CHAPTER-7

SUMMARY

&

CONCLUSION

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Summary and Conclusion

7.1 Summary:

Consistent epidemic elevations of TIIDM in today's modern times are a most prominent health concern worldwide. Amongst all the developing countries India would be leading in the occurrence of this metabolic disease. It is a comprehensive effect of various metabolic diseases caused by multiple intrinsic and extrinsic factors. Various extrinsic and intrinsic factors like physical activities, dietary components, environmental pollutants, pathogenic infections and oxidative stress, elevated glucose, lipids, inflammation etc are responsible for occurrence of metabolic ailments associated with TIIDM.

Obesity is one of the most important factor responsible for insulin resistance and its associated metabolic complications. Later stage of obesity mediated insulin resistance leads to lipolysis where there is drastic weight loss. Causative factor for positive calorie imbalance starts in brain and molecular mechanism of insulin resistance in adipose tissue, which is a key player in regulation and maintenance of metabolic homeostasis in adipose tissue. Unlike western countries, India, due to diverse habitats and food intake with their preserved epigenetic modification, not only obese but lean people are also affected by insulin resistance.

Since decades, the pharmaceutical world is striving for discovery of sustainable drugs that would ameliorate the peripheral defects in insulin resistance with minimum adverse effects. Our Ayurveda has lucidly explained the therapeutic efficacies of the naturally occurring herbs and their active ingredients in ameliorating insulin resistance and metabolic disorders. Large numbers of herbal extracts and their active principal ingredients have demonstrated hypoglycemic and anti obesity potential for treating TIIDM, representing a valuable alternative for control of the disease. However, lack of understanding of mechanism precludes the use of these herbal molecules as a new class of therapeutic agents. Enicostemma littorale belonging to family Gentianaceae is being used by rural population for treatment of diabetes. Our lab has well reported anti-oxidant, hypolipidemic and anti-diabetic, activities of EL both in animals and human NIDDM patients.

TIIDM previously known as non-insulin dependent diabetes mellitus (NIDDM) is widely associated with hampered insulin signaling due to defect in insulin receptors or its downstream signaling. In this study, we made an attempt to unravel the effective role of swertiamarin by comparing its effects with those of aqueous EL extract in alleviating insulin resistance in Type II Diabetic Rat model (Chapter 3). NA-STZ model mimics the non-obese diabetes mellitus condition. These animals were orally fed with EL extract and swertiamarin. We investigated hypolipidemic and insulin sensitizing effect in NA-STZ induced experimental non insulin dependent diabetes mellitus (NIDDM) rats; swertiamarin (50 mg/kg/day) and aqueous extract (15 grams dried plant equivalent extract/kg/day) were administered to rats orally for 40 days. In diabetic rats, swertiamarin enhanced insulin sensitivity and glucose metabolism by restoring enzyme activity of G6Pase and HMG-CoA reductase to normal levels and restoring gene expression levels of PEPCK, GK, GLUT-2, PPAR-γ, Leptin, Adiponectin, LPL, SREBP-1c and Glut4. We for the first time reported a significant role of SM as a regulator of gene expression under the control of transcriptional factors like peroxisome proliferator-activated receptor PPAR-γ, thus confirming that swertiamarin improves insulin sensitivity and modulates carbohydrate and fat metabolism and is better candidate for designing therapeutics in order to ameliorate TIIDM.

Further study was to understand molecular mechanism of swertiamarin using in vitro models. In vivo results motivated us to design the study for elucidating its the molecular mechanism at cellular and molecular level in liver, adipose and muscle in order to understand its major role in glucose and fat metabolism in tissue specific manner (Chapter 4).

Dysregulation of fat metabolism is one of the major cause of hepatic damage. The hepatocytes start accumulating free fatty acids and are converted into signet like structure, a state called as non-alcoholic fatty liver which further hampers glucose, glycogen and fat metabolism. In order to target this, HepG2 cells were selected as the *in vitro* model for studying the role of swertiamarin on hepatic steatosis (Chapter 4A). Elevated TG accumulation with increased expression of the key enzymes like SREBP-1c, PPAR-γ, ACC-1, FAS and CPT-1 has been observed in oleic acid treated HepG2 cells. Swertiamarin treated group showed reduced expression levels of SREBP-1c and PPAR-γ along with increased levels of insulin signaling protein depicting that swertiamarin treatment abolishes fat accumulation in hepatocytes and reduces hepatic insulin resistance condition. Swertiamarin also targeted PPAR-α, the major transcriptional factor regulating carbohydrate and lipid

homeostasis in hepatocytes. Thus, it can be presumed that swertiamarin has modulatory effects on proteins of PPAR family.

Skeletal muscle plays a major role in energy homeostasis. Dramatic changes in regulation of energy homeostasis in peripheral insulin resistance are found in skeletal muscle and hence, it is evidently important to understand role of swertiamarin in ameliorating insulin resistance in skeletal muscle. L6 myocytes were taken as the in vitro skeletal muscle model for studying TNF-α induced insulin resistance (Chapter 4B). The major enzymes GP and G6Pase were restored by swertiamarin treatment. It was also found that there was marked increase in the expression of IR, tyrosine phoshorylation of IRS-1, PI(3)K and Akt along with reduction in stress kinases like P38 MAPK and Erk1/2. Hence, swertiamarin potentially promoted glycogen storage and ameliorated stress mediated insulin resistance in skeletal muscle, which mimicked normally maintained exercised muscles.

Another major tissue exhibiting insulin resistance is adipose tissue which plays an important role in TIIDM associated obesity. Hence, mature adipocytes differentiated from pre-adipocytes 3T3-L1 were selected as the in vitro model for the current study (Chapter 4C). These cells were treated with dexamethasone, a known glucocorticoid to make them insulin resistant which, is known to alter glucose and fat homeostasis.

Swertiamarin treatment increased the gene expressions of the key proteins like aP2, FAS, ACC-1 and CD36 that directly showed restoration of insulin sensitive adipocytes. This was supported by increased expression of an important adipokines adiponectin that functions as the marker of mature adipocyte and also plays an important role in energy homeostasis.. Also reduced ser phosphorylation at position 307 of IRS-1 with significant increase in PI(3)K and Akt showed that swertiamarin activated tyrosine and inhibited serine kinases thus, increasing insulin sensitivity, restoring fatty acid synthesis and fat metabolism. Increased adipogensis during insulin resistance conditions leads to hypertrophic adipocytes that attracts activated macrophages and increases secretion of pro-inflammatory cytokines like TNF-α which further hampers fat metabolism and increases peripheral insulin resistance by free fatty acid penetration. Swertiamarin was potent enough to ameliorate insulin resistance in TNF-α treated adipocytes. Apart from increased insulin signaling proteins like IRS-1, PI3K, Akt, various isoforms of PKCs called as serine/threonine kinases those involved in negative regulation were found to be downregulated. Major stress kinase MAPK p38 and Erk1/2 were reduced in swertiamarin treatment with elevated insulin sensitivity and glucose uptake. The

overall study on all the three cell lines, showed decreased insulin resistance in peripheral tissues like liver, adipocyte and skeletal muscle with swertiamarin treatment thus clearly depicts swertiamarin as a potent insulin sensitizer (Chapter 4).

Based on the above observations in glucocorticoid and TNF-α mediated insulin resistance in adipocytes, swertiamarin treatment was used to study its anti-obesity effect on adipogenesis (Chapter 5). It was found that the major transcriptional factors C/EBP-β and PPAR-γ were drastically reduced. Hence, the results concluded that swertiamarin markedly reduced the differentiation and maturation of adipocytes which was regulated by PPARγ2 dependent gene expression.

There are other known factors to regulate adipogensis and PARP is amongst them which, is the DNA repair protein that inhibits adipogenesis (Chapter 6). It is known that PARP-1-/- mice display reduced fat mass deposition and decrease in adipocyte size, it was suggested that PARP-1 is mandatory for complete adipocyte differentiation. In the present study we tried to explore the role of PARP along with swertiamarin.

Treatment with PARP inhibitor, PJ-34 inhibited polymer formation which was normally induced on 3rd day of adipogenesis and remains till day 7 of adipogenesis. PARP inhibitor treatment did not change C/EBP-β protein expression although activity caused decrease in PPAR-γ protein expression. Swertiamarin and Activin A did not show reduction in polymer formation (PARylation) but are regulating PPAR-γ protein expression. Better efficacy of swertiamarin was observed when the cells were treated with PARP inhibitors. PARP-1 protein level was depleted by antisense (siRNA) technique that showed inhibition of adipocyte differentiation. These studies shows specific role of PARP-1 mediated PAR formation, and interaction with smad proteins, playing a major role in adipogenesis.

Conclusively swertiamarin has proved its insulin sensitizing, antiadipogenic and glucose modulatory activity by majorly targeting PPAR-γ and PPAR-α, thus potentiating it as a better drug for TIIDM complications.

7.2 Conclusion:

Following conclusions were drawn from the present study:

1) Swertiamarin an active lead compound from *Enicostemma littorale* markedly decreased hyperglycemia and hyperlipidemia *in vivo* in experimental non-insulin dependent diabetes mellitus. Glucose and lipid metabolism were restored in peripheral tissues like liver and adipose tissues and regulates gene expression by targeting PPAR-γ & improved insulin sensitivity in TIIDM.

2) Swertiamarin restored the levels of key genes involved in glucose and fat metabolism. TNF-α induced HepG2 directly indicates progression of non alcoholic fatty liver disease like condition. It was noted that fatty acid production was reduced as the expression levels of the key enzymes involved in lipogenesis were declined with restored insulin signaling causing enhanced glucose uptake. Thus, swertiamarin explicitly ameliorated hepatosteatosis and can be an effective therapy for the non-alcoholic fatty liver disease (NAFLD).

Swertiamarin treatment of TNF-α mediated insulin resistance in L6 myocytes increases key insulin signaling proteins with reduced expression of the stress kinases P38 MAPK and Erk1/2 proving that swertiamarin potentially improves skeletal muscle insulin resistance and ameliorates glucose and glycogen metabolism.

Swertiamarin ameliorated dsyregulated fat metabolism in dexamethasone treated adipocytes by altering insulin sensitivity. Inflammation mediated insulin resistance in obese adipocytes was ameliorated when treated with swertiamarin by restoring the levels of the genes and key proteins involved in insulin signaling. Serine/threonine kinases were reduced which conclusively depicts reduction in inflammation mediated insulin resistance.

3) Enhanced adipogensis leads to hypertrophic adipocytes those resembling the fat cells in obesity and leading to peripheral insulin resistance. Swertiamarin inhibits adipogenesis with decreased expression of the key transcriptional factors and the downstream genes involved in lipogenesis and maturation of adipocytes hence, recommended as an anti-obesity drug.

4) Understanding the role of SM and poly(ADP-ribose) polymerase-1 (PARP-1) in adipogenesis. PARP-1 inhibition and depletion reduces adipogenesis independent of SM activity. Exogenously inhibited PARylation of proteins suppresses PPAR-γ2 transcription by inhibiting Smad2/3 phosphorylation through its interaction with Smad4 and by sequestering C/EBP-β from the PPAR-γ2 promoter. Thus, PARP activity positively regulates

adipogenesis, and its inhibition holds promising future possibilities for the treatment of obesity and type II diabetes.

The major targets of swertiamarin were found to be IRS-1, PI(3)K, PPAR-γ and PPAR-α. This is the first report stating that this glycoside has anti-adipogenic activity and more than one target in modulation of insulin sensitivity and associated carbohydrate and lipid metabolism, making it an incredible drug for ameliorating TIIDM associated complications.