

TABLE OF CONTENTS

	Page
ACKNOWLEDMENT	iii
ENGLISH ABSTRACT	iv
THAI ABSTRACT	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
ABBREVIATIONS	xiv
CHAPTER I INTRODUCTION	1
1.1 Statement of the problems	1
1.2 Literature reviews	4
1.2.1 Multistep chemicals carcinogenesis	4
1.2.2 Carcinogenicity test	8
1.2.3 Diethylnitrosamine and hepatocarcinogenesis	13
1.2.4 Cancer chemoprevention and carcinogenesis	14
1.2.5 Xenobiotic-metabolizing enzymes	16
1.2.6 Effect of flavanones on xenobiotic-metabolizing enzymes	24
1.2.7 Pinocembrin	27
1.3 Objectives	28
CHAPTER II MATERIALS AND METHODS	29
2.1 Chemicals and instruments	29
2.2 Animals	29
2.3 Extraction and isolation of pinocembrin from <i>Boesenbergia</i> <i>pandurata</i> (Roxb.) Schltr. rhizome.	29
2.4 Mutagenicity study of pinocembrin in rat liver	30

	Page
2.5 Partial hepatectomy	32
2.6 Isolation of hepatocytes	32
2.7 Microscopic observation and micronucleus determination	33
2.8 Inhibitory effect of pinocembrin on diethylnitrosamine-induced micronucleated hepatocyte formation in rat	33
2.9 Preventive effect of pinocembrin on 30 mg/kg bw of diethylnitrosamine-induced micronucleated hepatocyte formation in rat	34
2.10 Protective effect of pinocembrin on 20 mg/kg bw of diethylnitrosamine-induced micronucleated hepatocyte formation in rat	34
2.11 Effect of pinocembrin on promotion stage in diethylnitrosamine-induced rat hepatocarcinogenesis	38
2.12 Immunohistochemistry for GST-P	39
2.13 Quantitative assessment of GST-P positive foci	39
2.14 Determination of lipid peroxidation by thiobarbituric acid reactive substances assay	42
2.15 Determination of the expression and activities of phase I and phase II xenobiotic-metabolizing enzymes	43
2.16 Statistical analysis	50
CHAPTER III RESULTS	51
3.1 Mutagenicity study of pinocembrin in rat liver	51
3.2 Effect of pinocembrin on lipid peroxidation in rat liver	51
3.3 Effect of pinocembrin on xenobiotic-metabolizing enzymes in rat liver	52
3.4 Inhibitory effect of pinocembrin on diethylnitrosamine-induced micronucleated hepatocyte formation in rat	57
3.5 Preventive effect of pinocembrin on diethylnitrosamine-induced micronucleus formation in rat liver	57

	Page
3.6 Effect of pinocembrin on promotion stage in diethylnitrosamine-induced rat hepatocarcinogenesis	62
CHAPTER IV DISCUSSION AND CONCLUSION	69
REFERENCES	74
APPENDICES	89
APPENDIX A	90
APPENDIX B	93
APPENDIX C	95
APPENDIX D	102
CURRICULUM VITAE	106

LIST OF TABLES

Table	Page
3-1 Mutagenicity of pinocembrin in rat liver	53
3-2 Effect of pinocembrin on the expression of cytochrome P450 isoenzymes and cytochrome P450 reductase in rat liver.	55
3-3 Effect of pinocembrin on the activities of some phase I and phase II enzymes in rat liver	56
3-4 Inhibitory effect of pinocembrin on diethylnitrosamine-induced micronucleus formation in rat liver	59
3-5 Preventive effect of pinocembrin on 30 mg/kg bw of diethylnitrosamine-induced micronucleated hepatocyte formation in rat	60
3-6 Protective effect of pinocembrin on 20 mg/kg bw of diethylnitrosamine-induced micronucleus formation in rat liver	61
3-7 General appearance of rats in medium-term carcinogenicity experiment	64
3-8 Relative organ weight of rats in medium-term carcinogenicity experiment	65
3-9 Blood biochemicals analysis of rats in medium-term carcinogenicity experiment	66
3-10 Lipid peroxidation of rats in medium-term carcinogenicity experiment	67
3-11 Number and the distribution of size of GST-P positive foci of rats in medium-term carcinogenicity experiment	68

LIST OF FIGURES

Figure	Page
1-1 Overview of genotoxic and non-genotoxic effects of carcinogens	7
1-2 Mechanisms of multistage carcinogenesis	7
1-3 A schematic diagram show the origin of micronucleus	9
1-4 Standard protocol of the medium-term liver bioassay	10
1-5 Hypothetical model for the development of GST-P positive lesions	12
1-6 Regulation of the GST-P gene expression in normal liver cells and in the pre-neoplastic lesion	12
1-7 Biotransformation of diethylnitrosoamine and mechanism of DNA-adduct formation	13
1-8 Role of dietary detoxifying enzyme inducers in chemoprevention	15
1-9 Major detoxification activities in drug metabolism	17
1-10 The microsomal NADPH-cytochrome P450 reductase system	18
1-11 The pathway of heme degradation in mammalian cells	19
1-12 NADPH: quinone oxidoreductase 1	21
1-13 Consequences of quinone metabolism	21
1-14 Role of UDP-glucuronyltransferase	22
1-15 Role of glutathione-S-transferase	23
1-16 The basic structure of flavonoids	25
1-17 Chemical structures of some representative flavanone	25
1-18 Flavanones that block or suppress multistage carcinogenesis	26
1-19 Structure of pinocembrin	27
2-1 The protocol for mutagenicity study of pinocembrin in male Wistar rat	31

Figure	Page
2-2 The protocol for inhibitory effect of pinocembrin on DEN-induced micronucleus formation in rat liver	35
2-3 The protocol for preventive effect of pinocembrin on 30 mg/kg bw of DEN-induced micronucleus formation in rat liver	36
2-4 The protocol for protective effect of pinocembrin on 20 mg/kg bw of DEN-induced micronucleus formation in rat liver	37
2-5 The protocol for the effect of pinocembrin on promotion stage in diethylnitrosamine-induced rat hepatocarcinogenesis	40
2-6 The procedure of GST-P immunohistochemistry in rat liver	41
2-7 MDA-TBA adduct	42
2-8 The preparation of microsomal and cytosolic fractions obtained from rat liver	44
2-9 SDS-PAGE and Western blot procedures	46
3-1 Effect of pinocembrin on lipid peroxidation	54
3-2 Western blot analysis of liver microsomes from rat treated with various doses of pinocembrin.	54
3-3 Growth curve of rats in medium-term carcinogenicity experiment	63
S-1 Extraction scheme of <i>Boesenbergia pandurata</i>	103
S-2 Isolation scheme of pinocembrin	104

ABBREVIATIONS

β - NADPH	β -nicotinamide adenine nucleotide phosphate (Reduced form)
$^{\circ}\text{C}$	degree celcius
μg	microgram
μl	microliter
μM	micromolar
μm	micrometer
A	Ampere
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BSA	bovine serum albumin
bw	body weight
CDNB	1-Chloro-2, 4-dinitrobenzene
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	copper sulphate pentahydrate
CYP	cytochrome P450
DAB	3, 3'-diaminobenzidine
DCPIP	2, 6-dichlorophenolindophenol
DEN	diethylnitrosamine
DI	deionized water
DTT	dithiothreitol
DW	distilled water
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid

FAD	flavin adenine dinucleotide
g	gram
GSH	glutathione (Reduced form)
GST-P	glutathione-S-transferase placental form
H ₂ O ₂	hydrogen peroxide
HCl	hydrochloric acid
i.g.	intragastium
i.p.	intraperitoneum
IgG	immunoglobulin G
KCl	potassium chloride
KCN	potassium cyanide
kg	kilogram
KH ₂ PO ₄	potassium dihydrogen phosphate
KOH	potassium hydroxide
L	liter
M	molar
mA/cm ²	milli ampere per square centimeter
MDA	malondialdehyde bis(dimethyl acetal)
mg	milligram
MgCl ₂	magnesium chloride
min	minute
ml	milliliter
mm	millimeter
mM	milli molar
MNHEPs	micronucleated hepatocytes
NaCl	sodium chloride
NaHCO ₃	sodium bicarbonate
NaH ₂ PO ₄	sodium dihydrogen phosphate
Na ₂ HPO ₄	sodium hydrogen phosphate
Na ₂ CO ₃	sodium carbonate
NaOH	sodium hydroxide

NSS	normal saline solution
nm	nanometer
PBS	phosphate buffer saline
PC	pinocembrin
PH	partial hepatectomy
PMSF	phenylmethanesulphonylfluoride
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TCA	trichloroacetic acid
v/v	volume by volume
w/v	weight by volume