5

SUMMARY AND CONCLUSION

Diabetes is a metabolic disorder where human body does not produce sufficient insulin or properly use it, a hormone that is required to convert sugar, starch and other food into energy. Diabetes results in abnormal levels of glucose in the bloodstream. The increasing prevalence of Type 2 Diabetes Mellitus and the side effects observed with the commercially available antidiabetic drugs requires investigation of new therapeutic approaches for controlling postprandrial glucose levels.

The use of carbohydrate digestive enzyme inhibitors from natural resources could be a possible strategy to block dietary carbohydrate absorption with less adverse effects than synthetic drugs in the treatment of diabetes. Potent inhibitors found in vegetables, herbs and fruits might be effective for the treatment of diabetes. Hence, the present study was designed to compare the antidiabetic potential of alpha amylase inhibitors in *Momordica charantia* and *Trigonella foenum graecum*.

Objectives of the Study

- *In vitro* alpha amylase inhibitory and antioxidant activities of *Momordica charantia* and *Trigonella foenum graecum*
- Antidiabetic potential of plant extracts on streptozotocin induced diabetic rats
- Phytochemical analysis, isolation and characterization of active principles responsible for alpha amylase inhibition
- *In silico* molecular docking studies of compounds identified with pancreatic alpha amylase enzyme

The plant materials used in the present study *Momordica charantia* and *Trigonella foenum graecum* were collected from local market identified and authenticated by botanist from Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The study was carried out in four phases.

Experimental Design and Findings of the Study

Phase I

In Phase I, the selected plant parts *Momordica charantia* flesh (MCF), *Momordica charantia* seeds (MCS), *Trigonella foenum graecum* leaves (TGL) and *Trigonella foenum*

graecum seeds (TGS) were extracted with different solvents (petroleum ether, chloroform, ethyl acetate, ethanol, acetone and water). *In vitro* alpha amylase inhibitory activity of MCF, MCS, TGL and TGS in all the six extracts were determined by assaying the inhibitory activity of the enzyme and the inhibition was found to be higher in ethyl acetate extracts of MCF (93%), MCS (94%) and TGS (91%) and ethanol extract of TGL (80%) when compared to petroleum ether, chloroform, acetone and aqueous solvents. Hence, ethyl acetate extracts of MCF, MCS, TGS and ethanol extract of TGL were chosen for further studies. The mechanism of inhibition and enzyme kinetics were studied by the method of Dixon and Cornish - Bowden plot. Alpha-amylase inhibition was found to be dose dependant. Mechanism of inhibition showed that the type of inhibition in *Momordica charantia* was non competitive type in both the MCF and MCS extracts where as in *Trigonella foenum-graecum,* the mechanism was found to be competitive type in TGL and mixed inhibition in TGS.

Total antioxidant potential of the selected plant parts were determined by FRAP assay and other radical scavenging activities namely DPPH, ABTS, nitric oxide, hydroxyl, superoxide radicals and inhibition of *in vitro* lipid peroxidation. Total antioxidant potential and inhibition of lipid peroxidation were highest in MCS followed by TGS when compared to MCF and TGL. DPPH, nitric oxide and superoxide radical scavenging activities were higher in the seeds of MCS and TGS when compared to MCF and TGL. ABTS and hydroxyl radical scavenging activities were higher in MCF and TGL. Since, alpha amylase inhibitory and antioxidant activities were found to be higher in seeds of *Momordica charantia* and *Trigonella foenum-graecum* extracts, they were chosen for further *in vivo* studies.

Phase II

In Phase II, antidiabetic potential of seeds of *Momordica charantia* and *Trigonella foenum-graecum* extracts were evaluated in Streptozotocin – Nicotinamide (STZ-NIC) administered diabetes induced rats. Acute oral toxicity studies revealed that the extracts were safe and non toxic up to a maximum dose of 2000 mg/kg body weight. Since no mortality was observed in acute toxicity studies, 1/5th and 1/10th of the highest dose (2000mg/kg b.w) were chosen for performing Oral Glucose Tolerance Test (OGTT) in normal rats. In OGTT, MCS, TGS and glibenclamide treated rats significantly prevented a rise of the blood glucose level compared to the control group. Both the seed extracts seemed to possess glucose lowering effect indicating a better glucose utilization capacity.

Diabetes Mellitus was induced by a single intraperitoneal injection of STZ-NIC and rats with blood glucose concentration more than 250mg/dl were used for further study. The rats were divided into seven groups and treated with MCS, TGS and glibenclamide for of 21 days. Rats treated with MCS 400 mg/kg b.w and TGS 400 mg/kg b.w. showed significant decrease in the levels of blood glucose monitored at weekly intervals. Rats treated with plant extracts and glibenclamide showed a significant increase ($p<0.05$) in the total protein content and in liver glycogen. Total cholesterol, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were found to be significantly decreased ($p<0.05$) and high density lipoprotein was significantly increased ($p<0.05$) in plants and glibenclamide treated rats when compared to STZ-NIC induced diabetic rats.

There was a significant improvement in the activities and the levels of enzymic and non enzymic antioxidants catalase, glutathione peroxidase, superoxide dismutase, vitamins C, E and reduced glutathione (GSH) on treatment with 400mg /kg b.w of the plant extracts and glibenclamide. The rats treated with glibenclamide, MCS and TGS showed significant reduction ($p<0.05$) in lipid peroxidation, activities of hepatic enzymes glucose -6-phosphatase, fructose 1, 6-diphosphatase and significant increase in glucose -6-phosphate dehydrogenase activity.

Haematological evaluations revealed that there was significant improvement in the levels of RBCs and its indices upon treatment with glibenclamide, MCS and TGS. The results of white blood cell count showed a significant increase and the lymphocyte percentage were decreased in diabetic group when compared to the control group. There were no differences in the percentage of monocytes, eosinophils and polymorphs. Histopathology results showed that the islets demonstrated the recovery of damaged ones and an improvement in the number of β cells after treatment with the plant extracts. It can thus be proved that *Momordica charantia* and *Trigonella foenum graecum* seed extracts have a therapeutic effect that alleviates Diabetes Mellitus.

Phase III

In Phase III, analysis of the plant extracts showed the presence of various phytochemicals namely flavonoids, phenols, tannins, terpenoids, steroids and saponins. HPTLC analysis showed the presence of flavonoids corresponding to quercetin, rutin and kaempferol standards. The phenols present were confirmed using resorcinol, catechol, gallic acid and hydroquinone as reference standards.

TLC profile of MCS and TGS showed four and five visible bands respectively. Alpha amylase inhibitory potentials of MCS showed highest inhibition in one of the TLC fractions with 96 % inhibition and in TGS one of the TLC fractions showed 94% inhibition. FT-IR analysis of MCS confirmed the presence of alcohols, phenols, alkanes, alkynes, aldehydes, aromatic compounds, aromatic amines and aromatic hydrocarbons. TGS extracts showed the presence of esters, aliphatic compounds, primary alcohols, phenols, aromatic amines, cyclic and primary alcohols.

GCMS analysis of potent TLC fractions of MCS and TGS revealed the presence of eight and seven major compounds respectively. The compounds 4-di- tert- butylphenol, 1-nonadecene and 2[(Decahydro-5, 5, 8a -trimethyl-2-methylene-1-naphthalenyl) methyl 2, 5-cyclohexadiene-1,4-dione were present in both the extracts. Other compounds identified were 1-methyl-4-(1-methylethenyl)cyclohexene,2,6,10–trimethyldodecane (farnesane), nona hexacontanoic acid methyl ester, 1,2-benzenedicarboxylic acid,11-methylspirostan-3,11-diol, 1-heptacosanol, i-propyl 5,9,17-hexacosatrienoate, 1-phenanthrene carboxylic acid, 1, 2, 3, 4, 4a, 9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-,methyl ester (dehydroabietic acid) and *2,5-Di-tert*-butylhydroquinone.

Phase IV

In Phase IV, *in silico* docking analysis of the identified compounds showed the binding energies ranging from - 8.59 Kcal/mol to - 4.43 Kcal/ mol with human pancreatic alpha amylase (HPA) and - 6.02 Kcal/mol to -2.50 Kcal/ mol with porcine pancreatic alpha amylase (PPA) as target proteins. The ligand molecules were more potent in binding to HPA than to PPA. The plant compounds 11-methylspirostan-3,11-diol, dehydroabietic acid, farnesane, 2,4-di-tert-butylphenol and 1-nonadecene showed better interactions with low docking energy and high binding ability compared to other compounds.

Conclusion

The present study emphasizes the potential of *Momordica charantia* and *Trigonella foenum graecum* seed *extracts* to inhibit pancreatic alpha- amylase, an enzyme that is responsible for the digestion of starch. The seeds of both the plants were found to be effective in terms of antioxidant potential and are expected to act through different mechanisms. *In vivo* studies demonstrated the blood glucose- lowering effect by the plant extracts. Presence of phytochemicals namely flavonoids, phenols, tannins, terpenoids, steroids and saponins might be responsible for antidiabetic and antioxidant activities. *In silico* docking studies of bioactive compounds identified from the seeds of *Momordica charantia* and *Trigonella foenum graecum* with human pancreatic alpha

amylase and porcine pancreatic alpha-amylase enzymes as target proteins showed good binding affinity and might act as potential inhibitors. Hence, inhibition of pancreatic alpha-amylase inhibitor compounds obtained from *Momordica charantia* and *Trigonella foenum graecum* seeds might be a promising strategy in the management and prevention of Diabetes.

Scope for Future Research

The bioactive compounds identified in the study can be isolated, purified, tested in animal models and human subjects. The compounds can be assessed for the inhibitory action on other carbohydrate metabolizing enzymes namely alpha - glucosidase, dipeptidyl peptidase (DPP- 4 inhibitors) and fructose 1-6 diphosphatase. This may help in the formulation of new drugs for the treatment of Diabetes Mellitus.