

## Table of Contents

Particulars	Page No.
<i>Certificates</i>	
<i>Acknowledgement</i>	
<i>List of Abbreviations and Symbols</i>	
<i>List of Figures</i>	
<i>List of Tables</i>	
<i>Preface</i>	
<b>1. Introduction</b>	1-7
<b>2. Literature review</b>	9
2.1. Diabetes Mellitus	9
2.2. Epidemiology of diabetes	10
2.3. Diabetic cardiomyopathy	12
2.3.1. Morphology and functional alterations of heart in diabetic cardiomyopathy	13
2.3.2. Dysfunction of excitation-contraction coupling of diabetic myocardium	14
2.3.3. Metabolic derangements in diabetic myocardium	15
2.3.4. Remodeling of extracellular matrix in diabetic hearts	16
2.3.5. Abnormalities in the microvasculature	16
2.3.6. Diabetes-induced changes in myocardial tolerance against ischemia/reperfusion injury	17
2.4. Mechanisms responsible for diabetic cardiomyopathy	18
2.4.1. Oxidative, nitrosative and nitrative stress	18
2.4.2. Inflammation in diabetic cardiomyopathy	21
2.4.3. Defective intracellular protective mechanisms in diabetes	24
2.5. Principal targets for the prevention of diabetic cardiomyopathy	25
2.5.1. AMP-activated protein kinase (AMPK)	26
2.5.2. Nuclear factor-erythroid (NF-E) 2-related factor 2 (Nrf2)	31
2.5.3. Hemoxygenase-1 (HO-1)	35
2.6. Need and Scope of Herbal Medicine for diabetic cardiomyopathy	38
2.7. Pterostilbene	38
2.7.1. Structure and history	38
2.7.2. Sources of PT	40
2.7.3. Effects of PT on metabolic and cardiovascular diseases	40
2.7.3.1. Antidiabetic mechanism of PT	40
2.7.3.2. Antihyperlipidemic mechanism of PT	43
2.7.3.3. Antiatherosclerotic mechanism of PT	45
2.7.3.4. Infarct sparing mechanism of PT	47
2.7.3.5. Antihypertensive mechanism of PT	48

2.7.3.6. Antihematologic action of PT	48
2.7.4. Safety	50
<b>3. Objectives</b>	51
<b>4. Materials and Methods</b>	53
4.1. Ethical approval	53
4.2. Drugs, chemicals and antibodies	53
4.3. Equipment and software	55
4.4. Animal husbandry	55
4.5. Experimental groups	56
4.5.1. Experimental groups for objective-I	56
4.5.2. Experimental groups for objective-II	57
4.5.3. Experimental groups for objective-III	58
4.5.3.1. <i>In vivo</i> experiments	58
4.5.3.2. <i>In vitro</i> experiments	59
4.6. Experimental design	61
4.6.1. Study design for objective-I	61
4.6.2. Study design for objective-II	62
4.6.3. Study design for objective-III	62
4.7. Methods	63
4.7.1. Diabetes induction by high fructose diet (for experiments related to Objective-I and Objective-II)	63
4.7.2. Diabetes induction by intravenous streptozotocin injection (for experiments related to Objective-III)	63
4.7.3. Myocardial ischemia-reperfusion by coronary artery ligation model (Objective-III)	64
4.7.4. Histological evaluation	66
4.7.5. Primary adult rat cardiomyocytes isolation and hypoxia/reoxygenation (HR)	68
4.7.5.1. Primary rat cardiomyocytes isolation	68
4.7.5.2. Hypoxia/Reoxygenation (HR) of Cardiomyocytes	73
4.7.5.3. Extraction of primary rat cardiomyocytes from the plates (cell scraping)	74
4.7.5.4. Estimation of protein concentration of cardiomyocyte suspension	75
4.7.6. Determination of heart rate and blood pressure	76
4.7.7. Oral glucose tolerance test (OGTT)	76
4.7.8. Collection of samples and preparation of tissue homogenates	77
4.7.8.1. Preparation of cardiac and liver homogenates	77
4.7.8.2. Preparation of cardiomyocyte sample	77
4.7.9. Paraffin embedding	78
4.7.10. Hematoxylin & Eosin (H&E) staining	78
4.8. Biochemical estimations	79

4.8.1.	Estimation of fasting blood glucose (FBG), fasting serum insulin (FSI) and insulin sensitivity index (ISI) and homeostasis model of insulin resistance (HOMA-IR)	79
4.8.2.	Estimation of glycated haemoglobin (HbA1c), hydrogen sulfide, peroxynitrite and uric acid	80
4.8.3.	Estimation of lipid profile, cardiovascular risk indices and antiatherogenic index	80
4.8.4.	Detection of oxidative stress and antioxidant markers	81
4.8.5.	Determination of proinflammatory cytokines by ELISA	81
4.8.6.	Measurement of plasma creatine kinase-MB and free 8-isoprostane levels	81
4.8.7.	Determination of LDH levels in diabetic rats and primary rat cardiomyocytes	82
4.8.7.1.	Procedure for <i>in vitro</i> experiments	82
4.8.7.2.	Procedure for <i>in vivo</i> experiments	82
4.8.8.	Determination of cardiac cell viability	83
4.8.9.	Determination of apoptotic cell death in diabetic IR hearts and primary rat cardiomyocytes	84
4.8.9.1.	For adherent cells/ <i>in vitro</i> studies	84
4.8.9.2.	For <i>in vivo</i> studies	85
4.8.10.	Determination of reactive oxygen species (ROS)	86
4.8.11.	Reverse transcriptase-polymerase chain reaction (RT-PCR)	86
4.8.12.	Western blotting	88
4.9	Statistical analysis	92
<b>5. Results</b>		93
5.1.	To explore the efficacy of PT on insulin resistance, metabolic syndrome and hepatic oxidative stress in high fructose (65%) diet-induced type 2 diabetic rats (Objective-I).	93
5.1.1.	Effect of PT treatment on body weight and weight gain of fructose-fed diabetic rats	93
5.1.2.	Effect of PT treatment on fasting blood glucose (FBG) levels of fructose-fed rats	94
5.1.3.	Effect of PT treatment on OGTT of fructose-fed rats	95
5.1.4.	Effect of PT treatment on insulin sensitivity of fructose-fed diabetic rats	96
5.1.5.	Effect of PT treatment on blood pressure of fructose-fed diabetic rats	97
5.1.6.	Effect of PT treatment on lipid profile, cardiovascular risk indices and antiatherogenic index (AAI) of fructose-fed diabetic rats	98
5.1.7.	Effect of PT treatment on glycated haemoglobin, uric acid, peroxynitrite and hydrogen sulfide levels in fructose-fed diabetic rats	100

5.1.8.	Effect of PT treatment on liver thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and glutathione (GSH) levels of fructose-fed diabetic rats	101
5.2.	To investigate the therapeutic potency and signalling mechanism of pterostilbene against diabetes induced-cardiac oxidative stress, inflammation and mitochondrial impairment in fructose-fed diabetic rats (Objective-II)	103
5.2.1.	Effect of PT treatment on the MAP, heart rate, body weight and hypertrophy index in the fructose-fed diabetic rats	103
5.2.2.	Effect of PT treatment on glycemic control and cardiac injury markers in fructose-fed diabetic rats	104
5.2.3.	Effect of PT treatment on the myocardial oxidative stress in fructose-fed diabetic rats	105
5.2.4.	Effect of PT treatment on the antioxidant defence system in the myocardium of fructose-fed diabetic rats	106
5.2.5.	Effect of PT treatment on the myocardial inflammation in fructose-fed diabetic rats	108
5.2.6.	Effect of PT treatment on the myocardial NF- $\kappa$ B expression and inflammasome in fructose-fed diabetic rats	110
5.2.7.	Effect of PT treatment on mitochondrial biogenesis in the hearts of fructose-fed diabetic rats	112
5.2.8.	Effect of PT treatment on the mRNA expression of Nrf2, HO-1 in cardiac tissues of fructose-fed diabetic rats	114
5.2.9.	Effect of PT on AMPK/Nrf2/HO-1 signalling pathway in fructose-fed diabetic rats	114
5.3.	To investigate the cardioprotective potential and mechanistic pathway of pterostilbene against myocardial ischemia-reperfusion injury in streptozotocin-induced diabetic rats (Objective-III)	117
5.3.1.	Effect of PT treatment on general characteristics of streptozotocin diabetic rats	117
5.3.2.	Effect of PT treatment on post-ischemic myocardial injury in streptozotocin-induced diabetic rats	118
5.3.3.	Effect of PT treatment on the phosphorylation of AMPK in diabetic rats	121
5.3.4.	Effect of PT treatment on cardiomyocytes viability exposed to hypoxia-reoxygenation under high glucose condition	123
5.3.5.	Effect of PT treatment on the phosphorylation of AMPK in primary cardiomyocytes subjected to HG+HR	127
5.3.6.	Effect of PT treatment on apoptosis in primary cardiomyocytes subjected to HG+HR	129
<b>6. Discussion</b>		135
6.1.	To explore the efficacy of PT on insulin resistance, metabolic	135

	syndrome and hepatic oxidative stress in high fructose (65%) diet-induced type 2 diabetic rats (Objective-I).	
6.2.	To investigate the therapeutic potency and signalling mechanism of pterostilbene against diabetes induced-cardiac oxidative stress, inflammation and mitochondrial impairment in fructose-fed diabetic rats (Objective-II).	143
6.3.	To investigate the cardioprotective potential and mechanistic pathway of pterostilbene against myocardial ischemia-reperfusion injury in streptozotocin-induced diabetic rats (Objective-III).	151
<b>7. Conclusion</b>		157
<b>8. References</b>		159
<b>List of Publications</b>		