ANTI-ATHEROSCLEROTIC AND HEPATOPROTECTIVE EFFECTS OF TRIHONEY IN HYPERCHOLESTEROLEMIC RABBITS

BY

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ABSTRACT

Cardiovascular diseases are major contributor to morbidity and mortality worldwide. Atherosclerosis is a leading cause to cardiovascular diseases in addition to its pathogenic association with non-alcoholic fatty liver disease (NAFLD). Protection against atherosclerosis and NAFLD constitutes a global aim. Modern trend has emerged to reintroduce natural products such as honey for management of these metabolic epidemics because of the less side effects perhaps. In the present study, Trihoney was investigated for its anti-atherosclerotic and hepatoprotective effects in diet induced hypercholesterolemic rabbits model. Forty-eight male New Zealand white rabbits were randomly assigned to one of 6 groups. First group was fed only commercial rabbit diet, second group was fed commercial rabbit diet with 0.6g of Trihoney/kg/day, third group was fed 1% cholesterol diet, fourth and fifth groups were fed 1% cholesterol diet with 0.3 and 0.6 g of Trihoney/kg/day while the last group was fed 1% cholesterol diet plus 2mg of atorvastatin/kg/day. Experiment continues for 12 weeks duration. Blood samples were withdrawn before and after the experimental period. Aorta and liver were harvested and processed for homogenate and histopathological studies. In the first phase, Trihoney was investigated for its lipid lowering and anti-inflammatory effects through analysis of serum lipids [total cholesterol (TC), low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), triglycerides (TG) and TC/HDL risk ratio] and by assay of serum pro-atherogenic inflammatory cytokines [interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α)]. The results showed that Trihoney had significant lipid lowering and marked anti-inflammatory effects. In the second phase, Trihoney was assessed for antioxidant function by analysing serum and aorta homogenate for superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA), in addition to analysing serum for oxidised-LDL (Ox-LDL). Results showed that Trihoney exerted significant antioxidant effects systemically as well as locally in the aorta. In the third phase, Trihoney was investigated of its effects on the atherosclerotic plaques, inflammatory adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and on homocysteine. Results showed that Trihoney had significant anti-inflammatory and vascular protective functions. In the fourth phase, Trihoney was examined for hepatoprotective function against NAFLD through histopathological study and *via* assay of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin (T. Bil.), alkaline phosphatase (ALP), fasting glucose, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR). In addition to antioxidant assay of liver homogenate for SOD, GPx and MDA. Results showed that under status of sustained hypercholesterolemia, Trihoney was able to normalise hepatic function in NAFLD induced hypercholesterolemia, Trihoney showed no effect on fasting glucose, insulin and HOMA-IR, Trihoney exhibited significant antioxidant effect against hepatic oxidative stress and it was protective against progression of NAFLD to non-alcoholic steatohepatitis (NASH). Accordingly, Trihoney has a potential protective role against atherosclerosis and NAFLD through hypocholesterolemic, antioxidant and anti-inflammatory functions. Further studies may be needed to explore possible molecular mechanisms underlying those health beneficial properties of Trihoney.

خلاصة البحث

تعتبر أمراض القلب والأوعية الدموية المساهم الرئيسي في الوفايات على مستوى العالم. ويعد تصلب الشرايين (Atherosclerosis) سببا مباشرا لأمراض القلب والأوعية الدموية بالأضافة الى ارتباطه الممرض بمرض الكبد الدهني غير الكحولي (NAFLD). حديثا أعتبر العمل على الوقاية من مرض تصلب الشرايين ومرض الكبد الدهني غير الكحولي هدفا عالميا. هناك ظهور لتوجه حديث لإعادة المنتجات الطبيعية مثل العسل واستخدامها في علاج هذه الأوبئة الأيضية. في هذه الدراسة تم التحقق من الأثر الوقائي للعسل الثلاثي ضد مرض تصلب الشرايين ومرض الكبد الدهني غير الكحولي باستخدام نموذج غذائي مفرط الكوليستيرول في الأرانب النيوزيلاندية البيضاء. تم توزيع 48 أرنبا ذكرا الى 6 مجموعات. تم تغذية المجموعة الأولى بالغذاء التجاري للأرانب والمجموعة الثانية بالغذاء التجاري للأرانب بالأضافة الى جرعة يومية من العسل الثلاثي بمقدار 0.6 جم/كجم/يوم. أما المجموعة الثالثة فتم تغذيتها فقط بالغذاء المرتفع الكوليستيرول والمجموعتين الرابعة و الخامسة تم تغذيتهما بالغذاء مرتفع الكوليستيرول بالأضافة الى جرعة يومية من العسل بمقدار 0.3 و0.6 جم/كجم/يوم على التوالي. أما المجموعة السادسة تم تغذيتها بالغذاء المرتفع الكوليستيرول بالأضافة الى جرعة يومية من دواء الأتورفاستاتين المخفض للدهون بمقدار 2 ملجم/كجم/يوم. استمرت التجربة لمدة 12 أسبوعا.تم اختبار معدل الدهون و وظائف الكبد والأنزيمات المضادة للأكسدة وكذلك تم اختبار المؤشرات اللألتهابية الدالة على تصلب الشرايين في مصل الدم. وتمت دراسة مضادات الأكسدة في كل من نسيجي الشريان الأورطي والكبد وتم ايضا اجراء دراسة نسيجية على كل من الشريان الأورطي والكبد باستخدام تقنيات متعددة لصبغ الأنسجة. أظهرت النتائج التأثير الوقائي للعسل الثلاثي ضد مرض تصلب الشرايين وضد مرض الكبد الدهني غير الكحولي عن طريق خفض معدل الدهون والحفاظ على وظائف الكبد في معدلها الطبيعي بالأضافة الى الدور الوقائي المضاد للأكسدة وخفض مؤشرات التهاب تصلب الشرايين. كما أظهر العسل الثلاثي دورا وقائيا على مستوي خفض لويحات تصلب الشرايين والتركيب النسيجي للكبد. بناء على ذلك فأن للعسل الثلاثي دورا وقائيا ضد تصلب الشرايين ومرض الكبد الدهني غير الكحولي من خلال خفض معدل الدهون و الألتهابات. بالإضافة إلى قدرة العسل الثلاثي على إخماد الأكسدة المصاحبة لهذه الأمراض. قد تكون هناك حاجة الى دراسات مستقبلية لأستكشاف اليات عمل العسل الثلاثي على المستوي الجزيئي و الجيني.

APPROVAL PAGE

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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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Signature..... Date .....

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LIST OF ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
ABC	ATP-binding cassette
ACC	American College of Cardiology
ACS	Acute coronary syndrome
AHA	American heart association
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASCVDs	Atherosclerotic cardiovascular diseases
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BMI	Body mass index
CAT	Catalase
CBB	Coomassie Brilliant Blue
CCl_4	Carbon tetrachloride
Cd	Cadmium
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CHF	Congestive heart failure
cm	Centimetre
COX-1	Cyclooxygenase -1
COX-2	Cyclooxygenase -2
CRP	C-Reactive protein
CSF	Colony-stimulating factor
CVDs	Cardiovascular diseases
DALY	Disability-adjusted life year loss
DASH	Dietary advice to stop hypertension
DNA	Deoxyribonucleic acid
DNL	De novo lipogenesis
DNS	Department of nutrition sciences
DPX	Distyrene Plasticizer Xylene
EF	Extra cellular fat
EL	External elastic lamina
EMA	European medicines agency
EPCs	Endothelial progenitor cells
ER	Endoplasmic reticulum
FAO	Food and agriculture organization
FDA	Food and drug administration
FFA	Free fatty acids
FM	Foamy macrophage
FSH	Follicle stimulating hormone
FSMCs	Foamy smooth muscle cells
g	Gram
GGT	Gamma-glutamyl transferase
GIT	Gastrointestinal tract
GNI	General national income

GPx	Glutathione peroxidase
H&E	Haematoxylin and eosin
H_2O_2	Hydrogen peroxide
HDL-c	High density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
HRP	Horseradish peroxidase
hs-CRP	High-sensitivity C reactive protein
ICAM-1	Intercellular adhesion molecule-1
IDL	Intermediate low-density lipoprotein
IHD	Ischaemic heart disease
IIUM	International Islamic University Malaysia
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
Kcal	Kilocalorie
kg	Kilogram
L	Littre
LDL-c	Low-density lipoprotein cholesterol
LDL-R	Low-density lipoprotein receptor
LFT	Liver function test
LH	Luteinizing hormone
LOX-1	Lectin-like oxidized low-density lipoprotein receptor-1
LPC	Lysophosphatidylcholine
LXRα	Liver X receptors
M	Mean
MDA	Malondialdehyde
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MDR TB	Multidrug resistance tuberculosis
mg	Milligram
mL	Millilitre
µL	Microliter
µm	Micrometre
mm	Millimetre
mmol	Millimole
µmol	Micromole
MMP-1	Metalloproteinase-1
MMP-9	Metalloproteinase-9
MNC	Mononuclear cell
mRNA	Messenger ribonucleic acid
MRSA	Methicillin-resistant *S. aureus*
MT	Masson's trichrome
mU	Milliunit
NADPH	Nicotinamide adenine dinucleotide phosphate
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NBF	Neutral buffer formalin
NCCFN	National coordinating committee on food and nutrition Malaysia
NCDs	Noncommunicable diseases
NCEP	National Cholesterol Education Program

NF-κB	Nuclear factor kappa B
NLRP3	Nod-like receptor protein 3
NO	Nitric oxide
NZW	New Zealand white rabbits
O_2^-	Superoxide anion
OD	Optical density
$ONOO^{\cdot-}$	Peroxynitrite
Ox-LDL	Oxidised low-density lipoprotein
PBS	Phosphate-buffered saline
PBUH	Peace and blessings upon him
PDGF	Platelets derived growth factor
PKC	Phosphate kinase C
PPAR-α	Peroxisome proliferator-activated receptor-alpha
PCSK-9	Proprotein convertase subtilisin/kexin type-9
RDI	Recommended daily intake
RM	Ringgit Malaysia
ROS	Reactive oxygen species
RSM	Response Surface Methodology
sdLDL	Small-density low-density lipoprotein
siRNA	Small interfering RNA molecules
SMCs	Smooth muscle cells
SOD	Superoxide dismutase
SR	Scavenger receptor
SREBP1c	Sterol regulatory element-binding protein 1c
SREBP2	Sterol regulatory element-binding protein 2
TA	Tunica adventitia
T. Bil	Total bilirubin
TC	Total cholesterol
TC/HDL	Cardiovascular risk ratio
TG	Triglycerides
TGF-β	Transforming growth factor-beta
TI	Tunica intima
TIA	Transient ischemic attack
TM	Tunica media
TMAO	Trimethylamine N-oxide
TNF-α	Tumour necrosis factor-alpha
TPC	Total phenolic content
TXNIP	Thioredoxin-interacting protein
U	Unit
UN	United nations
US	United states
US FDA	United States food and drug administration
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very-low density lipoprotein
WHO	World health organization

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
≥	Equal to or greater- than sign
±	Plus-minus sign
°	Degree sign