Chapter 9

Summary and Conclusions

The aim of this research work was to determine the effect of dietary polyphenols from faba bean on diabetic mellitus. India is rich in a variety of flora and fauna. Faba beans are utilized as a crop for human consumption and belong to pulse family. The present studies explore the therapeutic potentials of polyphenols extracted from faba beans in the treatment of type 2 diabetes which is a metabolic disorder and its prevalence is very high in developing as well as in developed countries. DM is characterized by high glucose levels in the body. Oxidative stress also plays a critical role in the pathogenesis of diabetes mellitus and leads to accumulation of free radical in the human body that results in oxidative stress. Dietary polyphenols have antioxidant property and may be an alternative strategy for the diabetic patient. Based on the available literature, the work was undertaken to inhibit some key enzymes such as alpha-amylase, alpha-glucosidase and xanthine oxidase by polyphenols from beans for addressing diabetes mellitus disorders.

Extraction and purification of polyphenols from faba bean depend upon nature and type of solvents. Solvents with increasing polarities (hexane, dichloromethane, chloroform-methanol, ethanol, acetone, and water) were used to obtain extracts with different metabolite profiles. TLC, FTIR, phytochemical screening detected the presence of phenolic compounds in seed extract. HPLC analysis indicated the presence of gallic acid and catechin which was further validated by HR-LCMS. HR-LCMS provides abundant information for the structural elucidation of the compounds like gallic acid, catechin, epicatechin, ellagic acid. Mass spectroscopy of fraction 5 and 8 detected the presence of gallic acid and catechin. Acetone extract of faba bean (*Vicia faba* L.) was

found to be highest with total phenol and flavonoid content among all extracts. Free radical scavenging activity was found to be 86.47% for acetone extract, and 97.36% for ascorbic acid respectively. The IC_{50} value of ascorbic acid and acetone extract was found to be 9 μg/mL ± 0.20 and 30 μg/mL ± 0.21.

For the development of the effective drug, knowledge of the interaction between phenolic compounds and digestive enzymes is very necessary. The molecular docking tool can be exploited to study the interaction between a small molecule and a receptor at the atomic level, which may give the insight to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes. The docking studies showed that the van der Waals, electrostatic and desolvation energies play an important role in binding. Galloyl groups in polyphenols are responsible for hydrophobicity and therefore, polyphenols can interact with enzymes through hydrophobic association.

Faba bean seeds had catechin, epicatechin, gallic acid, and ellagic acid which on molecular docking study revealed that binding was significant with xanthine oxidase by binding energies were –7.78, –6.11, –6.39, –5.78 kcal/mol respectively. Allopurinol drug on molecular docking with xanthine oxidase energy showed binding energy of –4.94 kcal/mol. Gallic acid, ellagic acid, catechin, epicatechin (polyphenols) and allopurinol bind other than catalytic residues (Glu-1261) of xanthine oxidase. The presence of a benzopyran ring in their basic nucleus would have contributed to its XO-inhibitory activity. *In vitro* and *in silico* analysis recommended that mode of enzyme inhibition was of mixed type. Antioxidant activity may be due to combined effects of all the phytochemicals constituents such as alkaloids, flavonoids, terpenoids, or acting independently may be responsible for such activity. These experimental outcomes

indicated that faba bean (*Vicia faba* L.) polyphenols showed strong antioxidant properties and might be used as potential natural drugs against oxidative diseases.

The combined effects of hydrophobic interaction and hydrogen bond formation between polyphenols and the porcine-alpha amylase and alpha-glucosidase could contribute to control postprandial hyperglycemia in type 2 diabetic patients. Acetone extract and ethanol also possessed the highest inhibitory potential against porcine α-amylase (IC_{50} value of 2.94 mg/mL and 2.83 mg/ml) and alpha-glucosidase. Kinetic analysis revealed that the acetone displayed a mixed mode of inhibition towards α-amylase and alpha-glucosidase. *In-silico* analysis was in agreement with *in-vitro* studies in which phenolic compounds (catechin, epicatechin, gallic acid, and epicatechin) showed more negative free energy with respect to standard drug (acarbose) and bound with catalytic residues and other than the catalytic residue of α-amylase and alpha-glucosidase. These results might be due to the combined effects of phytochemicals present in seed extract or acting separately. Molecular dynamics simulation studies of alpha-amylase, alpha-glucosidase and xanthine oxidase with gallic acid, ellagic acid, catechin, and epicatechin showed interaction with the catalytic and noncatalytic residues. Moreover, a combined molecular docking and molecular dynamics simulation studies revealed the predicted residues that may hold favorable polyphenolic-specific interactions. The probable binding modes of the gallic-acid and catechin from this study may extend the knowledge of the alpha-glucosidase, alpha-amylase xanthine oxidase with polyphenols interactions and offered the way to design the analogs of acetone-extract with reduced toxicity. Any nanoscopic differences in the cell surface morphology of the yeast cells were studied by using atomic force microscopy (AFM) and these results showed that acetone fraction of seed extracts was reducing the effect of oxidative stress in terms of cell roughness and mean cell volume parameters. SEM studies investigate the change in

cells surface morphology with and without the presence of H_20_2 and it was found that cell shape and cell membrane were deformed due to hydrogen peroxide treatments. Propidium iodide staining, DAPI staining, DNA fragmentation assay, ROS measurement confirmed that oxidative stress takes place in the yeast cell and effect of seed extract was also very effective for reducing stress condition. On the basis of cell viability assays, it could be inferred that oxidative stress caused significant death in yeast cells. Oxidative stress defense might be the result of the antioxidant effect of seed extract. 2-NBDG is transported into *S. cerevisiae* through glucose transporters, therefore, the glucose uptake activity in yeast can be directly evaluated by measuring the incorporation of 2-NBDG into the cell as fluorescence intensity. The hypoglycemic effect exhibited by the crude *seed* extract is mediated by increasing glucose adsorption, decreasing glucose diffusion rate and at the cellular level by promoting glucose transport across the cell membrane as revealed by simple *in vitro* model of yeast cells.

Propidium iodide staining, DAPI staining, ROS measurement confirmed that oxidative stress takes place in 3T3-L1 cell line and seed extract was effective for reducing oxidative stress environment. Propidium Iodide cannot cross the membrane of live cells, making it useful to differentiate necrotic apoptotic and healthy cells. Flow cytometry study also confirmed that seed extract was highly effective for reducing cell death. Extract and insulin enhance glucose uptake in 3T3-L1 cells with respect to control. 2-NBDG is transported into 3T3-L1 cell line through glucose transporters and that glucose uptake activity in yeast can be directly evaluated by measuring the incorporation of 2-NBDG into the cell in terms of fluorescence intensity. Extracts with NBDG increased the glucose uptake in cells effectively as compared with NBDG only. Computational biology approaches might be influencing the effects of dietary polyphenols from faba bean on the

modulation of metabolic diseases by testing the *in vitro* study. Dietary polyphenols from faba bean may act as a lead compound for drug discovery.