6. SUMMARY AND CONCLUSION

In the present study, marker based approach was used to standardize *G. sylvestre* extract using HPLC-ESI-MS/MS method. Considering technical difficulties with gymnemic acids, gymnemagenin (GMG) was used as marker compound for indirect measurement of gymnemic acids in *G. sylvestre* samples. As a prerequisite, a sensitive, rapid with high sample throughput HPLC-ESI-MS/MS method was developed and validated for quantitative estimation of GMG. The method was successfully applied for important single as well multi-herb formulations of *G. sylvestre* for estimation of GMG. This method could be used as a quality control tool for *G. sylvestre* and their marketed formulations.

Further, GMG was identified and quantified in rat plasma after oral administration of *G. sylvestre* alcoholic extract using sensitive and selective HPLC-ESI-MS/MS based method. This suggested that gymnemic acids from *G. sylvestre* extract undergoes hydrolysis in GIT resulting in the production of hydrolyzed product, GMG could be considered as bioactive pro-drug. However, further investigations on identification of presence of any traces of gymnemic acids and other constituents from *G. sylvestre* after oral administration using global metabolites profiling approach in plasma samples are required.

Considering *G. sylvestre*, as a bioactive prodrug, GMG was isolated from hydrolysed *G. sylvestre* alcoholic extract and chemical structure was confirmed on the basis of IR, MS and NMR data. Isolated GMG was evaluated for its absolute bioavailability after oral and *i.v.* administration using single dose administration at 100 mg/kg. The results indicated poor oral bioavailability of GMG (6.21%) could be because of poor aqueous solubility, less absorption or rapid metabolism. This has stimulated to design strategies to overcome the poor oral bioavailability and therefore, the pharmacological efficacy. Strategies such as nanonization and formation of water soluble salts of GMG were undertaken. PLGA based nanoparticles were prepared and characterized for particle size, zeta potential, polydispersibility index and entrapment efficiency. There was improvement in the above properties of PLGA based GMG nanoparticles. Na and K salts of GMG were synthesized and chemically characterized using IR, MS and NMR. These salts significantly improved the water solubility of GMG. The developed GMG nanoparticles were evaluated for their comparative bioavailability in normal rats. The results suggested that PLGA based nanoparticles improved the AUC\(_0-t\) and C\(_{\text{max}}\) significantly as compared to parent GMG molecule, resulting in improved oral
bioavailability. These observations were further assessed for comparative anti-hyperglycemic and anti-hyperlipidemic activities in STZ induced diabetic rats. GMG, GMG nanoparticles and GMG salts significantly reduces FBGL, GHbA1c, TG and TC and enhances serum insulin level as compared to untreated diabetic rats after 14 days of oral administration. The anti-hyperglycemic and anti-hyperlipidemic potentials were found to be K-GMG>Na-GMG>GMG nanoparticles>GMG alone>MET. Further, the structural resemblance and docking studies with agonists and antagonists indicated that GMG acts like Mefepristone (a potent hepatic non selective Glucocorticoid antagonist) and since it has no toxic effects on blood and organs till 5000 µg/mL, it might be the potent hepato-selective glucocorticoid passive antagonist. Therefore, GMG, GMG nanoparticles and GMG salts could be potential novel candidates in the management of diabetes and need further investigations using detailed chronic toxicity studies and controlled clinical trials.

In the present study, PLGA based *G. sylvestre* extract nanoparticles have also been developed and characterized for particle size, zeta potential, entrapment efficiency and release studies. The result suggested the improved physicochemical properties. Comparative oral bioavailability of GMG after administering extract nanoparticles and parent extract were studied in normal rats. Extract nanoparticles resulted significant increase in \( C_{\text{max}} \) and \( \text{AUC}_{0-1} \) leading to improvement in absorption and thus the bioavailability. This observation further evaluated for comparative anti-hyperglycaemic and ant-hyperlipidemic activities in STZ induced diabetic rats. Results indicated that nanonization of extract significantly improved the anti-hyperglycaemic and anti-hyperlipidemic effects as compared with parent extract.

There are increasing incidences of herb-drug interactions leading to beneficial or unwanted effects. Therefore, in this work, effect of *G. Sylvestre* extracts on pharmacokinetics and pharmacodynamic of conventionally used oral hypoglycaemic, glimepiride (GLM) was studied in STZ induced rats. Sub-chronic co-administration of extract and GLM for 28 days resulted significant increase in anti-hyperglycaemic and anti-hyperlipidemic activities leading to pharmacodynamic interactions. This suggested that extract or the GLM could be administered at lowered dose or need to be monitored for possible hypoglycaemia if administered chronically. However, this observation warranted further studies after chronic co-administration as controlled trials or case studies.