# **CONTENTS**

	Page
Acknowledgement	d
Abstract in Thai	f
Abstract in English	i
List of Tables	q
List of Figures	r
List of Abbreviations	u
Statement of Originality in Thai	W
Statement of Originality in English	X
Chapter 1 Introduction	1
1.1Statement of the problems	1
1.2 Literature reviews	3
1.2.1 Ovarian cancer statistics and epidemiology	3
1.2.2 Classification and histological subtypes	4
1.2.3 Ovarian cancer diagnosis, staging and treatment	6
1.2.4 Chemotherapeutic drugs used in ovarian cancer	7
1.2.5 Platinum drug resistance mechanisms in ovarian cancer	8
Decreased intracellular drug accumulation and	9
platinum resistance	
Intracellular drug inactivation and platinum resistance	10
3) Alteration in apoptotic pathways and platinum	10
resistance	
4) Alteration in intracellular signaling pathways and	13
platinum resistance	

5) Inflammatory cytokine and chemokine networks and	14
platinum resistance	
6) Increased DNA repair and platinum resistance	18
1.2.6 Drug resistance mechanisms in human ovarian cancer cell	20
lines (SKOV3, A2780 and A2780/cis)	
1.2.7 Role of natural products in the treatment of cancer	20
1.2.8 Alkaloids from Stephania venosa	21
1.2.9 Effects of alkaloids from Stephania venosa	22
1.3 Objectives	23
	2.4
Chapter II Materials and Methods	24
2.1 Reagents and chemicals	24
2.2 Preparation of alkaloids from <i>Stephania venosa</i>	24
2.3 Ovarian cancer cell lines and maintenance	24
2.4 Patients and samples	25
2.5 Primary cell culture from solid tumor tissues of ovarian cancer	25
patients	
2.6 Treatment with alkaloids and drugs	27
2.7 Measurement parameter	27
2.7.1 MTT cell viability assay	27
2.7.2 Combination index analysis	28
2.7.3 Guava apoptotic assay	28
2.7.4 Determination of IL-6 by ELISA assay	29
2.7.5 Tumor cell invasion assay	29
2.7.6 Colony formation assay	30
2.7.7 Gelatin zymography	30
2.7.8 Extraction of whole cell proteins and nuclear proteins	31
2.7.9 Western blotting	31
2.7.10 Statistical analysis	32
Chapter III Results	33

3.1 Sensitivity to platinum drugs in ovarian cancer cells	33
3.2 Cytotoxic effects of alkaloids from Stephania venosa in ovarian	34
cancer cells	
3.3 Effects of alkaloids on cisplatin sensitivity in ovarian cancer cells	36
3.3.1 Effects of alkaloids on cisplatin sensitivity in SKOV3 cells	36
3.3.2 Effects of alkaloids on cisplatin sensitivity in A2780 cells	39
3.3.2 Effects of alkaloids on cisplatin sensitivity in A2780/cis	42
cells	
3.4 Effect of CN and OMBC on SKOV3 cells apoptosis	44
3.4.1 Effect of CN and OMBC on SKOV3 cells apoptosis	44
accessed by flow cytometry	
3.4.2 Effect of CN and OMBC on SKOV3 cells apoptotic	48
proteins expression by Western blotting	
3.5 Effect of CN and OMBC on SKOV3 cell survival proteins	50
expression	
3.5.1 Effect of CN and OMBC on cisplatin-induced anti-	50
apoptotic and survival proteins	
3.5.2 Effect of CN and OMBC on cisplatin-induced IL-6	52
expression	
3.6 Signaling molecules and transcription factors induced by	54
cisplatin treatment in SKOV3 cells	
3.7 Akt/NF-κB signaling pathway is concerned with cisplatin	57
resistance in SKOV3 cells	
3.7.1 Effect of PI3K inhibitor on cisplatin-augmented NF-κB	57
activity in SKOV3 cells	
3.7.2 Effect of PI3K inhibitor on cisplatin sensitivity in SKOV3	58
cells	
3.8 Effect of CN and OMBC on Akt/NF-κB signaling in SKOV3	59
cells	
3.8.1 Effect of CN and OMBC on cisplatin-augmented Akt	59
activity in SKOV3 cells	

3.8.2 Effect of CN and OMBC on cisplatin-augmented NF-κB	61
activity in SKOV3 cells	
3.9 Determination of IL-6 production from ovarian cancer cells	63
3.10 Effects of neutralization and addition of exogenous IL-6 in	64
SKOV3 cells	
3.10.1 Effects of neutralization of IL-6 on platinum sensitivity in	64
SKOV3 cells	
3.10.2 Effects of addition of exogenous IL-6 on platinum	65
sensitivity in SKOV3 cells	
3.11 Effect of crebanine on IL-6 induced SKOV3 cells survival and	66
proliferation	
3.11.1 Effect of crebanine on IL-6 induced anti-apoptotic	66
proteins and survival proteins expression by Western	
blotting	
3.11.2 Effect of crebanine on IL-6 induced SKOV3 cells colony	67
formation by clonogenic assay	
3.12 Effect of crebanine on IL-6 induced SKOV3 cells invasion and	69
migration	
3.12.1 Effect of crebanine on IL-6 induced SKOV3 cells	69
invasion detected by using Transwell invasion chamber	
3.12.2 Effect of crebanine on IL-6 induced SKOV3 cells	71
invasion and migration proteins expression	
3.13 Phosphorylation of STAT3 in SKOV3 cells was suppressed by crebanine	73
3.14 Characteristics of the included patients and their response to	75
chemotherapy	
3.15 Platinum induced IL-6 production in ex-vivo studies	77
3.16 Effect of crebanine on platinum sensitivity in patients' samples	79
with high IL-6 production induced by carboplatin	
Chapter IV Discussion	81

Conclusion 87	7
Further Studies 89	9
References 90	0
List of publications 10	)2
Appendix	
Appendix A 102	)3
Appendix A  Appendix B  Appendix C  102  103	)5
Appendix C	)7
Curriculum Vitae	2
CAT UNIVERSITA	
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright <sup>©</sup> by Chiang Mai University All rights reserved	

# LIST OF TABLES

P	Page
Table 3.1 Sensitivity to platinum drugs in ovarian cancer cell lines	33
Table 3.2 IC <sub>50</sub> of the alkaloids in ovarian cancer cell lines	34
Table 3.3 Combination index (CI) values of cisplatin and CN or OMBC in	39
SKOV3 cells	
Table 3.4 Characteristics of included patients	76
Table 3.5 The relationships of platinum-induced IL-6 production in ex-vivo	78
studies and the clinical responses to chemotherapy among	
ovarian cancer patients	
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright <sup>©</sup> by Chiang Mai University All rights reserved	

# LIST OF FIGURES

	Page
Figure 1.1 Anatomy of female genital tract showing uterus, fallopian tubes	3
and ovaries	
Figure 1.2 The picture illustrates the different types of ovarian tumors	5
Figure 1.3 Structures of cisplatin and carboplatin	7
Figure 1.4 Schematic diagram shows the mechanisms of platinum	9
resistance in ovarian cancer	
Figure 1.5 Schematic representation of the intrinsic and extrinsic apoptotic	11
pathway	
Figure 1.6 Role of Akt and NF-κB signaling in drug resistance mechanism	12
of cancer cell	
Figure 1.7 Cisplatin-induced activation of PI3K/Akt and PI3K/JNK	13
signaling pathway	
Figure 1.8 Role of chemokines/cytokines in cancer growth and progression	14
Figure 1.9 IL-6 signaling cascade	15
Figure 1.10 Schematic illustration of IL-6 production by cancer cells upon	17
chemotherapy treatment	
Figure 1.11 Role of XRCC1 and ERCC1 in DNA repair pathways	19
Figure 1.12 Photograph of Stephania venosa which is known as Sabuleud	21
in Thailand	
Figure 1.13 Chemical structures of CN, OMBC, THP and NMTHP isolated	22
from S. venosa	
Figure 2.1 Steps involved in establishing primary cell culture from solid	26
tumor tissues from ovarian carcinoma patients	
Figure 3.1 Cytotoxic effect of CN, OMBC, THP and NMTHP in ovarian	35

cancer cell lines accessed by MTT assay	
Figure 3.2 Effects of CN, OMBC, THP and NM-THP on cisplatin drug	38
sensitivity in SKOV3 ovarian cancer cells	
Figure 3.3 Effects of CN, OMBC, THP and NM-THP on cisplatin drug	41
sensitivity in A2780 ovarian cancer cells	
Figure 3.4 Effects of CN, OMBC, THP and NM-THP on cisplatin drug	43
sensitivity in A2780/cis ovarian cancer cells	
Figure 3.5 Effect of CN on SKOV3 cells apoptosis	45
Figure 3.6 Effect of OMBC on SKOV3 cells apoptosis	47
Figure 3.7 CN and OMBC enhanced cisplatin sensitivity in SKOV3 cells	49
via induction of apoptosis-induced cell death	
Figure 3.8 CN and OMBC enhanced cisplatin sensitivity in SKOV3 cells	51
via induction of anti-apoptotic and survival proteins expression	
Figure 3.9 CN and OMBC enhanced cisplatin sensitivity in SKOV3 cells	53
via inhibition of cisplatin-induced IL-6 production	
Figure 3.10 Cisplatin-induced augmentation of intracellular signaling	56
molecules and transcription factors in SKOV3 cells	
Figure 3.11 Effect of PI3K/Akt inhibitor on cisplatin-augmented NF-κB	57
activity in SKOV3 cells	
Figure 3.12 Effect of PI3K/Akt inhibitor on cisplatin sensitivity in SKOV3	58
Figure 3.13 Effect of CN and OMBC on cisplatin-augmented Akt activity	60
in SKOV3 cells	
Figure 3.14 Effect of CN and OMBC on cisplatin-augmented NF-κB	62
activity in SKOV3 cells	
Figure 3.15 Platinum-induced cytokines secretion in SKOV3 ovarian	63
cancer cells	
Figure 3.16 Effect of neutralization of IL-6 on platinum sensitivity in	64
SKOV3 cells	
Figure 3.17 Effect of addition of exogenous IL-6 on platinum sensitivity in	65
SKOV3 cells	
Figure 3.18 Effect of crebanine on IL-6 induced anti-apoptotic proteins and	66

survival proteins expression	
Figure 3.19 Effect of crebanine on IL-6 induced SKOV3 cells colony	68
formation by clonogenic assay	
Figure 3.20 Effect of crebanine on IL-6 induced SKOV3 cells invasion	70
Figure 3.21 Effect of crebanine on IL-6 induced MT1-MMP protein	72
expression by Western blotting and MMP-9 protein secretion	
by gelatin zymography	
Figure 3.22 Effect of crebanine on IL-6 induced expression of STAT3 and	74
STAT3 phosphorylation	
Figure 3.23 Effect of crebanine on platinum sensitivity in patients' samples	80
with high IL-6 production induced by carboplatin	
Figure 4.1 Scheme illustrates summary effects of aporphine alkaloids from	88
S. venosa as chemosensitizer and inhibitor of ovarian cancer	
Aggressiveness	

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

### LIST OF ABBREVIATIONS

7-AAD 7-aminoactinomycin D

Akt Protein kinase B

AP-1 Activator protein 1

APS Ammonium persulfate

BER Bas excision repair

BRAF B-raf proto-oncogene

BRCA Breast cancer susceptibility gene

CI Combination index

cIAP-2 Baculoviral IAP repeat-containing protein 3

Cisplatin Cis -diamminedichloroplatinum-II

CN Crebanine

CTR1 Copper transporter 1

DTT Dithiothreitol

EDTA Ethylene diamine tertaacetic acid

EGTA Ethylene glycol-bis (β-aminoethyl ether) tetraacetic acid

ERCC1 Excision repair cross complementation group 1

ERK Extracellular signal-regulated kinase

FBS Fetal bovine serum

HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

IL-6 Interleukin-6

JNK c-Jun N-terminal kinase

KCl Potassium chloride

kDa Kilodaltons

KRAS Kristen ras oncogene

MAPK Mitogen activated protein kinase

ml Mililiter

mM Milimolar

MMPs Matrix metalloproteinase

MRP Multidrug resistant related protein

MT1-MMP Membrane type 1 metalloproteinase

MTT 3-(4, 5-dimethylthiazol2-yl)-2, 5-diphenyltetrazolium bromide

NaCl Sodium chloride

NER Nucleotide excision repair

NF-κB Nuclear Factor kappa-light-chain-enhancer of activated B cells

NMTHP *N*-methyl tetrahydropalmatine

OMBC *O*-methylbulbocapnine

NP 40 Nonidet P-40

pg Picogram

PI3K Phosphatidylinositol 3-kinase

PMSF Phenylmethylsulfonyl fluoride

PS Phosphatidylserine

SDS Sodium dodecyl sulfate

STAT3 Signal transducer and activator of transcription 3

TBS Tris Buffered Saline

TEMED Tetramethylethylenediamine

THP Tetrahydropalmatine

TMB Tetramethylbenzidine

μg Microgram

XRCC1 X-ray repair cross-complementing group 1

by Chiang Mai University

# ข้อความแห่งการริเริ่ม

- 1) ปัญหาหลักของการรักษาโรคมะเร็งรังไข่คือการคื้อยาในกลุ่มแพลตตินัมและการกลับมาเป็น โรคซ้ำเนื่องมาจากข้อจำกัดของการรักษามะเร็งรังไข่ดังนั้นจึงเป็นที่น่าสนใจหากมืองค์ความรู้ที่ มีประสิทธิภาพในการรักษามะเร็งรังไข่ ดังนั้นการศึกษาครั้งนี้จึงมุ่งเน้นในการลดการคื้อยาและ ปรับปรุงการรักษามะเร็งรังไข่
- 2) การศึกษาครั้งนี้มุ่งเน้นในการศึกษาประสิทธิภาพการเพิ่มความไวต่อยาเคมีบำบัดของครีบานีน และอนุพันธ์ชนิด โอ-เมททิลบูโบแคฟนีนในเซลล์มะเร็งรังไข่ที่ดื้อยาเคมีบำบัด โดยครีบานีน และโอ-เมททิลบูโบแคฟทีนที่อาจจะสามารถใช้เป็นสารช่วยเสริมประสิทธิภาพการเพิ่มความ ไวของยาเคมีบำบัดชนิดแพลตตินัม
- จากผลการทคลองพบว่าผลของครีบานีนและโอ-เมททิลบูโบแคฟนีนในการเพิ่มความไวของยา เคมีบำบัคสามารถนำไปประยุกต์ใช้และพัฒนาเพื่อจุดประสงค์ในปรับปรุงการรักษามะเร็งรัง ไข่ได้ กล่าวอีกนัยหนึ่งคือ ผลการศึกษาในเซลล์มะเร็งที่แยกได้จากผู้ป่วยมะเร็งรังไข่เป็นองค์ ความรู้ที่ลึกกว่าเคิมที่จะช่วยประเมินการตอบสนองต่อยาเคมีบำบัคสำหรับผู้ป่วยมะเร็งรังไข่แต่ ละราย

ลิ**ปสิทธิ์มหาวิทยาลัยเชียงใหม**่ Copyright<sup>©</sup> by Chiang Mai University All rights reserved

## STATEMENT OF ORIGINALITY

- 1. The major problems in the management of ovarian cancer is the platinum drug resistance and recurrence. Because of the limited efficacy of current treatments for advanced ovarian cancer, novel and more effective therapies are investigated. Thereby, investigations aiming to overcome the drug resistance and to improve the therapeutic strategies for the disease are still necessary and interesting.
- 2. This thesis was proposed for the chemosensitizing effects of CN and OMBC on ovarian cancer cells. The study results presented that CN and OMBC would be an effective adjuvant agent to sensitize the platinum-based chemotherapeutic drugs.
- 3. By the study results, advantage effects of CN and OMBC would be further applied and developed in aiming to improve the therapeutic strategies for ovarian cancer. On the other hand, the current results of *ex-vivo* studies from tumor tissue of ovarian cancer patients serves as the basis for a deeper investigation into drug responses and the establishment of personalized cancer therapy for ovarian cancer patients.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright<sup>©</sup> by Chiang Mai University All rights reserved