CHEMOPREVENTIVE EFFECTS OF SELECTED HERBAL PLANTS AGAINST CARBON TETRACHLORIDE-MEDIATED OXIDATIVE TISSUE DAMAGE IN RATS

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BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2011

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ABSTRACT

CHEMOPREVENTIVE EFFECTS OF SELECTED HERBAL PLANTS AGAINST CARBON TETRACHLORIDE (CCl_4)-MEDIATED OXIDATIVE TISSUE DAMAGE IN RATS

Oxidative damage of biomolecules is implicated in the pathogenesis of various chronic diseases including cancer. This has led to intensive investigation aimed at reducing the extent of such oxidative injury. The present study was aimed to evaluate the antioxidant and chemopreventive effects of selected herbal plants against carbon tetrachloride (CCl_4)-mediated oxidative tissue damage in rats. Herbal plants viz *Aloe vera*, *Andrographis paniculata*, *Cymbopogon citratus* and *Morinda citrifolia* were selected and evaluated for their total phenolic and 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity. Based on the results of *in vitro* studies, *A. paniculata* and *C. citratus* were selected further for *in vivo* studies due to their higher total phenolics and antioxidant activity. Rats were pretreated with ethanolic extract of *A. paniculata* and *C. citratus* accordingly to the selected doses (100 mg/kg b.w., 200 mg/kg b.w. and 300 mg/kg b.w.) for 14 days followed by two dosage of CCl_4 (1.2 ml/kg b.w.) via oral (gavage) on days 13 and 14. All of these animals were sacrificed 24 hours after the last dose of CCl_4 or saline. Blood, liver and kidney tissues were taken quickly for biochemical and histopathological studies to assess the derangement in the functioning of liver and kidneys. Challenge of CCl_4 induced oxidative stress both in the liver and kidneys, as evident from augmentation in lipid peroxidation (TBARS) which was accompanied by a decreased in antioxidant enzymes activities and depletion of glutathione reduced level. Parallel to these changes, CCl_4 enhanced hepatic damage as evidenced by a sharp increased in serum transaminases. However, blood urea nitrogen and serum creatinine were not elevated significantly. Additionally, serum biochemistry oxidative stress markers were consistent with the hepatic and renal histopathological studies. In liver, most of these changes were significantly alleviated by pretreatment of animals with 300 mg/kg b.w. *A. paniculata* and 200 mg/kg b.w. *C. citratus*. The ability of *A. paniculata* and *C. citratus* to scavenge the DPPH radical was determined and had an EC_{50} value of 583.60 and 994.77 µg/ml respectively. In addition, the antioxidant activity was closely related to the total phenolic content as evident by *A. paniculata* and *C. citratus* showing the value of 65.37 and 30.74 mg GAE/g of extract. In contrast, the changes in the kidney were not significantly alleviated by the pretreatment with *A. paniculata* and *C. citratus* extracts. Present results indicate that the hepatoprotective effects of *A. paniculata* and *C. citratus* might be ascribable to its antioxidant and free radical scavenging properties. We concluded that *A. paniculata* and *C. citratus* could be used as hepatoprotective agents and possess the potential to be used to treat or prevent degenerative diseases where oxidative stress is implicated.

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ABSTRAK

Kerosakan oksidatif biomolekul menyebabkan pelbagai patogenesis penyakit kronik termasuk kanser. Ini membawa kepada penyelidikan intensif ditumpukan untuk mengurangkan kesebaran kerosakan oksidatif. Kajian ini adalah bertujuan untuk menilai antioksidan dan kesan pencegahan kemo dengan menggunakan tumbuhan herbal yang dipilih terhadap kerosakan tisu oksidatif akibat pendedahan carbon tetrachloride (CCl_4) pada tikus. Aloe vera, Andrographis paniculata, Cymbopogon citratus dan Morinda citrifolia telah dipilih dan dinilai jumlah kandungan fenolik dan aktiviti penyingkiran radikal 2,2-diphenyl-2-picrylhydrazyl (DPPH). Berdasarkan keputusan in vitro, A. paniculata dan C. citratus mengandungi jumlah kandungan fenolik dan aktiviti antioksidan yang lebih tinggi dan dipilih seterusnya untuk kajian in vivo. Tikus diberikan rawatan ekstrak etanol A. paniculata dan C. citratus berdasarkan dos yang dipilih (100 mg/kg berat badan, 200 mg/kg berat badan dan 300 mg/kg berat badan) selama 14 hari dan diikuti dengan dua kali rawatan CCl_4 (1.2 ml/kg berat badan) melalui mulut (gavage) pada hari ke-13 dan 14. Dua puluh empat jam selepas kali terakhir CCl_4 atau saline, tikus dibunuh. Darah, hati dan ginjal dikeluarkan dengan segera untuk analisis biokimia dan histopatologi untuk menilai perubahan fungsi hati dan ginjal. Tikus yang diberi rawatan CCl_4 mengalami tekanan oksidatif pada kedua-dua hati dan ginjal dengan menunjukkan perubahan dalam lipid peroxidation (TBARS) bersama penurunan aktiviti enzim antioksidan serta penurunan tahap glutathione. Selaras dengan perubahan itu, rawatan CCl_4 meningkatkan kerosakan hati dengan menunjukkan peningkatan yang jelas pada serum transaminase. Namun demikian, nitrogen urea darah dan kretinin tidak menunjukkan peningkatan yang signifikan. Tambahan pula, serum penunjuk tekanan oksidatif adalah selaras dengan histopatologi hati dan ginjal. Dalam hati, kebanyakan perubahan jelas dikurangkan oleh pemberian rawatan 300 mg/kg b.w. A. paniculata dan 200 mg/kg b.w. C. citratus. Keupayaan A. paniculata dan C. citratus untuk menyingkirkan radikal DPPH telah dinilaikan dan menunjukkan nilai EC_{50} 583.60 dan 994.77 µg/ml masing-masing. Selain itu, aktiviti antioksidan adalah berkaitan rapat dengan kandungan fenolik dengan A. paniculata dan C. citratus menunjukkan sebanyak 65.37 dan 30.74 mg GAE/g ekstrak masing-masing. Sebaliknya, tiada perbezaan signifikan pada ginjal apabila rawatan ekstrak A. paniculata dan C. citratus diberikan. Keputusan pada kajian ini menunjukkan bahawa kesan perlindungan hepatik A. paniculata dan C. citratus mungkin disebabkan oleh antioksidan dan keupayaan menyingkirkan radikal bebas. Kajian ini merumuskan bahawa A. paniculata dan C. citratus berupaya digunakan sebagai agen perlindungan hepatik dan berpotensi untuk mengubati atau mencegah penyakit berkaitan tekanan oksidatif.

UMS UNIVERSITI MALAYSIA SABAH

TABLE OF CONTENTS

Page

TITLE i

DECLARATION ii

CERTIFICATION iii

ACKNOWLEDGEMENT iv

ABSTRACT v

ABSTRAK vi

LIST OF CONTENTS

LIST OF TABLES xi

LIST OF FIGURES xiii

LIST OF PHOTOS xiv

LIST OF ABBREVIATIONS xvi

LIST OF APPENDIX xxi

CHAPTER 1: INTRODUCTION 1

1.1 Background of the Study 1

1.2 Hypothesis 4

1.3 Objective 4

UMS
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CHAPTER 2: LITERATURE REVIEW 5
2.1 Carbon tetrachloride 5
2.1.1 Introduction 5
2.1.2 Mechanism Action 6
2.1.3 Carbon Tetrachloride Toxicity 10
2.2 *Aloe vera* 13
2.2.1 Introduction 13
2.2.2 Traditional Uses 14
2.2.3 Chemical Constituents 15
2.2.4 Biological Effects 16
2.3 *Andrographis paniculata* 18
2.3.1 Introduction 18
2.3.2 Traditional Uses 20
2.3.3 Chemical Constituents 21
2.3.4 Biological Effects 21
2.4 *Cymbopogon citratus* 25
2.4.1 Introduction 25
2.4.2 Traditional Uses 26
2.4.3 Chemical Constituents 26
2.4.4 Biological Effects 27
2.5 *Morinda citrifolia* 30
2.5.1 Introduction 30
2.5.2 Traditional Uses 32
2.5.3 Chemical Constituents 33
2.5.4 Biological Effects 35

CHAPTER 3: METHODOLOGY 38
3.1 Plant Materials 38
3.2 Chemicals 42
3.3 Preparation of Ethanolic Extract 43
3.4 Determination of Total Phenolic Content 43
3.5 Determination of 2,2-Diphenyl-2-picrylhydrazy (DPPH) Assay 44
3.6 Animals 45
3.6.1 Treatment Conditions 45
3.6.2 Collection of Serum and Tissue 46
3.7 Determination of Final Body Weight 47
3.8 Assessment of Liver and Kidney Index 47
3.9 Serum Biochemistry 48
3.9.1 Determination of Serum Alanine 48

UMS UNIVERSITI MALAYSIA SABAH

Aminotransferase (ALT)
3.9.2 Determination of Serum Aspartate Aminotransferase (AST) 48
3.9.3 Determination of Serum Urea Nitrogen 49
3.9.4 Determination of Serum Creatinine 50
3.9.5 Determination of Serum Lactate Dehydrogenase (LDH) Activity 51
3.10 Preparation of Tissues Post Mitochondrion Supernatant (PMS) 51
3.11 Determination of Total Protein Contents 52
3.12 Biochemical Analysis 53
3.12.1 Determination of Reduced Glutathione (GSH) 53
3.12.2 Determination of Lipid Peroxidation (LPO) 54
3.12.3 Determination of Catalase (CAT) Activity 55
3.12.4 Determination of Glutathione Peroxidase (GPx) Activity 56
3.12.5 Determination of Glutathione Reductase (GR) Activity 57
3.12.6 Determination of Quinone Reductase (QR) Activity 58
3.12.7 Determination of Glutathione-S-transferase (GST) Activity 59
3.12.8 Determination of Glucose-6-phosphate Dehydrogenase (G6PD) Activity 60
3.12.9 Determination of γ-Glutamyl Transpeptidase (γ-GT) activity 61
3.13 Histopathological Examination 62
3.14 Statistical Analysis 65

CHAPTER 4: RESULTS 66
4.1 Extraction Yield and Total Phenolic Content 66
4.2 2,2-Diphenyl-2-picrylhydrazyl (DPPH) Assay 67
4.3 Effects of *A. paniculata* Extract on Carbon Tetrachloride (CCl_4)-Mediated Oxidative Tissue Damage in Rats 68
4.3.1 Body Weight 68
4.3.2 Liver and Kidney Index 69
4.3.3 Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH) 70

UMS UNIVERSITI MALAYSIA SABAH

4.3.4 Blood Urea Nitrogen and Creatinine 71
4.3.5 Lipid Peroxidation (LPO) 72
4.3.6 Reduced Glutathione (GSH) 73
4.3.7 Quinone Reductase (QR) 74
4.3.8 Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Glutathione-S-transferase (GST) 75
4.3.9 Catalase (CAT), Glucose-6-phosphate Dehydrogenase (G6PD) and γ-Glutamyl Transpeptidase (γ-GT) 78
4.3.10 Histopathological Examination 81
4.4 Effects of *C. citratus* Extract on Carbon Tetrachloride (CCl_4)-Mediated Oxidative Tissue Damage in Rats 88
4.4.1 Body Weight 88
4.4.2 Liver and Kidney Index 89
4.4.3 Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH) 90
4.4.4 Blood Urea Nitrogen and Creatinine 91
4.4.5 Lipid Peroxidation (LPO) 92
4.4.6 Reduced Glutathione (GSH) 93
4.4.7 Quinone Reductase (QR) 94
4.4.8 Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Glutathione-S-transferase (GST) 95
4.4.9 Catalase (CAT), Glucose-6-phosphate Dehydrogenase (G6PD) and γ-Glutamyl Transpeptidase (γ-GT) 98
4.4.10 Histopathological Examination 101

CHAPTER 5: DISCUSSION 108
5.1 Extraction Yield and Total Phenolic Content 108
5.2 Free Radical Scavenging Activity 109
5.3 Effects of *A. paniculata* and *C. citratus* Extracts on Body Weight, Liver Index and Kidney Index in Rats 110
5.4 Effects of *A. paniculata* and *C. citratus* Extracts against CCl_4-Mediated Oxidative Hepatic and Renal Damage in Rats 111

UMS UNIVERSITI MALAYSIA SABAH

CHAPTER 6: CONCLUSION 124

REFERENCES 127

APPENDIX 153

LIST OF TABLES

		Page
Table 3.1	Parts, stages of maturity and sizes of plants used	39
Table 3.2	Procedure steps for tissue processing	63
Table 3.3	Procedure steps for auto-staining	64
Table 4.1	Extraction yields and total phenolic contents of *A. vera*, *A. paniculata*, *C. citratus* and *M. citrifolia* plant extracts	66
Table 4.2	DPPH free radical scavenging activity of *A. vera*, *A. paniculata*, *C. citratus* and *M. citrifolia* plant extracts compared with ascorbic acid	67
Table 4.3	Effects of *A. paniculata* extract on initial and final body weight	68
Table 4.4	Effects of *A. paniculata* extract on liver and kidney index	69
Table 4.5	Effects of *A. paniculata* extract on serum ALT, AST and LDH	70
Table 4.6	Effects of *A. paniculata* extract on blood urea nitrogen and creatinine	71

UMS
UNIVERSITI MALAYSIA SABAH

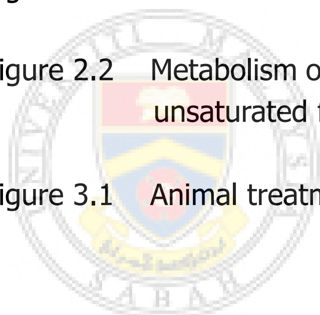
Table 4.7	Effects of *A. paniculata* extract on hepatic and renal LPO	72
Table 4.8	Effects of *A. paniculata* extract on hepatic and renal GSH	73
Table 4.9	Effects of *A. paniculata* extract on hepatic and renal QR	74
Table 4.10	Effects of *A. paniculata* extract on hepatic GPx, GR and GST	76
Table 4.11	Effects of *A. paniculata* extract on renal GPx, GR and GST	77
Table 4.12	Effects of *A. paniculata* extract on hepatic CAT, G6PD and γ-GT	79
Table 4.13	Effects of *A. paniculata* extract on renal CAT, G6PD and γ-GT	80
Table 4.14	Effects of *C. citratus* extract on initial and final body weight	88
Table 4.15	Effects of *C. citratus* extract on liver and kidney index	89
Table 4.16	Effects of *C. citratus* extract on serum ALT, AST and LDH	90
Table 4.17	Effects of *C. citratus* extract on blood urea nitrogen and creatinine	91
Table 4.18	Effects of *C. citratus* extract on hepatic and renal LPO	92
Table 4.19	Effects of *C. citratus* extract on hepatic and renal GSH	93
Table 4.20	Effects of *C. citratus* extract on hepatic and	94

UMS
UNIVERSITI MALAYSIA SABAH

	renal QR	
Table 4.21	Effects of *C. citratus* extract on hepatic GPx, GR and GST	96
Table 4.22	Effects of *C. citratus* extract on renal GPx, GR and GST	97
Table 4.23	Effects of *C. citratus* extract on hepatic CAT, G6PD and γ-GT	99
Table 4.24	Effects of *C. citratus* extract on renal CAT, G6PD and γ-GT	100

LIST OF FIGURES

		Page
Figure 2.1	Chemical structure of carbon tetrachloride	5
Figure 2.2	Metabolism of carbon tetrachloride (RH represents unsaturated fatty acids)	8
Figure 3.1	Animal treatment	45


UMS
UNIVERSITI MALAYSIA SABAH

LIST OF PHOTOS

Page

Photo 2.1 *Aloe vera* 13

Photo 2.2 *Andrographis paniculata* 19

Photo 2.3 *Cymbopogon citratus* 25

Photo 2.4 *Morinda citrifolia* 31

Photo 3.1 *Aloe* vera 40

Photo 3.2 *Andrographis paniculata* 40

Photo 3.3 *Cymbopogon citratus* 41

Photo 3.4 *Morinda citrifolia* 41

Photo 4.1 Histopathological sections of liver. 82
(A) Control; (B) CCl_4; (C) *A. paniculata* 100 mg/kg b.w. and CCl_4; (D) *A. paniculata* 200 mg/kg b.w. and CCl_4; (E) *A. paniculata* 300 mg/kg b.w. and CCl_4.
Sections stained with haematoxylin and eosin.
Black arrow, fatty changes.
Red arrow, infiltration of inflammatory cells.
Magnification 100X.

UNIVERSITI MALAYSIA SABAH

Photo 4.2	Histopathological sections of kidney. (A) Control; (B) CCl_4; (C) *A. paniculata* 100 mg/kg b.w. and CCl_4; (D) *A. paniculata* 200 mg/kg b.w. and CCl_4; (E) *A. paniculata* 300 mg/kg b.w. and CCl_4. Sections stained with haematoxylin and eosin. Black arrow, necrosis. Blue arrow, glomerular atrophy. Magnification 100X.	85
Photo 4.3	Histopathological sections of liver. (A) Control; (B) CCl_4; (C) *C. citratus* 100 mg/kg b.w. and CCl_4; (D) *C. citratus* 200 mg/kg b.w. and CCl_4; (E) *C. citratus* 300 mg/kg b.w. and CCl_4. Sections stained with haematoxylin and eosin. Black arrow, fatty changes. Red arrow, infiltration of inflammatory cells. Magnification 100X.	102
Photo 4.4	Histopathological sections of kidney. (A) Control; (B) CCl_4; (C) *C. citratus* 100 mg/kg b.w. and CCl_4; (D) *C. citratus* 200 mg/kg b.w. and CCl_4; (E) *C. citratus* 300 mg/kg b.w. and CCl_4. Black arrow, necrosis. Blue arrow, glomerular atrophy. Sections stained with haematoxylin and eosin. Magnification 100X.	105

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LIST OF ABBREVIATIONS

Abs	Absorbance
Δ Abs	Changes of absorbance
ABTS	2,2-Azinobis (3-ethyl benzothiazoline-6-sulphonic acid)
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	One-way analysis of variance
A. paniculata	*Andrographis paniculata*
AST	Aspartate aminotransferase
A. vera	*Aloe vera*
BCA	Bicinchoninic acid
BCA1	Bicinchoninic acid protein assay kit
BHT	Butylated hydroxyl toluene
BSA	Bovine serum albumin
CAPE	Caffeic acid phenethyl ester
CAT	Catalase
CB2	Cannabinoid 2
$\bullet CCl_3$	Trichloromethyl radical
CCl_4	Carbon tetrachloride
C. citratus	*Cymbopogon citratus*
$CCl_3OO\bullet$	Trichloromethyl peroxyl radical
CD^{8+}	Cluster of differentiation 8
CDNB	1-Chloro-2,4-dinitrobenzene
cm	Centimeter
cm^{-1}	Per centimeter
CO_2	Carbon dioxide

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Cu^+	Copper (I) ion
Cu^{2+}	Copper (II) ion
CYP2B1	Cytochrome P450 2B1
CYP2B2	Cytochrome P450 2B2
CYP2E1	Cytochrome P450 2E1
$cyp2e1^{-/-}$	CYP2E1 knockout
$cyp2e1^{+/+}$	CYP2E1 wild-type
CYP3A	Cytochrome P450 3A
DIAP	14-Deoxy-11,12-didehydroandrographolide
DCIP	Dichlorophenolindophenol
df	Dilution factor
DMSO	Dimethyl sulfoxide
DPPH	1,1-Diphenyl-2-picryl-hydrazyl or 2,2-diphenyl-2-picrylhydrazyl
DPX	Di-N-butyle phthalate in xylene
DNA	Deoxyribonucleic acid
DTNB	5,5′-Dithio-bis-2-nitrobenzoic acid
EC_{50}	Concentration giving 50% inhibition
EDTA	Ethylenediamine tetra acetic acid
FAD	Flavin adenine dinucleotide
FDA	Food and Drug Administration
g	Gram
G6PD	Glucose-6 phosphate dehydrogenase
GAE	Gallic acids equivalents
g/cm^3	Gram per cubic centimeter
GC-MS	Gas chromatography-mass spectrometry
g/l	Gram per liter
g/mol	Gram per mole
GPx	Glutathione peroxidase
GR	Glutathione reductase
G-SDNB	1-Chloro-2,4-dinitrobenzene conjugate
GSH	Reduced glutathione
GSSG	Glutathione oxidized
GST	Glutathione-S-transferase
GS-TNB	Mixed disulphide (between reduced glutathione and 5-thionitrobenzoic acid)
γ-GT	γ-Glutamyl transpeptidase
h	Hour
H^+	Hydrogen ion
HCl	Hydrogen chloride
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography

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H&E	Haematoxylin and eosin
H_2O	Water
H_2O_2	Hydrogen peroxide
IFN-γ	Interferon-gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL -4	Interleukin-4
IL-6	Interleukin-6
IU/L	International units per liter
KCl	Potassium chloride
LC-MS	Liquid chromatography-mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LPO	Lipid peroxidation
m	Meter
M	Molarity
M^{-1}	Per Molarity
Mac-1	Macrophage-1
M. citrifolia	*Morinda citrifolia*
MDA	Malondialdehyde
mg	Miligram
Mg^{2+}	Magnesium ion
$MgCl_2$	Magnesium chloride
mg/dl	Miligram per deciliter
mg/kg b.w.	Miligram per kilogram body weight
mg/ml	Miligram per mililiter
min	Minute
ml	Mililiter
ml/kg b.w.	Mililiter per kilogram body weight
mm	Milimeter
mM	Milimolarity
mol/l	Mole/liter
N	Normality
n	Number
Na_2CO_3	Sodium carbonate
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide reduced
NADP	Nicotinamide adenine dinucleotide phosphate

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$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate reduced
NaOH	Sodium hydroxide
NF-kappa B	Nuclear factor kappa-light-chain-enhancer of activated B cell
NH_3	Ammonia
NH_4^+	Ammonium ion
$(NH_2)_2CO$	Ammonium carbonate
NK	Natural killer
nm	Nanometer
nmol	Nanomole
No.	Number
O_2	Oxygen
$O_2\bullet^-$	Superoxide radical
•OH	Hydroxyl radical
ONOO-	Peroxynitrite radical
ORAC	Oxygen radical absorbance capacity
p	Probability
pH	Negative decimal logarithm of the hydrogen ion activity in a solution
PMS	Post-mitochondrion supernatant
PO_4^{3-}	Phosphate ion
POD	Peroxidase
PyOD	Pyruvate oxidase
QR	Quinone reductase
R•	Alkoxyl radical
RH	Unsaturated fatty acids
ROO•	Peroxyl radical
ROS	Reactive oxygen species
rpm	Revolutions per minute
RSA	Radical scavenging activity
s	Second
SD	Standard deviation
$SnCl_2$	Stannous chloride
SO_3^{2-}	Sulphate ion
SOD	Superoxide dismutase
SPSS	Statistical package for the social sciences
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
TNB	5-Thionitrobenzoic acid

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TNF-α	Tumor necrosis factor-alpha
TRAP	Total radical trapping antioxidant parameter
tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloric acid buffer
UMS	Universiti Malaysia Sabah
USA	United State of America
VLDL	Very low-density lipoprotein
v/v	Volume per volume
WHO	World Health Organization
w/v	Weight per volume
µg/ml	Microgram per mililiter
µl	Microliter
µm	Micrometer
µmol	Micromole
°C	Degree Celsius
%	Percent

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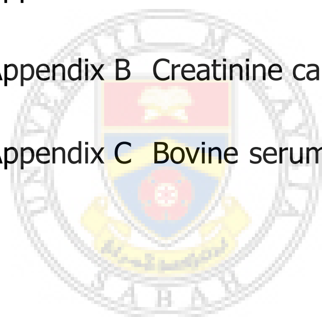
LIST OF APPENDIX

		Page
Appendix A	Gallic acid calibration curve	153
Appendix B	Creatinine calibration curve	154
Appendix C	Bovine serum albumin calibration curve	155



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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Reactive oxygen species (ROS) such as $O_2\bullet^-$, H_2O_2 and $\bullet OH$, are continuously generated inside the human body. They are generated due to the exposure of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer. However, the ROS generated are being detoxified by the antioxidant in the body under normal circumstances. There is homeostasis equilibrium between the ROS generated and the antioxidants present. Imbalance equilibrium between the formation and inactivation of these ROS causes the detrimental effects. However, this equilibrium is affected and favoring the increase of ROS formation that culminates in oxidative stress. This phenomenon is due to ROS overproduction and/or inadequate antioxidant defense. The ROS attack to various biomolecules including proteins, lipids, mitochondria, lipoproteins and DNA which ultimately induce oxidative damage (Farber, 1994). This oxidative damage is an important etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in ageing process (Hogg, 1998; Pong, 2003).

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Liver and kidney disorders are the serious health problem in the worldwide. The Global Burden of Disease: 2004 Update (2008) reported that liver cirrhosis, nephritis and nephrosis were categorized in the 20 leading causes of deaths in 2004 for all WHO member state. In Peninsular Malaysia, liver cancer was the sixth most common cancers among population in 2006. There were 793 cases per