TABLE OF CONTENTS

	pages
ACKNOWLEDGMENTS	iii
ABSTRACT (IN ENGLISH)	iv
ABSTRACT (IN THAI)	v
TABLE OF CONTENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	X
LIST OS ABBREVIATIONS	xiii
CHAPTER I INTRODUCTION	1
1. Carcinogenesis	1
2. Normal and cancer cell biochemistry and physiology	3
2.1 Cellular energetic state	3
2.2 Cellular redox state	5
2.2.1 Cellular oxidants and antioxidants	5
2.2.2 The beneficial functions of reactive oxygen species	10
pyright (ROS) Chiang Mai Univer	
2.3 Cellular oxidative stress	e 13
2.4 Growth/ death	14
3 Flavonoids	18

2	4. Objectives	20
CHAPTER	R II MATERIALS AND METHODS	21
	1. Cell lines and culture conditions	21
	2. Mn-SOD cDNA transfection of rat cells	21
	3. SOD activity gel assay	22
	4. Cell growth assay	23
4	5. Quercetin treatment	23
	6. Determination of reactive oxygen species production	23
1 500°	7. Immunofluorescent staining for 4-hydroxynonenal	24
	8. Immunofluorescent staining for microtubule-associated protein	
	light chain 3 (MAP-LC3)	25
9	9. Cell staining procedures with Hoechst 33342	25
	10. Statistic Analysis	26
CHAPTER	R III RESULTS	27
1	1. Selection of stable Mn-SOD transfected clones	27
2	2. Effects of <i>Mn-SOD</i> gene transfect on growth rate	28
dan	3. Intracellular reactive oxygen species (ROS _i)	30
opyrig	4. Determination of 4-HNE Protein adducts	33
	5. Quercetin inhibits cell growth	39
(6. Quercetin induced autophagy	40
5	7. Determination of pycnotic nuclei in apoptotic cells	44
CHAPTER	R IV DISCUSSION	45

CHAPTER V CONCLUSION	49
REFERENCES	50
APPENDIX ON SIELLE OF THE SECOND OF THE SECO	64
CURRICULUM VITAE	70

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

LIST OF TABLES

Table	page
1. Principal cellular anti-oxidants that scavenge or inactivate	
excessive ROS and thereby protect cells from oxidative damage.	8
2. The specific growth rate of RGM1, RGK1 and Mn-SOD transfected	
cells	31

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

LIST OF FIGURES

Figure	Page
1. The generation of ROS by mitochondria.	6
2. ROS generated by microsomal mono-oxygenases, which have cytochrome	e
P450 as a central link and ROS generated by phagocytes kill infectious	S
microorganisms and cancer cells.	7
3. Schematic representation of apoptosis. ROS generated by mitochondria are	e
essential mediators of apoptosis.	12
4. The non-denatured gel of Mn-SOD activity assay.	28
5. Growth curves of RGM1, Mn-SOD transfected RGM1 clone no. 6 and Mn-	
SOD transfected RGK1 clone no. 10 cells	29
6. Confocal micrographs of untreated RGM1 (i) and RGK1 (iv) and treated with	
$100~\mu\text{M}$ (ii, v) and $200~\mu\text{M}$ (iii, vi) quercetin, respectively. Cells were stained	
with HPF at 24 hours after treatments according to materials and methods.	31
7. Confocal micrographs of untreated Mn-SOD transfected RGM1 clone no. 6 (i)31
and Mn-SOD transfected RGK1 clone no. 10 (iv) and treated with 100 μM (ii	, I 1.
v) and 200 μM (iii, vi) quercetin, respectively. Cells were stained with HPF a	t
24 hours after treatments according to materials and methods.	31
8. Effects of Mn-SOD and exogenous quercetin (QT) on ROS _i ; for Mn-SOD in	1
RGM1 and RGK1 cells (a) and for indicated concentration of querceting	1
demined at 2 hours (b) and 24 hours (c).	33

9. Confocal micrographs of Mn-SOD transfected RGM1 clone no. 6 in the	
presence of 0.01% (v/v) DMSO for 2 and 24 hours.	34
10. Confocal micrographs of RGM1, RGK1, Mn-SOD transfected RGM1 clone	
no. 6 and RGK1 clone no. 10 cells in the presence of 0.01% (v/v) DMSO for 2	
and 24 hours.	35
11. Confocal micrographs of RGM1 and Mn-SOD transfected RGM1 clone no. 6	
cells in the presence of indicated concentration of quercetin for 2 and 24 hours	36
	30
12. Confocal micrographs of RGK1 and Mn-SOD transfected RGK1 clone no. 6	
cells in the presence of indicated concentration of quercetin for 2 and 24 hours	37
13. The 4-HNE protein adduct contents without (a, b) and with indicated the	
concentration of quercetin (c, d) determined at 2 and 24 hours of RGM1, Mn-	
SOD transfected RGM1, RGK1 and Mn-SOD transfected RGK1 cells.	38
14. The effects of quercetin on the specific growth rate in RGM1, Mn-SOD	
transfected RGM1 clone no. 6, RGK1 and Mn-SOD transfected RGK1 clone	
no. 10 cells.	39
15. Light micrographs of RGM1 and RGK1 cells without (i, iii) and treated with	
quercetin 200 μM (ii, iv). The arrows show vacuole-like structures in the	
Cytoplasm. by Chiang Mai University	40
16. Characterization of autophagic cells by immunofluorescence technique,	
using goat anti-MAP-LC3 IgG as primary antibody and Alexa Fluor 488	
donkey anti goat IgG as secondary antibody. Confocal micrographs of RGM1	
and RGK1 without (i, iii) and treated with quercetin 200 μM (ii, iv)	42

17. Determination of autophagic cells by using goat anti-MAP-LC3 IgG as primary antibody and Alexa Fluor 488 donkey anti goat IgG as secondary antibody. Confocal micrographs of Mn-SOD transfected RGM1 and Mn-SOD transfected RGK1 cells without (i iii) and treated with quercetin 200 μM (ii, iv).

18. The relative MAP-LC3 staining intensity in RGM1, RGK1 and their corresponding Mn SOD transfected cells (a) and the presence of quercetin for 24h (b) using goat anti-MAP-LC3 IgG as primary antibody and Alexa Fluor 488 donkey anti goat IgG as secondary antibody

43

44

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

ABBREVIATIONS

ROS reactive oxygen species

ROS_i intracellular reactive oxygen species

ATP adenosine triphosphate

O₂ superoxide anion radicals

OH hydroxyl radicals

H₂O₂ hydrogen peroxide

ETC electron transport chain

SOD superoxide dismutase

Mn-SOD manganese superoxide dismutase

CuZn-SOD copper-zinc superoxide dismutase

EC-SOD extracellar superoxide dismutase

CAT catalase

AO anti-oxidant

RGM1 rat normal gastric mucosal cell line

RGK1 N-methyl-N'-nitro-N-nitrosoguanidine induced gastric

cancer cell line

MNNG N-methyl-N'-nitro-N-nitrosoguanidine

NBT nitro blue tetrazolium

TEMED N,N,N',N'-Tetramethylethylenediamine

HPF hydroxyphenyl fluorescein

4-HNE 4-hydroxynonenal

MAP-LC3 microtubule-associated protein light chain 3

PBS phosphate-buffered saline

γ specific growth rate

