

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	xii
LIST OF FIGURES	xiv
ABBREVIATION	xvii
CHAPTER I: INTRODUCTION	
1.1 Statement and significance of the problem	1
1.2 Literature review	
1.2.1 Functions of the kidney	3
1.2.2 Renal dialysis	4
1.2.3 The relevance of iron in biology	5
1.2.4 Human iron metabolism	5
1.2.5 Iron absorption	7
1.2.6 Proteins involved in iron transport and storage	9
1.2.7 Cellular iron acquisition from transferrin	12
1.2.8 Regulation of iron levels in the intracellular labile pool	15
1.2.9 Plasma iron transport and turnover	19
1.2.10 Iron overload	20
1.2.11 Iron-catalyzed generation of free radicals	20
1.2.12 Changes in plasma iron and NTBI formation in iron overload	22
1.2.13 Iron status in end-stage renal disease (ESRD) patients	23
1.2.14 Non-transferrin bound iron in the plasma of hemodialysis patients	27
1.2.15 Deferiprone	27
1.2.16 Curcumin	30
1.3 Objectives	37

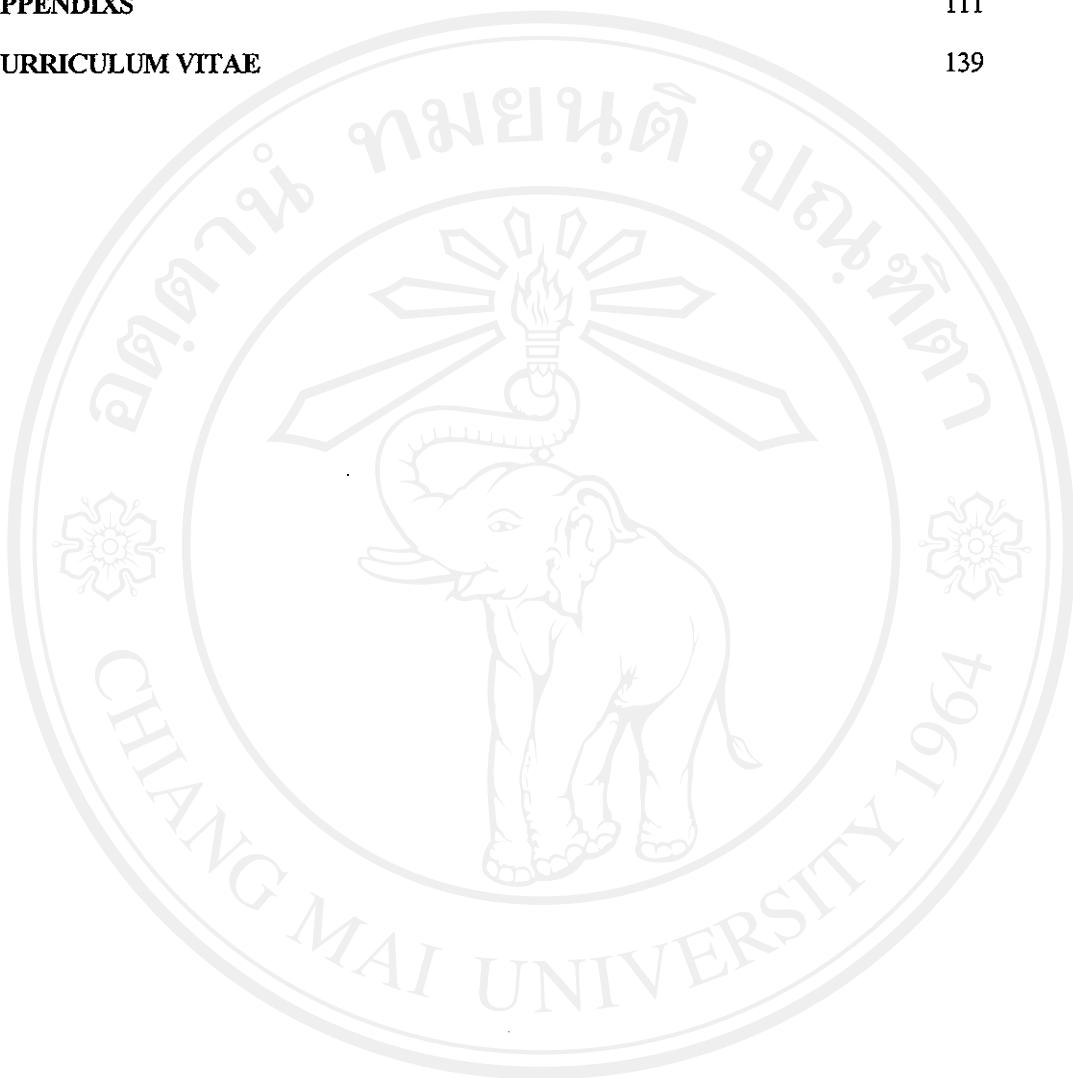
CHAPTER II: MATERIALS AND METHODS

2.1 Chemicals and Reagents	38
2.2 Patients	38
2.3 Blood samples	38
2.4 Determination of renal function	38
2.5 Determination of red blood cell concentration	39
2.5.1 Hemoglobin determination	39
2.5.2 Hematocrit determination	40
2.6 Determination of iron overload status	40
2.6.1 Plasma iron determination	40
2.6.2 Total iron-binding capacity determination (TIBC)	41
2.6.3 Percentage of transferrin saturation determination	42
2.7 Determination of plasma non-transferrin bound iron (NTBI)	42
2.8 Determination of oxidative stress status	44
2.8.1 Determination of oxidative stress in RBC by flow cytometry	44
2.8.2 Determination of thiobarbituric acid-reactive substances (TBARS) in plasma	45
2.9 Effect of hemodialysis on NTBI level	45
2.9.1 Effect of <i>in vivo</i> hemodialysis by hemodialyzer	45
2.9.2 Effect of hemodialysis <i>in vitro</i>	46
2.9.3 Effect of curcumin and deferiprone on <i>in vitro</i> hemodialysis	46
2.10 Effect of curcumin on iron-binding activity <i>in vitro</i>	47
2.10.1 Spectral analysis	47
2.10.2 Dose-response formation curcumin-iron complex	47
2.10.3 Time course of curcumin-iron complex formation <i>in vitro</i>	47
2.11 Efficacy of plasma NTBI removal by deferiprone and curcumin <i>in vitro</i>	48
2.12 Effect of curcumin on oxidative stress in whole blood	48
2.13 Effect of curcumin on oxidative stress in RBC suspension	48

CHAPTER III: RESULTS

3.1 Flow cytometric analysis of free radicals in erythrocytes from end-stage renal disease patients	50
3.2 Measurement of NTBI using the NTA/HPLC method	54
3.3 Characteristics of ESRD patients	60
3.4 Oxidative stress in ESRD'S RBCs	64
3.5 Correlation between parameters of iron overload and oxidative stress in blood of ESRD patients	67
3.6 Effect of curcumin on oxidative stress in whole blood	67
3.7 Effect of curcumin on oxidative stress in RBC suspension	73
3.8 Effect of <i>in vivo</i> hemodialysis on plasma NTBI levels	78
3.9 Effect of <i>in vitro</i> hemodialysis on plasma NTBI levels	80
3.10 Effect of curcumin and deferiprone to remove plasma NTBI during <i>in vitro</i> hemodialysis	82
3.11 Iron-binding activity of curcumin <i>in vitro</i>	84
3.11.1 Spectrum analysis of binding of curcumin with ferric nitrate	84
3.11.2 Spectrum analysis of binding of curcumin with ferric citrate	85
3.12 Effect of iron concentrations on curcumin-iron complex formation	86
3.12.1 Effect of ferric nitrate concentration	86
3.12.2 Effect of ferric citrate concentration	87
3.13 Effect of curcumin concentration on curcumin-iron complex formation	88
3.13.1 Effect of curcumin concentration on curcumin-ferric nitrate complex formation	88
3.13.2 Effect of curcumin concentration on curcumin-ferric citrate complex formation	89
3.14 Kinetic study of curcumin and iron binding	90
3.14.1 Time course of curcumin-ferric complex formation <i>in vitro</i>	90
3.14.2 Time course of curcumin-ferric citrate complex formation <i>in vitro</i>	91
3.15 Effect of curcumin and deferiprone on the removal of plasma NTBI	92

CHAPTER IV: DISCUSSION AND CONCLUSION	95
REFERENCES	101
APPENDIXS	111
CURRICULUM VITAE	139

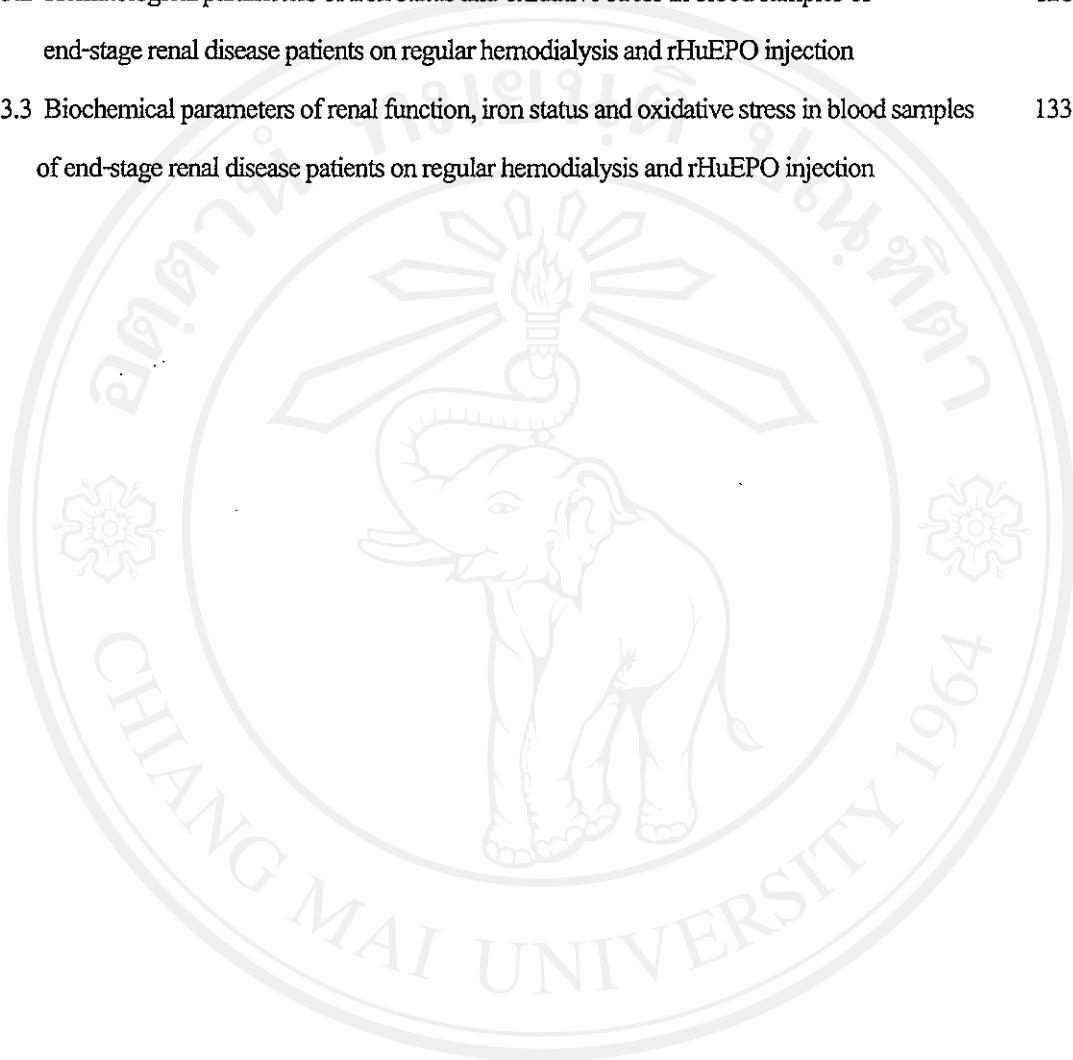


ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright[©] by Chiang Mai University
All rights reserved

LIST OF TABLES

TABLE	PAGE
1.1 Biochemical activities of curcumin	35
3.1 Quality control of flow cytometric measurements	54
3.2 Clinical history of normal volunteers and ESRD patients on regular hemodialysis	60
3.3 Hematological parameters of normal volunteers and ESRD patients on regular hemodialysis	62
3.4 Biochemical parameters of normal volunteers and ESRD patients on regular hemodialysis	63
3.5 Determination of oxidative stress in whole blood of ESRD patients	68
3.6 Determination of oxidative stress in red blood cell suspension of ESRD patients	73
3.7 Effect of <i>in vivo</i> hemodialysis for 4 hours on plasma NTBI levels in ESRD patients	78
3.8 Effect of <i>in vitro</i> hemodialysis for 4 hours on NTBI levels in whole blood of ESRD patients chelated with 100 μ M L1	80
3.9 Effect of curcumin and deferiprone on <i>in vitro</i> hemodialysis for 4 hours to remove NTBI levels in whole blood of ESRD patients	82
3.10 <i>In vitro</i> removal of NTBI by curcumin and deferiprone in plasma of four ESRD patients	92
S-1.1 Clinical history of healthy volunteers	117
S-1.2 Hematological parameters of iron status and oxidative stress in blood samples of healthy volunteers.	118
S-1.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of healthy volunteers.	119
S-2.1 Clinical history of end-stage renal disease patients on regular hemodialysis and intermittent blood transfusions	120
S-2.2 Hematological parameters of iron status and oxidative stress in blood samples of end-stage renal disease patients on regular hemodialysis and intermittent blood transfusions	121
S-2.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of end-stage renal disease patients on regular hemodialysis and intermittent blood transfusions	122

S-3.1 Clinical study of end-stage renal disease patients on regular hemodialysis and rHuEPO injection	123
S-3.2 Hematological parameters of iron status and oxidative stress in blood samples of end-stage renal disease patients on regular hemodialysis and rHuEPO injection	128
S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of end-stage renal disease patients on regular hemodialysis and rHuEPO injection	133



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright[©] by Chiang Mai University
All rights reserved

LIST OF FIGURES

FIGURE	PAGE
1.1 Normal kidney function	3
1.2 Hemodialysis procedure	4
1.3 Distribution of iron in adults	6
1.4 Iron transport across the intestinal epithelium	8
1.5 The transferrin cycle	14
1.6 Translational control of ferritin synthesis in response to iron	17
1.7 Control of degradation of transferrin receptor (TfR) mRNA in response to iron	18
1.8 Structure of Deferiprone	28
1.9 Turmeric plant and turmeric powder	30
1.10 Chemical structures of curcuminoids	31
1.11 Chemical structures of curcumin	32
1.12 Proposed biotransformation and metabolism of curcumin in mouse plasma	34
2.1 Diagram of plasma NTBI quantification using the NTA chelation/HPLC-based assay	43
3.1 Flow cytometric patterns of oxidative stress in erythrocytes	51
3.2 Quality control of flow cytometric measurements of fluorescence intensity	53
3.3 HPLC chromatograms of NTBI in plasma from normal volunteers, β -thalassemia patients and ESRD patients	55
3.4 Calibration curve of NTBI assayed using the NTA chelation/HPLC technique	59
3.5A Distribution of oxidative stress expressed as fluorescence intensity in erythrocytes from normal volunteers, ESRD-T, ESRD-E	65
3.5B Distribution of oxidative stress expressed as fluorescence intensity with H_2O_2 stimulation in erythrocytes from normal volunteers, ESRD-T, ESRD-E	66
3.6A Determination of oxidative stress in whole blood of ESRD patients treated with curcumin, Fe^{2+} -EDTA and curcumin with Fe^{2+} -EDTA	69

3.6B Determination of oxidative stress in whole blood of ESRD patients treated with curcumin, Fe ²⁺ -EDTA and curcumin with Fe ²⁺ -EDTA (% of control)	70
3.7A Determination of oxidative stress in whole blood of ESRD patients treated with curcumin, Fe ²⁺ -EDTA and curcumin with Fe ²⁺ -EDTA.	71
3.7B Determination of oxidative stress in whole blood of seven ESRD patients treated hydrogen peroxide, curcumin, Fe ²⁺ -EDTA and curcumin with Fe ²⁺ -EDTA (% of control)	72
3.8A Determination of oxidative stress in red blood cell suspension of ESRD patients treated curcumin in the presence and absence of Fe-EDTA	74
3.8 B Determination of oxidative stress in red blood cell suspension of ESRD patients treated curcumin in the presence and absence of Fe-EDTA (% of control)	75
3.9A Determination of oxidative stress in red blood cell suspension of ESRD patients treated curcumin in the presence and absence of Fe-EDTA with hydrogen peroxide stimulation	76
3.9B Determination of oxidative stress in red blood cell suspension of ESRD patients treated curcumin in the presence and absence of Fe-EDTA with hydrogen peroxide stimulation (% of control)	77
3.10 Effect of <i>in vivo</i> hemodialysis for 4 hours on NTBI levels in ESRD patients	79
3.11 Effect of <i>in vitro</i> hemodialysis for 4 hours on NTBI levels in ESRD patients chelated with L1	81
3.12 Effect of curcumin and deferiprone on <i>in vitro</i> hemodialysis for 4 hours to remove NTBI levels in ESRD patients chelated with curcumin and L1 (%decreased NTBI)	83
3.13 Spectrum of curcumin-ferric complex formation (ferric nitrate form)	84
3.14 Spectrum of curcumin-ferric complex formation (ferric citrate form)	85
3.15 Interaction of curcumin solution with ferric nitrate solution different concentration	86
3.16 Interaction of curcumin solution with ferric citrate solution different concentration	87
3.17 Interaction of ferric-nitrate solution with curcumin different concentration	88
3.18 Interaction of ferric-citrate solution with curcumin different concentration	89
3.19 Time-course of ferric nitrate binding to curcumin	90
3.20 Time-course of ferric citrate binding to curcumin	91
3.21 <i>In vitro</i> removal NTBI by deferiprone and curcumin in plasma of ESRD patients	93

3.22 <i>In vitro</i> removal NTBI by deferiprone and curcumin in plasma of ESRD patients (%decreased)	94
4.1 Overview of Iron overload and oxidative stress in ESRD patients on regular hemodialysis	100



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright[©] by Chiang Mai University
All rights reserved

ABBREVIATIONS

%	Percent
°C	Degree Celsius
µg	Microgram
µM	Micromolar
µL	Microlitre
mg	Milligram
mM	Millimolar
nm	Nanometre
BUN	Blood urea nitrogen
CO ₂	Carbon dioxide
DCFH-DA	Dichlorofluorecein dicetate
DFP	Deferiprone
EDTA	Ethylenediamine tetraacetic acid
Fe ²⁺	Ferrous iron
Fe ³⁺	Ferric iron
Fe-NTA	Ferric-nitrilotriacetate
ESRD	End-stage renal disease
ESRD-E	End-stage renal disease (receive rHuEPO)
ESRD-T	End-stage renal disease (receive blood transfusion)
F	Female
FI	Fluorescence intensity
FI*	Fluorescence intensity with H ₂ O ₂ stimulation
H ₂ O ₂	Hydrogen peroxide
4-HNE	4-Hydroxynonenal
HPLC	High performance liquid chromatography
L1	Deferiprone

M	Molarity
M	Male
MDA	Malondialdehyde
NA	Not available
NTBI	Non-transferrin bound iron
IRE	Iron responsive element
IRP	Iron responsive protein
kD	Kilodalton
LIP	Labile iron pool
OH [•]	Hydroxyl radical
PBS	Phosphate buffer saline
Pt	Protein
PI	Plasma iron
RBC	Red blood cell
rpm	Revolution per minute
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rHuEPO	Recombinant Human Erythropoietin
SD	Standard deviation
SEM	Standard error of measurement
Tf	Transferrin
TIBC	Total iron binding capacity