-demographic data, general conditions and genitalia examination were done to exclude structural or other treatable causes. The respondents were underwent confirmatory seminal fluid analysis (SFA) and the baseline blood investigations (FBC, RFT and LFT) with hormonal profiles (Total testosterone, FSH, and LH) were taken in early morning. The IIEF-5 questionnaire Malay version was

given to the respondents in this visit. The respondents who fulfilled the criteria were randomised into two groups to either Tualang honey group or Tribestan group.

The respondents in the Tualang honey group were instructed to take 20 grams of Tualang honey early in the morning 30 minutes before breakfast daily for 12 weeks. Meanwhile, the respondents in the Tribestan group were instructed to take 250 mg Tribestan, for three times daily after meals for the same period of time. These medications were dispensed to the respective group during this visit with amount of medication given was calculated based on the next follow up visit. The IIEF-5 questionnaire was also given to the respondents during this visit.

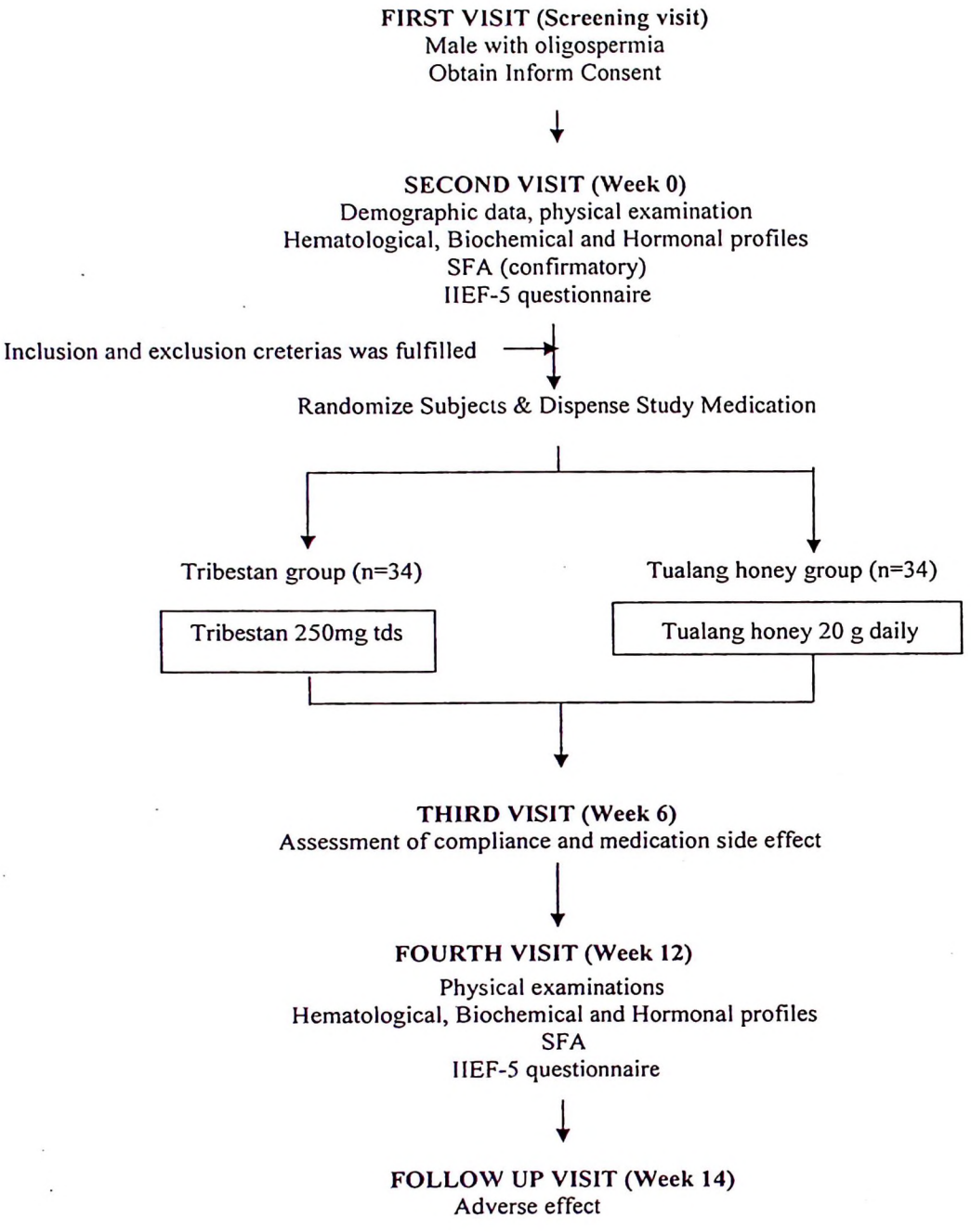
In the third visit, compliance towards medication and medication side-effects were evaluated. The respondents were required to bring back the remaining Tualang honey or Tribestan for compliance assessment. Medications were supplied to the respondents to be completed for a total of 12 weeks.

In the fourth visit, all the respondents were re-examined for their general conditions and blood investigations including FBC, RFT, LFT, total testosterone, FSH and LH were repeated. SFA was repeated and erectile function was re-evaluated using IIEF-5.

During the fifth visit i.e. about two weeks after completion of the study, the respondents were contacted asking on any other adverse effects.

Figure 3.1 showed flow chart of the study.

Figure 3.1 : Flow chart of the study



3.8 Statistical analysis

The data were analysed using Statistical Package for Social Sciences (SPSS) 18.0 under license of USM, Malaysia.

In descriptive analysis, mean (SD, standard deviation) was described for continuous variables of normality distributed data while frequency and percent (%) were described for categorical data. Socio-demographics and baseline medical characteristics were compared in between two groups using chi-square tests for categorical data and ANOVA for numerical data. Paired–t test was used for comparisons between pre and post interventions in sperm concentration, motility and morphology of Tualang honey and Tribestan group. ANOVA was used for comparison post intervention outcomes of sperm concentration, motility and morphology, erectile function and hormonal profiles in between two groups. After potential cofounders were controlled, significant different in the outcome of sperm concentration, motility and morphology, erectile function and hormonal profiles in between two groups for was analysed using ANCOVA analysis. Repeated measure ANOVA was used to analyse outcome of safety profiles in both groups. The P value < 0.05 was used as a statistically significant.

CHAPTER 4

RESULTS

A total of 34/34 (100.0%) respondents in the Tualang Honey group and 32/34 (94.1%) respondents in the Tribestan group completed the study. Two respondents in Tribestan group not completed the study.

4.1 Socio-demographic and baseline medical characteristics

Mean age of the respondents in Tualang honey and Tribestan group was 34.0 and 34.9 years respectively. Most the respondents in this study were Malay with only six respondents in Tualang honey group and three respondents in Tribestan group were non-Malays. More than 70% of the respondents in both groups were non smoker. The respondents in Tualang honey group and Tribestan group had mean systolic blood pressure and diastolic blood pressure in normotensive range. Nevertheless, mean body mass index (BMI) of the respondents in both groups were overweight (BMI>25 kg/m^2) with 27.4 kg/m2 in Tualang honey group and 26.2 kg/m^2 in Tribestan group.

Mean sperm concentration was 12.4 $x10^6$ /mL in Tualang honey group and 12.9 x 10^6 /mL in Tribestan group. There were no statistically significant different of mean sperm concentration in between these groups. Sperm motility and sperm morphology in Tualang honey group were 38.3 and 65.1% respectively as compare to Tribestan group which were 40.4 and 63.0%. No statistical significant difference of these sperm parameters (sperm concentration, sperm motility and sperm morphology) was founded in between both groups.

Table 4.1 Socio-demographic and baseline medical characteristics

Variable	Tualang honey (n=34)		Tribestan (n=32)		*P* value
	mean(SD[a])	n(%)	mean(SD[a])	n(%)	
Socio-demographic					
Age (years)	34.0 (4.87)		34.9 (6.89)		0.524[b]
Marriage duration (years)	4.5 (2.50)		6.1 (4.31)		0.072[b]
Number of children	0.2 (0.39)		0.3 (0.67)		0.585[b]
Number of partner	1.0 (0.17)		1.1 (0.25)		0.526[b]
Race					
Malay		28 (82.4)		29(90.6)	0.328[c]
Non-Malay		6 (17.6)		3(9.4)	
Education level					
Primary and secondary school		19 (55.9)		14(43.8)	0.325[c]
College and university		15 (44.1)		18(56.3)	
Smoking status					
Non-smoker		24 (70.6)		23(71.9)	0.908[c]
Smoker		10 (29.4)		9(28.1)	
Physical examination					
SBP (mmHg)	126.2(13.34)		121.8 (10.20)		0.137[b]
DBP (mmHg)	80.7(10.04)		76.6 (7.49)		0.063[b]
BMI (kg/m^2)	27.4 (3.56)		26.2 (3.49)		0.173[b]
Size right testes (mL)	18.0 (2.90)		16.6 (3.40)		0.081[b]
Size left testes (mL)	17.9(2.93)		16.4 (3.75)		0.084[b]
Sperm quantity and quality					
Sperm concentration(x10^6/ml)	12.4 (4.58)		12.9 (4.99)		0.632[b]
Sperm motility(%)	38.3(20.25)		40.4 (23.69)		0.692[b]
Sperm morphology(%)	65.1(23.97)		63.0 (28.30)		0.746[b]
Erectile function					
IIEF 5 score	20.1 (2.91)		20.8 (3.47)		0.362[b]
Grading of erectile function					0.041[c]
No dysfunction		19 (55.9)		24(75.0)	
Mild dysfunction		15 (44.1)		6(18.8)	
Mild to Moderate dysfunction		0 (0.0)		2(6.2)	
Hormonal profile					
LH (IU/L)	6.0 (7.11)		5.1 (2.79)		0.514[b]
Testosterone (nmol/L)	15.1 (4.58)		15.5 (6.60)		0.745[b]
FSH (IU/L)	6.4 (2.86)		7.3 (3.98)		0.337[b]

[a] Standard deviation
[b] ANOVA
[c] Chi squared test.

Mean IIEF-5 score among the respondent in both groups showed mild erectile dysfunction (score 17-21) with 20.1 score in Tualang honey group and 20.8 score in Tribestan group. However, no statistical significant differences of mean IIEF-5 score in between both groups. Grading of severity erectile dysfunction among the respondents in between Tualang honey and Tribestan group showed statistically significant difference (P = 0.041).

Mean hormonal profile of LH, testosterone and FSH among the respondents in both groups were quite similar. The value of LH, testosterone and FSH were 6.0 IU/L, 15.1 nmol/L and 6.4 IU/L respectively in Tualang honey group and 5.1 IU/L, 15.5 nmol/L and 7.3 IU/L respectively in Tribestan group.

4.2 Sperm parameters

ANOVA showed no significant difference of the outcome comparing Tualang honey and Tribestan groups in term of mean sperm concentration, sperm motility and sperm morphology and ANCOVA showed no significant difference comparing both groups when age, smoking status and baseline sperm concentration, motility or morphology were included in the model (Table 4.2).

Table 4.2 Sperm parameters between Tualang honey and Tribestan

Variable	ANOVA				ANCOVA			
	Mean (SD[a])		F stat[b]	*P* value	EMM[c] (95% CI[d])		F stat[b]	*P* val.
	Tualang Honey	Tribestan			Tualang Honey	Tribestan		
Sperm concentration ($x10^6$/ml)	31.4 (29.12)	25.3 (25.14)	0.23	0.632	30.9 (21.27, 40.53)	23.7 (13.76, 33.71)	1.18	0.28
Sperm motility (%)	48.1 (15.12)	49.8 (16.62)	0.18	0.670	47.2 (41.44, 52.93)	48.9 (42.94, 54.80)	0.18	0.67
Sperm morphology (%)	79.2 (14.80)	77.3 (10.86)	0.35	0.558	79.3 (74.53, 84.13)	77.2 (72.23, 82.16)	0.42	0.51

[a] Standard deviation
[b] F statistic
[c] Estimated marginal mean
[d] Confidence interval

There were statistically significant difference between pre- and post-intervention of sperm concentration ($p<0.001$), sperm motility ($p=0.015$) and sperm morphology ($p=0.008$) in Tualang honey group. However, paired t-test only showed statistically significant difference between pre and post-intervention of sperm concentration ($p=0.007$) and sperm morphology ($p=0.009$) (Table 4.3).

Table 4.3 Sperm parameters in Tualang honey and Tribestan group

Variable	Tualang Honey			Tribestan		
	Mean (SD[a])		*P* value	Mean (SD[a])		*P* value
	Pre-intervention	Post-intervention		Pre-intervention	Post-intervention	
Sperm concentration ($x10^6$/ml)	12.4 (4.58)	31.4 (29.12)	0.000	12.9 (4.99)	25.3 (25.14)	0.007[c]
Sperm motility (%)	38.2 (20.25)	48.1 (15.12)	0.015	40.4 (23.69)	49.8 (16.62)	0.066[c]
Sperm morphology (%)	65.1 (24.97)	79.2 (14.80)	0.008	63.0 (28.30)	77.3 (10.86)	0.009[c]

[a] Standard deviation
[c] Paired-t test

4.3 Erectile function

ANOVA showed no significant difference in the outcome of mean IIEF-5 score in between Tualang honey and Tribestan groups and ANCOVA showed no significant difference in IIEF-5 score between Tualang honey and Tribestan when age, smoking status, SBP, DBP, Total Testosterone and baseline IIEF-5 score were included in the model (Table 4.4).

Table 4.4 Erectile function between Tualang honey and Tribestan

Variable	ANOVA				ANCOVA			
	Mean (SD[a])		F stat[b]	*P* value	EMM[c] (95% CI[d])		F stat[b]	*P* value
	Tualang honey	Tribestan			Tualang honey	Tribestan		
IIEF-5 score	20.6 (2.81)	21.9 (2.96)	3.44	0.068	20.7 (19.94, 21.43)	21.6 (20.87, 22.42)	3.42	0.070

[a] Standard deviation
[b] F statistic
[c] Estimated marginal mean
[d] Confidence interval

4.4 Hormonal profiles

ANOVA showed no significant difference in hormonal profile i.e. Luteinizing Hormone, total testosterone and Follicular Stimulating Hormone comparing in between Tualang honey and Tribestan groups. Meanwhile, ANCOVA showed no significant difference in these outcome when age and baseline hormonal level were included in the model (Table 4.5).

There were decreased level of mean LH among the respondent in Tualang Honey and Tribestan groups post intervention but no changes in mean of Testosterone and FSH.

Table 4.5 Hormonal profile between Tualang honey and Tribestan

Variable	ANOVA				ANCOVA		
	Mean (SD[a])		F stat[b]	*P* value	EMM[c] (95% CI[d])		F stat[b]
	Tualang honey	Tribestan			Tualang honey	Tribestan	
LH(IU/L)	4.4 (1.87)	4.8 (2.19)	0.73	0.397	4.4 (3.69, 5.07)	4.8 (4.09, 5.52)	0.72
Testosterone(nmol /L)	14.8 (5.64)	14.5 (5.74)	0.34	0.565	15.0 (13.60, 16.32)	14.3 (12.89, 15.69)	0.47
FSH(IU/L)	6.7 (3.48)	7.2 (4.19)	0.94	0.337	7.1 (6.48, 7.67)	6.8 (6.16, 7.39)	0.49

[a] Standard deviation
[b] F statistic
[c] Estimated marginal mean
[d] Confidence interval

Normal value :
LH, Luteinizing Hormone 1-12 IU/L; Testosterone, Testosterone hormone 6-30 nmol/L; FSH, Follicular Stimulating Hormone 1-12 IU/L.

4.5 Safety profiles and adverse effects

All safety profiles of Tualang honey and Tribestan were normal in ranges. Repeated Measure ANOVA showed no significant difference comparing Tualang honey and Tribestan group in haematological, renal and liver profiles (Table 4.6). There were no adverse effects reported from all the respondents throughout the study.