6. Overall Discussion

Aim of the present research work was to systematically investigate the phytochemical and pharmacological aspects of two selected plants *Tinospora sinensis* and *Chonemorpha fragrans*. The antidiabetic activity studies revealed the potential of these traditionally used plants in the future development of new antidiabetic leads.

The total ethanolic extracts of both the plants exhibited significant antioxidant activity in models like DPPH, ABTS radical scavenging assay, nitric oxide scavenging assay, ferric reduction capacity by orthophenantroline and total antioxidant activity by phosphomolybdinum method. Since oxidative stress has been implicated in the pathology of many diseases like diabetes, these results suggests the potential of these plants in the treatment of diabetes.

An *in vitro* glucose uptake study was carried out on the total ethanolic extracts of both the plants using cultured 3T3 adipocyte cell lines. The results have demonstrated the ability of the plant extracts to stimulate the glucose uptake in the absence of insulin. Standard pioglitazone exhibited 88.45 % glucose uptake at a concentration of 100 µg/mL whereas the CFTE exhibited 23.56 % and TSTE showed 17.19 % at the same concentration as that of standard. These results suggest that mechanism underlying the antidiabetic activity may be attributed to other molecular pathways in addition to the above mentioned ones.

Acute toxicity studies revealed the safety of the ethanolic extracts up to 2000 mg/kg body weight as per the OECD guidelines and the LD_{50} of the tested extract was higher than the dose. Based on this, two doses were selected for the *in vivo* antidiabetic study i.e. 200 mg/kg and 400 mg/kg body weight.

The normoglycemic study demonstrated that the glibenclamide showed a significant reduction (p<0.01) in blood glucose level at a dose of 0.60 mg/kg body weight when compared to the normal control after 4 hr of treatment, whereas the total ethanolic extracts of *T. sinensis* and *C. fragrans* did not show a significant reduction of blood sugar in normal rats (p>0.05)
The results of the OGTT showed that the blood glucose levels of all the extract treated groups and glibenclamide treated groups showed a very steep decrease in the glucose level. The ethanolic extract of the plants at a dose of 200 mg/kg and 400 mg/kg showed a significant decrease (P < 0.05) in blood glucose levels when compared to the control. The extracts were found to reduce the sugar levels in a dose dependant manner.

The antidiabetic activity study was carried out on streptozotocin-nicotinamide model. The diabetic rats showed a significant elevation in fasting blood glucose levels (p<0.001) when compared to normal rats. The normoglycemic rats continued to maintain their basal blood glucose levels throughout the experiment period. The fasting blood glucose levels of the diabetic rats were elevated throughout the study but the diabetic symptoms were found to be deteriorating during the treatment period. While the TSTE and CFTE treated groups at the dose of 200 mg/kg and 400 mg/kg exhibited significant lowering of blood glucose levels in STZ-NA treated rats on 7th day, 14th day and 21st day. The results of the study have shown a significant difference between the initial and final fasting blood glucose levels of *T. sinensis*, *C. fragrans* and glibenclamide treated diabetic rats. In the present study the body weights of streptozotocin-nicotinamide injected rats were found to be statistically less (P < 0.05) as compared to normal rats and associated with observations of diabetic symptoms like polyuria, polydipsia and polyphagia.

Hypercholesterolemia is one of the major problems associated with diabetes. In our study there was a marked decrease in triglycerides in extract treated animals. Treatment with extracts exhibited significant lowering of VLDL-C levels in STZ-NA diabetic rats at the end of treatment period. Extracts showed significant elevation of HDL cholesterol levels when compared to the diabetic group. Similarly there was a significant reduction in the elevated concentration of LDL cholesterol when compared with diabetic control at the end of treatment period. This improvement in the lipid profile of extract treated diabetic animals suggests the hypolipidemic activity of the extracts.
Hepatic serum markers like ALT and AST were estimated on day 21 and used for the evaluation of hepatic damage. Streptozocin treatment has a significant role in the alteration of liver functions since the activity of AST and ALT were slightly higher than those of normal value. Treatment with CFTE and TSTE showed a significant reduction of AST on comparison with diabetic control. Diabetic control showed a significant increase in creatinine and urea levels compared to control animals. The elevation of serum urea and creatinine are significant markers related to renal dysfunction in diabetic hyperglycemia. Treatment with extracts significantly decreased serum creatinine levels. But the extracts did not show any significance change in urea as compared with diabetic controls.

In diabetes there is an increased glycation of a number of proteins including haemoglobin. Haemoglobin is highly susceptible to non-enzymatic glycation. In diabetic condition, the excess of glucose present in the blood reacts with haemoglobin to form glycated haemoglobin, which has altered affinity for oxygen. The glycated haemoglobin levels in the diabetic control group were significantly high as compared to the control group. Glycated haemoglobin was significantly increased in diabetic rats and this increase was directly related to elevated fasting blood glucose levels. \( \text{HbA1c} \) levels are monitored as a reliable index of glycaemic control in diabetes. Administration of the extracts to diabetic rats prevented the increase in glycosylated haemoglobin significantly and this could be due to decrease in glucose levels.

In an attempt to identify the exact molecular mechanism we have evaluated the alpha amylase inhibitory potential of the total ethanolic as well as the fractions of both the plants. Among the two plants \( T. \text{sinensis} \) exhibited a significant activity compared to \( C. \text{fragrans} \). The total ethanolic extract of \( C. \text{fragrans} \) roots showed 50 % inhibition at a concentration of 287.5 \( \mu \text{g/mL} \). The petroleum ether, chloroform extract, ethyl acetate and residual alcoholic extracts showed 50 % inhibition at a concentration of 197.5, 42.5, 135, 47.5 \( \mu \text{g/mL} \) respectively. The chloroform extract exhibited the maximum activity followed by residual alcoholic. The total ethanolic extract of \( T. \text{sinensis} \) showed 50% inhibition at a concentration of 82.5 \( \mu \text{g/mL} \). The petroleum ether, chloroform, ethyl acetate and residual alcoholic fractions showed
50 % inhibition at a concentration of 47.5 µg/mL, 262.5 µg/mL, 22.5 µg/mL and 110 µg/mL respectively. The ethyl acetate extract exhibited a significant activity (IC\textsubscript{50} 22.5 µg/mL) when compared to standard acarbose which showed 50 % inhibition at 25.0 µg/mL concentration. The significant results justify the traditional use of these plants as an antidiabetic agent since the α-amylase inhibitors reduce postprandial hyperglycaemia by suppressing hydrolysis of starch and this has been found to be useful in the control of \textit{Diabetes mellitus}.

This triggered us to further investigate the phytoconstituents of \textit{T. sinensis}, which is responsible for the activity. The total ethanolic and ethyl acetate fractions were subjected to UPLC-QTOF MS/MS analysis. Many phytochemicals were tentatively identified by the molecular mass and the fragmentation pattern obtained. Compounds like naringenin 7-O- glucoside, luteolin 7-O-glucoside/kaempferol 3-O-glucoside, naringenin, luteolin, kaempferol, rhamnetin, myricetin 3-O-glucoronide, jatrorrhizine, 7-O-methyl cyanidin 3-O-galactoside and kaempferide were tentatively identified. Among these, compounds like kaempferol, and naringenin were reported to have antidiabetic property.

Further investigations resulted in the isolation and structural elucidation of compounds like 1-octacosanol, stigmasterol, ferulic acid derivative, coumaric acid derivative, naringenin and kaempferol methyl ether from various fractions of \textit{T. sinensis}. The structures of all these compounds were confirmed by UV, IR, NMR and mass spectroscopical analysis.

The various fractions of the \textit{C. fragrans} root were also subjected to column chromatography. The isolated compounds were only subjected to IR spectral studies to identify the functional groups.

All the isolated compounds from \textit{T. sinensis} were subjected to \textit{in vitro} alpha glucosidase inhibitory activity study. The isolated compound naringenin (TS-7) was demonstrated to have a potent α-glucosidase enzyme inhibitory activity with an IC\textsubscript{50} value of 4.75 µg/mL which was comparable with the conventional antidiabetic drug, acarbose. Among the other isolated compounds kaempferol methyl ether (TS-8) showed an IC\textsubscript{50} value of 19.56 µg/mL. Octacosanol (TS-1) and stigmasterol (TS-2)
showed an IC$_{50}$ value of 82.62 µg/mL and 76.09 µg/mL respectively. Compounds TS-4 and TS-5 exhibited least inhibitory activity among the isolated compounds IC$_{50}$ value of 161.59 µg/mL and 172.60 µg/ml respectively.

Since the compound naringenin was found to be a very good alpha glucosidase inhibitor we have selected this compound for the further study in order to understand the molecular mechanism underlying. Naringenin (TS-7) was subjected to the PPAR$\gamma$ gene expression studies. The L6 cell lines were treated with test substance and standard drug rosiglitazone to study the PPAR$\gamma$ gene expression. The test substance naringenin, at a concentration of 100 µg/mL and 50 µg/mL showed 0.08 folds and 0.04 folds up-regulation respectively, of PPAR$\gamma$ gene expression compared to control. The standard drug rosiglitazone up-regulated PPAR$\gamma$ expression by 0.15 folds as compared to control. These results indicated the usefulness of naringenin as a potential candidate for the development of a therapeutically useful PPAR$\gamma$ agonist.

In the course of our investigation on naringenin, it was also learned that other workers, also made studies on the mechanisms such as stimulation of glucose uptake in L6 myotubes and significantly increased AMPK phosphorylation. Naringenin showed insulin like effect to decrease apoliprotein B secretion in hepatocytes. However their studies were confined to the above mechanisms and not on PPAR$\gamma$ gene expression. Its worthiness as a potential antidiabetic agent merits further pharmacological/clinical investigation.