

**THE EFFECTS OF TUALANG HONEY ON MALE
REPRODUCTIVE SYSTEM OF HIGH CHOLESTEROL
DIET RAT MODEL**

BY

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the degree of Master of Medical Sciences**

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ABSTRACT

Hypercholesterolaemia is a recognised factor associated with male infertility. However, currently, there is limited therapy available. Tualang honey (TH) is a type of Malaysian polyfloral wild honey produced by the rock bee (*Apis dorsata*) that has been proven to exert both anti-inflammatory and anti-oxidative effects. This study aimed to determine the effects of TH on the male reproductive system of high cholesterol diet (CD) administered rats. Fifty-four Sprague Dawley rats weighing 200 - 250 gm were randomly divided into the standard diet (control) group (n=6), mixed cholesterol diet (MCD) group, and high cholesterol diet (HCD) group (n=24 each). The six rats in the control group were fed with standard commercial rat pellet while the 48 rats in the MCD and HCD groups were fed with a 12% cholesterol diet for 16 weeks. At the end of the 16 weeks, the 24 rats in the MCD group, divided into four subgroups (group M1, M2, M3 and M4) were continued on the standard diet in addition to oral administration of distilled water, 1.2, 2.4, and 3.0 g of honey per kg body weight daily respectively as a supplement for 4 weeks. The other 24 rats in the HCD group were also divided into four subgroups (group H1, H2, H3 and H4) and continued on the 12% high cholesterol diet in addition to oral administration of distilled water, 1.2, 2.4, and 3.0 g of honey per kg body weight daily respectively for 4 weeks. The rats in the control group were continued with commercial rat pellets without honey supplementation. At the end of the 4 weeks, all rats were sacrificed and for each of the rat blood specimen was taken for biochemical analysis while the left testis and epididymis were collected for histology and sperm analysis. The follicular stimulating hormone (FSH) level, relative epididymal weight and all the sperm parameters (sperm concentration, the percentage of total sperm motility, progressive motility, normal sperm morphology, and viability) of rats in group H1 were significantly reduced compared to the control. All the sperm analysis parameters of group M1 also demonstrated significant reduction compared to the control. On the contrary, all TH supplemented groups demonstrated significant improvement in the sperm parameters. The higher the dosage of TH in both HCD and MCD groups, the greater the improvements in the sperm concentrations, sperm viability, and normal sperm morphology. For the testicular histological analysis, the mean Johnsen score was 10 for all the groups indicating no abnormalities. In conclusion, the TH supplementation improved the sperm analysis results in the HCD animal model.

خلاصة البحث

من المعروف ارتباط عامل ارتفاع الكوليسترول في الدم بالعقم عند الذكور, ولا يتوفر له حاليا الا علاج محدود فقط. ان عسل توالانج من أنواع العسل البريه الماليزيه المتعددة الأزهار والمنتج من النحلة الصخرية المعروفه علميا (Apis dorsata) والمثبت فعاليته كمضاد للالتهابات ومضاد للأكسده. الغايه من هذه الدراسه تحديد تأثير عسل توالانج على الجهاز التناسلي الذكري في الجرذان المختبريه على نظام غذائي عالي الكوليسترول. تم تقسيم أربعة وخمسون جرذ نوع (Sprague Dawley) بوزن يتراوح من 200 - 250 غرام عشوائيا مجموعاه ضابطه بعدد 6 ومجموعه ذات نظام غذائي مختلط الكوليسترول (MCD) وكذلك مجموعاه ذات نظام غذائي عالي الكوليسترول (HCD) بعدد 24 لكل منهما. المجموعه الضابطه عدد 6 غذيت بحبيبات تجاريه قياسييه مخصصه للجرذان. أما 48 جرذ في المجموعتين الأخيرين فقد تم اعطائهم غذاء يحوي 12% كوليسترول لمدة 16 أسبوع. في نهاية مدة 16 أسبوع, تم تقسيم 24 جرذ في مجموعاه الغذاء المتنوع الى 4 مجاميع صغيره (M1, M2, M3, M4), تم اكمال تغذيتهم بالغذاء القياسي واطافه والماء المقطر العسل بنسب 1.2, 2.4, و3 غرام / كغم وزن الجسم على التوالي لمدة 4 أسابيع. اما المجموعه الأخرى من الجرذان في مجموعاه الغذاء عالي الكوليسترول عك وعددهم 24 تم أيضا تقسيمهم الى أربعة مجاميع صغيره (H1, H2, H3, H4) وتم الاستمرار في تغذيتهم بطعام عالي الكوليسترول 12% بالاطافه الى الماء المقطر, العسل بنسب 1.2, 2.4, و3 غرام / كغم وزن الجسم على التوالي لمدة 4 أسابيع. اما الجرذان في المجموعه الضابطه فقد تم الاستمرار في اعطائهم الحبيبات الغذائيه التجاريه القياسييه المخصصه للجرذان بدون أضافه العسل. في نهاية الأسابيع الأربعة تمت التضحيه بجميع الجرذان واخذت عينات دم من كل منها لفحوصات الكيمياء الحيويه وكذلك اخذت الخصيه اليسرى والبربخ للفحص النسيجي وفحص الحيوانات المنويه. انخفض بدرجة ذات مغزى احصائي مستوى الهرمون (FSH) المنبه للجريب, الوزن النسبي للبربخ وجميع العوامل المتغيره للحيوان المنوي (تركيزه, النسبه الكليه

لحركته وحركته التقدميه, وكذلك شكله وحيويته) في جميع الجرذان في المجموعه H1 مقارنة بالمجموعه الضابطه. جميع تحاليل الحيوانات المنويه للمجموعه M1 بينت انخفاض ذو مغزى احصائي لهذه المتغيرات مقارنة بالمجموعه الضابطه. بالعكس من هذا, جميع مجموعات الجرذان المعالجه بالعسل توالانج ظهر عليها تحسن ذو مغزى احصائي لجميع تحاليل الحيوانات المنويه المنوه عنها سابقا. كلما ازدادت جرعة عسل توالانج في المجموعتين ذات النظام الغذائي مختلط الكوليسترول وكذلك المجموعه ذات النظام الغذائي عالي الكوليسترول كلما ازداد تحسن مستوى العوامل المتغيره للحيوان المنوي من حيث تركيزه, شكله وحيويته. اما بالنسبة الى تحليل الفحص النسيجي للخصية, فان معدل "درجة جونسون" فكانت 10 لكل مجاميع الجرذان مما يعني عدم وجود اي خلل بين للعيان. ختاماً فان عسل توالانج كعنصر غذائي مكمل قد حسن من متغيرات تحليل الحيوان المنوي في الجرذان التجريبيه.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Medical Sciences.

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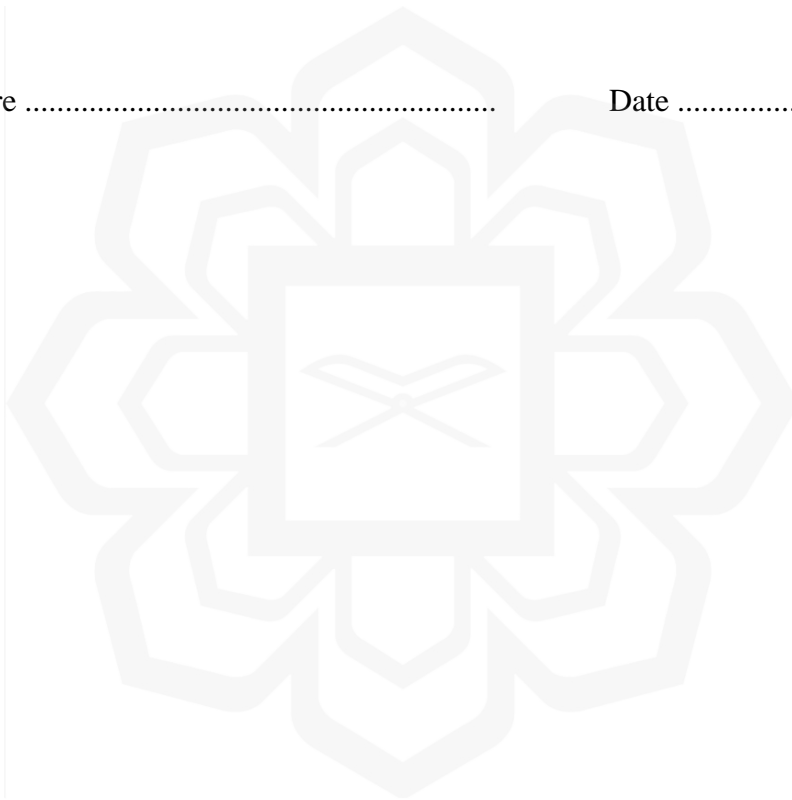
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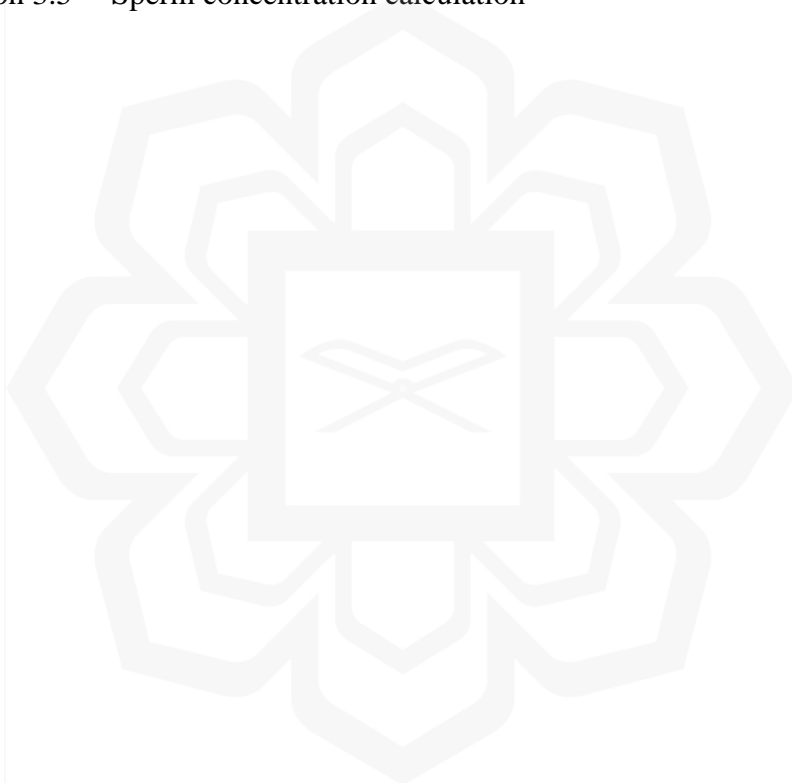
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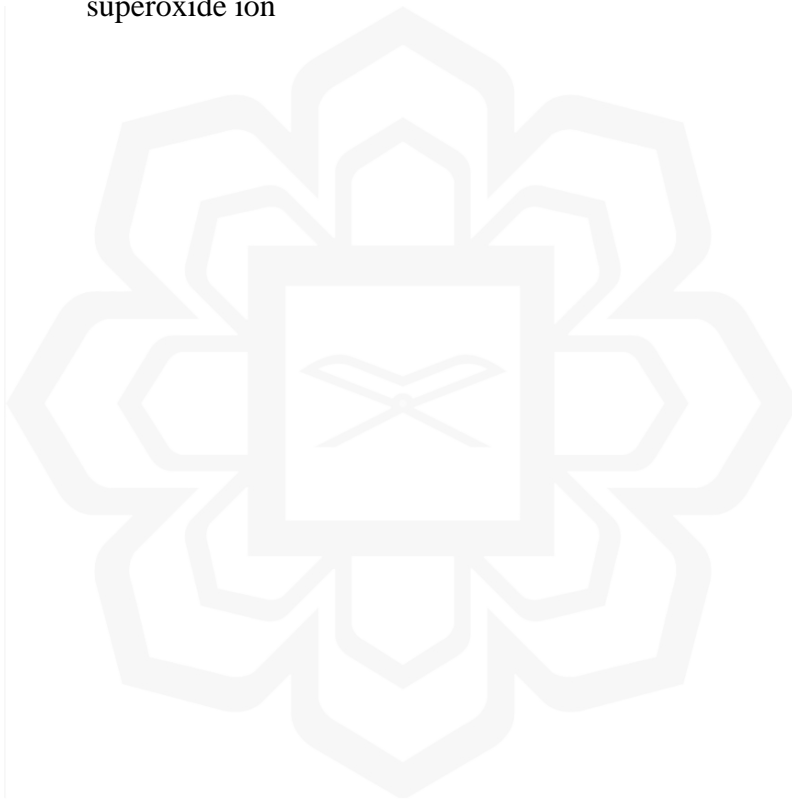
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LIST OF SYMBOLS

%	percentage
<	less than
>	greater than
≥	greater than or equal to
=	equal
/	per
-	minus
+	plus
±	plus minus
°C	degree celcius
µg	microgram
µL	microliter
µm	micrometer
µmol	micromolar
Ca ²⁺	calcium ion
cm	centimeter
CO ₂	carbon dioxide
df	degree of freedom
g	gram
h	hour
H ₂ O ₂	hydrogen peroxide
g/kg	gram per kilogram

m	meter
mg	milligram
mg/kg	milligram per kilogram
mL	milliliter
n	number
NaCl	sodium chloride
O ₂	oxygen
O ₂ ⁻	superoxide ion



LIST OF ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
AMPK	5' adenosine monophosphate-activated protein kinase
ANOVA	One-way analysis of variance
BMI	Body mass index
C	Control
CA	Caffeic acid
cAMP	Cyclic adenosine monophosphate
CFTR	Cystic fibrosis transmembrane conductance regulator
CKD	Chronic kidney disease
CPG	Clinical practice guidelines
CV	Cardiovascular
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate Polystyrene Xylene
DW	Distilled water
ELISA	Enzyme-linked immunosorbent assay
FAMA	Malaysian Federal Agricultural Marketing Authorities
FAS	Fatty acid synthase
FFA	Free fatty acid
FOS	Fructooligosaccharides
FSH	Follicle-stimulating hormone
GA	Gallic acid
GFR	Glomerular filtration rate
GnRH	Gonadotropin-releasing hormone
HCD	high cholesterol diet
hCG	Human chorionic gonadotropin
HDL-c	High-density lipoprotein cholesterol
HIV	Human Immunodeficiency Virus
hMG	Human menopausal gonadotropin
HPG	Hypothalamic-pituitary-gonadal
HRP	Horseradish peroxidase
HSDA	Sodium N-(2-hydroxyl-3-sulphopropyl)-3,5-dimethoxyaniline
IACUC	Institutional Animal Care and Use Committee
ICSI	intracytoplasmic sperm injection
IUM	International University Islamic Malaysia
IVF	<i>in-vitro</i> fertilization
Km	Conversion factor for rats
LDL-c	Low-density lipoprotein cholesterol
LED	Light-emitting diode
LH	Luteinizing hormone
MCD	Mixed cholesterol diet
ME	Malic enzyme
mRNA	Messenger ribonucleic acid
NHMS	National Health and Morbidity Surveys

NPR	Non-progressive motility
P-CA	p-coumaric acid
PKA	Protein kinase A
PR	Progressive motility
PUFA	Polyunsaturated fatty acids
r-hFSH	Recombinant human FSH
ROS	Reactive oxygen species
SBH	Stingless bee honey
SD	Standard deviation
SERM	Selective oestrogen receptor modulator
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
SREBPF-1c	Sterol regulatory element-binding protein 1c
T/E	Testosterone to Estradiol
TC	Total cholesterol
TG	Triglycerides
TH	Tualang honey
TLC	Therapeutic lifestyle changes
TMB	Tetramethylbenzidine
Urinary-hFSH	Purified urine human FSH
VLDL	Very-low-density lipoprotein
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Cholesterol is one of the key molecules in mammalian physiology. It is essential for cell functions and integrity as it plays a role in maintaining the permeability and fluidity of the cell membrane. It is also a common precursor for steroid hormones synthesis such as testosterone and oestrogen. Cholesterol homeostasis is strictly regulated at the cellular level to balance the synthesis and catabolism of cholesterol in order to assure normal cellular processes. The main source of cholesterol in our body is food. Hence, excessive intake of cholesterol may induce hypercholesterolaemia. This will disturb cholesterol homeostasis in the body which may cause adverse effects including cardiovascular complications, obesity, metabolic disorders, and infertility (Pushpendra & Jain., 2015; Sèdes et al., 2018).

The prevalence of hypercholesterolaemia is on the rise in young people in both developed and developing countries. The World Health Organization (WHO) survey in 2008 estimated that the global prevalence of hypercholesterolaemia among adults to be 39%, with South East Asia region recorded a prevalence of 29%. According to the National Health and Morbidity Surveys (NHMS) in Malaysia, the prevalence of hypercholesterolaemia had risen to 38.1% in 2019. The prevalence of hypercholesterolaemia generally exhibits an increasing with age (NHMS, 2019).

In relation to the male reproductive system, cholesterol is deemed to be important not only for its role as the common precursor for steroid hormone synthesis but also because it is the modulator of sperm functions. Cholesterol homeostasis regulation is crucial for post-testicular sperm maturation. Hypercholesterolaemia has

been shown to adversely affect the post-testicular maturation and normal male reproductive functions and therefore predisposes to infertility. Nevertheless, the exact mechanisms are still poorly understood (Whitfield et al., 2015).

Infertility is defined by the inability to conceive after 12 months of regular, unprotected intercourse. From an epidemiological study in 2018, infertility affects 15% to 20% of couples which involves 48.5 million couples worldwide (Alahmar et al., 2018). Surprisingly, almost 50 % are attributed solely to male factors. It has been demonstrated that 65% of infertile men had hypercholesterolaemia with or without triglyceridaemia (Sèdes et al., 2018).

Currently, there are treatments available for male infertility, for example, anti-oestrogen like tamoxifen and clomiphene citrates, aromatase inhibitors, and gonadotropins. However, they have various side effects (European Association of Urology Guidelines on Male Infertility, 2018). The current modern treatments are also less accessible especially for those in the rural areas due to the high costs coupled with the need for long-term treatment. In addition to the modern treatments, herbs and traditional medicine such as *Eurycoma Longifolia* ('Tongkat Ali'), *Tribestan*, *Ashwagandha*, and Manuka honey are also used as an aphrodisiac to improve male reproductive functions.

Tualang honey is a wild poly-floral honey produced by rock bees (*Apis dorsata*) which build hives high up in the branches of the Tualang tree (*Kompassia excelsa*). It owns a high reputation in Malaysia due to its medicinal benefits. Tualang honey is collected only by authorised bee hunters under the supervision of the Malaysian Federal Agricultural Marketing Authorities (FAMA). It contains approximately 200 substances such as sugars (fructose, glucose, maltose, and sucrose), a small number of other components such as organic acids, vitamins, minerals, proteins, flavonoids, phenolic