SUMMARY & CONCLUSION

CHAPTER 6

Chapter 6: Summary and Conclusions

Regenerative medicine and exploring novel bioactive are the two major areas being elucidated for effective diabetes therapy in present thesis. Bioactive swertisin showed a promising glucose-lowering effect. Hence, here we mechanistically dissected the glucose lowering property of swertisin. A systematic *in silico, in vitro,* and *in vivo* approach was directed for target analysis of swertisin. We then moved to secretome therapeutics and studied the effect of secretome on islet differentiation and functionality. The secretory nature of skeletal muscle has made it an integral part of the inter organ crosstalk network. A pathological condition like insulin resistance (IR), alters the secretome including myokine panel since it is the primary defect seen in skeletal muscle. In the present study, we have explored the interaction between skeletal muscle and pancreatic islet β cells by trophic effects under normal and insulin resistance conditions, not only in terms of islet functionality but also islet neogenesis or islet differentiation. Adipose derived stem cells (ADSC) secrete a large array of factors in the extracellular milieu that exhibit regulatory effects on other tissues including pancreatic islets. In the present study, the role of hADSC secretome was also studied on the differentiation of islets.

- In Chapter 3 we studied identification of molecular targets of swertisin in glucose homeostasis and islet differentiation & functionality
- *In silico* pharmacokinetic analysis of swertisin exhibits low absorption, moderate metabolism and a low clearance rate. It demonstrates moderate drug-likeliness and follows Lipinski's rule. Swertisin shows mild toxicity, is cardio-protectant and not a skin-irritant which is a good pharmacophore drug candidate for diabetes management.
- Sodium Glucose Cotransporter Class Protein has been identified as one of the targets of swertisin, we proceeded for molecular docking and simulations studies. Human SGLT2 structure was modelled with C-score of -0.68 [range: -2 to 5] and evaluated by the Ramachandran Plot with 92.2% residues in the favoured region, 5.4% residues in the allowed region, and 2.4% residues in the outlier region.

- The docking score for the Swertisin-hSGLT2-interaction -8.5 kcal/mol and -8.7 kcal/mol for canagliflozin which is a commercially available SGLT2 inhibitor, used a positive comparator throughout the first objective.
- The overall RMSD comparison of Cα backbone showed that the SGLT2-swertisin, was much more stable during the entire simulations compared to apo-protein (SGLT2) and reference inhibitor (SGLT2-canagliflozin). The radius of gyration of SGLT2, SGLT2-canagliflozin and SGLT2-swertisin shows a minor difference, which shows that the binding of ligands did not affect the compactness of the protein. Moreover, compared to SGLT2, SGLT2-canagliflozin and SGLT2-swertisin showed a lesser deviation in SGLT2.From dynamics cross-correlation matrix, it is clearly evident that residue interaction in SGLT2 was similar with that found with SGLT2-swertisin. The eigen data for SGLT2-swertisin had a steep deep, indicating early stable complex formation compared to SGLT2-canagliflozin.
- The marked observation that emerged from our *in vitro* sodium dependent glucose uptake experiment was that the potency of SGLT2 inhibition by swertisin (7.5 µg/ml) was significantly effective at a lower dose as against canagliflozin (13 µg/ml).
- Our findings confirm that swertisin does not interfere with non-sodium dependent GLUT transporters and SGLT1, further strengthens the selective inhibition for SGLT2.
- Reduced SGLT2 expression under the effect of swertisin is associated with differential expression of some protein kinases which interpreted that, swertisin does not affect PKC expression in acute treatment duration but increases expressions of pp38MAPK and ERK1/2.
- *In vivo* STZ induced diabetic Balb/c mice, when treated with of swertisin, demonstrated striking reduction in fasting blood glucose which is at par with canagliflozin.
- Body parameters are important for diabetogenic and metabolic assessment. A similar contribution of swertisin like canagliflozin for weight management is noteworthy in our *in vivo* study which was co-related with chow and water intake.

- The most striking observation of SGLT2 inhibition is glucosuria along with low proteinuria which was observed with swertisin treatment. Creatinine and Urea levels were within permissible range, ruling out the possibility of kidney dysfunction and generating a strong possibility of effects emerging exclusively from SGLT2 inhibitory action.
- The most remarkable observation to emerge from these data was that swertisin was able to downregulate the protein expression of SGLT2 along with upregulation of PKC in contrast to canagliflozin which makes it an excellent pharmacophore agent.
- **This is the first report of SGLT2 inhibitory property of swertisin.**

In the next chapters, we focused on organ crosstalk by trophic factors. In chapter 4 we explored the Effect of secretome of skeletal Muscle on islet differentiation & functionality and in Chapter 5 we demonstrated effect of secretome of Adipose derived Stem cells on islet differentiation & functionality

- Diabetes Mellitus is attributed by insulin resistance and ultimately pancreatic beta cell dysfunction. Here we have studied crosstalk between Skeletal Muscle and Pancreatic Islet in terms of Trophic effects (via Secretome).
- C2C12 is a myoblast cell line, which differentiates rapidly, forming contractile myotubes, used as our *in vitro* model for skeletal muscle. Myotube formation was confirmed by increased expressions of myogenin, desmin, and αSMA.
- Mature myotubes were subjected to TNF-α treatment. Reduced key insulin signaling proteins IR, IRS-1, p-Akt/Akt and *GLUT4* confirmed insulin resistant condition. Myokine gene profile demonstrated variable expression of selected myokines like *IL6, IL13, IL15, IL10, CXCL1, CCL3, FGF21* under insulin resistant condition.
- Conditioned media (secretome) of control and insulin resistant myotube (C2C12 myotube) were subjected to pancreatic resident endocrine progenitor cells (PREP) during islet differentiation. We found a significantly lower yield of islets in those treated with IR muscle secretome in comparison to islets treated with IS muscle secretome.
- Influence of control and insulin resistant skeletal muscle secretome on islet differentiation and functionality parameters were studied. Differential expression of key transcription

factors HNF-3β, NGN-3, PAX4, Nkx 6.1, Nestin, NeuroD, PDX- 1, Maf-A, and GLUT-2 were observed.

- ILCCs functionality and viability were critically hampered under IR muscle secretome. ILCCs had compromised yield and morphometry, lower expression of C-peptide and Glucagon with increased ROS activity and cell death parameters. Functionality of ILCC were well corelated with myokine and protein profile of control and insulin resistant skeletal muscle secretome
- **This is the first *in vitro* report to the best of our knowledge in terms of effect of muscle secretome on islet differentiation. This report will widen our knowledge of skeletal muscle therapeutics along with its restoring capacities on islet functionality and differentiation.**
- Adipose derived stem cells (ADSC) also secrete a large array of factors in the extracellular milieu that exhibit regulatory effects on other tissues including pancreatic islets.
- The microenvironment of metabolically compromised (obese) human ADSCs (hADSCs) has been studied on islet functionality and differentiation of islets and compared with control hADSC secretome.
- Expression of key transcription factors like HNF-3B, NGN-3, NeuroD, PDX- 1, Maf-A, and GLUT-2 involved in development were differentially regulated in obese hADSC secretome as compared to control hADSC secretome.
- Islet like cell clusters (ILCCs) functionality and viability were critically hampered under obese hADSC secretome with compromised yield, morphometry, lower expression of C-peptide and Glucagon as well as higher ROS activity and cell death parameters.
- **This study provides considerable insights on the regulating effect of altered hADSC secretome under a metabolically compromised condition. This is the first *in vitro* report to best of our knowledge.**
- **Thus overall this study provides considerable insights on three major findings which are (1) Swertisin as a potent SGLT2 inhibitor and ideal candidate for diabetes therapy (2) exploring the use of muscle and hADSC secretome in islet differentiation and (ii)**

understanding the regulating effect of altered muscle and hADSC secretome under a metabolically compromised condition and their cautious therapeutic use.