

Table of Contents

Sr. No.	Title	Page No.
CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE		1-66
1	Introduction	2
1.1	Diabetes Mellitus	2
1.2	Classification	2
1.3	Epidemiology of diabetes mellitus	3
1.4	Diagnostic criteria for diabetes mellitus	4
1.5	Pathogenesis of diabetes mellitus	5
1.5.1	Pathogenesis of type 1 diabetes (T1D)	5
1.5.1.1	Role of genetic factors in T1D	7
1.5.2	Pathogenesis of type 2 diabetes (T2D)	9
1.5.2.1	Role of genetic factors in T2D pathogenesis	10
1.5.2.2	Role of cytokines, oxidative stress, ER stress and inflammation in T2D	14
1.5.2.3	Obesity-induced inflammation and insulin resistance	16
1.5.2.3.1	Insulin signaling and mechanism of insulin resistance	18
1.5.2.3.2	Pro-inflammatory adipokine: Leptin (LEP)	21
1.5.2.3.3	Leptin resistance	22
1.6	Management of Diabetes Mellitus (DM)	24
1.6.1	Management of type 1 diabetes (T1D)	25
1.6.2	Management of type 2 diabetes (T2D)	26
1.7	β -cell regeneration	31
1.7.1	β -cell proliferation	31
1.7.2	β -cell neogenesis	32
1.7.3	β -cell transdifferentiation	33
1.7.4	Role of biomolecules in β -cell regeneration	35
1.8	Melatonin	36
1.9	γ -Aminobutyric acid (GABA)	40
1.10	References	45
OBJECTIVES		67-68
CHAPTER 2. TO ASSESS THE EFFICACY OF MELATONIN, GABA AND COMBINATION THERAPY IN AMELIORATION OF DIABETIC MANIFESTATIONS IN STREPTOZOTOCIN INDUCED T1D MOUSE MODEL		69-83
2.1	Introduction	70
2.2	Materials and methods	71
2.2.1	Animals	71
2.2.2	Induction of T1D and treatment	71
2.2.3	Intraperitoneal glucose tolerance test (IPGTT)	72
2.2.4	Plasma Insulin Levels	72
2.2.5	Pancreatic tissue preparation and immunohistochemistry-immunofluorescence (IHC-IF) analysis	72

2.2.6	Statistical Analyses	73
2.3	Results	73
2.3.1	Assessment of body weight, fasting blood glucose, and glucose tolerance	73
2.3.2	Assessment of Pancreatic β -cell regeneration and Apoptosis	75
2.4	Discussion	76
2.5	References	80
CHAPTER 3. TO ASSESS THE EFFICACY OF MELATONIN, GABA AND COMBINATION THERAPY IN THE AMELIORATION OF DIABETIC MANIFESTATIONS IN HIGH FAT DIET (HFD) INDUCED T2D MOUSE MODEL		84-109
3.1	Introduction	85
3.2	Materials and methods	86
3.2.1	Animals	86
3.2.2	Induction of T2D and treatment	86
3.2.3	Glucose tolerance and insulin tolerance tests	87
3.2.4	Metabolic and biochemical parameters	87
3.2.5	Gene expression analyses	87
3.2.6	Droplet digital PCR (ddPCR)	88
3.2.7	Glucoregulatory enzyme activity and liver glycogen content	89
3.2.8	Mitochondrial oxygen consumption rate (OCR)	90
3.2.9	Western Blot Analysis	90
3.2.10	Immunohistochemistry-immunofluorescence (IHC-IF) analysis	91
3.2.11	Statistical Analyses	92
3.3	Results	92
3.3.1	Assessment of metabolic profile	92
3.3.2	Assessment of plasma lipid profile, and insulin, leptin & melatonin levels	93
3.3.3	Assessment of key glucoregulatory enzyme gene expression & specific activity, and liver glycogen content	94
3.3.4	Gene expression analysis of mitochondrial biogenesis markers in skeletal muscle and lipid metabolism markers, <i>MTNR1B</i> , & <i>GLUT4</i> in Adipose Tissue	96
3.3.5	Estimation of mitochondrial respiratory control ratio (RCR) of complexes I-IV in skeletal muscle	97
3.3.6	Protein expression analysis for insulin signaling pathway in skeletal muscle	98
3.3.7	Assessment of β -cell mass and islet number in pancreas	99
3.4	Discussion	100
3.5	References	104
CHAPTER 4. TO ASSESS GENOTYPE-PHENOTYPE CORRELATION OF LEPTIN (LEP) AND ITS RECEPTOR (LEPR) IN GUJARAT T2D PATIENTS AND CONTROLS		110-135
4.1	Introduction	111
4.2	Materials and methods	112
4.2.1	Study subjects	112
4.2.2	Anthropometric parameters, lipid profiling, and DNA extraction	112

4.2.3	Genotyping of <i>LEP</i> and <i>LEPR</i> polymorphisms by PCR-RFLP	113
4.2.4	Estimation of <i>LEP</i> & <i>LEPR</i> transcript levels	113
4.2.5	Estimation of <i>LEP</i> & <i>LEPR</i> protein levels	114
4.2.6	Statistical analyses	115
4.2.7	Bioinformatics analysis	115
4.3	Results	115
4.3.1	Baseline characteristics	115
4.3.2	Association of <i>LEP</i> and <i>LEPR</i> polymorphisms with T2D	116
4.3.3	Haplotype analysis	120
4.3.4	Linkage disequilibrium (LD) analysis	120
4.3.5	Correlation of <i>LEP</i> and <i>LEPR</i> polymorphisms with FBG, BMI and plasma lipids	121
4.3.6	Assessment of <i>LEP</i> and <i>LEPR</i> transcript levels from PBMCs	121
4.3.7	Estimation of plasma protein levels of leptin and sOb-R	122
4.3.8	Plasma protein levels of leptin and sOb-R and their correlation with metabolic profile	123
4.3.9	Bioinformatics analysis	124
4.4	Discussion	124
4.5	References	128
CHAPTER 5. CONCLUSIONS		136-143
<i>Appendix</i>		
<i>List of Publications</i>		
<i>List of Oral/Poster Presentations</i>		
<i>Reprints of the Publications</i>		
<i>Ph.D. Thesis Synopsis</i>		

List of Tables

Sr. No.	Title	Page No.
CHAPTER 1. INTRODUCTION AND REVIEW OF LITERATURE		
1.1	Summary of genetic loci associated with risk of T2D	12
1.2	Characteristics and side effects of the most widely used antidiabetic drugs	27
1.3	Comparisons of initial combination therapy <i>versus</i> monotherapy with respect to the risks of hypoglycemia and other adverse effects	29
1.4	Therapeutic effects of melatonin on metabolic profile in diabetes model	38
1.5	Therapeutic effects of GABA on metabolic profile, β -cell regeneration and apoptosis in a diabetes model	43
CHAPTER 2. TO ASSESS THE EFFICACY OF MELATONIN, GABA AND COMBINATION THERAPY IN AMELIORATION OF DIABETIC MANIFESTATIONS IN STREPTOZOTOCIN INDUCED T1D MOUSE MODEL		
2.1	List of antibodies used for the IHC studies	73
CHAPTER 3. TO ASSESS THE EFFICACY OF MELATONIN, GABA AND COMBINATION THERAPY IN THE AMELIORATION OF DIABETIC MANIFESTATIONS IN HIGH FAT DIET (HFD) INDUCED T2D MOUSE MODEL		
3.1	List of primers used for the transcript analysis	88
3.2	List of antibodies used for the immunoblot analysis	91
CHAPTER 4. TO ASSESS GENOTYPE-PHENOTYPE CORRELATION OF LEPTIN (LEP) AND ITS RECEPTOR (LEPR) IN GUJARAT T2D PATIENTS AND CONTROLS		
4.1	Primers and restriction enzymes used for genotyping for <i>LEP</i> and <i>LEPR</i> polymorphisms and expression	114
4.2	Baseline characteristics of diabetic and non-diabetic individuals from Gujarat population	116
4.3	Distribution of genotype and allele frequencies of <i>LEP</i> and <i>LEPR</i> polymorphisms in T2D patients and controls	119
4.4	Distribution of haplotype frequencies of <i>LEPR</i> polymorphisms in T2D patients and controls	120
4.5	Genotype-phenotype correlation of <i>LEP</i> and <i>LEPR</i> polymorphisms with BMI, FBG and plasma lipid profile	121
4.6	Correlation analysis of plasma protein levels of leptin and sOb-R with the metabolic profile	124
4.7	<i>In-silico</i> prediction results for <i>LEPR</i> Q223R A/G polymorphism	124

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	Proposed mechanism for the pathogenesis of T1D	7
1.4	Role of genes and environmental factors in developing obesity-insulin resistance and T2D	10
1.5	Cellular stress, insulin resistance and β -cell apoptosis	15
1.6	Obesity-induced macrophage infiltration into adipose tissue convicts inflammation and insulin resistance	17
1.7	Direct interaction of insulin signaling and inflammatory pathways and their role in insulin resistance	20
1.8	Mechanism of obesity and leptin resistance mediated insulin resistance	23
1.9	Glucose metabolism and targeted therapies for T2D management	26
1.10	Lineage decisions during pancreas development & Potential strategies for regenerating β -cells	35
1.11	Activation of intracellular signaling pathways by melatonin receptors.	37
1.12	Role of GABA in the regulation of pancreatic cell function	42
CHAPTER 2. TO ASSESS THE EFFICACY OF MELATONIN, GABA AND COMBINATION THERAPY IN AMELIORATION OF DIABETIC MANIFESTATIONS IN STREPTOZOTOCIN INDUCED T1D MOUSE MODEL		
2.1	Assessment of Body Weight, Fasting Blood Glucose levels, and Glucose Tolerance	74
2.2	Assessment of pancreatic β -cell regeneration and apoptosis	76
2.3	Effect of melatonin, GABA, and combination therapy in amelioration of STZ-induced T1D manifestations in a mouse model	80
CHAPTER 3. TO ASSESS THE EFFICACY OF MELATONIN, GABA AND COMBINATION THERAPY IN THE AMELIORATION OF DIABETIC MANIFESTATIONS IN HIGH FAT DIET (HFD) INDUCED T2D MOUSE MODEL		
3.1	Evaluation of bodyweight, blood glucose levels, food and water intake, IPGTT and IPITT	93
3.2	Evaluation of plasma lipid profile, insulin, leptin, and melatonin levels	94
3.3	Gene expression and enzyme activities of glucoregulatory enzymes and glycogen content in the liver	95
3.4	Gene expression of mitochondrial biogenesis markers in skeletal muscle, lipid metabolism markers, and absolute gene quantification of <i>MTNR1B</i> and <i>GLUT4</i> in adipose tissue	97
3.5	Mitochondrial respiratory control ratio (state 3/state 4) in the skeletal muscle	98
3.6	Protein expression of the insulin signaling pathway in skeletal muscle	99
3.7	Pancreatic β -cell mass and islet number	100
3.8	Effect of melatonin, GABA, and combination therapy in amelioration of HFD-induced T2D manifestations in a mouse model	104

CHAPTER 4. TO ASSESS GENOTYPE-PHENOTYPE CORRELATION OF LEPTIN (LEP) AND ITS RECEPTOR (LEPR) IN GUJARAT T2D PATIENTS AND CONTROLS		
4.1	PCR-RFLP analyses of <i>LEP</i> and <i>LEPR</i> polymorphisms	117
4.2	Confirmation of genotyping results by Sanger's sequencing of PCR products	118
4.3	Linkage disequilibrium (LD) block	120
4.4	<i>LEP</i> and <i>LEPR</i> transcript levels in PBMCs of T2D patients and controls	122
4.5	Plasma protein levels of leptin and sOb-R in T2D patients and controls	123
4.6	Role of leptin (LEP) and leptin receptor (LEPR, sOb-R) and altered adipokine levels in T2D	128
CHAPTER 5. CONCLUSIONS		
5.1	Summary	138
5.2	Possible mode of action of melatonin, GABA and their combinations in the amelioration of diabetes manifestations in the peripheral tissues (pancreas, liver, skeletal muscle and adipose tissue)	140