RENOPROTECTIVE ROLE OF TUALANG HONEY AGAINST HIGH CHOLESTEROL DIET INDUCED ACUTE KIDNEY DISEASES IN FEMALE RATS

BY

ZENAB B. HAMAD MOHAMED

A dissertation submitted in fulfilment of the requirement for the degree of Master of Medical Sciences

Kulliyyah of Medicine
International Islamic University Malaysia

MARCH 2017

ABSTRACT

BACKGROUND: Many researches have proven that there exists a complex association between progressive renal damage and hypercholesterolemia. However, there was little information about the early effect of hypercholesterolemia on the kidney. Additionally, although there is a growing insight into the causes and mechanisms of these diseases, preventive and therapeutic measures are still few. Hence, the aim of this study is to determine the acute and sub-acute effects of high cholesterol diet on the kidney, and to examine the protective role of tualang honey against these disease. **METHODOLOGY**: Fifteen female Sprague-Dawley rats were randomly divided into three groups: control group, fed with commercial rat pellet; high cholesterol diet group (HCD), fed with 12% cholesterol diet with 0.3% cholic acid, and HCD with tualang honey supplements at 1.4 g/kg/day orally group (HCD+TH). Biochemical analyses for lipid profile and renal function test were performed at completed 48 hours (day 3), day 7, and day 42 of the experiment. The rats were sacrificed at completed day 42 and the kidneys were harvested and subjected to histopathological examination. The data were analysed using ANOVA and LSD Post-Hoc test. **RESULTS**: Consumption of 12% cholesterol diet for six weeks resulted in an increment of the mean serum creatinine level of HCD and HCD+TH groups to 1.5 times the control level at the completed day 7. Also overall both the mean serum creatinine and blood urea levels were higher in HCD group than the control group. The mean TC showed an increasing trend throughout the experiment with the level being significantly higher than the control group at the completed day 42. A significantly higher mean serum LDL-c in the HCD group as compared to the control at the completed day 42 was also documented. The mean serum TG levels were higher than that of the control group at the completed 48 hours and day 7. The mean serum HDL-c showed a significant reduction in HCD group than the control group at the completed day 42. With tualang honey supplementation, the mean serum creatinine level showed significant reduction at 48 hours in the HCD+TH group as compared to the HCD group. There was also a reduction in the mean serum creatinine level at the completed day 42. As for the lipid profile, honey supplementation significantly reduced the mean TG and vLDL-c at the completed day 7 as compared to HCD group. Histopathologically the kidneys exhibited segmental mesangial hypercellularity and mesangial matrix expansion of almost all the glomeruli in both HCD and HCD+TH groups. **CONCLUSION**: The 12% cholesterol diet utilized in this study caused acute and sub-acute kidney injuries in the animal model while tualang honey at 1.4 g/kg/day orally exhibited lipid lowering activities and some degree of renoprotective effect against high cholesterol diet induced kidney injury.

ملخص البحث

العديد من الأبحاث اثبتت وجود علاقة معقدة بين زيادة نسبة الكوليستيرول في الدم وحدوث تلف الكلى مع عدم وجود معلومات كافية على التأثيرات الأولية لإرتفاع كوليستيرول الدم على الكلى. لذلك فإن الهدف من هذه الدراسة هو تحديد إمكانية الإصابة بأمراض الكلية الحادة وتحت الحادة عند تناول الأغذية المحتوية على نسبة عالية من الكوليستيرول. بالإضافة إلى دراسة القدرة الوقائية لعسل التوالنق ضد الإصابة بهذه الأمراض. خمس عشرة أنثى من جرذان السبراغ داولي قسمت عشاوئيا إلي ثلاث مجموعات: المجموعة المحتكم إليها وتم إطعامها بغذاء الجرذان المتوفر تجاريا، مجموعة الغذاء عالي الكوليستيرول وتم إطعامها بغذاء يحتوي على 12% كوليستيرول و 0.3% حمض الكوليك ، مجموعة الغذاء عالي الكوليستيرول مع العسل وتم إطعامها بالغذاء المحتوي على 12% كوليستيرول مع إعطائها عسل التوالنق بجرعة 1.4 جم/كجم يوميا. تم قياس وظائف الكلى ومستوى الدهون في الدم بعد 48 ساعة (اليوم الثالث) ثم بعد 7 أيام ثم بعد 42 يوما وهو وقت نهاية الدراسة وتشريح الجرذان. عند ذلك الوقت تم إستئصال الكلى وتجهيزها لفحص أنسجتها. مجموعة الغذاء عالي الكوليستيرول و مجموعة الغذاء عالي الكوليستيرول مع العسل أظهرتا إرتفاعا في معدل الكرياتينين بزيادة تساوي 1.5 معدله في المجموعة المحتكم إليها بعد 7 أيام. كما أن معدلات الكرياتنين وبولينا الدم في القياسات الثلاث للدراسة أظهرت إرتفاعا في مجموعة الغذاء عالي الكوليستيرول عند مقارنتها بالمجموعة المحتكم إليها. أما بالنسبة لمعدلات الدهون فقد بينت إرتفاعا ذو دلالة إحصائية في معدلات الكوليستيرول الكلي و الكوليستيرول الضار في مجموعة الغذاء عالي الكوليستيرول بعد 42 يوما. كما كانت معدلات الدهون الثلاثية والكوليستيرول منخفض الكثافة (بعد 48 ساعة وبعد 7 أيام) أعلى من معدلاتها في المجموعة المحتكم إليها. أما الكوليستيرول النافع فقد بين إنخفاضا في معدله بعد 42 يوما. بينما المجموعة التي تم تزويدها بالعسل أظهرت إنخفاضا ذو دلالة إحصائية في معدل الكرياتينين في الدم بعد 48 ساعة عند مقارنتها بمجموعة الغذاء عالي الكوليستيرول، هذا الإنخفاض ظهر أيضا بعد 42 يوما. كذلك أدى إستخدام العسل إلى إنخفاض معدل الدهون االثلاثية والكوليستيرول منخفض الكثافة إنخفاضا ذو دلالة إحصائية بعد 7 أيام. نتائج الشرائح المصبوغة لنسيج الكلى بينت وجود زيادة في عدد خلايا المسراق واتساع مادتها الخلوية في مجموعتي الغذاء عالي الكوليستيرول و الغذاء عالي الكوليستيرول مع العسل. طبقا للنتائج المذكورة تم استنتاج ان الغذاء المحتوي على 12% كوليستيرول قادر على إحداث الإصابة بأمراض الكلى الحادة وإحداث اضطراب في مستوى الدهون في الدم. وكذلك تم استنتاج أن عسل التوالنق له بعض الفوائد الوقائية من الإصابة بأمراض الكلى كما له القدرة على تنظيم الدهون في الدم.

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.

……..
Roslina Abdul Rahim
Supervisor

……..
Naznin Muhammad
Co-Supervisor

……..
Nor Zamzila Abdullah
Co-Supervisor

I certify that I have 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.

……..
Ahmed Kaid Naji Allow
Internal Examiner

This dissertation was submitted to the Department of Basic Medical Sciences and is accepted as a fulfilment of the requirements for the degree of Master of Medical Sciences.

……..
Zunariah Buyong
Head, Head of Basic Medical Sciences

This dissertation was submitted to the Kulliyyah of Medicine and is accepted as a fulfilment of the requirements for the degree of Master of Medical Sciences.

……..
Azmi Md Nor
Dean, Kulliyyah of Medicine

DECLARATION

I hereby declare that this dissertation is the result of my own investigation, 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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ACKNOWLEDGEMENTS

In the name of Allah the Entirely Merciful, Especially Merciful and May His peace and blessings be upon our beloved Prophet Mohammad. Foremost, I want to offer this endeavor to our Allah Almighty for the wisdom he bestowed upon me, the strength, peace of my mind and good health in order to finish this project as a requirement for the degree of Master of Medical Sciences in Physiology (MMDSc).

I would like to dedicate this work to my parents; to my father who I adore and he has left me forever, my father thank you so much for everything you have done to grow me up and upbringing me sound breeding to reach this level, my father you will stay in my heart forever and I will not stop praying for you and may Allah gather me with you in His paradise. To my mother who I adore may Allah protect you and give you the health and the strength. Mum thank you so much for your patience and abundant emotions that you have carried to me. Mum I miss you in my pilgrimage and may Allah gather me with you soon.

I would like to express my deepest gratitude towards my family for the encouragement which helped me in completion of this work. My beloved and supportive husband, Hamad Abdulsalam Alfarisi who is always by my side when times I needed him most and helped me a lot in making this study, and my lovable children, Hothaifa and Mohammed who served as my inspiration to pursue this undertaking and their endurance with me for a lot of the hard days during my master journey. I would like to extend the heartfelt gratitude to all of my siblings for their continuous support and motivation.

Special thanks and appreciation to my eldest brother Hamad Bubaker Hamad for his continuous caring, support and encouragement since my under graduation till this moment. My brother you behave with me as if I am your daughter and I will not disappoint your hopes.

I would like to express my sincere appreciation to my supervisor, Asst. Prof. Dr. Roslina Abdul Rahim and Co- supervisors, Professor Naznin Muhammad and Assoc. Prof. Dr. Norzamzila Abdullah, for their constant guidance, encouragement, and unwavering support with which this thesis becomes a reality.

Special thanks and appreciation to Professor Pakeer Othman for his motivation, patience and wisdom through which we could broad our knowledge and creativity. I would like also to thank Madam Jarifah Aman, the executive officer of the office of deputy dean, for her hard work and support.

All the thanks to the pharmacophysiology lab technicians, Haj Suhaimi Hashim, brothers Zulnizam Azdan, Mohd Saiful Fairul, and Mohd Fadhlilullah. And histopathologylogy lab technicians, Brother Mohd Hanif Kasmuri and Sister Wan Norulkhamisa. I would like also to thank my friends, and my lecturers at the Internatinal Islamic University Malaysia for their encouragement and moral support. My thanks and appreciations go to all people who have willingly helped me out with their abilities. Thanks all of you.

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LIST OF ABBREVIATIONS

AKD	Acute kidney diseases and disorders
AKI	Acute kidney injury
ANOVA	Analysis of variance
ATN	Acute tubular necrosis
ATP	Adenosine triphosphate
BUN	Blood urea nitrogen
CAM-1	Cell adhesion molecule-1
Cl^-	Chloride ion
CO	Cardiac output
CKD	Chronic kidney disease
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
DPX	depax
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
ER	Estrogen receptor
FAMA	Malaysian Federal Agricultural Marketing Authorities
g	gram
GFR	Glomerular filtration rate
GSH	Glutathion
H_2O_2	Hydrogen peroxide
HCD	High cholesterol diet
HDL-c	High density lipoprotein cholesterol
H&E	Haematoxylin and Eosin
HFD	High fat diet
IACUC	Institutional Animal care and Use Committee
ICU	Intensive care unit
IIUM	International University Islamic Malaysia
IL-18	Interleukin 18
IL-10	Interleukin 10
IL-6	Interleukin 6
IMT	Integrated Multisensor Technology
K^+	Potassium ion
KDIGO	Kidney Disease: Improving Global Outcomes
Kg	Kilogram
KIM-1	Kidney injury molecule-1
Km	Factor for dose translation
LDL-c	Low density lipoprotein cholesterol
L-FABP	Liver-Type Fatty Acid Binding Protein
MDA	Malondialdehyde
mRNA	Messenger ribonucleic acid
Na^+	Sodium ion
NADH	Nicotinamide adenine dinucleotide hydride
NAFLD	Nonalcoholic fatty liver disease
NaOH	Sodium hydroxide

NGAL	Neutrophil gelatinase-associated lipocalin
NK	Natural killer
NKT	Natural killer T
NO	Nitric oxide
O_2•−	Superoxide ion
OH•	Peroxyl radical
ONOO−	Peroxynitrite
ORG	Obesity related glomerulopathy
OS	Oxidative stress
PAI-1	Plasminogen activator inhibitor
PCO	Protein carbonyl
PEG	polyethylene glycol
RFT	Renal function test
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RRT	Renal replacement therapy
RT-PCR	Real time polymerase chain reaction
SCr	Serum creatinine
SD	Standard deviation
TAM	Tamoxifen
TC	Total cholesterol
TG	Triglyceride
TGF-β	Transforming growth factor-beta
TNF-α	Tumour necrosis factor-α
™	Trade mark sign
vLDL-c	Very low density lipoprotein cholesterol
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

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Kidneys are vital organs because they perform several important functions essential for life, including: body fluids, electrolyte, and blood pressure regulation, excretion of metabolic end products, and erythrocyte production. Twenty five percent of cardiac output (CO) and 7% of daily energy expenditure are the requirements of human kidneys to accomplish their different functions (Sureshbabu, Ryter, & Choi, 2015).

Kidney diseases include acute kidney injury (AKI), sub-acute kidney injury, and chronic kidney disease (CKD). For AKI more than 35 definitions have been used in the literature. This results in confusion and ill-defined association between acute renal dysfunction and morbidity and mortality (Mandelbaum et al., 2011). AKI is characterized by rapid and sometimes fatal loss of kidney function, leading to inability to maintain body fluids, electrolytes and acid-base homeostasis, and causing accumulation of end products of nitrogen metabolism (urea) and creatinine, or reduction in urine output, or both (Bellomo, Kellum, & Ronco, 2012). It is also defined as an abrupt reduction in the glomerular filtration rate (GFR) which results in accumulation of nitrogenous waste products, mainly creatinine and blood urea nitrogen (BUN) (Basile, Anderson, & Sutton, 2012).

However the preferred definition and staging system of AKI is the most recent proposed definition by the Kidney Disease: Improving Global Outcomes (KDIGO). According to KDIGO guidelines (2012), AKI defined as an increase in serum creatinine (SCr) by ≥0.3 mg/dl (≥26.5 μmol/l) within 48 hours; or increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7

days; or urine volume < 0.5 ml/kg/h for 6 hours (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group, 2012). KDIGO guidelines proposed the term, acute kidney diseases and disorders (AKD), to include any decline in renal function occurring in less than three months. Disorders that evolve over more than 48 hours, but generally under than three months are referred to as sub-acute kidney injury. AKD includes both AKI and sub-acute kidney injury, and there is considerable overlap in an acute and sub-acute presentation (Pedram Fatehi, 2016).

The incidence of AKD is on the rise in both developed and developing countries (Lameire et al., 2013), and it has been proven that diet and lifestyle have an important role in its development. High cholesterol diet (HCD) has been documented to causes elevation of blood pressure and induce renal injury (Al-Rejaie, Abuohashish, Alkhamees, Aleisa, & Alroujayee, 2012). Researchers have proven that there exists a complex association between progressive renal damage and hypercholesterolemia (Ghada, 2014). These findings are of concerns as approximately 50% of the middle-aged adult population have been shown to have total cholesterol levels above the normal range (Chade et al., 2005). Most previous studies however focused on the impact of chronically high blood cholesterol levels on the renal tissue. One of them revealed that hypercholesterolemia resulted in the development of focal glomerulosclerosis and proteinuria that rapidly progressed to renal failure (Deepa & Varalakshmi, 2006).

In essence, there is minimal information available in the literature with regard to early effects of hypercholesterolemia on the kidney (Abdel-Hafez, Othman, & Seleim, 2011). Also although there is a growing insight into the causes and mechanisms of the kidney diseases, preventive and therapeutic measures are still few (Lameire et al., 2013). Hence the aim of this study is to determine the acute and sub-

acute effects of high cholesterol diet on the kidney, and to examine the protective role of tualang honey against high cholesterol diet induced AKD.

1.2 PROBLEM STATEMENT

Diet and lifestyle have important roles in kidney disease development. Despite an increasing incidence of AKD in both high and low-income countries and a growing insight into the aetiologies and the pathogenesis of the disease, few preventive and therapeutic options exist. Tualang honey which is known for its anti-inflammatory and anti-oxidant activities may have protective effects against AKD since inflammation and oxidative stress damage have been implicated in the pathogenesis of several kidney diseases.

1.3 SIGNIFICANCE OF THE RESEARCH

1. This research can form a foundation for future research to be undertaken to study the impact of high cholesterol diet on the development of acute kidney injury.
2. This research explores the role of tualang honey as a prophylaxis against HCD induced acute kidney diseases.

1.4 OBJECTIVES

1.4.1 General objective

1. To study the acute and sub-acute effects of high cholesterol diet on the kidney in rat animal model.

2. To investigate the protective effects of orally administered tualang honey against high cholesterol diet induced acute kidney diseases in rat animal model.

1.4.2 Specific objectives

1. To determine the effects of high cholesterol diet on the body and relative kidney weights.
2. To determine the effects of high cholesterol diet on the renal and lipid profiles, and renal histology.
3. To determine the protective effects of tualang honey against high cholesterol diet induced changes of body and relative kidney weights.
4. To determine the protective effects of tualang honey against high cholesterol diet induced changes of renal and lipid profiles, and renal histology.

1.5 RESEARCH HYPOTHESES

1. Supplementation of the rats with high cholesterol diet will increase their weight and change their relative kidney weight.
2. Supplementation of the rats with high cholesterol diet will cause abnormalities in the lipid profile and induce acute kidney diseases.
3. Supplementation of the rats with high cholesterol diet along with tualang honey will maintain the animal weight and the relative kidney weight within the normal.
4. Supplementation of the rats with high cholesterol diet along with tualang honey will ameliorate renal and lipid profiles changes, and renal histopathological changes.

CHAPTER TWO

LITERATURE REVIEW

2.1 ACUTE KIDNEY DISORDERS (AKD)

2.1.1 Overview

Acute kidney diseases and disorders include any decline in the renal function or kidney damage occurring in less than three months. Acute kidney injury is a subset of AKD. Thus, the definitions for AKD include criteria for the AKI as well as other criteria. Both AKI and AKD can also occur in patients with CKD (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group, 2012).

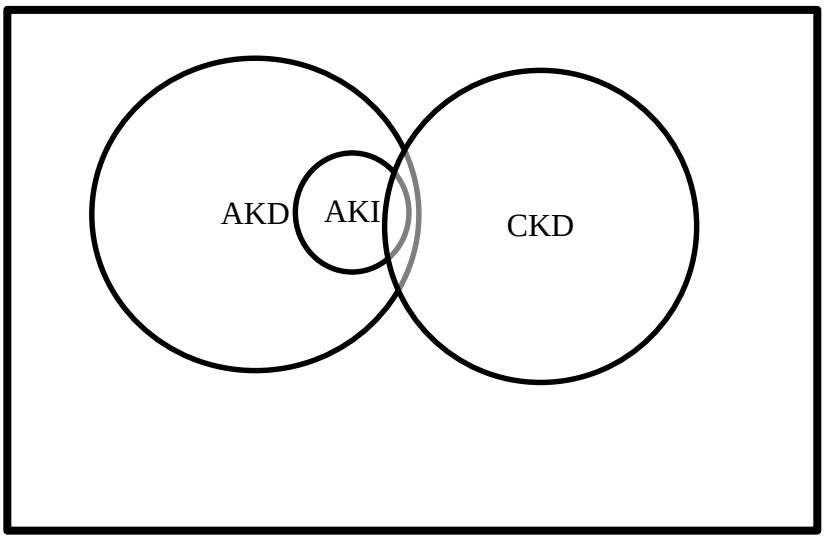

Figure 2.1: Overview of AKI, CKD, and AKD. Overlapping ovals show the relationships among AKI, AKD, and CKD. AKI is a subset of AKD. Both AKI and AKD without AKI can be superimposed upon CKD. AKD, acute kidney diseases and disorders; AKI, acute kidney injury; CKD, chronic kidney disease. Adapted from (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group, 2012).