

## TABLE OF CONTENTS

	<b>Page</b>
ABSTRACT (IN THAI)	i
ABSTRACT (IN ENGLISH)	iv
DEDICATION	vii
ACKNOWLEDGEMENTS	viii
LIST OF TABLES	xii
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xix
CHAPTER I INTRODUCTION	1
1. Rationale and Background	1
2. Objectives of the Study	3
3. Scope and Limitation of the Study	4
4. Hypothesis	5
5. Anticipated Outcomes	7
CHAPTER II LITERATURE REVIEWS	9
1. Diabetes Mellitus and Complications	9
2. Nerve Injury	12
3. Sciatic Nerve	14
4. Free Radicals and Antioxidants	15
5. Blood-Nerve Barrier	16
6. Diabetic Wound	17
7. Wound Healing or Wound Repair	19
8. Hydroxyproline	21
9. Transdermal Route Administration	21
10. Rodent Model of Diabetes	23
11. Quercetin	24
12. Tomato	26
13. Zein Based Nanofibers	27

## TABLE OF CONTENTS (Cont.)

		<b>Page</b>
CHAPTER III	THE DEVELOPMENT AND THE ASSESSMENT CHRONIC TOXICITY OF THE NATURAL PRODUCTS LOADED NANOFIBER	29
	1. Introduction	29
	2. Materials and Method	31
	3. Results	35
	4. Discussion and Conclusion	55
CHAPTER IV	IN VIVO EFFECT OF NATURAL PRODUCTS LOADED ZEIN BASED POLYMER ON NEUROPATHY IN DIABETIC CONDITION	57
	1. Introduction	57
	2. Materials and Method	58
	3. Results	69
	4. Effect of Quercetin Loaded Zein Based Nanofiber on the Functional Recovery of Sciatic Nerve of Diabetic Rats	93
	5. Effect of Tomato Extract Loaded Zein Based Nanofiber on the Functional Recovery of Sciatic Nerve of Diabetic Rats	95
	6. Conclusion	98
CHAPTER V	WOUND HEALING EFFECT OF NATURAL PRODUCTS LOADED WITH ZEIN BASED NANOFIBER MATS IN STREPTOZOTOCIN- DIABETIC RATS	99
	1. Introduction	99
	2. Materials and Methods	99
	3. Results	107
	4. Wound Healing Activity of Quercetin-Loaded Nanofiber in Diabetic Condition	127

**TABLE OF CONTENTS (Cont.)**

	<b>Page</b>
5. Wound Healing Activity of Tomato Extract-Loaded Nanofiber in Diabetic Condition	129
6. Conclusion	130
REFERENCES	131
APPENICIES	145
RESEARCH PUBLICATIONS	209
VITAE	213

## LIST FO TABLES

		<b>Page</b>
Table 2-1	Rodent models that have been used to study diabetes type I	23
Table 3-1	The antioxidant activity determined via DPPH and FRAP assays and the suppression activity effect of aldose reductase of vitamin C, quercetin, tomato extract and the nanofiber mats loaded either with various concentration of quercetin or with various concentration of tomato extract	38
Table 3-2	The effect 6-month administration of zein based nanofiber mat via transdermal route on toxicity signs of male rats (1)	46
Table 3-3	The effect 6-month administration of zein based nanofiber mat via transdermal route on toxicity signs of female rats (2)	47
Table 3-4	The effect 6-month administration of 15% quercetin loaded zein based nanofiber mat via transdermal route on toxicity signs of male rats	48
Table 3-5	The effect 6-month administration of 15% quercetin loaded zein based nanofiber mat via transdermal route on toxicity signs of female rats	49
Table 3-6	The relative organ weight of female rats which received 15% quercetin loaded zein based nanofiber mat via transdermal route for 6 months	51
Table 3-7	The relative organ weight of male rats which received 15% quercetin loaded zein based nanofiber mat via transdermal route for 6 months	52
Table 3-8	Biochemical changes of biomarkers indicated liver and kidney functions of female rats (N=15/group) Data were expressed as mean±SEM	53

## LIST OF TABLES (Cont.)

		<b>Page</b>
Table 3-9	Biochemical changes of biomarkers indicated liver and kidney functions of male rats (N=15/group) Data were expressed as mean±SEM	53
Table 3-10	Blood hematological values changes of female rats (N=15/group). Data were expressed as mean±SEM	54
Table 3-11	Blood hematological values changes of female rats (N=10/group). Data were expressed as mean±SEM	55
Table 4-1	Effect of nerve crush injury and diabetes mellitus on sciatic function index evaluated by walking track analysis	73
Table 4-2	Effect of quercetin loaded zein based nanofiber on sciatic nerve function index of diabetic rats of various treatment groups evaluated by walking track analysis (n=8/group). The SFI are regarded as normal when they are within the range of -10 to 10%	74
Table 4-3	Effect of quercetin loaded zein based nanofiber mat on walking pattern of diabetic rats with crush injury (n=8/group)	75
Table 4-4	Effect of quercetin loaded zein based nanofiber mat on muscle power of diabetic rats with crush injury (n=8/group)	76
Table 4-5	Effect of quercetin loaded zein based nanofiber mat on paw withdrawal latency of diabetic rats with crush injury (n=8/group)	77
Table 4-6	Effect of quercetin loaded zein based nanofiber mat on oxidative stress markers of sciatic nerve of diabetic rats with crush injury	81

## LIST OF TABLES (Cont.)

		<b>Page</b>
Table 4-7	Effect of tomato extract loaded zein based nanofiber on sciatic nerve function index of diabetic rats of various treatment groups evaluated by walking track analysis (n=8/group) The SFI are regarded as normal when they are within the range of 10 to 10%	85
Table 4-8	Effect of tomato extract loaded zein based nanofiber mat on walking pattern of diabetic rats with crush injury (n=8/group)	86
Table 4-9	Effect of tomato extract loaded zein based nanofiber mat on muscle power of diabetic rats with crush injury (n=8/group)	87
Table 4-10	Effect of tomato extract loaded zein based nanofiber mat on paw withdrawal latency of diabetic rats with crush injury (n=8/group)	88
Table 5-1	The effect of quercetin-loaded nanofiber mats on the healing process of wound in diabetic rats evaluated by using wound adsorption score as index	110
Table 5-2	The effect of quercetin-loaded nanofiber mats on the healing process of wound in diabetic rats evaluated by using wound healing index as indicator	111
Table 5-3	The effect of quercetin loaded zein based nanofiber mats on percent of wound contraction of diabetic rats	112
Table 5-4	Effect of quercetin loaded zein based nanofiber mat on content of hydroxyproline in granulation tissue in diabetic rats	114

## LIST OF TABLES (Cont.)

		<b>Page</b>
Table 5-5	Effect of quercetin loaded zein based nanofiber mat on content of hydroxyproline in granulation tissue in diabetic rats	115
Table 5-6	Effect of quercetin extract loaded zein based nanofiber mat on hydroxyproline content and oxidative stress markers including MDA level and the activities of SOD, CAT and GPx in the wound area of diabetic rats	116
Table 5-7	Effect of tomato loaded nanofiber mats on wound adsorption score of diabetic rats	120
Table 5-8	The effect of tomato-loaded nanofiber mats on wound healing process of diabetic rats evaluated by using wound healing index as indicator	121
Table 5-9	The effect of tomato loaded zein based nanofiber mats on percent of wound contraction of diabetic rats	122
Table 5-10	Effect of zein based nanofiber mats loaded with tomato at concentrations of 5%, 10% and 15% on collagen thickness of diabetic rats	124
Table 5-11	Effect of tomato loaded zein based nanofiber mat on content of hydroxyl proline in granulation tissue in diabetic rats	125
Table 5-12	Effect of tomato extract loaded zein based nanofiber mat on hydroxyl proline content and oxidative stress markers including MDA level and the activities of SOD, CAT and GPx in the wound area of diabetic rats	126

## LIST FO FIGURES

	<b>Page</b>	
Figure 2-1	Sciatic nerve	14
Figure 2-2	The phases of coetaneous wound healing	20
Figure 2-3	Schematic representation of the “brick and motar” model of the stratum corneum, lipid bilayer organization and possible pathways	22
Figure 2-4	Structure of Quercetin 2-(3, 4-dihydroxyphenyl)- 3,5,7-trihydroxy- 4 <i>H</i> -chromen- 4-one	25
Figure 2-5	Tomato ( <i>Solanum lycopersicum</i> Linn.)	26
Figure 3-1	Schematic diagram shows the experimental protocol of the determination of chronic toxicity of quercetin-loaded nanofiber mats	35
Figure 3-2	Morphology of zein based nanofiber and zein based nanofiber loaded with 5%, 10% and 15% quercetin	39
Figure 3-3	Morphology of zein based nanofiber and zein based nanofiber loaded with 5%, 10% and 15% tomato extract.	41
Figure 3-4	Entrapment efficiency of quercetin loaded zein based nanofiber	43
Figure 3-5	Cumulative release of quercetin from quercetin-loaded zein based nanofiber mats	44
Figure 3-6	Histopathological changes of vital organs such as liver, kidney and spleen of male and female rats which subjected to a 6-month administration of either zein based nanofiber mat or 15% quercetin loaded zein based nanofiber mat in comparison with control rats	50
Figure 4-1	Schematic diagram showing experimental protocol for the determination of the effect of nerve crush injury on sciatic function index of diabetic rats	60

## LIST FO FIGURES (Cont.)

		<b>Page</b>
Figure 4-2	Schematic diagram showing experimental protocol for the determination of the effect of zein based nanofiber mats loaded with various concentrations of quercetin in diabetic nerve crush injury	61
Figure 4-3	Schematic diagram showing experimental protocol for the determination of the effect of zein based nanofiber mats loaded with various concentrations of tomato extract in diabetic nerve crush injury	63
Figure 4-4	Walking Track Test (De medinacelli method). The equation was used for calculating sciatic function index.	64
Figure 4-5	The motor nerve conduction velocity study	66
Figure 4-6	Effect of quercetin loaded zein based nanofiber mat on nerve conduction velocity (NCV) of diabetic rats with crush injury (n=8/group)	78
Figure 4-7	Effect of quercetin loaded zein based nanofiber mat on axon density of sciatic nerve of diabetic rats with crush injury	80
Figure 4-8	Effect of quercetin loaded zein based nanofiber on the level of ERK1/2 and pERK1/2 in sciatic lesion nerve of diabetic rats	82
Figure 4-9	Effect of tomato extract loaded zein based nanofiber mat on nerve conduction velocity (NCV) of diabetic rats with crush injury (n=8/group)	89
Figure 4-10	Effect of tomato extract loaded zein based nanofiber mat on axon density of sciatic nerve of diabetic rats with crush injury	90

## LIST FO FIGURES (Cont.)

		<b>Page</b>
Table 4-11	Effect of tomato extract loaded zein based nanofiber mat on oxidative stress markers of sciatic nerve of diabetic rats with crush injury (n=8/group)	92
Figure 4-12	Schematic diagram shows the possible underlying mechanism of quercetin loaded zein based nanofiber on the functional recovery of sciatic nerve of diabetic rats	95
Figure 4-13	Schematic diagram shows the possible underlying mechanism of tomato extract loaded zein based nanofiber on the functional recovery of sciatic nerve of diabetic rats	97
Figure 5-1	Schematic diagram showing experimental protocol for the determination of the effect of zein based nanofiber mats loaded with various concentrations of quercetin in diabetic wound	102
Figure 5-2	Schematic diagram showing experimental protocol for the determination of the effect of zein based nanofiber mats loaded with various concentrations of tomato extract in diabetic wound	103
Figure 5-3	Photographs of skin at the wound area of diabetic rats which received zein based nanofiber mats loaded with various concentrations of quercetin which stained with Masson's trichrome at 40X magnification	113
Figure 5-4	Effect of zein based nanofiber mats loaded with quercetin at concentrations of 5%,10% and 15% on collagen thickness of diabetic rats	114
Figure 5-5	Photographs of skin at the wound area of diabetic rats which received zein based nanofiber mats loaded with various concentrations of tomato extract which stained with Masson's trichrome at 40X magnification	124

## LIST OF ABBREVIATIONS

AODM	Adult onset diabetes mellitus
AGE	Advanced glycation end-products
BBB	Blood–Brain Barrier
BNB	Blood-Nerve Barrier
BRB	Blood-Retinal-Bbarrier
BW	Body weight
Ca <sup>2+</sup>	Calcium ion
CAT	Catalase
Cu <sup>2+</sup>	Copper ion
CCI	Chronic constriction injury
Da	Dalton
DM	Diabetic mellitus
DCCT	The Diabetes Control and Complications Trial Research Group
dl	Decilitre
EDL	Extensor digitorum longus muscle
eNOS	Endothelial NOS
ETC	Electron transport chain
Fe <sup>2+</sup>	Ferric ion
FADH <sub>2</sub>	Flavin adenine dinucleotide (reduced)
GFs	Ggrowth factors
GPx	Glutathione peroxidase
GSH	Glutathione
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
iNOS	Inducible NOS
IDDM	Insulin dependent diabetes mellitus
kg	Kilogram
MDA	Malondialdehyde

**LIST OF ABBREVIATIONS (Cont.)**

mg	Milligram
min	Minute
NCI	Nerve crush injury
NIDDM	Non-insulin dependent diabetes mellitus
NGF	Nerve growth factor
NO	Nitric oxide
nm	Nanometer
NGF	Nerve growth factor
(O <sup>2-</sup> )	The superoxide anion
(OH <sup>·</sup> )	Hydroxyl radical
QC	Quercetin (3, 5, 7, 3, 4-pentahydroxyflavone)
STZ	Streptozotocin
SEM	Standard error of mean
SOD	Superoxide dismutase
ROS	Reactive oxygen species
µg	Microgram
µm	Micrometer