

## List of Figures

Figure 1.1 Anatomical organization of the pancreas depicting exocrine and endocrine sections with various cell types .....	6
Figure 1.2 Mechanism of glucose-stimulated insulin secretion in pancreatic islet $\beta$ cells .....	8
Figure 1.3 Pancreatic organogenesis demonstrating islet cell development.....	11
Figure 1.4 Key transcription factors involved in different steps of Pancreatic islet $\beta$ -cell formation .....	12
Figure 1.5 Risk Factors associated with the pathogenesis of Diabetes Mellitus .....	19
Figure 1.6 Mechanism of $\beta$ cell destruction in T1DM.....	21
Figure 1.7 Different cell sources for islet neogenesis.....	28
Figure 1.8 Small molecules induced MSC differentiation to $\beta$ cells .....	30
Figure 1.9 Properties of Enicostemma Littorale, Swertisin and swertiamarin (Our Lab Reports).....	35
Figure 1.10 Insulin signaling pathway.....	37
Figure 1.11 Physiological insulin resistance and insulin dependent tissues.....	39
Figure 1.12 Effects of various myokines on different organs.....	45
Figure 1.13 Site of action for glucose-lowering medications.....	48
Figure 1.14 Glucose reabsorption by SGLT1/2 through a proximal tubule epithelial cell in nephron .....	52
Figure 1.15 Mechanism of action of SGLT2 inhibitors.....	54
Figure 3.1 I-TASSER protocol for protein structure and function prediction.....	65
Figure 3.2 <i>In silico</i> plan of work for molecular docking of swertisin .....	67
Figure 3.3 <i>In vitro</i> plan of work for Sodium dependent glucose uptake .....	70
Figure 3.4 Figure showing schematic <i>in vivo</i> plan of work .....	72
Figure 3.5 Distribution of predicted targets of swertisin in different protein classes by SwissTarget Prediction tool.....	82
Figure 3.6 Graphical representation of pair-wise alignment of human SGLT2 structure against 3DH4.....	85
Figure 3.7 Graphical representation of pair-wise alignment of human SGLT2 structure against 2QX2.....	85
Figure 3.8 Figure showing ligand binding site residues of the PDB Hit used for template threading of hSGLT2 by I-TASSER.....	88

Figure 3.9 Computationally modelled human SGLT2 structure using I-TASSER .....	89
Figure 3.10 Ramchandran plot analysis of modelled human SGLT2 structure.....	90
Figure 3.11 Swertisin interacts with key residues within the active site of hSGLT2 by molecular docking. 3D and 2D diagram of molecular docking interaction of Swertisin-hSGLT2.....	91
Figure 3.12 3D and 2D diagram of molecular docking interaction of Canagliflozin-hSGLT2....	92
Figure 3.13 Molecular Dynamics Simulations of SGLT2 in apo and inhibitor bound complexes, computing the deviation (nm) versus function of time (100 ns). RMSD of the protein Ca backbone atoms of SGLT2 (black), SGLT2-canagliflozin (red), and SGLT2-swertisin (green) .....	94
Figure 3.14 Molecular Dynamics Simulations of SGLT2 in apo and inhibitor bound complexes, computing the residue-wise RMSF deviations (nm). RMSF deviation plot of the protein Ca backbone atoms of SGLT2 (black), SGLT2-canagliflozin (red), and SGLT2-swertisin (green). 95	95
Figure 3.15 Molecular Dynamics Simulations of SGLT2 in apo and inhibitor bound complexes, computing the radius of gyration (nm) versus function of time (100 ns). RoG of the protein Ca backbone atoms of SGLT2 (black), SGLT2-canagliflozin (red), and SGLT2-swertisin (green). 96	96
Figure 3.16 Intra and Inter hydrogen bonds of SGLT2 in apo and inhibitor bound complexes: (a) Intra H-bond formation plot computed versus function of time (100 ns) between protein and inhibitor in SGLT2 (black), SGLT2-canagliflozin (red), and SGLT2-swertisin (green). (b) Number of hydrogen bonds computed versus function of time (100 ns) between SGLT2 and inhibitor in SGLT2 (black), SGLT2-canagliflozin (red), and SGLT2-swertisin (green) .....	99
Figure 3.17 Dynamics cross-correlation for SGLT2, SGLT2-canagliflozin and SGLT2-swertisin complexes. DCCM was calculated according to time average of C $\alpha$ atoms. The whole range of correlation from - 1 to +1 is represented in three ranges: cyan color corresponding to positive correlation values ranging from 0.25 to 1; magenta color corresponding to negative correlation values ranging from - 0.25 to - 1; and white color corresponding to weak or no-correlation values ranging from - 0.25 to +0.25. The extent of correlation or anti-correlation is indicated by variation in the intensity of respective cyan or magenta color in SGLT2 (a), SGLT2-canagliflozin (b), and SGLT2-swertisin (c) .....	102
Figure 3.18 PCA analysis for SGLT2 (a), SGLT2-canagliflozin (b), and SGLT2-swertisin (c). In all three sections, the PC1, PC2 and PC3 eigen values are plotted against each other and the fourth section indicates the plot of population variance and eigenvalue rank.....	103

Figure 3.19 MM-PBSA Calculation for binding free energy. The total binding free energy for all the SGLT2-inhibitor complexes calculated for last 50 ns stable trajectory for a total of 200 frames, each at 250 ps interval..... 104

Figure 3.20 SGLT2 specific inhibition by swertisin affects sodium dependent glucose uptake *in vitro* in HEK293 cell line. Sodium dependent glucose uptake assay was performed in the HEK293 cell line. Swertisin treatment was given at varying doses and uptake inhibition of 2-NBDG was performed in (B) sodium buffer (C) sodium free buffer and (D) sodium buffer with 10  $\mu$ M cytochalasin B (GLUT inhibitor) for 60 min. Canagliflozin was taken as a positive control. Results are represented as % Fluorescence intensity per total DNA  $\pm$  SEM, N=3. Significance is expressed as p-value \*\*\* <0.001, \*\*\*\* <0.0001 control vs treatment groups. G=Glucose, S=Swertisin, C=Cytochalasin B ..... 105

Figure 3.21 Representative time dependent fluorescence imaging of uptake was performed. HEK293 cells were incubated in sodium buffer in the absence (Control) and presence of 7.5 $\mu$ g/ml swertisin with 10  $\mu$ M cytochalasin B for 10 min in presence of 2-NBDG (green) (Magnification:20X). ..... 106

Figure 3.22 Sodium dependent glucose uptake demonstrating unaltered SGLT1 inhibition by swertisin. Sodium dependent glucose uptake assay was performed in the Caco2 cell line. Swertisin treatment was given at varying doses and uptake inhibition of 2-NBDG was performed in (B) sodium buffer (C) sodium free buffer and (D) sodium buffer with 10  $\mu$ M cytochalasin B (GLUT inhibitor) for 60 min. Canagliflozin was taken as a positive control. Results are represented as % Fluorescence intensity per total DNA  $\pm$  SEM, N=3. G=Glucose, S=Swertisin, C=Cytochalasin B ..... 107

Figure 3.23 Swertisin selectively regulates SGLT2 expression. Time dependent protein expression of SGLT2 and regulating factors in the HEK293 cell line were studied. Western Blot analysis of proteins PKC, pp38 MAPK, ERK1/2, and SGLT2 along with densitometric analysis normalized to beta-actin are expressed as arbitrary unit $\pm$  S.E.M. N=3, Significance is expressed as p-value \*<0.05, \*\* <0.01, \*\*\* <0.001 control vs treatment groups ..... 109

Figure 3.24 Figure showing H&E staining of pancreatic islets from control, diabetic, swertisin and canagliflozin treatment groups..... 110

Figure 3.25 (A) Graph representing fasting blood glucose at different days of treatment for control, diabetic, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are

represented as mean± SEM. \*<0.05, \*\* <0.01, \*\*\* <0.001 Control vs treatment groups #<0.05, ## <0.01, ### <0.01 Diabetic vs treatment groups (N=8-12). (B) Graph representing blood glucose levels for oral glucose tolerance test over 2H for control, diabetic, swertisin and canagliflozin treated STZ diabetic BALB/c mice. Data are represented as mean± SEM. \*<0.05, \*\* <0.01, \*\*\* <0.001, \*\*\*\* <0.0001 Diabetic vs control, #<0.05, ## <0.01, ##### <0.0001 Diabetic vs swertisin treatment. Diabetic vs canagliflozin treatment +<0.05, ++ <0.01, +++ <0.001, ++++ <0.0001 (N=8)..... 112

Figure 3.26 Swertisin impacts physiological and metabolic parameters in STZ induced diabetic mice Graphs representing different parameters (A) Body weight (B) Chow intake (C) water intake (D) urine output for control, diabetic control, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are represented as mean± SEM. \*<0.05, \*\* <0.01, \*\*\* <0.001, \*\*\*\* <0.0001 Control vs treatment groups ##### <0.0001 Diabetic control vs treatment groups (N=8) ..... 113

Figure 3.27 Graphs representing different parameters like proteinuria and glucosuria for control, diabetic control, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are represented as mean± SEM. \*\*\*\* <0.0001 Control vs treatment groups #<0.05, ### <0.001, ##### <0.0001 Diabetic control vs treatment groups. Swertisin treatment vs canagliflozin treatment +++ <0.001 (N=8) ..... 114

Figure 3.28 Graphs representing different parameters serum creatinine, urine creatinine and creatinine clearance for control, diabetic control, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are represented as mean± SEM. \*<0.05 (N=8) ..... 115

Figure 3.29 Graphs representing different parameters serum urea (K) urine urea (L) urea clearance for control, diabetic control, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are represented as mean± SEM. \*<0.05, \*\* <0.01, \*\*\* <0.001, \*\*\*\* <0.0001 Control vs treatment groups #<0.05, ### <0.001, ##### <0.0001 Diabetic control vs treatment groups (N=8) ..... 116

Figure 3.30 SGLT2 and PKC expression is reduced by swertisin in mice kidney. Western Blot analysis of proteins PKC and SGLT2 along with densitometric analysis normalized to respective internal control beta-actin Data are represented as mean± SEM. p \*<0.05, \*\*\* <0.001, \*\*\*\* <0.0001 Control vs treatment groups ## <0.01, ##### <0.001 Diabetic control vs treatment groups. Swertisin treatment vs canagliflozin treatment + <0.05, ++++ <0.0001 (N=3) ..... 117

Figure 3.31 Immunohistochemistry was performed in mice kidney. Reduced SGLT2 expression was observed (N=3) .....	118
Figure 3.32 Staining of mice kidney and intestine by H&E for analysis of cytoarchitecture ....	119
Figure 3.33 Graphical Summary of Chapter 3 .....	125
Figure 4.1 <i>In vitro</i> Plan of work insulin resistant model generation and secretome collection....	132
Figure 4.2 <i>In vitro</i> Plan of work of differentiation of PREP into ILCCs.....	133
Figure 4.3 Differentiation of C2C12 myoblast into myotubes. Day wise maturation of myoblast into myotube. Visualization of myotube formation by May-grunwald giemsa staining .....	136
Figure 4.4 Confirmation of C2C12 myoblast into myotubes. Confirmation by gene expression of myogenin (Data is expressed as Fold change $\pm$ S.E.M. N=3), protein expression of desmin (Densitometric analysis was done normalized to beta-actin and data expressed as arbitrary unit $\pm$ S.E.M. N=3). Significance is expressed as p-value $* < 0.05$ and immunocytochemistry of $\alpha$ -SMA was performed (magnification 20x).....	137
Figure 4.5 Confirmation of insulin resistance in C2C12 myotube Myotubes were treated with TNF- $\alpha$ to make <i>in vitro</i> insulin resistant model and confirmation of insulin resistant condition was done by Gene expression of Insr (Expression of Insr was evaluated both by RT-PCR and dd-PCR), Irs1, Glut4 was done (Data is expressed as Fold change $\pm$ S.E.M. N=3). Significance is expressed as p-value $* < 0.05$ , $** < 0.01$ , $*** < 0.001$ .....	138
Figure 4.6 Confirmation of insulin resistance in C2C12 myotube Myotubes were treated with TNF- $\alpha$ to make <i>in vitro</i> insulin resistant model and confirmation of insulin resistant condition was done by Protein expression of IR, IRS1, pAkt/Akt key insulin signaling proteins (Densitometric analysis was done normalized to beta-actin and expressed as arbitrary unit $\pm$ S.E.M. N=3). Significance is expressed as p-value $* < 0.05$ , $** < 0.01$ .....	139
Figure 4.7 Myokine gene expression in C2C12 myotube under insulin resistance. Differential gene expression of IL6, IL13, IL15, IL10, CX3CL1, CXCL1, FGF21 myokines were performed in control and IR C2C12 myotubes. Data is expressed as Fold change $\pm$ S.E.M. N=3. Significance is expressed as p-value $* < 0.05$ , $** < 0.01$ , $**** < 0.0001$ .....	141
Figure 4.8 Characterization of differentially regulated proteins from secretomes. Chromatogram of secretomes of C2C12 myotube from control and IR groups. ....	142

Figure 4.9 Characterization of differentially regulated proteins from secretomes. Graph showing Abundance and Classification of proteins present in secretomes of C2C12 myotubes from control and IR groups .....	144
Figure 4.10 Representative images of temporal differentiation of ILCC from day 0 to day 4. PREPs were subjected to control and IR secretomes of C2C12 myotubes and ILCCs were differentiated. ....	145
Figure 4.11 Functional analysis of ILCCs under the influence of C2C12 myotube secretome. PREPs were subjected to control and IR secretomes of C2C12 myotubes and ILCCs were differentiated. Functional parameters like DTZ staining (magnification 20X), Yield and Morphometric analysis was done. Data is expressed as Mean $\pm$ S.E.M. N=3. Significance is expressed as p-value * $<0.05$ ** $<0.01$ .....	146
Figure 4.12 Functional analysis of ILCCs under the influence of C2C12 myotube secretome. Functional parameters like Immunocytochemistry of C-peptide and Glucagon were performed (magnification 63X).....	148
Figure 4.13 Islet integrity parameter of ILCCs under the influence of C2C12 myotube secretome was done. DCFDA analysis for ROS measurement was done. Data is expressed as Mean $\pm$ S.E.M. N=3. Significance is expressed as p-value * $<0.05$ .....	149
Figure 4.14 Islet survival parameter by FDA PI staining was performed (magnification 20X).	150
Figure 4.15 Islet survival parameter by Annexin PI was performed (magnification 20X). .....	151
Figure 4.16 Western blotting of Parp1 and Caspase3 was done for cell death evaluation. Densitometric analysis of cleaved PARP-1 and CASP-3 was done normalized to Beta-actin and data expressed as arbitrary unit $\pm$ S.E.M. N=3, Significance is expressed as p-value * $<0.05$ , ** $<0.01$ , *** $<0.001$ , **** $<0.0001$ .....	152
Figure 4.17 Temporal analysis of proteins involved in islet differentiation of ILCCs under the influence of C2C12 myotube secretome Differentiation of ILCC took place from day 0 to day 4 from PREPs with secretomes of C2C12 myotube. HNF-3B, NGN-3, Nestin, Pax4, Nkx6.1, NeuroD1, PDX-1, MaF-A and GLUT-2 expression was checked for islet differentiation key proteins. Densitometric analysis was done normalized to Beta-actin and expressed as arbitrary unit $\pm$ S.E.M. N=3, Significance is expressed as p-value * $<0.05$ , ** $<0.01$ , *** $<0.001$ , **** $<0.0001$ .....	153
Figure 4.18 Summary of Chapter 4.....	160

Figure 5.1 <i>In vitro</i> Plan of work for hADSC secretome collection .....	165
Figure 5.2 <i>In vitro</i> Plan of work of differentiation of PREP into ILCCs.....	166
Figure 5.3 HPLC Chromatogram of secretome from Control hADSC .....	168
Figure 5.4 HPLC Chromatogram of secretome from Obese hADSC.....	168
Figure 5.5 HPLC Chromatogram of secretome from Control and Obese hADSC. The graph represents the specific peak difference between the secretome. Distinct peaks in comparison to the vehicle control and secretomes were highlighted with blue arrow.....	169
Figure 5.6 Differential characterization of protein from secretomes Chromatogram of secretomes of hADSC from control and obese.....	171
Figure 5.7 Differential characterization of protein from secretomes) Graph showing abundance and classification of proteins present in secretomes of hADSC from control and obese. ....	172
Figure 5.8 Functional analysis of ILCCs in the presence of hADSC conditioned media ILCCs were differentiated from PREPs in presence of control and obese secretomes of hADSC. Representative images of temporal differentiation of ILCC from day 0 to day 4 with control and obese hADSC secretomes.....	173
Figure 5.9 Functional analysis of ILCCs in the presence of hADSC conditioned media ILCCs were differentiated from PREPs in presence of control and obese secretomes of hADSC. Functional parameters like DTZ staining, Yield and Morphometric analysis was done (magnification 20X) .....	175
Figure 5.10 Immunocytochemistry of C-peptide and Glucagon were done in ILCC (magnification 63X). .....	176
Figure 5.11 Islet survival and integrity parameter of ILCCs in the presence of hADSC conditioned media ILCC were differentiated from day 0 to day 4 under the influence of secretomes of hADSC. Parameter like ROS measurement by DCFDA analysis.....	177
Figure 5.12 Islet survival and integrity parameter of ILCCs in the presence of hADSC conditioned media ILCC were differentiated from day 0 to day 4 under the influence of secretomes of hADSC. Islet survival parameter by FDA PI staining was done.....	178
Figure 5.13 PARP-1 and CASP-3 expression by western blotting were done to check islet cell death. Densitometric analysis was done normalized to Beta-actin and expressed as arbitrary unit±	

S.E.M. N=3, Significance is expressed as p-value \*<0.05, \*\* <0.01, \*\*\* <0.001, \*\*\*\* <0.0001  
 ..... 179

Figure 5.14 Temporal analysis of proteins involved in islet differentiation Differentiation of ILCC  
 took place from day 0 to day 4 from PREPs with secretomes of hADSC. Western blotting of HNF-  
 3B, NGN-3, NeuroD1, PDX-1, Maf-A and GLUT-2. Densitometric analysis was done normalized to  
 Beta-actin and expressed as arbitrary unit± S.E.M. N=3, Significance is expressed as p-value  
 \*<0.05, \*\* <0.01, \*\*\* <0.001, \*\*\*\* <0.0001..... 181

Figure 5.15 Summary of Chapter 5..... 187

### List of Tables

Table 3.1 *In silico* pharmacokinetic analysis demonstrating Absorption, Distribution, Metabolism,  
 Elimination and Toxicity analysis of swertisin by ADMETLab ..... 80

Table 3.2 Predicted targets of swertisin by SwissTargetPrediction tool ..... 83

Table 3.3 PASS analysis of swertisin. Activities with  $P_a > P_i$  are only considered for a Homology  
 Modelling of hSGLT2 compound.  $P_a$  (probability "to be active") calculates the chance that the  
 compound under query resembles the structures of molecules, which are the most typical in a sub-  
 set of "actives" in PASS training set.  $P_i$  (probability "to be inactive") calculates the chance that the  
 compound under query resembles the structures of molecules, which are the most typical in a sub-  
 set of "inactives" in PASS training set..... 84

Table 3.4 NCBI-Protein BLAST showing percent identity of human SGLT2 structure against  
 3DH4 and 2QX2 ..... 85

Table 3.5 EMBOSS-pair wise alignment of human SGLT2 structure with 3DH4 and 2QX2..... 86

Table 3.6 I-TASSER predicted models of threaded hSGLT2 structures with respective C-scores  
 and TM-scores. \*NA: Not available ..... 87

Table 3.7 Summary of H-bond occupancy between SGLT2 and SGLT2 complexes with  
 canagliflozin and swertisin ..... 100