TABLE OF CONTENTS

| | PAGE |
|---|------|
| ACKNOWLEDGEMENTS | iii |
| ABSTRACT | iv |
| LIST OF TABLES | xiii |
| LIST OF FIGURES | xv |
| ABBREVIATIONS | xvii |
| CHAPTER I: INTRODUCTION | |
| 1.1 Statement and significance of the problem | 1 |
| 1.2 Literature reviews | 4 |
| 1.2.1 The clinical problem and phenotype of multidrug resistance | 5 42 |
| 1.2.2 Multidrug resistance mediated by P-glycoprotein (Pgp) | 8 |
| 1.2.3 Pgp gene family | 10 |
| 1.2.4 Tissue distribution and overexpression of Pgp in cancer cell | 10 |
| 1.2.5 Structure of Pgp | 12 |
| 1.2.6 The mechanism of action of Pgp | 14 |
| 1.2.7 Reversal of Pgp mediated MDR by chemosensitiser | 17 |
| 1.2.8 Green tea | 19 |
| 1.2.9 Green tea in cancer | 22 |
| 1.2.10 Isolation and characterization of human MDR KB-V1 cell line | 24 |
| 1.3 Objectives | 26 |
| CHAPTER II: MATERIALS AND METHODS | |
| 2.1 Chemicals and reagents 2.2 Green tea flavonoids | 27 |
| 2.2 Green tea flavonoids | 27 |
| 2.3 Cell culture | 27 |
| 2.4 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay | 27 |
| 2.5 Accumulation and efflux of Rhodamine123 | 28 |
| 2.6 Accumulation and efflux of ³ H-vinblastine | 29 |
| 2.7 Plasma membrane preparation | 29 |

| 2.8 Protein determination | 30 |
|---|-------------|
| 2.9 Western blot analysis and ECL detection | 31 |
| 2.10 Statistical analysis | 32 |
| 2.11 Cytotoxicity of green tea flavonoids in KB-V1 and KB-3-1 cell lines | 32 |
| 2.12 Effect of green tea flavonoids on Pgp mediated drugs transport in KB-V1 | 32 |
| and KB-3-1 cell lines | |
| 2.12.1 Effect of green tea flavonoids on Rh123 accumulation and efflux | 32 |
| 2.12.2 Effect of green tea flavonoids on radiolabeled drug accumulation | 33 |
| and efflux | |
| 2.13 Effect of green tea flavonoids on Pgp expression (protein level) in KB-V1 | 33 |
| cell line | |
| 2.14 Effect of green tea flavonoids on cytotoxicity of chemotherapeutic drugs | 34 |
| (MDR phenotype) in KB-V1 and KB-3-1 cell lines | |
| 2.14.1Effect of co-incubation of green tea flavonoids on cytotoxicity of | 34 |
| chemotherapeutic drugs | 6 |
| 2.14.2 Effect of pre-incubation of green tea flavonoids on cytotoxicity of | 34 |
| chemotherapeutic drugs | |
| CHAPTER III: RESULTS | |
| 3.1 Cytotoxicity of green tea flavonoids in KB-V1 and KB-3-1 cell lines | 35 |
| 3.2 Effect of green tea flavonoids on Pgp mediated drug transport | |
| 3.2.1 Effect of green tea flavonoids on Rh123 accumulation and efflux | 39 |
| 3.2.2 Effect of green tea flavonoids on ³ H-vinblastine accumulation | 47 |
| and efflux | |
| 3.3 Effect of green tea flavonoids on Pgp expression (Pgp level) in KB-V1 cell line | 55 |
| 3.3.1 Effect of ECG and EGCG on Pgp level in KB-V1 cell line at 2 h. | - 55 SITY - |
| 3.3.2 Effect of green tea flavonoids on Pgp level in KB-V1 cells at 48 h. | . 55 |
| 3.4 Effect of green tea flavonoids on cytotoxicity of chemotherapeutic drugs | 63 |
| (MDR phenotype) in KB-V1 and KB-3-1 cell lines | |

| CHAPTER IV: DISCUSSION AND CONCLUSION | 78 |
|---|-----|
| 4.1 Effects of green tea flavonoids on Pgp mediated drugs transport in KB-VI | 79 |
| and KB-3-1 cell lines | |
| 4.2 Effect of green tea flavonoids on Pgp expression (protein level) in KB-V1 | 80 |
| cell line | |
| 4.3 Effect of green tea flavonoids on cytotoxicity of chemotherapeutic drugs | 80 |
| (MDR phenotype) in KB-V1 and KB-3-1 cell lines. | |
| 4.4 The possible mechanism of desensitizing effect to chemotherapeutic drug | 81 |
| by EGCG in KB-V1 and KB-3-1 | |
| REFERENCES | 84 |
| APPENDIX | 95 |
| VITA | 107 |
| | |
| | |
| | |
| | |
| | |
| | |
| | |
| | |

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

LIST OF TABLES

| TA | TABLE | |
|-----|--|----|
| 1. | The various mechanisms and phenotypes of MDR | 7 |
| 2. | Nomenclature of multidrug resistance genes | 10 |
| 3. | Cellular localization of Pgp in tissues, which are important for drug | 11 |
| | disposition and effects | |
| 4. | Compounds which interact with Pgp | 14 |
| 5. | Selected phamacological agents with ability reverse MDR | 19 |
| 6. | Flavonoids content of 1.25% water extract of green tea and black tea | 22 |
| 7. | Properties of MDR KB cell lines | 26 |
| 8. | Preparation of bovine serum albumin standard solution | 30 |
| 9. | Cytotoxicity of green tea flavonoids in KB-V1 cells | 36 |
| 10. | IC ₂₀ and IC ₅₀ values of green tea flavonoids on cytotoxicity of KB-V1 cells | 36 |
| 11. | Cytotoxicity of green tea flavonoids in KB-3-1 cells | 37 |
| 12. | IC ₂₀ and IC ₅₀ values of green tea flavonoids on cytotoxicity of KB-3-1 cells | 38 |
| 13. | Effect of green tea flavonoids on Rh123 accumulation in KB-V1 and KB-3-1 cells | 45 |
| 14. | Effect of green tea flavonoids on Rh123 efflux in KB-V1 and KB-3-1 cells | 46 |
| 15. | Effect of green tea flavonoids on ³ H-vinblastine accumulation in KB-V1 | 53 |
| | and KB-3-1 cells | |
| 16. | Effect of green tea flavonoids on ³ H-vinblastine efflux in KB-V1 and KB-3-1 cells | 54 |
| 17. | Effect of catechin on vinblastine cytotoxicity in KB-V1 cells | 64 |
| 18. | Effect of EC on vinblastine cytotoxicity in KB-V1 cells | 65 |
| 19. | Effect of ECG on vinblastine cytotoxicity in KB-V1 cells | 66 |
| 20. | Effect of EGC on vinblastine cytotoxicity in KB-V1 cells | 67 |
| 21. | Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells | 68 |
| 22. | Effect of EGCG on vinblastine cytotoxicity in KB-3-1 cells | 69 |
| 23. | Effect of EGCG on doxorubicin cytotoxicity in KB-V1 cells | 70 |
| 24. | Effect of EGCG on doxorubicin cytotoxicity in KB-3-1 cells | 71 |
| 25. | Effect of EGCG on colchicine cytotoxicity in KB-V1 cells | 72 |

| 26. | Effect of EGCG on colchicine cytotoxicity in KB-3-1 cells | 73 |
|-----|---|----|
| 27. | Effect of EGCG on paclitaxel cytotoxicity in KB-V1 cells | 74 |
| 28. | Effect of EGCG on paclitaxel cytotoxicity in KB-3-1 cells | 75 |
| 29. | Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells for 24 h incubation | 76 |
| 30. | Effect of EGCG on pre-incubation of EGCG on vinblastine cytotoxicity in | 77 |
| | KB-V1 cells | |

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

LIST OF FIGURES

| FI | GURE | PAGE |
|-----|--|------|
| 1. | The resistance development during cancer chemotherapy | 5 |
| 2. | Factors contribute to clinical MDR in patients with cancer | 6 |
| 3. | Pgp function in the plasma membrane of a cancer cell during chemotherapy | 8 |
| 4. | Pgp expression in tumor prior to receiving chemotherapy and after therapy | 9 |
| 5. | Two-dimensional hypothetical model of human Pgp structure based on a | 13 |
| | hydropathy plot analysis of primary amino acid sequence | |
| 6. | A proposed scheme for the catalytic cycle of ATP hydrolysis by Pgp | 16 |
| 7. | Functional representation of Pgp | 18 |
| 8. | Nuclear structure and numbering system of bioflavonoids | 20 |
| 9. | Structure of green tea flavonoids | 21 |
| 10. | Flow diagram showing the steps for increasing vinblastine resistance in | 25 |
| | the MDR KB-V1 cell line | |
| 11. | The principle of enhanced chemiluminescence (ECL) system | 32 |
| 12. | Cytotoxiciy of green tea flavonoids in KB-V1 cells | 35 |
| 13. | Cytotoxiciy of green tea flavonoids in KB-3-1 cells | 37 |
| 14. | Effect of catechin on Rh123 accumulation (A) and efflux (B) in | 40 |
| | Pgp expressing KB-V1 cell line | |
| 15. | Effect of EC on Rh123 accumulation (A) and efflux (B) in | 41 |
| | Pgp expressing KB-V1 cell line | |
| 16. | Effect of EGC on Rh123 accumulation (A) and efflux (B) in | 42 |
| | Pgp expressing KB-V1 cell line | |
| 17. | Effect of EGCG on Rh123 accumulation (A) and efflux (B) in | 43 |
| | Pgp expressing KB-V1 and KB-3-1 cell lines | |
| 18. | Effect of ECG on Rh123 accumulation (A) and efflux (B) in | 44 |
| | Pgp expressing KB-V1 and KB-3-1 cell lines | |
| 19. | Effect of catechin on ³ H-vinblastine accumulation (A) and efflux (B) | 48 |
| | in KR-V1 cell line | |

| 20. | Effect of EC on 'H-vinblastine accumulation (A) and efflux (B) | 49 |
|-----|---|-----------|
| | in KB-V1 cell line | |
| 21. | Effect of EGC on ³ H-vinblastine accumulation (A) and efflux (B) | 50 |
| | in KB-V1 cell line | |
| 22. | Effect of EGCG on ³ H-vinblastine accumulation (A) and efflux (B) | 51 |
| | in KB-V1 and KB-3-1 cell lines | |
| 23. | Effect of ECG on ³ H-vinblastine accumulation (A) and efflux (B) | 52 |
| | in KB-V1 and KB-3-1 cell lines | |
| 24. | Pgp level in KB-V1 cells cultured in 100,200 and 300 μM ECG for 2 h | 56 |
| 25. | Pgp level in KB-V1 cells cultured in 100,200 and 300 μ M EGCG for 2 h | 57 |
| 26. | Pgp level in KB-V1 cells cultured in 50 and 100 μM catechin for 48 h | 58 |
| 27. | Pgp level in KB-V1 cells cultured in 50 and 100 μM EGC for 48 h | 59 |
| 28. | Pgp level in KB-V1 cells cultured in 50 and 100 μM EGCG for 48 h | 60 |
| 29. | Pgp level in KB-V1 cells cultured in 50 and 100 μM EC for 48 h | 61 |
| 30. | Pgp level in KB-V1 cells cultured in 50 and 100 μM ECG for 48 h | 62 |
| 31. | Effect of catechin on vinblastine cytotoxicity in KB-V1 cells | 64 |
| 32. | Effect of EC on vinblastine cytotoxicity in KB-V1 cells | 65 |
| 33. | Effect of ECG on vinblastine cytotoxicity in KB-V1 cells | 66 |
| 34. | Effect of EGC on vinblastine cytotoxicity in KB-V1 cells | 67 |
| 35. | Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells | 68 |
| 36. | Effect of EGCG on vinblastine cytotoxicity in KB-3-1 cells | 69 |
| 37. | Effect of EGCG on doxorubicin cytotoxicity in KB-VI cells | 70 |
| 38. | Effect of EGCG on doxorubicin cytotoxicity in KB-3-1 cells | 71 |
| 39. | Effect of EGCG on colchicine cytotoxicity in KB-V1 cells | 72 |
| 40. | Effect of EGCG on colchicine cytotoxicity in KB-3-1 cells | 73 SITY - |
| 41. | Effect of EGCG on paclitaxel cytotoxicity in KB-V1 cells | 74 |
| 42. | Effect of EGCG on paclitaxel cytotoxicity in KB-3-1 cells | 75 |
| 43. | Effect of EGCG on vinblastine cytotoxicity in KB-V1 cells for 24 h incubation | 76 |
| 44. | Effect of pre-incubation of EGCG on vinblatine cytotoxicity in KB-V1 cells | 77 |

ABBREVIATIONS

% Percent °C Degree Celsius μCi Microcurie μg Microgram μl Microlitre μM Micromolar μm Micrometre **ABC** ATP-binding cassette APS Ammonium persulphate bp Base pair BSA Bovine serum albumin cDNA Complementary DNA Ci Curie cm Centimetre cm^2 Square centrimetre CO₂ Carbon dioxide CuSO₄ Copper sulfate DDH₂O Double distilled water **DMEM** Dulbecco's modified Eagle's medium **DMSO** Dimethyl sulfoxide dpm Disintegration per minute EC Epicatechin **ECG** Epicatechin gallate **ECL** Enhanced chemiluminescence **EDTA** Ethylenediaminetetraacetic acid **EGC** Epigallocatechin

Epigallocatechin gallate

EGCG

xviii

ELISA Enzyme linked immunosorbent assay **FCS** Fetal calf serum Gram g Giggabecquerel (1.0x10⁹ becquerel) GBq Hour h **HBSS** Hanks' balance salt solution N-2-hydroxyethylpiperazine-N-2-**HEPES** ethanesulfonic acid IC_{20} Inhibitory concentration at 20% growth Inhibitory concentration at 50% growth IC_{50} KCl Potassium chloride kDa Kilodalton Kg Kilogram KH₂PO₄ Monobasic potassium phosphate KOH Potassium hydroxide Monoclonal antibody Mab **MDR** Multidrug resistance mdr-1 Multidrug resistance gene The isoforms of mdr-1 gene in human MDR-1, MDR-3 The isoforms of mdr-1 gene in rodents mdr-1a, mdr-1b Milligram mg Magnesium chloride MgCl, Minute Millilitre ml Millimole 3-(4,5 dimethylthiazole-2yl)-2,5 MTT diphenyltetrazolium bromide) Na,CO, Sodium carbonate Dibasic sodium phosphate Na,HPO4

Sodium chloride

NaCl

NaH₂PO₄ Monobasic sodium phosphate

NaHCO₃ Sodium bicarbonate

NaOH Sodium hydroxide

ng Nanogram

nm Nanometre

PBS Phosphate buffer saline

Pgp P-glycoprotein

PMSF Phenylmethyl sulfonyl fluoride

POPOP 1,4-bis[2-(5-phenyloxazolyl)]benzene

PPO 2,5-Diphenyloxazole

Rh123 Rhodamine123

rpm Revolution per minute

SDS-PAGE Sodium dodecyl sulfate-polacrylamide gel

Electrophoresis

TEMED N,N,N,N,-tetramethyl ethylene-diamine

THF Tetrahydrofuran

TM Transmembrane

Tris-base Tris (hydroxymethyl aminomethane)

VBL Vinblastine

Ver Verapamil

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