SUMMARY

Diabetes has become the next most widespread epidemic after cancer, the pathogenesis of type 2 diabetes mellitus (T2DM) is complex and involves the interaction of genetic and environmental factors. A number of environmental factors have been shown to play a role in the development of the disease, particularly excessive caloric intake leading to obesity and a sedentary lifestyle. Patients with T2DM consistently demonstrate three cardinal abnormalities: defective insulin secretion, particularly in response to a glucose stimulus (β-cell dysfunction); resistance to action of insulin in the peripheral tissues, particularly muscle and fat but also liver (insulin resistance); and increased glucose production by the liver (hyperglycemia). The common forms of T2DM are polygenic in nature and are due to a combination of insulin resistance and abnormal insulin secretion. Therefore extensive research is being carried out to develop drugs for its therapeutics; however the drugs being used are not only expensive but also pose a treat of severe side effects. Therefore, probable use of vitamins as anti-diabetic agents is being largely worked upon as this will not do any harm to the patients concerned.

Recent studies have found that deficiency of Vitamin D results in reduction in insulin secretion and thus in hyperglycemia. Both insulin secretion and sensitivity depend upon intracellular calcium concentration also Vitamin D is one of the hormone which has been found to regulate calcium flux within the cells. In both observational and case control studies, an inverse relationship has been reported with level of 25(OH)Vit D and degree of glycemic control. Therefore, in the first chapter of the present study, an attempt is made to present scientific evidence of linkage of Type 2 Diabetes with Vitamin D levels in order to explore the possibility of Vit D as an adon therapy to the existing treatment to strive near normo-glycemia. However, in the second and third chapter of the thesis, effect of combination of vitamin D along with vitamin C and vitamin A has been explored.

Since, hyperglycemia, from a pathophysiological standpoint, serves as a root cause of all the underlying complications by mediating its effects in more than one ways, namely: 1. β-cell glucotoxicity; 2. increased polyol pathway flux; 3. increased
advanced glycation end product formation and 4. PKC activation, converging to ultimately give rise to the major player: oxidative stress. Once generated, the free radicals cause pancreatic β cell damage, thus adding to increased insulin resistance, mitochondrial dysfunction, lipid peroxidation, glucose oxidation, DNA damage and last but not the least, manifesting changes in the histology of diabetic tissues. Therefore, in order to stop the progression of the disease or to slow down the occurrence of diabetic complications, these reactive oxygen species (ROS) should either be quenched or their production must be stopped. To carry out these functions, antioxidants like vitamin C might be used however, it would be of double benefit if the cause as well as the effect of type 2 diabetes mellitus complications i.e. hyperglycemia as well as oxidative stress, are targeted simultaneously. To work out this strategy we used 1, 25 (OH)2 vitamin D3 against hyperglycemia and vitamin C to act against the oxidative stress in the second chapter.

In the last chapter of the study, we tried to see the combined effect of vitamin A and vitamin D on several diabetes related parameters as both these vitamins belong to the steroid family and have been found to share signalling pathways.

The results found in all the three cases i.e. in case of vitamin D alone and Vitamin D along with vitamin C and vitamin A, we observed that the most effective results and values closest to control levels were observed in case of vitamin D alone however the results of combination vitamin C with vitamin D proved to be better than vitamin C alone. Although the mode of action, the targets acted upon and the mechanism of action of both these vitamins is totally different from each other but given together, they have the potential to act upon the two biggest and most basic abnormalities of type 2 diabetes mellitus i.e. hyperglycemia and oxidative stress. This combination approach therefore, brings together the anti-hyperglycemic effect of vitamin D and the anti-oxidant effect of vitamin C, thus targeting both, the cause and the effect leading to type 2 diabetes mellitus and following complications.

In case of vitamin A with vitamin D, the results did not follow any pattern. In case of some parameters like the activity of glucose metabolic enzymes hexokinase, FBPase and G6Pase, vitamin A alone proved to be better in terms of the recovery of enzyme activities while dealing with the anti-oxidant parameters, the combination (vitamin
A+vitamin D) was more effective. Vitamin A supplementation has already been found to induce the expression of GLUT-2 in insulinoma cell line INS-1. All trans-retinoic acid induced GLUT-2 m-RNA expression was observed after 48 hrs of incubation. Another mechanism by which vitamin A increased glucose uptake may involve PKC and thus effecting insulin sensitivity positively. It has been found that all trans retinoic acid modulates the activity of PKC by directly binding to its isoymes. These PKC isoforms might play a role in ser/thr phosphorylation of insulin receptor substrate (IRS-1, IRS-2) and also inactivate NADH oxidase. This may inactivate and block the downstream signalling pathway of insulin action and free radical production. This effect of all trans-retinoic acid may prove helpful in insulin signalling pathway ultimately improving insulin sensitivity and reducing oxidative stress. However, the changes were not that significant in the group given the combination treatment i.e. vitamin A+ vitamin D, this could be due to the fact that both vitamin D and vitamin A involve RAR for their cellular action, the presence of both these vitamins together can lead to the decrease in the available RAR in the cell. As the number of available RAR decreases, neither of the vitamins (vitamin D or vitamin A) are probably able to carry out their molecular functions, thereby leading to poor results. This is the reason that combination of vitamin D and vitamin A showed mixed results depending upon the parameter analysed and the pathway used by the vitamins. The oxidative stress parameters showed better results in case of combination group when compared to vitamin A alone. This could be because vitamin A probably uses the PKC pathway to lower down the free radical production that inhibits NADH oxidase and thus lowers oxidative stress parameters. However in case of glucose metabolic enzymes both vitamin A and vitamin D probably mediate their effects via molecular mechanism involving RAR. Thus insufficiency of RAR in the cell decreases the effect of combination when compared to individual vitamins (A or D) results than in case of either of the vitamins alone.

In the end, we suggest that, these vitamins could be given as an add-on therapy to diabetic patients as they not only help in bringing down hyperglycemia and diabetes related complications but also are less costly, easily available, free from side effects and pose no threat of further complications.