CHAPTER 5

SUMMARY & CONCLUSIONS

Diabetes is a metabolic disorder affecting the metabolism of carbohydrates, fats, and proteins. It commonly impairs glucose homeostasis because either the body (i.e., beta cells in the pancreas) does not produce enough insulin (type-1 diabetes) or the cells do not respond to endogenously-produced insulin (type-2 diabetes). According to a recent report from the World Health Organization (2016), diabetes is the fastest growing chronic disease in the world; the number of diabetic patients is increasing particularly in the world’s middle-income countries where the rate increased from 4.7 to 8.5% between 1980 and 2014. Herbal products have attracted more attention than synthetic drugs for the treatment of human disease due to their safety profiles and price. The pharmacological activity of a medicinal plant is due to the presence of some bioactive compound which has same/more therapeutic index as compared to the plant. Some bioactive compounds are poorly bioavailable and they are not efficiently absorbed and/or distributed in the body. Hence, those drugs may not attain a high enough systemic concentration to exert their pharmacological effects. Thus, a novel drug delivery system can be built to enhance the absorption and bioavailability of such poorly bioavailable drugs. Polymeric nanoparticulate drug delivery systems have been widely studied as drug carrier owing to the properties of conversion of poorly-soluble and/or poorly-absorbed bioactive compounds into efficiently-deliverable drugs. These have received great attention now a days because they protect the active agents and have better circulation time than conventional small drug molecules. Moreover, nanoformulations have advantages over conventional formulations in many respects such as enhanced solubility and bioavailability, targeted drug delivery, sustained drug release, dose reduction, and the minimizing of possible side effects.

The present study was focused on enhance the efficacy and bioavailability of bioactive compounds which have been reported for antidiabetic activity. Glycyrrhizin and thymoquinone were selected as bioactive compounds for this purpose as they are hydrophilic and hydrophobic in nature respectively. Nanoformulations of bioactive compounds were synthesized, characterized and evaluated for anti-diabetic activity and further compared with standard anti-diabetic drug, metformin and its nanoformulation. Glycyrrhizin and thymoquinone are
bioactive constituent of *Glycyrrhiza glabra* roots (family - abaceae) and *Nigella sativa* seeds (family – anunculaceae) respectively. Glycyrrhizin as well as thymoquinone, both have been reported for anti-diabetic activity. Glycyrrhizin have been reported for their poor/low bioavailability after oral administration due to slow and incomplete absorption in the gastrointestinal tract. Similarly, thymoquinone has also been reported for its poor bioavailability. In order to enhance the bioavailability, polymeric nanoformulations were synthesized and characterized. However, till date, no report have been published on the anti-diabetic activity of nanoformulation of selected bioactive compounds. The various dose treatments of bioactive compounds and their nanoformulations were evaluated for anti-diabetic potential in nicotinamide-streptozotocin induced diabetic rats. Body weight, fasting blood glucose level, glycated hemoglobin and lipid profile were included in the present study as anti-diabetic parameters. Sub-effective doses of bioactive compounds and their combination as well as sub-effective doses of bioactive compound’s nanoformulations and their combination were also evaluated for their anti-diabetic activity. The following conclusions can be drawn from the present research work.

1. Glycyrrhizin-loaded NPs were synthesized via ionotropic gelation method using chitosan and gum arabic as polymers. A three-level two-factor factorial design of the Design Expert Software was used to optimize the process variables for achievement of minimum particle size and maximum encapsulation efficiency. The final optimized concentrations of chitosan and gum arabic were 1.0% w/v and 0.15% w/v respectively for the synthesis of both glycyrrhizin-loaded NPs. The same optimized concentrations were used for the synthesis of metformin-loaded NPs. The optimized nanoparticle formulations were found to be spherical in shape and size less than 200 nm as characterized by TEM. Various bioactive compound and its nanoformulations doses were evaluated for anti-diabetic effect and compared to diabetic control rats. Our results showed that glycyrrhizin and its NPs produced significant anti-hyperglycemic and anti-hyperlipidemic activity in type 2 diabetic rats comparable to standard anti-diabetic drug, metformin and its NPs. The NPs contained approximately 1/4th amount of pure glycyrrhizin and still exert better anti-diabetic effect in type-2 diabetic rats as compared to the diabetic control rats. NPs showed biocompatibility as compared to pure drug as determined by MTT assay on vero cell lines.

2. Thymoquinone-loaded NPs were prepared by the nanoprecipitation method, which requires solvent (aqueous) and non-solvent (organic) phases. A three-level three-factor ox-enhanced statistical design of Design Expert Software was used to optimize the
formulation ingredients for achievement of minimum particle size, minimum polydispersity, and maximum zeta potential. The optimized concentration of gum rosin, lecithin and polyvinyl alcohol were 0.08% w/v, 0.40% w/v and 0.26% w/v respectively for the synthesis of nanocapsules. The same optimized concentrations were used for the synthesis of metformin-loaded N s. The particle shape and size of both the nanoformulations was spherical and less than 100 nm respectively as predicted from T M analysis. Nanocapsules were evaluated for cytotoxicity screening on vero cell lines which illustrated that nanoformulation was less toxic as compared to their respective pure drug. Various thymoquinone and its N s doses were evaluated for anti-diabetic effect as compared to diabetic control rats. Our results showed that thymoquinone and thymoquinone-loaded N s induced a significant dose-dependent anti-hyperglycemic and anti-hyperlipidemic activity in type 2 diabetic rats comparable to standard anti-diabetic drug, metformin and its NPs. The N s contained half amount of pure thymoquinone and still exert better anti-diabetic effect in type-2 diabetic rats in comparison to pure thymoquinone.

Sub-effective doses of Glycyrrhizin-loaded NPs (10 mg/ g) and thymoquinone-loaded N s (10 mg/ g) and their combination were also included in the study, which was prepared by physical mixing of two nanoformulations. The results showed that combination of bioactive compounds and combination of bioactive compound-nanoformulations induced a significant anti-diabetic effect whereas the sub-effective doses of glycyrrhizin, thymoquinone and their nanoformulations have no effect on studied parameters. Moreover, combination of bioactive compound-nanoformulations have better antidiabetic potential as compared to pure drug, even at less amount of drug in the matrix of polymers.

Comparing the results of glycyrrhizin-loaded NPs and thymoquinone-loaded N s with their respective bioactive compounds i.e., glycyrrhizin and thymoquinone, showed that nanoformulation improved the bioavailability of the selected bioactive compounds which was the goal of the present investigation. This could be due to the encapsulation of hydrophilic/hydrophobic drug in suitable polymers which induced sustained release and enhances the availability of drug moiety at side of action. These all factors improve the anti-diabetic potential of bioactive compound-nanoformulations as compared to pure bioactive compounds.

In the present study, combined dose of glycyrrhizin-loaded NPs thymoquinone-loaded N s was also used to evaluate the effect of combination of drugs as compared to single
drug treatment. Sub-effective doses of bioactive compounds have no effect on studied anti-diabetic parameters. On the other hand, contrary to the single nanoformulation of bioactive compounds, a combination of nanoformulations was more effective and might reduce the drug load to the patients. Therefore, combination of bioactive compound-nanoformulations will be very helpful in future for the management of diabetes as compared to single drug treatment.

This investigation put a step forward in the direction of using new medicinal agent for the treatment of type-2 diabetes. Therefore, clinic trials should be further carried out on higher animals and voluntary diabetic patients, for the fulfillment of dream in the realistic world. Moreover, the work can be further extended for quantitative and qualitative analysis. Nanoformulations of bioactive compounds can be studied for its cellular uptake analysis to determine the quantitative acceptance of nanoformulation by the different organs. A pharmaco kinetic study can also be performed so as to establish a pharmaco kinetic and pharmacodynamic relationship for the nanoformulation which would confirm the effect of nanoformulations at lower dose. There should be some molecular pathways behind every improvement in anti-diabetic parameters. Therefore, further research may be carried out to explore the changes induced at molecular level by glycyrrhizin and thymoquinone for anti-diabetic effect. There are various bioactive compounds reported not only for anti-diabetic potential, but also for other pharmacological activities such as anti-malarial, nootropic, anti-depressant, anti-inflammatory, anti-convulsant, anti-oxidant, anti-anxiety, anti-HI, anti-viral, anti-tumor, and hepatoprotective, etc. However, the effects of nanoformulation of these bioactive compounds on other pharmacological activities have still not been evaluated. Therefore, nano-encapsulation of bioactive compounds in various suitable biocompatible and biodegradable polymers can be explored towards various diseases for better healthcare applications.