

TABLE OF CONTENTS

	Page
ABSTRACT (IN THAI)	i
ABSTRACT (IN ENGLISH)	iv
DEDICATION	vii
ACKNOWLEDGEMENTS	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xvii
CHAPTER I INTRODUCTION	1
1. Background and Rationale of the study	1
2. Objectives of the Study	3
3. Hypothesis	4
4. Anticipated Outcome	5
CHAPTER II LITERATURE REVIEWS	6
1. Diabetes Mellitus (DM)	6
2. Diabetic Complications	9
3. Mechanisms and Pathophysiology of Diabetic Complications	10
4. Endogenous Antioxidant System	16
5. Peripheral Nerve Injury	17
6. Nerve Injury Classifications	18
7. Neuropathic Pain	19
8. Plants and Nutritional Supplements	20
CHAPTER III THE DEVELOPMENT AND THE ASSESSMENT ACUTE TOXICITY OF THE NOVEL HERBAL RECIPE FROM THE SELECTED PLANTS	25
1. Introduction	25
2. Materials and Methods	26
3. Results	32
4. Discussion	43
5. Conclusion	44

TABLE OF CONTENTS (Cont.)

	Page
CHAPTER IV THE EFFECTS OF NOVEL HERBAL RECIPE (PWCG) ON DIABETIC NEUROPATHY	45
1. Introduction	45
2. Materials and method	46
3. Results	52
4. Discussion	66
5. Conclusion	69
CHAPTER V THE EFFECTS OF NOVEL HERBAL RECIPE (PWCG) ON DIABETIC CATARACT AND RETINOPATHY MODELS	70
1. Introduction	70
2. Materials and Methods	70
3. Results	77
4. Discussion	110
5. Conclusion	113
CHAPTER VI CONCLUSION	114
REFERENCES	116
APPENDICES	129
APPENDIX A Determination of Total Phenolic Compounds	130
APPENDIX B Determination of DPPH Radical Scavenging Activity	133
APPENDIX C Determination of Frap Activity	136
APPENDIX D Determination of Flavanoids Content	139
APPENDIX E Determination of Anthocyanin Contents	141
APPENDIX F Phosphate Buffer Saline Solution	143
APPENDIX G Hematoxilin and Eosin Staining	145
APPENDIX H Determination of Protein	148
APPENDIX I Determination of Lipid Peroxide Contents	151
APPENDIX J Determination of Superoxide Dismutase Activity	154
APPENDIX K Determination of Glutathione Peroxidase	157
APPENDIX L Determination of Catalase Activity	160
APPENDIX M Determination of Aldose Reductase Activity	163

TABLE OF CONTENTS (Cont.)

	Page
RESEARCH PUBLICATIONS	165
VITAE	167

LIST OF TABLES

	Page
Table 3-1 Gradient program of HPLC analysis of the novel herbal product containing the combination extract of purple waxy corn and ginger (PWCG)	30
Table 3-2 The antioxidative activity of the selected plants extract	34
Table 3-3 The antioxidative activity of the novel herbal recipe ratio	35
Table 3-4 Effect of PWCG on acute oral toxicity test in rats	39
Table 3-5 Relative organ weights, food and water consumptions of male and female Wistar rats treated with PWCG	40
Table 3-6 Hematological and blood biochemical parameters of male and female Wistar rats treated with PWCG	42
Table 4-1 Effect of PWCG on the sciatic function index evaluated by walking track analysis	57
Table 4-2 Effect of PWCG on the paw withdrawal threshold intensity in respond to mechanical stimuli evaluated by von Frey filament test	58
Table 4-3 Effect of PWCG on the paw withdrawal threshold latency in respond to temperature stimuli evaluated by hot plate test	59
Table 5-1 Effect of purple waxy corn grains extract (PWC) on lens opacity	80
Table 5-2 Effect of PWC on aldose reductase and oxidative stress markers in rat lens after the exposure to a 72 h glucose incubation	86
Table 5-3 Effect of PWCG on aldose reductase and oxidative stress Markers in rat lens after the exposure to a 72 h glucose incubation	93
Table 5-4 Opacity index of the lens of normal and diabetic rats (DM) which received either vehicle or PWCG	99

LIST OF FIGURES

	Page
Figure 2-1 Schematic diagram illustrating underlying mechanism of β -cell destruction in IDDM	7
Figure 2-2 Pathophysiology of Type 2 diabetes mellitus	8
Figure 2-3 Classification of cataract according to the location of the opacity	10
Figure 2-4 Schematic diagram illustrating multiple interacting pathways that resulting in the enhanced reactive oxygen species (ROS) which in turn induces the diabetic complications	11
Figure 2-5 Schematic diagram illustrating polyol pathway-induced oxidative stress	12
Figure 2-6 Schematic diagram illustrating advance glycation end products (AGE) pathway	14
Figure 2-7 Endogenous antioxidant systems	17
Figure 2-8 Schematic diagram showing the possible site of peripheral nerve injury	18
Figure 2-9 The classification of peripheral nerve injury according to Seddon's	19
Figure 2-10 Common symptoms of painful diabetic neuropathy including hyperalgesia and allodynia	20
Figure 2-11 <i>Zea mays</i> Linn. var. <i>ceratina</i> Kulesh or Purple waxy corn	21
Figure 2-12 <i>Zingiber officinale</i> Roscoe or Ginger	21
Figure 2-13 <i>Morus alba</i> Linn. or Mulberry	22
Figure 2-14 <i>Allium cepa</i> Linn. or Shallot or red onion	23
Figure 2-15 <i>Carica papaya</i> Linn. or Papaya	24
Figure 3-1 Fingerprint chromatogram of the combination extract Of purple waxy corn and ginger or PWCG	37
Figure 3-2 Histopathological changes of male and female Wistar rats treated with PWCG	41

LIST OF FIGURES (Cont.)

		Page
Figure 4-1	Schematic diagram showing experimental protocol for the determination of effect of a novel herbal recipe (PWCG) on diabetic neuropathy model	47
Figure 4-2	The effect of PWCG on fasting blood sugar levels	56
Figure 4-3	Effect of PWCG on sciatic nerve conduction velocity	60
Figure 4-4	Effect of PWCG on malondialdehyde (MDA) level in the lesion sciatic nerve	60
Figure 4-5	Effect of PWCG on superoxide dismutase (SOD) activity in the lesion sciatic nerve	62
Figure 4-6	Effect of PWCG on catalase (CAT) activity in the lesion sciatic nerve	63
Figure 4-7	Effect of PWCG on glutathione peroxidase (GPx) activity in the lesion sciatic nerve	64
Figure 4-8	Effect of PWCG on aldose reductase (AR) activity in the lesion sciatic nerve	65
Figure 4-9	Effect of PWCG on the density of myelinated fibers evaluated by using toluidine blue stain	66
Figure 5-1	Schematic diagram showing experimental protocol for the determination of effect of PWC and PWCG on diabetic cataract in Glucose-induced cataractogenesis model	72
Figure 5-2	Schematic diagram showing experimental protocol for the determination of effect of PWCG on diabetic cataract and retinopathy induced by STZ model	73
Figure 5-3	Anti-cataract effect of the extract of purple waxy corn grains after 72 hours incubation with glucose	80
Figure 5-4	The effect of purple waxy corn grains extract (PWC) on the level of malondialdehyde (MDA) level in lens exposed to high concentration of glucose	81

LIST OF FIGURES (Cont.)

		Page
Figure 5-5	The effect of purple waxy corn grains extract (PWC) on superoxide dismutase (SOD) activity in lens exposed to high concentration of glucose	82
Figure 5-6	The effect of purple waxy corn grains extract (PWC) on catalase (CAT) activity in lens exposed to high concentration of glucose	83
Figure 5-7	The effect of purple waxy corn grains extract (PWC) on glutathione peroxidase (GPx) in lens exposed to high concentration of glucose	84
Figure 5-8	The effect of purple waxy corn grains extract (PWC) on aldose reductase (AR) activity in lens exposed to high concentration of glucose	85
Figure 5-9	Anti-cataract effect of PWCG after 72 hrs incubation with glucose	87
Figure 5-10	The level of malondialdehyde (MDA) in lens after incubated 72 hours in the combination extract of purple waxy corn and ginger (PWCG)	88
Figure 5-11	The activity of superoxide dismutase (SOD) in lens after incubated 72 hours in the combination extract of purple waxy corn and ginger (PWCG)	89
Figure 5-12	The activity of catalase (CAT) in lens after incubated 72 hours in the combination extract of purple waxy corn and ginger (PWCG)	90
Figure 5-13	The activity of glutathione peroxidase (GPx) in lens after Incubated 72 hours in the combination extract of purple waxy corn and ginger (PWCG)	91
Figure 5-14	The activity of aldose reductase (AR) in lens after incubated 72 hours in the combination extract of purple waxy corn and ginger (PWCG)	92

LIST OF FIGURES (Cont.)

	Page
Figure 5-15 The average fasting blood glucose levels at 1-week, 5- week and 10-week intervention period	97
Figure 5-16 Representative photographs showing the effects of a 10-week treatment of PWCG on cataract genesis	98
Figure 5-17 Photographs of transverse sections of eye balls stained with haematoxylin and eosin-stained sections were determined the cataract severity at the end of experiment using light microscope	100
Figure 5-18 The effect of a 10-week treatment of PWCG on cataract in STZ-induced diabetic rats	101
Figure 5-19 Effect of a 10-week treatment with PWCG on retinal thickening and extracellular matrix in STZ-treated rats evaluated by haematoxylin and eosin-stained sections	102
Figure 5-20 The effect of a 10-week treatment of PWCG on total retinal thickness of retina (TRT)	103
Figure 5-21 The effect of a 10-week treatment of PWCG on the thickness of the retinal outer nuclear layer (ROT)	104
Figure 5-22 The effect of a 10-week treatment of PWCG on the number Of cells in the ganglion cell layer (NG) in retina	105
Figure 5-23 The effect of a 10-week treatment of PWCG on malondialdehyde 105 (MDA) level in lens of STZ-induced diabetic rats	106
Figure 5-24 The effect of a 10-week treatment of PWCG on superoxide dismutase (SOD) activity in lens of STZ-induced diabetic rats	107
Figure 5-25 The effect of a 10- week treatment of PWCG on catalase (CAT) activity in lens of STZ-induced diabetic rats	108
Figure 5-26 The effect of a 10-week treatment of PWCG on glutathione peroxidase (GPx) activity in lens of STZ-induced diabetic rats	109
Figure 5-27 The effect of a 10-week treatment of PWCG on aldose reductase (AR) activity in lens of STZ-induced diabetic rats	110

LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
μM	Micromolar
μg/ml	Microgram per milliliter
<i>A. cepa</i>	<i>Allium cepa</i> Linn
AGE	Advance glycation end products
AlCl ₃	Aluminum chloride
APC	Antigen-presenting cells
AR	Aldose reductase
Ca ²⁺	Calcium ion
CAT	Catalase
CCI	Chronic constriction injury
cm	Centimeter
<i>C. papaya</i>	<i>Carica papaya</i> Linn
Cu ²⁺	Copper ion
DI	Deionized
DM	Diabetes mellitus
DN	Diabetic neuropathy
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DTNB	5, 5'-dithiobis-2-nitrobenzoic acid (DTNB)
DTT	Dithiothreitol
EAS	Endogenous antioxidant systems
EC ₅₀	Half maximal efficient concentration
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric reducing antioxidant power
G	Gram
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
H&E	Haematoxylin and eosin

LIST OF ABBREVIATIONS (Cont.)

HCL	Hydrogen chloride
HDLC	High density lipoprotein
H ₂ O ₂	Hydrogen peroxide
HPLC	High performance liquid chromatography
HLAs	Human leukocyte antigens
IDDM	Insulin-dependent diabetes mellitus
IGT	Impaired glucose tolerance
IL-12	Interleukin-12
ITF	Intermediate toe spread factor
KCL	Potassium chloride
LD50	Median lethal dose
<i>M.alba</i>	<i>Morus alba</i> Linn
MDA	Malondialdehyde
mg	Milligram
mg.kg ⁻¹ BW	Milligram per kilogram body weight
mM	Millimolar
MHC	Major histocompatibility complex
MW	Molecular weight
Na ⁺	Sodium ion
NAD ⁺	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NBT	Nitrobluetetrazolium
NCV	Nerve conduction velocity
NIDDM	Noninsulin-dependent diabetes mellitus
NG	the number of cells in the ganglion cell layer
nM	Nanometer
NO	Nitric oxide
NOS	Nitric oxide synthase
OECD	Organization for Economic Co-operation and Development

LIST OF ABBREVIATIONS (Cont.)

ONL	Outer nuclear layer
PBS	Phosphate buffer saline
PL	Print length
PLF	Print length factor
PWC	Purple waxy corn
PWCG	Purple waxy corn and ginger
QE	Quercetin equivalent
RBCs	Red blood cells
ROS	Reactive oxygen species
ROT	Retinal outer nuclear layer thickness
SDH	Sorbital dehydrogenase
SEM	Standard error of mean
SFI	Sciatic function index
SOD	Superoxide dismutase
STZ	Streptozotocin
TBA	Thiobarbituric acid
TBRs	Thiobarbituric acid reacting substances
TCM	Traditional Chinese Medicine
TEP	1,3,3-tetra ethoxy propane
TPTZ	2,4,6-tripyridyls-triazine
TRP	Total retinal thickness
TS	Toe spread
TSF	Toe spread factor
XO	Xanthine oxidase
<i>Z.mays</i>	<i>Zea mays</i> Linn. var. <i>ceratina</i> Kulesh
<i>Z.officinale</i>	<i>Zingiber officinale</i>