CHAPTER IX

9.1 SUMMARY

Diabetes mellitus (DM) is the most common endocrine disorder caused either by insufficient insulin secretion or insulin resistance. The major cause of morbidity and premature death associated with diabetes are actually due to severe complications of this disorder. Existing oral anti-diabetic medications address hypoglycemia but leads to many side effects. Moreover, in addition to lack of efficacy and undesirable adverse effects of synthetic drugs, herbal drugs are prescribed throughout the world and frequently considered to be less toxic and free from side effects as compared to synthetic ones. However, the major drawback is that the quantity of herbal extract necessary for treatment is higher due to the degradation of different plant constituents in the gastro intestinal tract since they are very sensitive to the acidic pH of the stomach, which promotes the loss of the desired effect and a longer duration of treatment is needed due to the poor absorption of these constituents in the intestine. Recently, many studies focused on applying nanotechnology to plant extracts for the enhancement of solubility, bioavailability and sustained delivery. Hence, the present study was designed to incorporate nanotechnology to medicinal plants (MS and NC) and investigate the antidiabetic effect of Medicinal plants and prepared nanoparticles (GMSN and GNCN) by in vitro and in vivo methods.

- Preliminary physiochemical analysis (Ash values, extractive values, loss on drying) was performed for NC and MS as per standard procedures and the obtained results were presented. The phytochemical screening of MS and NC showed the presence of phenols, flavonoids, tannins, alkaloids, steroids and glycosides.
In vitro antidiabetic studies were carried out in ethanolic extract of MS and NC by alpha amylase and alpha glucosidase inhibition assay methods. Both extracts showed excellent antidiabetic effect. This may be due to the presence of high quantity of phenolic and flavonoids content in the ethanolic extract of MS and NC.

Gelatin encapsulated *Myxopyrum serratum* nanoparticles (GMSN) and Gelatin encapsulated *Nilgirianthus ciliatus* nanoparticles (GNCN) were prepared by solvent evaporation method. For each extract, five formulations (GMSN1, GMSN2, GMSN3, GMSN4, GMSN5, GNCN1, GNCN2, GNCN3, GNCN4 and GNCN5) were developed by varying the polymer concentration from 0.1% to 0.5% and characterized by various analytical techniques.

The DLS results showed that average particle size of all the formulation lies below 200 nm is suitable for drug delivery applications. The zeta potential values lies between -30 to +30 indicated more stability of nanoparticles. Among all the prepared nanoparticles GMSN2 and GNCN3 showed less particle size with higher entrapment efficiency. Hence GMSN2 and GNCN3 nanoparticles were selected for further study. The TEM and SEM images clearly described the monodispersed shape of nanoparticles.

The in vitro drug release study showed that nearly 80% of drugs released within 8 h and kinetics results revealed that all nanoparticles showed good linearity in zero order. The mechanism of drug release followed anomalous non-fickian diffusion ie, the increased diffusivity of drug from the matrix by solvent induced relaxation of the polymer.

The antioxidant properties of MS, NC, GMSN2 and GNCN3 were evaluated by means of DPPH and nitric oxide scavenging assay methods. In both the
methods, MS and GMSN2 showed good antioxidant activity due to the presence of large quantity of phenolic compounds.

- MTT assay was performed to check the cytotoxicity of MS, NC and nanoparticles GNCN3, GMSN2 in L6 and 3T3L1 cell lines. The results showed that both extracts and nanoparticles did not confer any toxicity up to 500µg/ml in both cell lines.

- *In vitro* antidiabetic effect of MS, NC, GMSN2 and GNCN3 was studied by glucose uptake assay method in L6 myoblasts. The basal and insulin stimulated glucose uptake assay was measured for MS, NC, GMSN2 and GNCN3. The study results clearly indicated that MS, NC, GMSN2 and GNCN3 stimulate the glucose uptake under *in vitro* conditions. This may be due to its effects on the various receptors located in the skeletal muscle L6 cells.

- The extract MS, NC and nanoparticles GNCN3, GMSN2 were also tested for their antidiabetic effect in antiadipogenic assay method. The inhibitory effect of plant extracts and its nanoparticles on lipid accumulation during adipogenesis was dose dependent and in both *in vitro* antidiabetic methods, MS and GMSN2 showed better results when compared to NC and GNCN3. So, MS and GMSN2 were selected for further study to screen their antidiabetic effect in HFD induced diabetes in C57BL/6J mice.

- *In vivo* antidiabetic activity of MS and GMSN2 were evaluated using high fat diet induced diabetes in C57BL/6J mice. HFD fed mice showed a significant weight gain and this weight gain remarkably reduced after treatment with MS and GMSN2 compared to the untreated groups. This finding confirmed that
MS and GMSN2 effectively preventing body weight gain and showed reduction in their body weight after 21 days of treatment.

- MS at 800 mg/kg b.wt and GMSN2 at 160 mg/kg b.wt significantly reversed the glucose, total cholesterol and triglycerides towards the normal levels in plasma compared to the untreated group which indicates the hypoglycemic, hypocholesterolmic potential of the plants.

- The antioxidants enzyme SOD, GSH, GPx levels and free fatty acid levels were determined in the liver. Treatment with MS and GMSN2 significantly increases the antioxidant enzyme levels and significantly reduces the TBARS levels in the liver to a greater extent that was comparable with standard drug metformin. MS and GMSN2 at dose of 800 mg/kg and 160 mg/kg b.wt significantly increased the antioxidant enzymes in the liver. This confirmed that MS and GMSN2 have significant antioxidant potential by scavenging the free radicals.

- The level of carbohydrate metabolizing enzymes in the liver was also studied. The glycolytic enzyme (glucokinase) level was increased effectively and significantly, the gluconeogenic enzymes (Glucose 6 phosphatase and fructose 1,6- bisphosphatase) level was also significantly brought back to normal level after treated with different doses of MS and GMSN2. The plant extract and nanoparticles could significantly restored the altered carbohydrate metabolizing enzyme levels suggesting its potential use as therapeutic agent in the management of type 2 diabetes.

- Histopathological studies of adipose tissues illustrated that MS and GMSN2 at the dose of 800 mg/kg and 160 mg/kg b.wt significantly reduced the inflammation. Moderate reduction was observed in low dose treated group.
Immunohistochemical studies of adipose tissues revealed MS and GMSN2 at the dose of 800 mg/kg and 160 mg/kg b.wt suppresses the expression of inflammatory markers IL-6 and TNF-α. While MS and GMSN2 at the dose of 400 mg/kg and 80 mg/kg b.wt showed moderate reduction in the expression of above markers. Thus, the present study demonstrates the anti-inflammatory property of MS and GMSN2 through the suppression of proinflammatory cytokines.

In vivo antidiabetic study revealed that GMSN2 at low dose (80 mg/kg and 160 mg/kg b.wt) significantly reverse all the biochemical parameters towards normal level similar to the effect produced by MS at high dose (400 mg/kg and 800 mg/kg b.wt). This confirmed the nanoparticles at low dose produces the more prominent antidiabetic effect in HFD induced diabetes in C57BL/6J mice.