

**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-1 β significantly. Atorvastatin reduced serum TNF- α and IL-1 β 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 تُبَيِّن فوائد العسل. أُجريت هذه الدراسة لتقييم التأثير الوقائي المحتمل للعسل الثلاثي ضد ضعف القدرة الإنجابية للذكور والناجمة عن إرتفاع كوليستيرول الدم في الأرانب ومقارنته بالأتورفاستاتين. ثمانية وأربعون من ذكور الأرانب البيضاء النيوزيلاندية قُسمت إلى 6 مجموعات. عُذِّت مجموعتان بغذاء الأرانب التجاري مع 0 و 0.6 جم/كجم/يوم من العسل على التوالي بينما عُذِّت المجموعات الأربعة الأخرى على غذاء عالي الكوليستيرول مع 0 و 0.3 و 0.6 جم/كجم/يوم من العسل و 2 جم/كجم/يوم من الأتورفاستاتين. قُسمت 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 دُرِس تأثير العسل على المؤشرات الحيوية الإلتهابية في مصل الدم. خفَّض كل من العسل والأتورفاستاتين من مستويات المؤشرات الحيوية الإلتهابية في مصل الدم. في المرحلة الثالثة، فُحص تأثير العسل على الهرمونات التناسلية الذكورية في مصل الدم والخصيتين، وعلى مؤشر مقاومة الإنسولين. حسَّن العسل خاصة بجرعة 0.6 جم/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 قُيِّم تأثير العسل على الحيوانات المنوية. العسل الثلاثي خاصة بجرعة 0.6 جم/كجم/يوم أثر إيجابياً على صفات الحيوانات المنوية بينما أحدث الأتورفاستاتين أسوأ النتائج. المرحلة الخامسة قُيِّمت تأثير العسل على التغيرات النسيجية في الخصيتين والبربخ. أحدث العسل الثلاثي تحسناً في التغيرات التنكسية للخصية وفي تكوين الحيوانات المنوية والحفاظ على الأنسجة الطبيعية للبربخ. التغيرات النسيجية في الخصيتين والبربخ كانت أكثر شدة في مجموعة الأتورفاستاتين. بناءً على ماسبق: أظهر العسل الثلاثي فوائده الصحية كخافض لكوليستيرول الدم ومعزز للإنزيمات المضادة للأكسدة، ومثبط للمؤشرات الحيوية الالتهابية، ومحسِّن للحيوانات المنوية والهرمونات التناسلية الذكورية ومخفف من التغيرات النسيجية للخصيتين والبربخ.

APPROVAL PAGE

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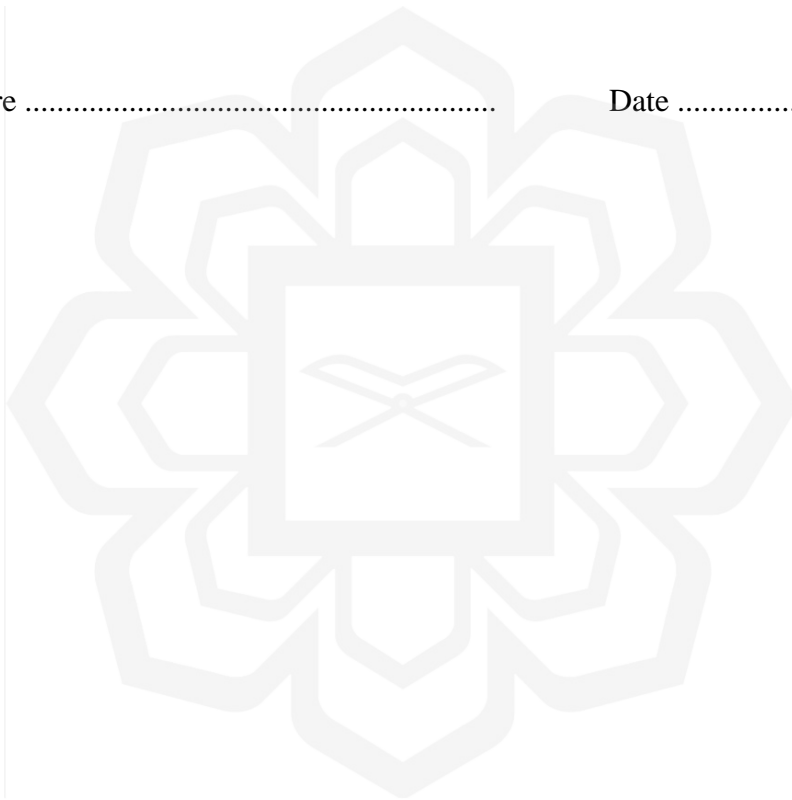
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DECLARATION

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

| | |
|--|----------|
| Abstract | ii |
| Abstract in Arabic | iii |
| Approval Page..... | iv |
| Declaration | v |
| Acknowledgements | vii |
| Table of Contents | viii |
| List of Tables | xv |
| List of Figures | xvii |
| List of Abbreviations | xix |
| List of Symbols | xxii |
| CHAPTER ONE: INTRODUCTION | 1 |
| 1.1 Background of the Study | 1 |
| 1.2 Statement of the Research Problem and Significance of the Study..... | 3 |
| 1.3 Research Objectives..... | 6 |
| 1.3.1 General Objective | 6 |
| 1.3.2 Specific Objectives | 6 |
| 1.4 Research Questions..... | 7 |
| 1.5 Research Hypothesis..... | 8 |
| CHAPTER TWO: LITERATURE REVIEW..... | 9 |
| 2.1 Male Infertility..... | 9 |
| 2.1.1 Overview and Epidemiology | 9 |
| 2.1.2 Normal Physiology of Male Reproductive System..... | 10 |
| 2.1.3 Causes of Male Infertility | 13 |
| 2.1.3.1 Azoospermia..... | 13 |
| 2.1.3.1.1 Obstructive Infertility..... | 14 |
| 2.1.3.1.2 Non-obstructive Infertility | 14 |
| 2.1.3.2 Coital Infertility | 15 |
| 2.1.3.3 Oxidative Stress and Male Infertility..... | 15 |
| 2.1.3.4 Inflammation and Male Infertility | 17 |
| 2.1.3.5 Nutrition and Male Infertility | 19 |
| 2.1.3.6 Hormonal Imbalance | 20 |
| 2.1.4 Evaluation of Male Infertility | 22 |
| 2.1.4.1 Semen Analysis | 22 |
| 2.1.4.1.1 Specialized Clinical Tests on Semen and Sperm..... | 23 |
| 2.1.4.2 Endocrine Evaluation | 23 |
| 2.1.4.3 Post Ejaculatory Urine Analysis..... | 24 |
| 2.1.4.4 Ultrasonography | 25 |
| 2.1.4.5 Testicular Biopsy..... | 25 |
| 2.1.4.6 Microbiologic Assessment | 25 |
| 2.1.4.7 Genetic Screening..... | 25 |
| 2.1.5 Treatment of Male Infertility | 25 |
| 2.1.5.1 Pharmacological Treatment of Male Infertility | 26 |
| 2.1.5.1.1 Hormonal Treatment..... | 26 |

| | |
|---|----|
| 2.1.5.1.2 Dopamine Agonists..... | 27 |
| 2.1.5.1.3 Aromatase Inhibitors..... | 27 |
| 2.1.5.1.4 Sympathomimetic Agents..... | 27 |
| 2.1.5.1.5 Selective Oestrogen Receptor Modulators..... | 28 |
| 2.1.5.2 Management of Oxidative Stress-Related Male Infertility..... | 28 |
| 2.1.5.2.1 Lifestyle Modification..... | 28 |
| 2.1.5.2.2 Antibiotics and Anti-Inflammatory Treatment of Infection/Inflammation..... | 29 |
| 2.1.5.2.3 Vitamins and Antioxidants..... | 29 |
| 2.1.5.3 Natural Products in Management of Male Infertility..... | 31 |
| 2.1.5.4 Sperm Retrieval and Assisted Reproductive Technology | 33 |
| 2.1.6 Hyperlipidaemia/ Hypercholesterolemia and Male Infertility..... | 34 |
| 2.1.6.1 Overview of Hyperlipidaemia..... | 34 |
| 2.1.6.2 Overview of Hypercholesterolemia..... | 35 |
| 2.1.6.3 Effects of Hyperlipidaemia on Semen Quality, Testes and Epididymides..... | 36 |
| 2.1.6.4 Hyperlipidaemia and Erectile Dysfunction..... | 38 |
| 2.1.6.5 Effects of Hypercholesterolemia on Semen Quality, Testes and Epididymides..... | 39 |
| 2.1.6.6 Hypercholesterolemia and Capacitation..... | 40 |
| 2.1.6.7 Effects of Hyperlipidaemia/ Hypercholesterolemia on Male Reproductive Hormones..... | 42 |
| 2.1.6.8 High Energy Diet/ Hypercholesterolemia-Induced Oxidative Stress and Male Infertility..... | 43 |
| 2.1.6.9 Hypercholesterolemia and Liver X Receptors..... | 47 |
| 2.1.6.10 Atorvastatin..... | 50 |
| 2.1.7 Obesity and Male Infertility..... | 51 |
| 2.1.7.1 Overview of Obesity..... | 51 |
| 2.1.7.2 Effects of Obesity on Semen Quality and Sperm Parameters..... | 52 |
| 2.1.7.3 Effects of Obesity on Male Reproductive Hormones..... | 54 |
| 2.1.7.4 Obesity and Increased Scrotal Temperature..... | 56 |
| 2.1.7.5 Obesity and Deoxyribonucleic Acid Fragmentation..... | 57 |
| 2.1.7.6 Insulin Resistance and Male Infertility..... | 57 |
| 2.1.7.7 Obesity Induced-Sleep Apnoea and Male Infertility..... | 59 |
| 2.1.7.8 Obesity and Erectile Dysfunction..... | 59 |
| 2.1.7.9 Management of Obesity-Induced Male Infertility..... | 60 |
| 2.1.8 Animal Models of Male Reproductive Disorders and Infertility..... | 60 |
| 2.1.8.1 Rabbits as a Model of Hypercholesterolemia..... | 61 |
| 2.1.8.2 Rabbits as a Model of Infertility..... | 62 |
| 2.2 Honey..... | 63 |
| 2.2.1 Definition and Composition of Honey..... | 63 |
| 2.2.2 Stingless Bees versus Honey Bees..... | 66 |
| 2.2.3 Trigona Honey..... | 67 |
| 2.2.4 Tualang Honey..... | 68 |
| 2.2.5 Apis Mellifera Honey..... | 68 |
| 2.2.6 Trihoney..... | 69 |

| | |
|---|------------|
| 2.2.7 Honey in Islamic Medicine | 71 |
| 2.2.8 Honey as a Food..... | 71 |
| 2.2.9 Antioxidant Activities of Honey | 72 |
| 2.2.10 Anti-Inflammatory Properties of Honey | 73 |
| 2.2.11 Honey and Infertility | 73 |
| 2.2.12 Medicinal Importance of Honey | 77 |
| 2.2.12.1 Antimicrobial Activities of Honey | 77 |
| 2.2.12.2 Wound Healing Properties of Honey..... | 79 |
| 2.2.12.3 Honey and Gastrointestinal Tract Diseases | 80 |
| 2.2.12.4 Honey and Cough in Children | 81 |
| 2.2.12.5 Honey and Cardiovascular Diseases' Risk Factors | 81 |
| 2.2.12.6 Hepatoprotective Effects of Honey | 82 |
| 2.2.12.7 Renoprotective Effects of Honey | 83 |
| 2.2.12.8 Antineoplastic and Antiproliferative Effects of Honey .. | 83 |
| 2.2.12.9 Honey and Eye Diseases..... | 84 |
| 2.2.12.10 Neuroprotective Potential of Honey | 85 |
| 2.2.12.11 Honey and Bone | 86 |
| CHAPTER THREE: METHODOLOGY..... | 88 |
| 3.1 Materials | 88 |
| 3.2 Sample Size Calculation | 88 |
| 3.3 Ethical Approval | 89 |
| 3.4 Preparation of 1% Cholesterol Diet..... | 89 |
| 3.5 Honey Dosage and Administration..... | 92 |
| 3.6 Atorvastatin Dosage and Administration..... | 92 |
| 3.7 Animal Grouping | 93 |
| 3.8 Blood Collection and Serum Separation | 96 |
| 3.9 Animal Sacrificing and Organ Harvesting | 96 |
| 3.10 Animal Handling Procedure and Experimental Design..... | 98 |
| 3.11 Preparation of Testicular Tissue Homogenate..... | 99 |
| 3.12 Protein Assay in Testicular Homogenate | 101 |
| 3.13 Statistical Analysis..... | 101 |
| CHAPTER FOUR: EFFECTS OF TRIHONEY ON SERUM LIPID PROFILE, SERUM AND TESTICULAR MALONDIALDEHYDE AND ANTIOXIDANT ENZYMES IN HIGH CHOLESTEROL DIET-FED MALE RABBITS | 102 |
| 4.1 Introduction..... | 102 |
| 4.2 Methodology..... | 103 |
| 4.2.1 Animal Weight and Daily Food Intake | 103 |
| 4.2.2 Blood Collection and Serum Separation..... | 103 |
| 4.2.3 Measurement of Lipid Profile Parameters | 104 |
| 4.2.4 Testicular Homogenate Preparation..... | 104 |
| 4.2.5 Protocols of Elisa Analysis for Serum and Testicular Homogenate..... | 105 |
| 4.2.5.1 Serum Malondialdehyde..... | 105 |
| 4.2.5.2 Testicular Malondialdehyde | 105 |
| 4.2.6 Protocols of Enzymes Assay for Serum and Testicular Homogenate..... | 106 |

| | | |
|--|---|------------|
| 4.2.6.1 | Superoxide Dismutase Activity Assay | 106 |
| 4.2.6.2 | Glutathione Peroxidase Assay | 107 |
| 4.2.6.2.1 | NADPH Standard Curve..... | 108 |
| 4.2.6.2.2 | Positive Control and Reagent Blank | 108 |
| 4.2.6.2.3 | Reaction Mix..... | 108 |
| 4.2.6.2.4 | Calculation of Results | 109 |
| 4.3 | Results | 109 |
| 4.3.1 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Animals' Weights, Weight Gain and Daily Food Intake..... | 109 |
| 4.3.2 | Baseline Lipid Profile | 112 |
| 4.3.3 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Lipid Profile After 12 Weeks | 112 |
| 4.3.4 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase..... | 114 |
| 4.3.5 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase..... | 116 |
| 4.4 | Discussion..... | 118 |
| 4.5 | Conclusion | 128 |
| | | |
| CHAPTER FIVE: EFFECTS OF TRIHONEY ON SERUM INFLAMMATORY BIOMARKERS IN HYPERCHOLESTEROLEMIC RABBITS | | 129 |
| 5.1 | Introduction..... | 129 |
| 5.2 | Methodology..... | 130 |
| 5.2.1 | Blood Collection and Serum Separation..... | 130 |
| 5.2.2 | Protocols of Elisa Analysis of Serum Pro-Inflammatory Biomarkers..... | 131 |
| 5.2.2.1 | Interleukin-6 and Tumour Necrosis Factor-Alpha | 131 |
| 5.2.2.2 | Interleukin-1 β | 131 |
| 5.3 | Results | 132 |
| 5.3.1 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum Levels of Pro-Inflammatory Cytokines..... | 132 |
| 5.4 | Discussion..... | 134 |
| 5.5 | Conclusion | 139 |
| | | |
| CHAPTER SIX: EFFECTS OF TRIHONEY ON MALE REPRODUCTIVE HORMONES AND INSULIN RESISTANCE IN HYPERCHOLESTEROLEMIC RABBITS | | 140 |
| 6.1 | Introduction..... | 140 |
| 6.2 | Methodology..... | 141 |
| 6.2.1 | Blood Collection and Serum Separation..... | 141 |
| 6.2.2 | Measurement of Fasting Blood Glucose, Fasting Serum Insulin and HOMA-IR..... | 141 |
| 6.2.3 | Testicular Homogenate Preparation..... | 142 |
| 6.2.4 | Protocols of Elisa Analysis | 142 |
| 6.2.4.1 | Serum Luteinizing Hormone and Follicle Stimulating Hormone | 142 |

| | |
|---|-----|
| 6.2.4.2 Serum and Testicular Testosterone..... | 143 |
| 6.3 Results | 144 |
| 6.3.1 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum and Intra-Testicular Testosterone..... | 144 |
| 6.3.2 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum Follicle Stimulating Hormone and Luteinizing Hormone | 145 |
| 6.3.3 Correlations of Serum Hormones with Serum Total Cholesterol and Low Density Lipoprotein Cholesterol..... | 147 |
| 6.3.4 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Fasting Blood Glucose, Fasting Serum Insulin and HOMA-IR... | 148 |
| 6.4 Discussion..... | 149 |
| 6.5 Conclusion | 155 |

CHAPTER SEVEN: EFFECTS OF TRIHONEY ON SPERM PARAMETERS IN HYPERCHOLESTEROLEMIC MALE RABBITS156

| | |
|--|-----|
| 7.1 Introduction..... | 156 |
| 7.2 Methodology..... | 157 |
| 7.2.1 Caudal Epididymal Sperm Analysis | 157 |
| 7.2.1.1 Analysis of Sperm Motility | 159 |
| 7.2.1.2 Analysis of Sperm Vitality and Morphology | 160 |
| 7.2.1.3 Analysis of Sperm Concentration..... | 161 |
| 7.3 Results | 162 |
| 7.3.1 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Sperm Motility..... | 162 |
| 7.3.2 Correlation of Sperm Motility with Serum Total Cholesterol and Low-Density Lipoprotein Cholesterol | 164 |
| 7.3.3 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Sperm Vitality..... | 165 |
| 7.3.4 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Sperm Morphology..... | 166 |
| 7.3.5 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Sperm Concentration | 167 |
| 7.3.6 Correlations of Sperm Parameters with Serum Total Cholesterol and Low-Density Lipoprotein Cholesterol..... | 170 |
| 7.3.7 Correlations Between Serum Hormones and Sperm Parameters | 171 |
| 7.4 Discussion..... | 173 |
| 7.5 Conclusion | 181 |

CHAPTER EIGHT: EFFECTS OF TRIHONEY ON TESTICULAR AND EPIDIDYMAL HISTOPATHOLOGICAL ALTERATIONS IN HYPERCHOLESTEROLEMIC MALE RABBITS183

| | |
|------------------------------------|-----|
| 8.1 Introduction..... | 183 |
| 8.2 Methodology..... | 184 |
| 8.2.1 Relative Organs Weight | 184 |
| 8.2.2 Tissue Histology | 184 |
| 8.2.2.1 Tissue Fixation | 184 |
| 8.2.2.2 Tissue Grossing | 185 |

| | |
|--|-----|
| 8.2.2.3 Tissue Processing | 185 |
| 8.2.2.4 Tissue Embedding | 186 |
| 8.2.2.5 Trimming and Sectioning | 186 |
| 8.2.2.6 Haematoxylin and Eosin Staining | 187 |
| 8.2.2.6.1 Deparaffinisation..... | 187 |
| 8.2.2.6.2 Hydration | 187 |
| 8.2.2.6.3 Haematoxylin and Eosin Stain..... | 187 |
| 8.2.2.6.4 Dehydration..... | 187 |
| 8.2.2.6.5 Mounting..... | 188 |
| 8.2.2.6.6 Slide Inspection and Picture Caption..... | 188 |
| 8.2.2.7 Masson’s Trichrome Staining..... | 188 |
| 8.2.2.7.1 Deparaffinisation..... | 188 |
| 8.2.2.7.2 Hydration | 189 |
| 8.2.2.7.3 Masson’s Trichrome Stain | 189 |
| 8.2.2.7.4 Dehydration..... | 189 |
| 8.2.2.7.5 Mounting..... | 189 |
| 8.2.2.7.6 Slide Inspection and Picture Caption..... | 190 |
| 8.2.2.8 Histological Measurements | 190 |
| 8.2.2.9 Spermatogenesis Evaluation..... | 190 |
| 8.3 Results | 191 |
| 8.3.1 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular and Epididymal Weights | 191 |
| 8.3.2 Correlations of Testicular and Epididymal Weights with Serum Total Cholesterol and Low-Density Lipoprotein Cholesterol..... | 194 |
| 8.3.3 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular and Epididymal Gross Morphology | 196 |
| 8.3.4 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular Histology | 197 |
| 8.3.4.1 Whole Testicular Sections from all Groups | 197 |
| 8.3.4.2 Control Group..... | 199 |
| 8.3.4.3 High-cholesterol Diet Group | 202 |
| 8.3.4.4 Atorvastatin Treated Group..... | 205 |
| 8.3.4.5 Trihoney (0.3 g/kg/day) Received Group..... | 207 |
| 8.3.4.6 Trihoney (0.6 g/kg/day) Received Group..... | 209 |
| 8.3.4.7 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on the Diameter of Seminiferous Tubules, Thickness of Tunica Albuginea and Johnsen’s Score | 211 |
| 8.3.5 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Epididymal Histology..... | 212 |
| 8.3.5.1 Control Group..... | 212 |
| 8.3.5.2 High Cholesterol Diet Group..... | 217 |
| 8.3.5.3 Atorvastatin Treated Group..... | 220 |
| 8.3.5.4 Trihoney (0.3 g/kg/day) Received Group..... | 223 |
| 8.3.5.5 Trihoney (0.6 g/kg/day) Received Group..... | 225 |
| 8.3.5.6 Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Epithelial Height and Diameter of Ductal Lumens of Caput and Cauda of the Epididymis..... | 228 |
| 8.4 Discussion..... | 231 |

| | |
|--|------------|
| 8.5 Conclusion | 239 |
| CHAPTER NINE: GENERAL CONCLUSION AND RECOMMENDATION | 240 |
| 9.1 General Conclusion | 240 |
| 9.2 Recommendation | 247 |
| REFERENCES..... | 248 |
| APPENDIX A: TRIHONEY | 294 |
| APPENDIX B: MATERIALS..... | 297 |
| APPENDIX C: JOURNAL ARTICLES..... | 302 |
| APPENDIX D: CONFERENCES AND SEMINARS..... | 304 |
| APPENDIX E: ACADEMIC SCHOLARSHIPS | 305 |



LIST OF TABLES

| <u>Table No.</u> | | <u>Page No.</u> |
|------------------|--|-----------------|
| 2.1 | Classification and Major Causes of Male Infertility | 15 |
| 2.2 | The Lower Reference Limit of Semen Parameters Based on WHO Laboratory Manual for the Examination and Processing of Human Semen | 23 |
| 2.3 | Composition of Trihoney | 70 |
| 3.1 | Composition of Coconut Oil | 90 |
| 3.2 | Animal Grouping of The Current Experiment | 94 |
| 3.3 | Nutritional Composition of Rabbit Pellet (Percentage/25kg) | 95 |
| 4.1 | Preparation of Samples and Blank for SOD Activity Measurement | 107 |
| 4.2 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Animals' Weights, Weight Gain and Daily Food Intake | 111 |
| 4.3 | Baseline Lipid Profile | 112 |
| 4.4 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Lipid Profiles After 12 Weeks | 114 |
| 4.5 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase | 116 |
| 4.6 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase | 118 |
| 5.1 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum Pro-Inflammatory Cytokines | 134 |
| 6.1 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum and Intra-Testicular Testosterone | 145 |
| 6.2 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Serum Follicle Stimulating Hormone and Luteinizing Hormone | 147 |

| | | |
|-----|--|-----|
| 6.3 | Correlations of Serum Hormones with Serum Total Cholesterol and Low-Density Lipoprotein Cholesterol | 148 |
| 6.4 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Fasting Blood Glucose, Fasting Insulin and HOMA-IR | 149 |
| 7.1 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on the Percentages of Sperm Motility | 164 |
| 7.2 | Correlation Coefficients Between Serum Lipid Profile and Sperm Motility | 165 |
| 7.3 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Percentages of Sperm Vitality, Sperm Morphology and Sperm Concentration | 168 |
| 7.4 | Correlation Coefficients Between Serum Lipid Profiles and Sperm Parameters | 171 |
| 7.5 | Correlations Between Serum Hormones and Sperm Parameters | 172 |
| 8.1 | Modified Johnsen's Score | 191 |
| 8.2 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Testicular and Epididymal Weights | 193 |
| 8.3 | Correlations of Serum Total Cholesterol and Low-Density Lipoprotein Cholesterol with Testicular and Epididymal Weights | 195 |
| 8.4 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Seminiferous Tubules Diameter, Tunica Albuginea thickness and Johnsen's Score | 212 |
| 8.5 | Effects of 1% Cholesterol Diet, Trihoney and Atorvastatin on Epithelial Height and Diameter of Ductal Lumen of Caput and Cauda of the Epididymis | 230 |

LIST OF FIGURES

| <u>Figure No.</u> | | <u>Page No.</u> |
|-------------------|--|-----------------|
| 2.1 | Anatomy of Testis Depicting the Site of Spermatogenesis. | 12 |
| 2.2 | Different Origins of Sperm Oxidative Stress | 16 |
| 2.3 | Management of Obesity-Induced Male Infertility | 60 |
| 2.4 | Overview of Male Reproductive System of Rabbits | 63 |
| 2.5 | Honey Comb (A) of Sting Bee Honey And Pot (B) of Stingless Bee Honey | 67 |
| 2.6 | Trihoney | 70 |
| 2.7 | Medicinal Importance of Honey | 87 |
| 3.1 | Preparation of 1% Cholesterol Diet | 91 |
| 3.2 | Animal Handling Procedure and Experimental Design | 98 |
| 3.3 | Preparation of Testicular Tissue Homogenate | 100 |
| 7.1 | The Procedure of Sperm Analysis | 159 |
| 7.2 | Eosin-Nigrosin Stain of Sperm Morphology and Vitality | 169 |
| 8.1 | Testicular and Epididymal Gross Morphology | 197 |
| 8.2 | Pictomicrograph of Testicular Whole Sections | 198 |
| 8.3 | Pictomicrograph of Testicular Sections from Control Group | 200 |
| 8.4 | Pictomicrograph of Testicular Sections from Commercial Pellet with Trihoney Group | 201 |
| 8.5 | Pictomicrograph of Testicular Sections from High-Cholesterol Diet Group | 203 |
| 8.6 | Pictomicrograph of Testicular Sections from High-Cholesterol Diet Group | 204 |
| 8.7 | Pictomicrograph of Testicular Sections from Atorvastatin Treated Group | 206 |
| 8.8 | Pictomicrograph of Testicular Sections from Trihoney (0.3 g/kg/day) Received Group | 208 |

| | | |
|------|---|-----|
| 8.9 | Pictomicrograph of Testicular Sections from Trihoney (0.6 g/kg/day) Received Group | 210 |
| 8.10 | Pictomicrograph of Sections of Caput Epididymis from Control Group | 213 |
| 8.11 | Pictomicrograph of Sections of Cauda Epididymis from Control Group | 214 |
| 8.12 | Pictomicrograph of Sections of Caput Epididymis from Commercial Pellet with Trihoney Group | 215 |
| 8.13 | Pictomicrograph of Sections of Cauda Epididymis from Commercial Pellet with Trihoney Group | 216 |
| 8.14 | Pictomicrograph of Sections of Caput Epididymis from High Cholesterol Diet Group | 218 |
| 8.15 | Pictomicrograph of Sections of Cauda Epididymis from High Cholesterol Diet Group | 219 |
| 8.16 | Pictomicrograph of Sections of Caput Epididymis from Atorvastatin Treated Group | 221 |
| 8.17 | Pictomicrograph of Sections of Cauda Epididymis from Atorvastatin Treated Group | 222 |
| 8.18 | Pictomicrograph of Sections of Caput Epididymis from Trihoney (0.3 g/kg/day) Received Group | 224 |
| 8.19 | Pictomicrograph of Sections of Cauda Epididymis from Trihoney (0.3 g/kg/day) Received Group | 225 |
| 8.20 | Pictomicrograph of Sections of Caput Epididymis from Trihoney (0.6 g/kg/day) Received Group | 226 |
| 8.21 | Pictomicrograph of Sections of Cauda Epididymis from Trihoney (0.6 g/kg/day) Received Group | 227 |

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 <i>Staphylococcus aureus</i> |
| CoQ10 | Coenzyme Q (10) |
| COX2 | Cyclooxygenase enzyme |
| CRP | C-Reactive protein |
| DFI | DNA fragmentation index |
| dH ₂ O | 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 |
| IUM | International Islamic University Malaysia |
| IL | Interleukin |
| IVF | <i>In vitro</i> fertilization |
| kg | Kilogram |
| LAC | L-acetyl-carnitine |
| LC | L-carnitine |
| LDH | Lactate dehydrogenase |
| LH | Luteinizing hormone |
| LXR _s | 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 |
| sreb1c | 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
XOD

World Health Organization
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, nonconceiving 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