EFFECTS OF TRIHONEY ON REPRODUCTIVE DYSFUNCTIONS IN HIGH CHOLESTEROL DIET-FED MALE RABBITS

BY

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ABSTRACT

Overconsumption of high-cholesterol diet induces hypercholesterolemia and disturbs cholesterol homeostasis in the body which adversely affects normal male reproductive functions. Use of honey has become of increasing interest due to the increase in the availability of evidence-based findings demonstrating the beneficial effects of honey in treating diverse diseases. The present study was undertaken to evaluate the potential protective effects of Trihoney (a mixture of Trigona, Mellifera and Tualang) against male reproductive dysfunctions in diet-induced hypercholesterolemic rabbits and compare its effects with atorvastatin. Forty-eight male New Zealand white rabbits at the age of 5 months were assigned into 6 groups. Two groups were fed commercial rabbit pellet and 0 and 0.6 g/kg/day of Trihoney respectively. The other four groups were fed 1% cholesterol diet and 0, 0.3, 0.6 g/kg/day of Trihoney, and 2 mg/kg/day of atorvastatin for 12 weeks. The study was planned in 5 distinct phases. The purpose of the first phase was to evaluate the effects of Trihoney on serum lipid profile and serum and testicular malondialdehyde (MDA) and antioxidant enzymes; superoxide dismutase (SOD) and glutathione peroxidase (GPx). Trihoney and atorvastatin reduced serum total cholesterol and LDL-c significantly. Trihoney was as effective as atorvastatin in the lipid lowering effect. Trihoney slightly reduced serum MDA but significantly enhanced serum SOD and GPx. It reduced testicular MDA and increased SOD significantly. Atorvastatin treatment significantly reduced serum and testicular MDA and enhanced serum and testicular SOD and GPx. In the second phase, the effect of Trihoney on serum inflammatory biomarkers was evaluated. Trihoney administration reduced serum levels of IL-6, TNF-α and IL-1β significantly. Atorvastatin reduced serum TNF-α and IL-1β significantly. In the third phase, the effects of Trihoney on serum and intra-testicular testosterone, serum FSH, serum LH, fasting insulin, fasting blood glucose and HOMA-IR were investigated. Trihoney particularly at the dose of 0.6 g/kg/day significantly improved serum and intra-testicular testosterone and serum FSH; whereas, atorvastatin showed no improvement in these hormones. Both Trihoney and atorvastatin showed no effects on fasting serum insulin, fasting blood glucose and HOMA-IR. The fourth phase was aimed to evaluate the effects of Trihoney on sperm parameters. Trihoney particularly at the dose of 0.6 g/kg/day improved the percentages of sperm motility and sperm with normal morphology as well as reduced the percentages of immotile sperm and sperm with abnormal morphology. Trihoney improved sperm concentration but with no statistical significant. Atorvastatin group showed the worst outcome of sperm parameters. In the fifth phase, the effects of Trihoney on testicular and epididymal histopathological changes were evaluated. Trihoney ameliorated the testicular degenerative changes, improved spermatogenesis and maintained the normal histology of the epididymis with an increase in the number of sperm in its tubules. Atorvastatin treated group showed severe testicular tubular degenerative changes and epididymal atrophy with fibrosis. In conclusion, Trihoney showed its potential health benefits as an effective hypocholesterolemic, anti-inflammatory and antioxidant agent. It was shown to improve sperm parameters and male reproductive hormones, and attenuate testicular and epididymal histopathological alterations in high-cholesterol diet fed male rabbits. Hence, Trihoney plays a favourable role on several mechanisms involved in combating hypercholesterolemia-induced male reproductive dysfunctions.

خلاصة البحث

يؤدي الإستهلاك المفرط لغذاء عالي الكوليسترول إلى إرتفاع كوليسترول الدم وإختلال توازن الكوليسترول في الجسم مما يؤثر سلباً على الوظائف التناسلية للذكور.أصبح إستخدام العسل ذا أهمية متزايدة بسبب زيادة توافر الدلائل العلمية التي تُبيّن فوائد العسل. أُجريت هذه الدراسة لتقييم التأثير الوقائي المحتمل للعسل الثلاثي ضد ضعف القدرة الإنجابية للذكور والناتجة عن إرتفاع كوليستيرول الدم في الأرانب ومقارنته بالأتورفاستاتين. ثمانية وأربعون من ذكور الأرانب البيضاء النيوزيلاندية قُسّمت إلي 6 مجموعات. غُذّيت مجموعتان بغذاء الأرانب التجاري مع 0 و0.6 جم/كجم/يوم من العسل على التوالي بينما غُذّيت المجموعات الأربع الأخرى على غذاء عالي الكوليستيرول مع 0 و0.3 و0.6 جم/كجم/يوم من العسل و2 مجم/كجم/يوم من الأتورفاستاتين. قُسِّمت هذه الدراسة إلى خمس مراحل. هدفت المرحلة الأولى لدراسة تأثير العسل على مستوى الدهون ومؤشر الإجهاد التأكسدي والإنزيمات المضادة للأكسدة في مصل الدم والخصيتين.كان تأثير العسل مساوٍ للأتورفاستاتين في خفض الكوليستيرول الكلي والكوليستيرول الضار. كانت الزيادة في الإنزيمات المضادة للأكسدة في مصل الدم أفضل في مجموعات العسل بينما أظهر الأتورفاستاتين أكثر تأثيراً في الخصيتين. في المرحلة الثانية دُرس تأثير العسل على المؤشرات الحيوية الإلتهابية في مصل الدم. خفّض كل من العسل والأتورفاستاتين من مستويات المؤشرات الحيوية الإلتهابية في مصل الدم. في المرحلة الثالثة، فُحص تأثير العسل على الهرمونات التناسلية الذكرية في مصل الدم والخصيتين، وعلى مؤشر مقاومة الإنسولين. حسَّن العسل خاصة بجرعة 0.6جم/كجم/يوم هرمون التستوستيرون وهرمون تحفيز الجريب. لم يُظهر الأتورفاستاتين أي تحسن في الهرمونات. لم يُؤثر العسل ولا الأتورفاستاتين على مؤشر مقاومة الإنسولين. في المرحلة الرابعة قُيّم تأثير العسل على الحيوانات المنوية. العسل الثلاثي خاصة بجرعة 0.6جم/كجم/يوم أثر إيجابياً على صفات الحيوانات المنوية بينما أحدث الأتورفاستاتين أسوأ النتائج. المرحلة الخامسة قيّمت تأثير العسل على التغيرات النسيجية في الخصيتين والبربخ. أحدث العسل الثلاثي تحسيناً في التغيرات التنكسية للخصية وفي تكوين الحيوانات المنوية والحفاظ على الأنسجة الطبيعية للبربخ. التغيرات النسيجية في الخصيتين والبربخ كانت أكثر شدة في مجموعة الأتورفاستاتين. بناءاً على ماسبق: أظهر العسل الثلاثي فوائده الصحية كخافض لكوليستيرول الدم ومعزز للإنزيمات المضادة للأكسدة، ومثبط للمؤشرات الحيوية الالتهابية، ومحسّن للحيوانات المنوية والهرمونات التناسلية الذكرية ومخفف من التغيرات النسيجية للخصيتين والبربخ.

APPROVAL PAGE

The thesis of Zenab B. Hamad Mohamed has been approved by the following:

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABCA1	ATP-binding transporters A1
ART	Assisted reproductive technology
BMI	Body mass index
ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CoQ10	Coenzyme Q (10)
COX2	Cyclooxygenase enzyme
CRP	C-Reactive protein
DFI	DNA fragmentation index
dH_2O	Deionized waster
DNA	Deoxyribonucleic acid
Fas	Fatty acid synthase
FSH	Follicle -stimulating hormone
g	Gram
GnRH	Gonadotropin releasing hormone
GPx	Glutathione peroxidase
GSH	Glutathione
GR	Glutathione reductase
GSSG	Oxidized glutathione
HCD	High cholesterol diet
hCG	Human chorionic gonadotropin
HDL-c	High density lipoprotein cholesterol
H&E	Haematoxylin and Eosin
HED	High-energy diet
hMG	Human menopausal gonadotropin
HOMA-IR	Homeostatic model assessment of insulin resistance
HPT-axis	Hypothalamic pituitary testicular axis
HRP	Horseradish peroxidase
HTF	Human tubal fluid
ICSI	Intracytoplasmic sperm injection
IIUM	International Islamic University Malaysia
IL	Interleukin
IVF	*In vitro* fertilization
kg	Kilogram
LAC	L-acetyl-carnitine
LC	L-carnitine
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
LXRs	Liver X receptors
M	Mean
MDA	Malondialdehyde
mg	Milligram
MM6	Monocytic cell line and precursor of macrophages
mL	Millilitre

µL	Microliter
mM	Millimole
µM	Micromole
µm	Micrometre
mmol/L	Millimole per litre
MT	Masson's Trichrome
NADP+	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NF-kB	Nuclear translocation of nuclear factor kappa B
ng/mL	Nano gram per millilitre
NO	Nitric oxide
NOI	Non-obstructive Infertility
NSAID	Nonsteroidal anti-inflammatory drugs
OI	Obstructive Infertility
PBS	Phosphate buffer saline
PBUH	Peace Be Upon Him
PC	Protein carbonyl
PG	Prostaglandin
Pg/mL	Pictogram per millilitre
PKA	Protein kinase A
PM	Progressive motility
RC	Reagent control
r-hFSH	Recombinant human FSH
rHuIL-6	Recombinant human interleukin-6
R/N	Reference number
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Revolution per minute
RSM	Response Surface Methodology
SC	Segar smoke
scd	Stearoyl Co-A desaturases
SD	Standard deviation
SDH	Sorbitol dehydrogenase
SERMs	Selective oestrogen receptor modulators
SMC	Smooth muscle cells
S/N	Serial number
SOD	Superoxide dismutase
srebp1c	Sterol response element binding protein-1c
STZ	Streptozotcin
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
TG	Triglycerides
TM	Total motility
TMB	Tetramethylbenzidine
TNF-α	Tumour necrosis factor- alpha
U/L	Activity unit per litre
US$	Dollars
VLDL	Very low density lipoprotein

WHO	World Health Organization
XOD	Xanthine oxidase

LIST OF SYMBOLS

-	Hyphen-minus
+	Plus sign
=	Equal sign
%	Percent sign
&	Ampersand
(	Left parenthesis
)	Right parenthesis
,	Comma
.	Full stop
/	Solidus
:	Colon
;	Semicolon
[	Left square bracket
]	Right square bracket
<	Less-than sign
>	Greater-than sign
±	Plus-minus sign
°	Degree sign

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Infertility is defined as the failure to conceive in sexually active, noncontracepting couples for a period of one year or more (Yilmaz et al., 2017). It is a common problem affecting 15% of couples of childbearing age, with detectable male factor in 30-50% of all infertile couples (Eisenberg et al., 2014; Oyeyipo et al., 2015). In 20% of couples, male factor is the only causative aetiology for infertility (Attaman et al., 2012). The prevalence of male infertility is on the rise globally and is of public concern owing to its socioeconomic burden (Michael et al., 2015). Due to environmental contamination and life style changes, infertility rate is going to increase in the future (Pushpendra & Jain, 2015). Lifestyle-related external factors including eating disorders can negatively affect spermatogenesis both at central and gonadal levels (Al Kushi et al., 2016). Poor dietary habits with high-fat or high-cholesterol intake are the main cause towards the development of hyperlipidaemia and hypercholesterolemia which are increasing in young people in both developed and developing nation (Aurelia Ouvrier et al., 2011; Onwe et al., 2015). Dyslipidaemia is a major risk factor for the development of cardiovascular complications. Its deleterious effects extend to affect the reproductive functions (Aurelia Ouvrier et al., 2011). The negative impact of hypercholesterolemia on male reproductive system and fertility has been reported in animal (Saez Lancellotti et al., 2010) and human (Schisterman et al., 2014). Hypercholesterolemia affects testicular structure and function, spermatogenesis, semen quality and ejaculatory function through disruption of hypothalamic-pituitary-testicular (HPT) axis, impairment of steroid hormone

biosynthesis, impairment of Sertoli and Leydig cells secretory functions, induction of oxidative stress and disruption of various testicular genes (Pushpendra & Jain, 2015). Furthermore, hypercholesterolemia affects structure and function of the epididymides (Aurelia Ouvrier et al., 2011).

Complementary and alternative medicine is widely used and rapidly growing in developing and developed countries. It is used by 80% of African population. In China, traditional medicine constitutes 40% of health care system delivered. In Malaysia, US$500 million is spent annually for this kind of care. Complementary and alternative medicine is used by 70% and 42% of population in Canada and United States respectively. The wide use of traditional medicine is attributed in developing countries to its affordability and accessibility, in Asia due to historical and cultural believes; whereas, in developed countries the main cause of increasing use of complementary and alternative medicine is the concern about the side effects of conventional medicine (WHO, 2002).

Honey is an important and unique natural product (Ramanauskiene et al., 2012). It has been used since ancient times as a therapeutic agent (Pyrzynska & Biesaga, 2009). Recently, the attention has been increased towards the use of honey for prevention and treatment of numerous diseases as well as for improving and maintaining the overall wellbeing (Inoue et al., 2005; Pyrzynska & Biesaga, 2009; Nweze et al., 2016). The medicinal importance of honey has been demonstrated in several previous studies. It has been reported to have antioxidant activity (Alvarez-Suarez et al., 2010), anti-inflammatory activity (Borsato et al., 2014) and Antihyperlipidaemic effect (Yaghoobi et al., 2008; Adnan, Sadiq & Jehangir, 2011). Traditionally, honey has been used in different cultures for enhancement of male fertility (Abdul-Ghani et al., 2008; Mohamed et al., 2012). It showed its ability to