8.1 Conclusion

Type 2 diabetes mellitus (T2DM) has been announced to have reached epidemic proportion by the World Health Organisation. Approximately 90-95% diabetic patients are of T2DM. It is well known that free fatty acids (FFA) are the major players in promoting the loss of insulin sensitivity causing insulin resistance and T2DM. Several reports demonstrated that increased plasma FFA level reduces insulin stimulated glucose uptake and thus impairs insulin sensitivity. Lowering of the increased plasma FFA levels in diabetic subjects causes improvement of insulin sensitivity. There are several commercially available antidiabetic drugs present in the market but none of these drugs address the pathogenesis of insulin resistance and T2DM. From the Table 2.2, it is clearly noted that the available drugs address the problem with increased insulin release or direct administration of insulin. Although dearth of insulin is not the actual problem of this disease but loss of insulin activity is the key event in insulin resistance. However, excess of insulin provides a relief. But whether this has a long term adverse effects is still not clear. Since, it is also responsible for other functions besides glucose regulation, an imbalance is expected to lead to certain unwanted effects. Only the TZD class of drugs increase the insulin sensitivity and that exactly address the T2DM. This class of drugs is responsible for the activation of PPARγ to stimulate adipogenic differentiation. This class of drugs increase the level of lipid uptake and decrease circulating FFA level and thus improves insulin signalling pathway. It also reduces the level of proinflammatory cytokines such as TNFα and IL6. But the use of these drugs for long period is known to bring about some adverse effects. So, there is a great unmet need to develop suitable therapeutics alternative for T2DM. Hence attempts have been made to avail such compound(s) from plant sources on the basis of traditional knowledge. Ethnobotanical approach and the traditional herbal medicines provide the much required platform for discovery and consideration in the development of new drugs. For thousands of years, natural products have been the basis of treating and preventing human diseases. Numerous novel drugs, which were discovered from natural sources, can be included among the most effective drugs available now for the treatment of human diseases.

In the process of screening for antidiabetic activity of potential plant based molecules, 20 plants were screened which have been traditionally used for the treatment of diabetes by the various ethnic groups of people in the northeastern part of India, especially in Assam. Among the screened plants, *Leucas aspera* (Willd.) Link. was found to be the most potent antidiabetic plant to improve palmitate induced impairment of insulin activity. The ethanolic extract of *L. aspera* leaf showed the best insulin sensitive activity in presence of FFA. To acquire the active fraction of *L. aspera* ethanolic leaf extract, the subfractionation was performed following the bioactivity guided approach using different solvents of different polarities. The fraction of the ethanolic leaf extract of *L. aspera* showed bioactivity assay improving FFA impaired insulin stimulated glucose uptake. For further purification of this active fraction, reverse phase HPLC was performed followed by bioactivity in improving insulin stimulated glucose uptake in presence of FFA. One HPLC purified fractions showed the most insulin sensitive activity at concentration of 20μg/ml in presence of FFA. Further characterization of this fraction using FTIR, NMR and HR-LCMS analysis, the compound was indicated as swietenine.

Depending on the various cell types, several pathways have been reported through which FFA induced insulin resistance occurs. Since the ethanolic fraction of *L. aspera* showed the insulin sensitivity, the molecular mechanism of its action was investigated. From the result obtained, it was revealed that the active fraction of *L. aspera* was found to be responsible for reducing the low grade inflammation induced by FFA in cultured L6 muscle cell. From the *in vivo* study, it was also observed that the active fraction of *L. aspera* could restore the phosphorylation of the important insulin signalling proteins in high fatty diet T2DM mice. It was also found to be responsible in suppressing the overexpression of proinflammatory cytokines in adipose tissues isolated from the HFD mice treated with *L. aspera* active fraction by reducing the expression of TLR4. From these observations it was hypothesised that the active fraction of *L. aspera* collectively reduce the level of proinflammatory cytokines by downregulating the expression of TLR4 and thereby FFA induced impairment of insulin stimulated glucose uptake could be reversed by the active fraction of *L. aspera*.

Several reports suggest that macrophage polarization and activation is another important reason for the development of insulin resistance and T2DM as it is responsible for severe inflammation at its M1 state. It has been reported that, in presence of FFA, macrophage cells attain its M1 state expressing M1 proinflammataory markers including TNFα, IL6, IL1β, etc., instead of M2 markers including IL4, IL13, etc. Since, lower expression level of proinflammatory cytokines were observed in the *L. aspera* treated HFD mice as well as in the FFA treated L6 skeletal muscle cell line, the effect of *L. aspera* in macrophage polarization and activation was investigated. From the RT-PCR analysis, it was observed that by the treatment of RAW264.7 murine macrophage cells by *L. aspera* active fraction in presence of FFA, the M2 markers were found to be overexpressed instead of the M1 macrophage markers. This confirms the reduced inflammation by the action of the *L. aspera* active fraction in the FFA treated cells. Interestingly, the reduced expression of TLR4 in *L. aspera* treated macrophage cells in presence of FFA suggested that due to the downregulation of TLR4 gene expression, macrophage cells could not attain the M1 proinflammatory state. So, a hypothesis was generated that *L. aspera* active fraction is responsible for the reduction of proinflammatory cytokines level by downregulating the expression of the TLR4 gene. Hence, lower level of secreted cytokines were observed in the blood plasma of *L. aspera* treated HFD mice in comparison to the HFD control mice

A polyphenol known as ferulic acid, isolated from *Hibiscus mutabilis* Linn. could also improve insulin sensitivity at 10μg/ml concentration. At this concentration of ferulic acid, the amount of glucose uptake by L6 myotubes in presence of FFA was found to be significantly high. On investigation of the molecular mechanism was found to lie in this phenomenon. It was observed that ferulic acid improved FFA induced downregulation of insulin receptor. In normal condition, the binding of HMGA1 to the promoter of insulin receptor, initiates the insulin receptor gene expression in association with some other proteins/ transcription factors. The binding of activated PKCε to HMGA1 interferes the binding of HMGA1 to the insulin resistance promoter resulting in insulin receptor gene downregulation. Ferulic acid reduced the activation of PKCε and hence it could not bind to the HMGA1 to interfere its binding to the insulin receptor

promoter site in the muscle cell. Like the *L. aspera* active fraction, it was also found to be a responsible factor in reducing inflammation in the adipose tissue of the HFD fed rat model. In the isolated adipose tissue, it was observed that, ferulic acid reduced the proinflammatory cytokines by reducing the expression of fetuin A gene and hence it improved the glucose uptake in the HFD rat maintaining blood glucose level under control in the HFD rat.

It has been observed that FFA induced oxidative stress is also a responsible factor for the etiology of insulin resistance and T2DM. Elevated ROS production, due to the mitochondrial dysfunction in presence of FFA was found to be a responsible factor for the development of oxidative stress. Several reports suggest the reduced activity of antioxidant enzymes in obese and T2DM patients. In the present study, the effects of FFA on the antioxidant gene expression as well as their activity in FFA treated L6 skeletal muscle cells were investigated. From the study, it was found that FFA alters antioxidant gene expression and their activity in a concentration as well as time dependent manner. From this result, it was hypothesised that along with mitochondrial dysfunction, FFA is also responsible for reduction of antioxidant expression and activity. So, this could be a good therapeutic target for the treatment of insulin resistance and T2DM.

The active fraction of *L. aspera* and ferulic acid showed the promising effect to increase the expression of these antioxidant genes as well as to modulate their activity in a concentration dependent manner. Since 10 μg/ml ferulic acid is sufficient for insulin sensitivity in presence of FFA, it was observed that 10 μg/ml ferulic acid could increase the antioxidant genes expression and modulate their activities.

In this study, an *in silico* based network pharmacology approach was used for the development of new drug entities for T2DM treatment. Due to the expensive and time consuming nature of the drug discovery process, *in silico* approach predicting drug target interaction provides a very useful and efficient information using network based poylpharmacology approach. On the basis of the advantages of combination therapy for the complex disease treatment, multiple drugs- multiple targets concept was used. A hypothesis was generated that the combination of different potential plant components of the plants used by various

ethnic groups for the treatment of diabetes would be more effective than their individual use. On the basis of molecular docking , network analysis and density functional theory, 3 compounds were identified as the most potent components targeting maximum 12 different proteins associated with insulin resistance and T2DM. The concoction of these 3 compounds at suitable ratio may reduce the diabetic complicacies owing a good therapeutic choice for the treatment of insulin resistance and T2DM.

8.2 Future Works:

- Validation of the molecular mechanism of the probable active compound swietenine in restoring the insulin sensitivity in FFA induced insulin resistance.
- Pharmacokinetic study of swietenine,
- Validation of *in silico* observations by *in vitro* experiments.