## **CONTENTS**

		Page
ACKNOWLED	OGEMENTS	iii
ABSTRACT (E	ENGLISH)	iv
ABSTRACT (T	THAI)	v
LIST OF TABI	LES	viii
LIST OF FIGU	URES	ix
LIST OF ABBI	REVIATIONS	xi
CHAPTER I	INTRODUCTION	1
CHAPTER II	OBJECTIVES	3
CHAPTER III	LITURATURE REVIEWS	4
3.1 Meth	amphetamine	4
3	.1.1 Mechanism of action of METH	6
3.2 Caffe	eine	7
3.3 Auto	phagy	9
3	.3.1 Signaling pathways regulating mammalian autophagy	10
	3.3.1.1 mTOR-dependent signaling	10
	3.3.1.2 mTOR-independent signaling	10
3	.3.2 Autophagy contributing to cell survival or cell death?	12
3	.3.3 Detection of autophagy	13
3.4 Apop	otosis	14
3	.4.1 Feature of apoptosis	14
3	.4.2 Mechanism of apoptosis	15
3	.4.3 Significant of apoptosis	16
3.5 Relat	tion of METH to Apoptosis and Autophagy	18
3.6 Relat	tion of caffeine to Apoptosis and Autophagy	21
CHAPTER IV	MATERIALS AND METHODS	23
4.1 Chen	nicals and Reagents	23

## **CONTENTS** (cont.)

	Page
4.2 Instruments	24
4.3 Cell cultures	24
4.3.1 Cell viability assay	24
4.3.2 Western blot analysis	25
4.3.3 Morphological detection	26
4.4 Statistical analysis	26
CHAPTER V RESULTS	27
CHAPTER VI DISCUSSION	49
CHAPTER VII CONCLUSION	55
REFERENCES	56
APPENDIX	63
BIOGRAPHY	69

### LIST OF TABLES

Table		Page
5.1	Effect of 3-MA on cell viability in the combination	45
	treatment of METH and caffeine.	

### LIST OF FIGURES

Figu	igure	
3.1	Structural diagram of Dopamine and Methamphetamine	6
3.2	Structural diagram of Adenosine and Caffeine	7
3.3	Model for autophagy	11
3.4	Diagrammatic representation of the event of apoptosis	15
3.5	Two main signaling cascades lead to apoptosis	17
3.6	Proposed model for METH-induced neurotoxicity	20
4.1	MTT reduction in live cells by mitochondrial reductase results	25
	in formation of insoluble formazan, characterized by high	
	absorptivity at 570 nm.	
5.1	The effect of METH on cell viability	28
5.2	The effect of caffeine on cell viability.	29
5.3	Effect of METH and caffeine co-treatment on the viability of	31
	SH-SY5Y cells.	
5.4	The effect of METH on LC3 expression.	32
5.5	Effect of METH-induced increase in LC3II protein levels and	34
	effect of 3-MA-induced induction of cleaved caspase-3 protein	
	levels in SH-SY5Y cultured cells.	
5.6	Effect of autophagy inhibitor, 3-MA, on cell viability in SH-SY5Y	36
	cultured cells.	
5.7	Effect of co-treatment with METH and caffeine on LC3II protein	38
	levels in SH-SY5Y cultured cells.	
5.8	Effect of co-treatment between METH and caffeine on p-mToR	40
	and p-4Ebp1 protein levels in SH-SY5Y cultured cells.	
5.9	Effect of co-treatment between METH and caffeine in the	42
	presence of ammonium chloride on LC3II protein levels in	
	SH-SY5Y cultured cells.	

# **LIST OF FIGURES (cont.)**

Figure		Page	
5.10	Effect of co-treatment between METH and caffeine on	43	
	cleaved caspase-3 protein levels in SH-SY5Y cultured cells.		
5.11	Effect of autophagy inhibitor, 3-methyladenine (3-MA),	45	
	on cell viability in SH-SY5Y cultured cells.		
5.12	Effect 3-MA induced induction of cleaved caspase-3 protein	46	
	levels in SH-SY5Y cultured cells treated with caffeine.		
5.13	Morphology of SH-SY5Y neuroblastoma cells.	48	

#### LIST OF ABBREVIATIONS

microliter μl micrometer μm micro molar μM

3-MA 3-Methyl adenine AD Alzheimer's disease

AIF apoptosis inducing factor **AMP** adenosine monophosphate

AMP-activated protein kinase **AMPK** 

APAF-1 apoptotic protease-activating factor 1

**APS** ammonium persulfate **ATG** autophagy-related gene ATP adenosine triphosphate Bcl-2 B-cell lymphoma 2 BH3

Bcl-2 homology 3

 $Ca^{2+}$ calcium CAF caffeine

BID

**CASPASE** cyteinyl aspartate specific protease **CMA** chaperone mediated autophagy

BH3 interacting-domain death agonist

CNS central nervous system

carbon dioxide  $CO_2$ 

DA dopamine

DAT dopamine transporter

**DMEM** Dulbecco's modified eagle's medium

**DMSO** dimethyl sulfoxide

DOC deoxylcholate

**ECL** enhancement chemiluninasence **EDTA** ethylenediaminetetraacetic acid

ER endoplasmic reticulum

### **LIST OF ABBREVIATIONS (cont.)**

ETC electron transport chain

FBS fetal bovine serum

 $\begin{array}{ll} g & gram \\ H_2O & water \end{array}$ 

H<sub>2</sub>O<sub>2</sub> hydrogen peroxideHCl hydrochloride

HRP horseradish peroxidase

hsc70 heat shock cognate protein of 70 kDa IC<sub>50</sub> half maximal inhibitory concentration IMPase inhibition of inositol monophosphatase IP<sub>3</sub> inositol and inositol 1,4,5-trisphosphate

KCl potassium chloride

kDa kilodalton kg Kilogram

KH<sub>2</sub>PO<sub>4</sub> potassium dihydrogen phosphate

l liter

LAMP lysosome-associated membrane protein

LBs lewy bodies

LC3 microtubule-associated protein 1 light chain 3

MAO monoamine oxidase

MEM minimum essential medium

METH methamphetamine

mg milligram
ml milliliter
mM millimolar
mm millimeter

MMP mitochondrial membrane permeabilization

mRNA messenger ribonucleic acid

mtDNA mitochondrial deoxyribonucleic acid

### **LIST OF ABBREVIATIONS (cont.)**

MTOR mammalian target of rapamycin

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

Na2HPO4 disodium hydrogen phosphate

NaDC sodium deoxycholate

NaF sodium fluoride NaOH sodium hydroxide NH4Cl ammonium chloride

nm nanometer

O2-· superoxide radical

PBS phosphate buffer saline

PBST phosphate buffer saline with tween-20

PD Parkinson's disease

PVDF polyvinylidenedifluoride

Rag Recombination activating genes

ROS reactive oxygen species
SDS sodium dodecyl sulfate

SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SEM the standard error of the mean
SNpc substantial nigra par compacta
TIDA Thanyalak institute for drug abuse

TNF tumor necrosis factor

TNFR tumor necrosis factor receptor

TRADD tumor necrosis factor receptor-associated death domain

U unit

UNODC The united nations office on drugs and crime

v volume

VMAT-2 vesicular monoamine transporter-2

w weight