Table of Contents

Sr. No.	Title	Page No.
CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE		**1-39**
1	Introduction	2
1.1	Diabetes Mellitus	2
1.2	Classification	2
1.3	Epidemiology	3
1.4	Diagnostic Criteria for Diabetes Mellitus	4
1.5	Pathogenesis of Diabetes Mellitus	5
1.5.1	Type 1 Diabetes Pathogenesis	5
1.5.2	Type 2 Diabetes Pathogenesis	6
1.5.2.1	Role of Genetic Factors in T2D pathogenesis	7
1.5.2.2	Cytokines, Oxidative Stress, ER Stress and Inflammation	8
1.5.2.3	Obesity and Insulin Resistance	10
1.5.2.3.1	Insulin Signalling	11
1.5.2.3.2	Tumour Necrosis Factor Alpha (TNF-α)	13
1.5.2.4	GPCRs in β-Cell Dysfunction and Insulin Resistance	14
1.5.2.4.1	Melatonin Receptors in β-cell Dysfunction and Insulin Resistance	15
1.6	Diabetes Management	16
1.6.1	Type 1 Diabetes Management	16
1.6.2	Type 2 Diabetes Management	17
1.7	β-Cell Regeneration	18
1.7.1	β-Cell Proliferation	18
1.7.2	β-Cell Neogenesis	19
1.7.3	β-Cell Transdifferentiation	20
1.7.4	Potent β-cell Regenerative Drugs	22
1.8	Melatonin: Hormone of Darkness with Pleiotropic Effects	22
1.9	Incretins and DPP-IV Inhibitors	24
1.10	References	26
OBJECTIVES		**40-41**
CHAPTER 2. INVESTIGATION OF GENOTYPE-PHENOTYPE CORRELATION OF *TNF-α* AND *MTNR1B* POLYMORPHISMS WITH T2D SUSCEPTIBILITY IN GUJARAT POPULATION		**42-68**
2.1	Introduction	43
2.2	Materials and Methods	44
2.2.1	Blood Collection, DNA Extraction, and Lipid Profiling	44
2.2.2	Genotyping of *MTNR1B* and *TNF-α* SNPs by PCR-RFLP	45
2.2.3	Estimation of *TNF-α* Transcript Levels	45
2.2.3.1	RNA Extraction and cDNA Synthesis	45
2.2.3.2	Real-Time PCR	46
2.2.4	Estimation of Plasma Melatonin, TNF-α and FFA Levels	47
2.2.5	Statistical Analyses	47
2.3	Results	47
2.3.1	Association of *TNF-α* and *MTNR1B* Polymorphisms with T2D	48
2.3.2	Haplotype Analyses of *TNF-α* and *MTNR1B* Polymorphisms	52

2.3.3	Linkage Disequilibrium Analyses of *TNF-α* and *MTNR1B* Polymorphisms	53
2.3.4	Correlation of *TNF-α* and *MTNR1B* Polymorphisms with FBG, BMI, and Plasma Lipid Profile	54
2.3.5	Plasma Melatonin Levels and their Correlation with FBG, BMI, and Plasma Lipid Profile	56
2.3.6	Relative Gene Expression of *TNF-α* and its Correlation with its Promoter Polymorphisms, BMI, FBG, and Plasma Lipid Profile	57
2.3.7	Plasma TNF-α Protein Levels and their Correlation with BMI, FBG, and Plasma Lipid Profile	58
2.3.8	Plasma Free Fatty Acid Levels and their Correlation with BMI, FBG, and Plasma Lipid Profile	58
2.4	Discussion	59
2.5	References	63
CHAPTER 3. *IN VITRO* EFFECT OF SITAGLIPTIN, MELATONIN, AND COMBINATION TREATMENT ON β-CELL PROLIFERATION IN MOUSE PANCREATIC ISLETS		**69-79**
3.1	Introduction	70
3.2	Materials and Methods	70
3.2.1	Islet Isolation and Dispersion	70
3.2.2	Islet Culture	71
3.2.3	Immunocytochemistry-Immunofluorescence (ICC-IF)	72
3.2.4	Statistical Analyses	72
3.3	Results	72
3.3.1	Assessment of β-cell Proliferation *In Vitro*	72
3.4	Discussion	75
3.5	References	77
CHAPTER 4. EFFECT OF SITAGLIPTIN, MELATONIN, AND COMBINATION TREATMENT ON PANCREATIC β-CELL REGENERATION IN STREPTOZOTOCIN (STZ)-INDUCED T1D MOUSE MODEL		**80-108**
4.1	Introduction	81
4.2	Materials and Methods	82
4.2.1	Animals	82
4.2.2	T1D Mouse Model Development and Drug Treatment	82
4.2.3	Intraperitoneal Glucose Tolerance Test	82
4.2.4	Plasma Insulin Levels	83
4.2.5	Immunohistochemistry-Immunofluorescence (IHC-IF)	83
4.2.6	Statistical Analysis	84
4.3	Results	84
4.3.1	Assessment of Body Weight, Fasting Blood Glucose, and Glucose Tolerance in Young T1D Mice	84
4.3.2	Assessment of β-Cell Proliferation in Young T1D Mice	85
4.3.3	Assessment of β-Cell Neogenesis in Young T1D Mice	87
4.3.4	Assessment of α- to β-Cell Transdifferentiation in Young T1D Mice	87
4.3.5	Assessment of β-Cell Apoptosis in Young T1D Mice	89
4.3.6	Assessment of Body Weight, Fasting Blood Glucose, and Glucose Tolerance in Old T1D Mice	92

4.3.7	Assessment of β-Cell Proliferation in Old T1D Mice	93
4.3.8	Assessment of β-Cell Neogenesis in Old T1D Mice	94
4.3.9	Assessment of α- to β-Cell Transdifferentiation in Old T1D Mice	96
4.3.10	Assessment of β-Cell Apoptosis in Old T1D Mice	97
4.4	Discussion	100
4.5	References	104
CHAPTER 5. EFFECT OF SITAGLIPTIN, MELATONIN, AND COMBINATION TREATMENT ON HIGH FAT DIET (HFD)-INDUCED T2D MOUSE MODEL		**109-135**
5.1	Introduction	110
5.2	Materials and Methods	111
5.2.1	Animals	111
5.2.2	Development of Diet-Induced Obese T2D Mouse Model and Drug Treatment	111
5.2.3	Glucose Tolerance Test and Insulin Tolerance Test	111
5.2.4	Metabolic and Biochemical Parameters	112
5.2.5	Gene Expression Analysis	112
5.2.6	Droplet Digital PCR (ddPCR)	113
5.2.7	Glucoregulatory Enzymes Activity and Liver Glycogen Content	114
5.2.8	Respiratory Control Ratio (RCR)	114
5.2.9	Western Blot Analysis	114
5.2.10	Immunohistochemistry-Immunofluorescence (IHC-IF)	115
5.2.11	Statistical Analyses	116
5.3	Results	116
5.3.1	Development of Diet-Induced Obese T2D Mouse Model	116
5.3.2	Analysis of Metabolic Parameters	117
5.3.3	Analysis of Plasma Lipid Profile, Insulin, Leptin and Melatonin levels	118
5.3.4	Analysis of Glucoregulatory Enzyme Activity, Liver Glycogen Content and Gene Expression	119
5.3.5	Absolute Gene Quantification of *MTNR1B*, *GLUT4* and *TNF-α* in Adipose Tissue	121
5.3.6	Assessment of Mitochondrial Respiratory Control Ratio (RCR) of Complexes I-IV	122
5.3.7	Protein Expression Analysis for Insulin Signalling Pathway in Skeletal Muscle	123
5.3.8	Assessment of β-cell Mass and Islet Number	124
5.4	Discussion	125
5.5	References	130
CHAPTER 6. EFFECT OF SITAGLIPTIN AND MELATONIN COMBINATION TREATMENT ON β-CELL PROLIFERATION IN HUMANIZED EUGLYCEMIC MOUSE MODEL OF ISLET TRANSPLANTATION		**136-143**
6.1	Introduction	137
6.2	Materials and Methods	138
6.2.1	Human Islets	138
6.2.2	Islet Transplantation	138
6.2.3	Euglycemic Human Islet Transplant Model	138

6.2.4	Statistical Analysis	139
6.3	Results	139
6.3.1	Assessment of β-cell Proliferation in the Human Islet Grafts	139
6.4	Discussion	139
6.5	References	141
CHAPTER 7. CONCLUSIONS		**144-149**
Appendix		
List of Publications		
List of Oral/Poster Presentations		
Reprints of the Publications		
Ph.D. Thesis Synopsis		

List of Tables

Sr. No.	Title	Page No.
CHAPTER 2. INVESTIGATION OF GENOTYPE-PHENOTYPE CORRELATION OF *TNF-α* AND *MTNR1B* POLYMORPHISMS WITH T2D SUSCEPTIBILITY IN GUJARAT POPULATION		
2.1	Primers and restriction enzymes used for *TNF-α* and *MTNR1B* SNPs genotyping, and *TNF-α* gene expression analysis	46
2.2	Baseline characteristics of diabetic and non-diabetic individuals from Gujarat population	48
2.3	Genotype and allele frequency distribution of *TNF-α* promoter polymorphisms in T2D patients and controls	50
2.4	Genotype and allele frequency distribution of *MTNR1B* polymorphisms in T2D patients and controls	51
2.5	Haplotype frequencies of *TNF-α* polymorphisms in T2D patients and controls	52
2.6	Haplotype frequencies of *MTNR1B* polymorphisms in T2D patients and controls	53
2.7	Genotype-phenotype correlation analyses of *TNF-α* polymorphisms with BMI, FBG, and plasma lipid profile	55
2.8	Genotype-phenotype correlation analyses of *MTNR1B* polymorphisms with BMI, FBG, and plasma lipid profile	56
2.9	Correlation analysis of plasma melatonin with BMI, FBG, and plasma lipid profile	57
2.10	Correlation analysis of *TNF-α* transcript levels, plasma TNF-α protein levels, and FFA concentrations with BMI, FBG, and plasma lipid profile	59

List of Figures

Sr. No.	Title	Page No.
CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE		
1.1	Worldwide prevalence of diabetes mellitus	4
1.2	Diagnostic criteria for diabetes mellitus	4
1.3	Effect of stressors on the β-cell in the pathogenesis of T2D	7
1.4	Cellular stress, insulin resistance and β-cell apoptosis	10
1.5	Role of inflammation in insulin resistance	13
1.6	Involvement of GPCRs in the development and/or progression of β-cell dysfunction, insulin resistance and obesity-induced T2D	15
1.7	Differentiation of pancreatic lineages and strategies for β-cell regeneration	22
1.8	Putative signalling pathways activated by MT1 and MT2 melatonin receptors	24
1.9	Mechanism of action of DPP-4 inhibitors and GLP-1RAs and its effect on pancreatic β-cell in animal models	26
CHAPTER 2. INVESTIGATION OF GENOTYPE-PHENOTYPE CORRELATION OF *TNF-α* AND *MTNR1B* POLYMORPHISMS WITH T2D SUSCEPTIBILITY IN GUJARAT POPULATION		
2.1	PCR-RFLP analysis of *TNF-α* -238 G/A, -308 G/A, -857 C/T and -863 C/A polymorphisms	49
2.2	PCR-RFLP analysis of *MTNR1B* -1193 C/T, 5' UTR G/C and intron C/G polymorphisms	51
2.3	Linkage Disequilibrium (LD) block of ***TNF-α*** polymorphisms	54
2.4	Linkage Disequilibrium (LD) block of *MTNR1B* polymorphisms	54
2.5	Plasma melatonin levels in controls and patients	57
2.6	*TNF-α* transcript levels	57
2.7	Plasma TNF-α protein levels	58
2.8	Plasma FFA levels	59
2.9	Schematic representation of role of *TNF-α* & *MTNR1B* polymorphisms in T2D pathogenesis in Gujarat population	63
CHAPTER 3. *IN VITRO* EFFECT OF SITAGLIPTIN, MELATONIN, AND COMBINATION TREATMENT ON β-CELL PROLIFERATION IN MOUSE PANCREATIC ISLETS		
3.1	The dose and time-dependent effect of sitagliptin and melatonin on β-cell proliferation under glucotoxic stress - representative images	73
3.2	The dose and time-dependent effect of sitagliptin and melatonin on β-cell proliferation under glucotoxic stress	74
3.3	The effect of sitagliptin and melatonin on β-cell proliferation under glucotoxic stress	74
3.4	The effect of sitagliptin and melatonin on β-cell proliferation under gluco-lipotoxic stress	75
3.5	Additive effect of sitagliptin, melatonin, and combination (S+M) on β-cell proliferation under glucotoxic and gluco-lipotoxic stresses *in vitro*	77

CHAPTER 4. EFFECT OF SITAGLIPTIN, MELATONIN, AND COMBINATION TREATMENT ON PANCREATIC β-CELL REGENERATION IN STREPTOZOTOCIN (STZ)-INDUCED T1D MOUSE MODEL		
4.1	Assessment of body weight, blood glucose levels, and glucose tolerance in young mice	85
4.2	Immunohistochemical analysis for pancreatic β-cell proliferation in young T1D mice as shown by $BrdU^+$ $insulin^+$ cells	86
4.3	Immunohistochemical analysis for pancreatic β-cell neogenesis in young T1D mice as shown by $PDX1^+$ $NGN3^+$ cells.	87
4.4	Immunohistochemical analysis for pancreatic α- to β-cell transdifferentiation in young T1D mice as shown by PAX^+ ARX^+ $insulin^+$ cells	89
4.5	Immunohistochemical analysis for pancreatic caspase-independent β-cell apoptosis in young T1D mice as shown by AIF^+ $insulin^+$ cells	90
4.6	Immunohistochemical analysis for pancreatic β-cell apoptosis as shown by $TUNEL^+$ $insulin^+$ cells in young T1D mice	92
4.7	Assessment of body weight, blood glucose levels, and glucose tolerance in old mice:	93
4.8	Immunohistochemical analysis for pancreatic β-cell proliferation in old T1D mice as shown by $BrdU^+$ $insulin^+$ cells	94
4.9	Immunohistochemical analysis for pancreatic β-cell neogenesis in old T1D mice as shown by $PDX1^+$ $NGN3^+$ cells	95
4.10	Immunohistochemical analysis for pancreatic α- to β-cell transdifferentiation in old T1D mice as shown by PAX^+ ARX^+ $insulin^+$ cells	96
4.11	Immunohistochemical analysis for pancreatic caspase-independent β-cell apoptosis in old mice as shown by AIF^+ $insulin^+$ cells	98
4.12	Immunohistochemical analysis for pancreatic β-cell apoptosis as shown by $TUNEL^+$ $insulin^+$ cells in old T1D mice	99
4.13	Effect of melatonin and sitagliptin on T1D manifestations in streptozotocin- induced T1D young and old mouse model	104
CHAPTER 5. EFFECT OF SITAGLIPTIN, MELATONIN, AND COMBINATION TREATMENT ON HIGH FAT DIET (HFD)-INDUCED T2D MOUSE MODEL		
5.1	Establishment of DIO T2D mouse model	117
5.2	Body weight, blood glucose levels, food and water intake, IPGTT and IPITT	118
5.3	Plasma lipid profile, insulin, leptin and melatonin levels	119
5.4	Gene expression and enzyme activities of glucoregulatory enzymes and glycogen content in the liver	120
5.5	Gene expression of mitochondrial biogenesis markers in skeletal muscle and lipid metabolism markers in adipose tissue	121
5.6	Absolute gene quantification of *MTNR1B*, *GLUT4*, and *TNF-α* in adipose tissue	122
5.7	Respiratory control ratio (state 3/state 4)	123
5.8	Protein expression of the insulin signalling pathway in skeletal muscle	124
5.9	β-cell mass and islet number	125

5.10	Effects of sitagliptin, melatonin and combination therapy in amelioration of T2D manifestations	130
CHAPTER 6. EFFECT OF SITAGLIPTIN AND MELATONIN COMBINATION TREATMENT ON β-CELL PROLIFERATION IN HUMANIZED EUGLYCEMIC MOUSE MODEL OF ISLET TRANSPLANTATION		
6.1	Immunofluorescence analysis for human β-cell proliferation in human islet graft as shown by $Ki67^+$ $insulin^+$ cells	139
6.2	Combination therapy of sitagliptin and melatonin induces human β-cell proliferation in euglycemic human islet transplant model in mice	141
CHAPTER 7. CONCLUSIONS		
7.1	Summary	146
7.2	Possible mode of action of sitagliptin (independent/via GLP-1), melatonin, and its combination in amelioration of diabetes manifestations in peripheral tissues (pancreas, liver, skeletal muscle, adipose tissue)	147