Summary and Conclusion

The alarming picture of the incidence and prevalence of diabetes worldwide is uncovered by the epidemiological studies of various agencies such as IDF, ADA and WHO. India, especially Kerala is becoming the global diabetic capital in recent years. Elucidating the causes of diabetes remains the key step towards eradicating the disease, but the prevention and amelioration of hyperglycemia and diabetes induced secondary complications is equally important for the millions of people who already living with the disease knowingly or unknowingly. Report from ministry of health, Govt. of India, says that ‘diabetes in India is no longer a disease of the affluent or a rich man’s disease; it is becoming a problem even among the middle income and poorer sections of the society’. It should be noted that poor diabetic subjects are more prone to secondary complications as they have less access to quality health care.

Traditional medicine or indigenous knowledge always serves as a low cost and easily available treatment option for a multifactorial disease like diabetes. Traditional formulations are much attractive due to their increased tolerance and synergistic effects, as they contain diverse kind of bioactives. Moreover, traditional medicines had paved way for development of the most effective modern drug like metformin from *Galega officinalis*. *Ayurveda*, a system of traditional medicine native to Indian subcontinent always plays major role in primary health care of both rural and urban populations of India.

*Symplocos cochinchinensis* (SC) is one of the key ingredients of *Nisakathakadi Kashayam*; a very effective *Ayurvedic* preparation for diabetes mentioned in the ancient script ‘*Sahasrayogam*’. But for wider acceptability of the health benefits of SC, a detailed scientific investigation on its mode of action on various biochemical targets relevant to diabetes is mandatory. But detailed study illustrating the mechanism of action of SC or its biochemical targets relevant to diabetes is not available in literature. Keeping this in mind, the main objective of the study was to see the various biochemical mechanisms responsible for its antidiabetic property by analysing its effects on selected biochemical and molecular targets relevant to diabetes using *in vitro* and *in vivo* models.

For this, bark of the plant was collected, identified and processed for extraction. The
ethanolic extract of the bark of SC (SCE) and its fractions (hexane, dichloromethane, ethyl acetate and 90% ethanol) were evaluated by various in vitro cell free and cell based methods against multiple targets relevant to diabetes such as the alpha glucosidase inhibition, glucose uptake, adipogenic potential, oxidative stress, pancreatic beta-cell proliferation, inhibition of protein glycation, protein tyrosine phosphatase-1B (PTP-1B) and dipeptidyl peptidase-IV (DPP-IV). HPLC analysis was also conducted to all the fractions for partial chemical characterization for its chemical composition. AAS analysis of SCE was conducted for mineral composition. The antihyperglycemic activity of SCE was confirmed by OSTT studies in normal, mild diabetic and severely diabetic streptozotocin (STZ) induced Sprague Dawley (SD) rats using acarbose and metformin as positive controls.

The beneficial effects of SCE have also been explored against hyperglycemia associated secondary complications in STZ-induced diabetic SD rat model. The experimental groups under this study consist of normal control (NC), N + SCE 500 mg/kg bwd, diabetic group (DC), D + metformin 100 mg/kg bwd, D + SCE 250 and D + SCE 500. SCE 250/500, metformin or vehicle were administered daily for 21 days and sacrificed on day 22. Oral glucose tolerance test, plasma insulin, % HbA1c, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, albumin and total protein were analysed. Aldose reductase (AR) activity in the eye lens, hepatic, renal oxidative stress and function markers; liver and muscle glycogen and histopathological alterations of pancreas were also checked in this STZ induced diabetic model.

Then, we analysed the therapeutic potential of SCE against diet induced insulin resistance model and the molecular mechanisms of its activity in SD rats. The experimental groups under this study consist of normal diet animal (ND), ND + SCE 500 mg/kg bwd, high fructose saturated fat diet (HFS), HFS + metformin 100 mg/kg bwd, HFS + SCE 250 and HFS + SCE 500. Initially the animals were kept under HFS diet for 8 weeks and at the end of 8 week period, animals of HFS group were found to be developed insulin resistance and dyslipidemia which was evident from oral glucose tolerance test, plasma insulin and lipid profiles. SCE 250/500, metformin or vehicle were administered daily for next 21 days and sacrificed on day 22. OGTT, ITT, plasma analysis of insulin, lipid profile, % HbA1c and uric acid were conducted. Liver glycolytic and gluconeogenic
enzyme activities, triglyceride and glycogen content in liver and muscle, plasma level of proinflammatory cytokines like MCP-1, IL-6, TNF alpha were also checked in HFS model. The mRNA expression of genes involved in the lipid metabolism like SCD-1, SREBP-1c, FAS and PPAR alpha were analysed using qRT-PCR. The hepatic expression levels of genes involved in carbohydrate metabolism and insulin signalling such as G6Pase, PEPCK, GLUT-2, Akt-2, GLUT-4, PTP-1B and SIRT-1 were also evaluated. The expression pattern of various proteins like IRS-1, AKT-2 and GLUT-2 in the liver; IRS-2, AKT-2 and GLUT-4 in the muscle and PPAR gamma, IRS-1, AKT-2, and GLUT-4 in adipose tissue were analysed by western blotting.

Overall results from in vitro cell free and cell line based studies revealed that among the five extracts analysed, SCE exhibited comparatively better activity via alpha glucosidase inhibition, insulin dependent glucose uptake 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, DPP-IV and PTP-1B inhibition. The in vivo studies involving OSTT after the oral administration of single dose of SCE confirmed the marked antihyperglycemic activity of SC in both STZ treated mild diabetic (SLM) and severely diabetic (STZ-S) SD rats. In SLM, treatment with 500 mg/kg bw of SCE reduced the whole glycemic response by 12.88% while acarbose and metformin caused 15.73% and 17.12% reduction respectively. SCE (500 mg/kg bw) treatment in STZ-S caused 23.48% improvement in blood glucose profile after 5 h of treatment and acarbose and metformin showed 30.27% and 33.18% respectively. These potential effects of SC contribute significantly to its antidiabetic property. SCE has been taken forward for the in vivo study as it exhibited better activity under in vitro assays, increased yield of more bioactive molecules and less toxicity of the solvent.

The long term (21 days) administration of SCE in STZ-induced diabetic SD rats showed that SCE can exert beneficial effects on liver, kidney, pancreas, eye lens and muscle against hyperglycemia induced secondary complications. Treatment with SCE protected from the deleterious alterations of biochemical parameters in a dose dependent manner including histopathological alterations in pancreas. SCE also exhibited of glucose lowering effect and decreased HOMA-IR, % HbA1c, lens aldose reductase activity, and
hepatic, renal oxidative stress and function markers compared to the diabetic control group. Considerable amount of liver and muscle glycogen was replenished by SCE treatment in STZ-induced diabetic SD rats.

The SCE was also found to be an effective agent against high fructose saturated fat diet model of insulin resistance and dyslipidemia in high fructose saturated fat fed SD rats. The study of multiple aspects of diabetes and insulin resistance displayed the antidiabetic potential of SCE via improving hepatic glucose homeostasis and lipid metabolism in the liver of rats. Increased insulin sensitivity was noticed in peripheral tissues like muscle and adipose by SCE administration which was the result of improved expression level and phosphorylation state of proteins involved in the insulin signalling pathway. SCE treatment reduced the level of serum proinflammatory cytokines and glycated hemoglobin which protected from further inflammatory response and tissue damage. SIRT-1 mediated down regulation or inhibition of the activity of PTP-1B enzyme may be one of the mechanisms behind the insulin sensitizing and improved hepatic glucose homeostatic property of SCE. The improvement of insulin resistance and hepatic steatosis by SCE may be due to the down regulation of SCD-1 gene expression which modulate SREBP-1c dependent and independent hepatic lipid accumulation.

The presence of known insulin sensitizers and AG inhibitors like phloretin 2’glucoside, oleanolic acid and beta-sitosterol in SC may be playing an important role in these multifaceted activities of SC with respect to diabetes. Furthermore, SCE does not affect these cited parameters in normal control rats. These findings indicate that SCE only affects the markers under diseased conditions and suggests that this herb is safe for consumption by healthy subjects.

The detailed investigation related to the antidiabetic effect of SC revealed its therapeutic potential against key biochemical targets like alpha glucosidase inhibition, oxidative stress induced diabetic secondary complications, diet induced insulin resistance and dyslipidimia. The present study also revealed presence of bioactives and minerals which are reported to have the therapeutic benefit to diabetes and other related complications. From overall results, it can be concluded that SC is the potential medicinal plant from Indian system of medicine to be exploited for the development of lead molecule or standardised extract for the control and management of diabetes. On the basis of these
preliminary investigations, SC is recommended for detailed future study on various models like *in vitro* and *in vivo* models for SGLT-2 inhibitors, db/db mice etc. using standardised extract or isolated molecules from this plant for the generation of new chemical entities for future drug.