

# CONTENTS

	Page
Acknowledgement	c
Abstract in Thai	d
Abstract in English	g
List of Tables	m
List of Figures	n
List of Abbreviations	q
List of Symbols	t
Chapter 1 Introduction	1
1.1 Statement of the problem	1
1.2 Literature reviews	3
1.2.1 Mechanism of chemical-induced carcinogenesis	3
1.2.2 Mutagenesis and carcinogenesis	5
1.2.3 Short-term mutagenicity tests	5
1.2.4 Xenobiotic metabolism	8
1.2.4.1 Phase I xenobiotic metabolizing enzymes	10
1.2.4.2 Phase II xenobiotic metabolizing enzymes	12
1.2.5 Toxicity of aflatoxin B <sub>1</sub>	15
1.2.6 Phytochemicals and chemoprevention	17
1.2.7 Modes of action of cancer chemopreventive agents	21
1.2.8 Purple rice bran	23
1.3 Objectives	25

	<b>Page</b>
Chapter 2 Materials and methods	26
2.1 Chemicals and instruments	26
2.2 Animals	26
2.3 Extraction and isolation of purple rice bran	26
2.4 Determination of major constituents of purple rice bran extracts	28
2.5 Identification of some phenolic compounds in purple rice bran extracts	29
2.5.1 Phenolic acids, flavonoids and anthocyanins	29
2.5.2 Gamma-oryzanol	29
2.5.3 Tocopherols and tocotrienols	29
2.6 Clastogenicity of purple rice bran extract	30
2.7 Partial hepatectomy	31
2.8 Liver perfusion and isolation of hepatocytes	31
2.9 Determination of micronucleus	34
2.10 Optimization of genotoxic concentration of AFB <sub>1</sub> induced liver micronucleus formation in rats	35
2.11 Anticlastogenicity of purple rice bran extract	35
2.12 Preparation of cytosolic and microsomal fractions	38
2.13 Determination of protein concentration	39
2.14 Determination of Phase I metabolizing enzyme activities	39
2.14.1 Cytochrome P450 isozymes 1A1 and 1A2	39
2.14.2 Cytochrome P450 isozyme 3A2	40
2.14.3 NADPH-cytochrome P450 reductase	41
2.15 Determination of Phase II metabolizing enzyme activities	42
2.15.1 Glutathione-S transferase	42
2.15.2 UDP-glucuronyltransferase	43
2.16 Determination of protein expression of phase I and II metabolizing enzymes	44
2.17 Statistical analysis	44

	<b>Page</b>
Chapter 3 Results	45
3.1 Evaluation of major constituents in purple rice bran extracts	45
3.2 Effect of purple rice bran extracts on micronucleus formation in rat liver	51
3.3 Effect of purple rice bran extracts on xenobiotic metabolizing enzymes	51
3.4 Optimization of AFB <sub>1</sub> on micronucleus formation in rat liver	59
3.5 Effect of purple rice bran extracts on micronucleus formation induced by AFB <sub>1</sub>	64
3.6 Inhibitory mechanism of purple rice bran extracts on xenobiotic metabolizing enzymes induced by AFB <sub>1</sub>	64
Chapter 4 Discussion and conclusion	74
References	78
Appendices	90
Appendix A	90
Appendix B	93
Appendix C	94
Appendix D	101
Curriculum Vitae	103

## LIST OF TABLES

	<b>Page</b>
Table 1 Major compounds in purple rice bran extracts	46
Table 2 Effect of purple rice bran extracts on body weight and micronucleus formation in rat liver	52
Table 3 Effect of purple rice bran extracts on the activities of xenobiotic metabolizing enzymes in rat liver	54
Table 4 Effect of purple rice bran extracts on the expression of xenobiotic metabolizing enzymes in rat livers using Western blot analysis	57
Table 5 Effect of AFB <sub>1</sub> administration on micronucleus formation in rat liver	60
Table 6 Effect of AFB <sub>1</sub> treatments on the activities of some major xenobiotic metabolizing enzymes in AFB <sub>1</sub> metabolism in rat liver	62
Table 7 Effect of purple rice bran extracts on micronucleus formation induced by AFB <sub>1</sub>	65
Table 8 Effect of purple rice bran extracts on the activities of phase I xenobiotic metabolizing enzymes activated by AFB <sub>1</sub> in rat liver	67
Table 9 Effect of purple rice bran extracts on the activities of phase II xenobiotic metabolizing enzymes activated by AFB <sub>1</sub> in rat liver	68
Table 10 Effect of purple rice bran extracts on the expression of xenobiotic metabolizing enzymes induced by AFB <sub>1</sub>	71
Table 11 Total phenolic compounds and flavonoids in purple rice bran extracts	102

## LIST OF FIGURES

	<b>Page</b>
Figure 1 Multistage carcinogenesis	3
Figure 2 Mechanism of carcinogens	4
Figure 3 Formation of liver micronucleus	7
Figure 4 Xenobiotic metabolism	9
Figure 5 Catalytic cycle of cytochrome P450	11
Figure 6 UDP-glucuronyltransferase reaction of bilirubin	13
Figure 7 Reaction of sulfotransferase	13
Figure 8 Reaction of glutathione <i>S</i> -transferase	14
Figure 9 Metabolism and toxicity of AFB <sub>1</sub>	16
Figure 10 Classification of dietary phytochemicals	19
Figure 11 Structures of phytochemical found in purple rice	20
Figure 12 Roles of phytochemical chemopreventive agents	22
Figure 13 The components of rice grain	24
Figure 14 Purple rice	24
Figure 15 The extraction of purple rice bran	27
Figure 16 Clastogenicity of purple rice bran extracts in liver of rats	30
Figure 17 Anatomy of rat liver	31
Figure 18 The procedure of partial hepatectomy	32
Figure 19 The procedure of liver perfusion and isolation of hepatocytes	33
Figure 20 The procedure of microscopic observation and micronucleus determination	34
Figure 21 Effect of AFB <sub>1</sub> treatment on liver micronucleus formation in rat	36
Figure 22 Anticlastogenicity of purple rice bran extracts	37
Figure 23 Preparation of cytosolic and microsomal fractions	38
Figure 24 Principle of the measurement of CYP 1A activity	39
Figure 25 Principle of the measurement of CYP 3A activity	40
Figure 26 The function of NADPH-cytochrome P450 reductase	41

	<b>Page</b>
Figure 27 The reaction of glutathione-S transferase	42
Figure 28 The reaction of UDP-glucuronyltransferase	43
Figure 29 Purple rice bran extracts	45
Figure 30 Chromatograms of standard phenolic acid and flavonoids and some phenolic acids in methanol extract of purple rice bran	47
Figure 31 Chromatograms of standard anthocyanins and some anthocyanins in methanol extract of purple rice bran	48
Figure 32 Chromatograms of standard gamma-oryzanol and some gamma- oryzanol in dichloromethane extract of purple rice bran	49
Figure 33 Chromatograms of standard tocols and some tocols in dichloromethane extract of purple rice bran	50
Figure 34 Microscopic morphology of hepatocytes	51
Figure 35 The number of hepatic micronuclei of purple rice bran extracts- treated rats	53
Figure 36 The activities of phase I xenobiotic metabolizing enzymes in rat livers treated by purple rice bran extracts	55
Figure 37 The activities of phase II xenobiotic metabolizing enzymes in rat livers treated by purple rice bran extracts	56
Figure 38 Protein expression of xenobiotic metabolizing enzymes in the livers of purple rice extracts treated rats	58
Figure 39 The frequencies of hepatic micronuclei induced by AFB <sub>1</sub>	61
Figure 40 The activities of some major xenobiotic metabolizing enzymes induced by AFB <sub>1</sub>	63
Figure 41 Inhibitory effect of purple rice bran extracts on micronucleus formation induced by AFB <sub>1</sub>	66
Figure 42 Effect of purple rice bran extracts on the activities of phase I xenobiotic metabolizing enzymes activated by AFB <sub>1</sub> in rat liver	69
Figure 43 Effect of purple rice bran extracts on the activities of phase II xenobiotic metabolizing enzymes activated by AFB <sub>1</sub> in rat liver	70

	<b>Page</b>
Figure 44 Protein expression of liver xenobiotic metabolizing enzymes activated by AFB <sub>1</sub> and treated with purple rice bran extracts	72
Figure 45 Effect of purple rice bran extract against AFB <sub>1</sub> treatment on the protein expression of xenobiotic metabolizing enzymes	73
Figure 46 The alternative extraction of purple rice bran	101



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright© by Chiang Mai University  
 All rights reserved

## LIST OF ABBREVIATIONS

°C	degree celcius
β-NADPH	β-nicotinamide adenine nucleotide phosphate (reduced form)
μg	microgram
μl	microliter
μM	micromolar
μm	micron
AFB <sub>1</sub>	aflatoxin B <sub>1</sub>
AlCl <sub>3</sub>	aluminium chloride
BSA	bovine serum albumin
bw	body weight
CaCl <sub>2</sub>	calcium chlorode
CDNB	1-chloro-2, 4-dinitrobenzene
cm	centimeter
CPR	NADPH-cytochrome P450 reductase
CuSO <sub>4</sub>	copper sulfate
CYP	cytochrome P450
DAPI	4', 6-diamidino-2-phenylindole dihydrochloride
DCPIP	2, 6-dichlorophenolindophenol
DI	deionized water
DMSO	dimethyl sulfoxide
DTT	dithiothreitol
DW	distilled water
EDTA	ethylene diamine tetraaceic acid
EGTA	ethylene glycol tetraacetic acid
FAD	flavin adenine dinucleotide
g	gram
GSH	glutathione (reduced form)



GST	glutathione- <i>S</i> transferase
hr	hour
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid
i.p.	intraperitoneum
IgG	immunoglobulin G
KCl	potassium chloride
KCN	potassium cyanide
kg	kilogram
KH <sub>2</sub> PO <sub>4</sub>	potassium dihydrogen phosphate
KNaC <sub>4</sub> H <sub>4</sub> O	potassium sodium tartrate
KOH	potassium hydroxide
L	liter
M	molar
mg	milligram
MgCl <sub>2</sub>	magnesium chloride
min	minute
ml	milliliter
mm	millimeter
mM	millimolar
MNHEPs	micronucleated hepatocytes
MI	mitotic index
N	normality
NaCl	sodium chloride
NaHCO <sub>3</sub>	sodium bicarbonate
NAH <sub>2</sub> PO <sub>4</sub>	sodium dihydrogen phosphate
Na <sub>2</sub> HPO <sub>4</sub>	disodium hydrogen phosphate
Na <sub>2</sub> CO <sub>3</sub>	sodium carbonate
Na <sub>2</sub> NO <sub>3</sub>	sodium nitrite
NaOH	sodium hydroxide
NSS	normal saline solution
nm	nanometer

PBS	phosphate-buffered saline
PF	perfusion
PH	partial hepatectomy
PMSF	phenylmethanesulphonylfluoride
PNP	<i>p</i> -nitrophenol
rpm	rounds per minute
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TCA	trichloroacetic acid
TEMED	tetramethylethylenediamine
UDP-GA	uridine 5'-diphospho-glucuronic acid
UGT	UDP-glucuronyltransferase

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
 Copyright© by Chiang Mai University  
 All rights reserved

## LIST OF SYMBOLS

$\alpha$  alpha

$\beta$  beta

$\delta$  delta

$\gamma$  gamma

$\lambda$  lamda

$\mu$  micro



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

Copyright© by Chiang Mai University

All rights reserved