Synopsis

Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia with disturbances in carbohydrate, protein and lipid metabolism resulting from defects in insulin secretion, insulin action or both. Currently there are 387 million people with diabetes worldwide and is expected to affect 592 million people by 2035. Insulin resistance in peripheral tissues and pancreatic beta cell dysfunction are the major challenges in the pathophysiology of diabetes. Diabetic secondary complications (like liver cirrhosis, retinopathy, microvascular and macrovascular complications) arise from persistent hyperglycemia and dyslipidemia can be disabling or even life threatening. Current medications are effective for control and management of hyperglycemia but undesirable effects, inefficiency against secondary complications and high cost are still serious issues in the present prognosis of this disorder. Hence the search for more effective and safer therapeutic agents of natural origin has been found to be highly demanding and attract attention in the present drug discovery research. The data available from Ayurveda on various medicinal plants for treatment of diabetes can efficiently yield potential new lead as antidiabetic agents. For wider acceptability and popularity of herbal remedies available in Ayurveda scientific validation by the elucidation of mechanism of action is very much essential. Modern biological techniques are available now to elucidate the biochemical basis of the effectiveness of these medicinal plants. Keeping this idea the research programme under this thesis has been planned to evaluate the molecular mechanism responsible for the antidiabetic property of Symplocos cochinchinensis, the main ingredient of Nishakathakadi Kashayam, a well-known Ayurvedic antidiabetic preparation. A general introduction of diabetes, its pathophysiology, secondary complications and current treatment options, innovative solutions based on phytomedicine etc has been described in Chapter 1.

The effect of Symplocos cochinchinensis (SC), on various in vitro biochemical targets relevant to diabetes is depicted in Chapter 2 including the preparation of plant extract. Since diabetes is a multifactorial disease, ethanolic extract of the bark of SC (SCE) and its fractions (hexane, dichloromethane, ethyl acetate and 90 % ethanol) were evaluated by in vitro methods against multiple targets such as control of postprandial hyperglycemia, insulin resistance, oxidative stress, pancreatic beta cell proliferation, inhibition of protein glycation, protein tyrosine phosphatase-1B (PTP-1B) and dipeptidyl peptidase-IV (DPP-
Among the extracts, SCE exhibited comparatively better activity like alpha glucosidase inhibition, insulin dependent glucose uptake (3 fold increase) in L6 myotubes, pancreatic beta cell regeneration in RIN-m5F and reduced triglyceride accumulation in 3T3-L1 cells, protection from hyperglycemia induced generation of reactive oxygen species in HepG2 cells with moderate antiglycation and PTP-1B inhibition. Chemical characterization by HPLC revealed the superiority of SCE over other extracts due to presence of bioactives (beta-sitosterol, phloretin 2’glucoside, oleanolic acid) in addition to minerals like magnesium, calcium, potassium, sodium, zinc and manganese. So SCE has been subjected to oral sucrose tolerance test (OGTT) to evaluate its antihyperglycemic property in mild diabetic and diabetic animal models. SCE showed significant antihyperglycemic activity in in vivo diabetic models.

Chapter 3 highlights the beneficial effects of hydroethanol extract of *Symplocos cochinchinensis* (SCE) against hyperglycemia associated secondary complications in streptozotocin (60 mg/kg body weight) induced diabetic rat model. Proper sanction had been obtained for all the animal experiments from CSIR-CDRI institutional animal ethics committee. The experimental groups consist of normal control (NC), N + SCE 500 mg/kg bwd, diabetic control (DC), D + metformin 100 mg/kg bwd, D + SCE 250 and D + SCE 500. SCEs and metformin were administered daily for 21 days and sacrificed on day 22. Oral glucose tolerance test, plasma insulin, % HbA1c, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein etc. were analysed. Aldose reductase (AR) activity in the eye lens was also checked. On day 21, DC rats showed significantly abnormal glucose response, HOMA-IR, % HbA1c, decreased activity of antioxidant enzymes and GSH, elevated AR activity, hepatic and renal oxidative stress markers compared to NC. DC rats also exhibited increased level of plasma urea and creatinine. Treatment with SCE protected from the deleterious alterations of biochemical parameters in a dose dependent manner including histopathological alterations in pancreas. SCE 500 exhibited significant glucose lowering effect and decreased HOMA-IR, % HbA1c, lens AR activity, and hepatic, renal oxidative stress and function markers compared to DC group. Considerable amount of liver and muscle glycogen was replenished by SCE treatment in diabetic animals. Although metformin showed better effect, the activity of SCE was very much comparable with this drug.
The possible molecular mechanism behind the protective property of *S. cochinchinensis* against the insulin resistance in peripheral tissue as well as dyslipidemia in *in vivo* high fructose saturated fat diet model is described in Chapter 4. Initially animal were fed a high fructose saturated fat (HFS) diet for a period of 8 weeks to develop insulin resistance and dyslipidemia. The normal diet control (ND), ND + SCE 500 mg/kg bwd, high fructose saturated fat diet control (HFS), HFS + metformin 100 mg/kg bwd, HFS + SCE 250 and HFS + SCE 500 were the experimental groups. SCEs and metformin were administered daily for the next 3 weeks and sacrificed at the end of 11th week. At the end of week 11, HFS rats showed significantly abnormal glucose and insulin tolerance, HOMA-IR, % HbA1c, adiponectin, lipid profile, liver glycolytic and gluconeogenic enzyme activities, liver and muscle triglyceride accumulation compared to ND. HFS rats also exhibited increased level of plasma inflammatory cytokines, upregulated mRNA level of gluconeogenic and lipogenic genes in liver. HFS exhibited the increased expression of GLUT-2 in liver and decreased expression of GLUT-4 in muscle and adipose. SCE treatment also preserved the architecture of pancreas, liver, and kidney tissues. Treatment with SCE reversed the alterations of biochemical parameters, improved insulin sensitivity by modifying gene expression in liver, muscle and adipose tissues. Overall results suggest that SC mediates the antidiabetic activity mainly via alpha glucosidase inhibition, improved insulin sensitivity, with antiglycation and antioxidant activities.

The conclusions of all the results described above and suggestions for future work are presented in last section of the thesis.