6. EXECUTIVE SUMMARY AND CONCLUSION

6.1 Introduction

According to recent estimates, the human population worldwide appears to be in the midst of a pandemic of diabetes. WHO indicates that Diabetes mellitus is one of the major diseases of high morbidity and mortality of our time and people in South East Asia and Western Pacific being most at risk. Finding an adequate treatment for this pathology especially to prevent complications of diabetes mellitus is an important goal in medicine. Natural remedies using medicinal plants would be a safe alternative in treating diabetes.

From the literature observed on Indian medicinal plants, it was observed that a combination consisting of *Momordica charantia, Aloe vera, Annona squamosa, Gymnema sylvestre* and *Scoparia dulcis* has not been explored so far for antidiabetic property. Hence an attempt has been made to elucidate an effective polyherbal combination for the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic loss.

6.2 Background

Despite the great strides that have been made in the understanding and management of this disease, serious problems like diabetic nephropathy, retinopathy and neuropathy continue to confront patients and physicians. There are many herbal remedies suggested for diabetes and diabetic complications. Major hindrance in these therapies to become part of modern medical practices is lack of scientific and clinical data proving their efficacy and safety. Hence there is a need for conducting elaborate studies including biological standardisation, pharmacological and toxicological evaluation and for studying the toxicity and safety of these herbal drugs.
As diabetes is a multifactorial disease leading to several complications, it demands a multiple therapeutic approach and hence the present study was designed. The plants selected for the study are potential antidiabetic drugs and some of them also possess hypolipidemic and antioxidant activity. It is postulated that when these selected plants are combined in appropriate proportion, they may evolve as an effective antidiabetic polyherbal combination without any side effects, which is the major drawback of modern medicine.

6.3 Methods

The five plants selected for study were the fruits of Momordica charantia (Cucurbitaceae), leaves of Aloe vera (Liliaceae), Annona squamosa (Annonaceae), Gymnema sylvestre (Asclepiadaceae) and the whole plant of Scoparia dulcis (Scrophulariaceae). These plants selected for the study were collected, identified and authenticated. Aqueous extracts of these five plants were prepared. The five plant extracts were combined in different ratios to arrive at fifteen combinations (PHC1-PHC15). All the fifteen combinations were subjected to in vitro antidiabetic (α-Glucosidase inhibitory activity and α-amylase inhibitory activity) and antioxidant activity (DPPH assay and Nitric oxide radical scavenging assay) in five different concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 mg/ml. Through statistical analysis, by determining IC50 and regression analysis two efficient combinations were selected. Since no two polyherbal combinations show equal efficacy in all the four in vitro assays, two modified polyherbal combinations such as MPHC1 and MPHC2 were prepared by slightly changing the proportions of the plant extracts and all the in vitro assays were repeated. The results of in vitro analysis of MPHC1 and MPHC2 showed that these two were better than the fifteen combinations and hence was selected for further analysis.
Acute oral toxicity study was conducted on Sprague dawley female rats according to the OECD test guideline 423- Acute toxic class method by administering MPHC\textsubscript{1} and MPHC\textsubscript{2}. The antidiabetic efficacy of the two selected polyherbal combinations MPHC\textsubscript{1} and MPHC\textsubscript{2} were evaluated in Streptozotocin-induced Sprague dawley diabetic model each in two different concentrations of 250 and 500mg/kg b.wt. The animals were induced diabetes through STZ administration. The treatment was conducted for fourteen days in seven groups of six animals each. Various biochemical parameters including fasting blood glucose level, lipid profile, parameters to study the liver and kidney function, carbohydrate metabolic enzymes and antioxidant enzymes activities were evaluated in the experimental rats on the last day of treatment. MPHC\textsubscript{1} and MPHC\textsubscript{2} were compared to the standard antidiabetic drug, metformin. Various organs like liver, pancreas, eyes, brain, kidneys, adrenal glands and heart from the experimental animals were evaluated for histopathological changes in these organs.

Using various bio-markers, plant extracts and the two polyherbal combinations MPHC\textsubscript{1} and MPHC\textsubscript{2} were quantified through HPTLC method. Based on in vivo studies on animals and HPTLC analysis MPHC\textsubscript{2} was selected and was subjected to in vitro molecular studies on 3T3 - L1 adipocytes. The expression of GLUT4 and PPAR\textgamma were analysed by using RT-PCR to study the molecular mechanism of action of the polyherbal combination.

6.4 Results

In the various in vitro analysis of the polyherbal combinations for antidiabetic and antioxidant activity, combinations with lower IC\textsubscript{50} values and with higher regression values had better antidiabetic and antioxidant activity. Thus, in all the in vitro assays, the modified polyherbal combinations MPHC\textsubscript{1} and MPHC\textsubscript{2} were found to have lower IC\textsubscript{50} and higher regression values when compared to other fifteen combinations.
MPHC$_1$ and MPHC$_2$ are polyherbal anti-diabetic combinations in appropriate proportion of the five plant extracts with maximum in vitro antidiabetic and antioxidant potentials with five plant extracts namely fruits of *Momordica charantia*, leaves of *Aloe vera*, *Annona squamosa*, *Gymnema sylvestre* and the whole plant of *Scoparia dulcis* in the ratio 9: 8: 8: 9: 8 and 8: 7: 10: 10: 7 respectively and were selected for further analysis in animal model.

In acute toxicity studies, LD$_{50}$ of the test drug was found to be greater than 2000mg/kg b wt. which falls in the “Category-5” or “unclassified” in accordance to the globally harmonized System as per OECD guidelines and no toxicological signs were observed.

The polyherbal combinations MPHC$_1$ and MPHC$_2$ exhibited significant hypoglycemic (p<0.01) and hypolipidemic effects (p<0.01) in experimentally induced hyperglycemic rats, with MPHC$_2$ showing better activity. Various biochemical parameters measured in the experimental rats also proved the hepatoprotective property exhibited by these two polyherbal combinations. Especially MPHC$_2$ in higher dose showed better activity (SGOT and SGPT levels) than metformin (p<0.001). The biochemical parameters for evaluating kidney function (BUN levels) also showed significant activity when compared to metformin (p<0.01).

These two combinations also elicit significant antioxidant effects (p< 0.01) in STZ-induced hyperglycemic rats as reflected by their ability to elevate the enzymatic antioxidants (SOD, CAT, GPx, GSH). SOD, CAT and GPx activity showed better activity than metformin (p<0.001). Gluconeogenic enzymes were normalized after MPHC$_2$ (higher dose) treatment showing significance p<0.01 when compared to hyperglycemic group. Hexokinase activity was significantly increased (p<0.01) in MPHC$_1$ and MPHC$_2$ (in higher dosages) treated groups indicating stimulation of glycolysis process by the polyherbal combinations. Liver glycogen content was
significantly increased (p<0.01) after the treatment with MPHC₁ and MPHC₂. The histopathological studies have shown that the combinations MPHC₁ and MPHC₂ ameliorate the STZ induced histological damage of pancreas.

The combination MPHC₂ in dose of 500mg/kg b wt. showed better antidiabetic and antioxidant activity when compared with MPHC₁. Quantification of bio markers in the polyherbal combinations through HPTLC studies also showed MPHC₂ to be a better combination possessing higher concentrations of the biomarkers. MPHC₂ was selected as the efficient combination and was hence subjected to in vitro molecular studies. There is a significant down-regulation of PPARγ expression (p<0.05) which is a well established transcriptional regulator of adipogenesis. Adipogenesis is a major mechanism leading to weight gain and obesity. Since MPHC₂ showed significant down regulation of PPARγ expression it may be considered to possess antiadipogenic property which substantiated the hypolipidemic activity of MPHC₂ observed in animal study. However, there is no significant change in the expression of GLUT4.

6.5 Conclusion

The polyherbal combination MPHC₂ is found to be an efficient hypoglycemic, hypolipidemic and antioxidant polyherbal combination proved through the various in vitro and in vivo studies performed, which is being reported for the first time in medicinal plant research. In vitro molecular studies also suggest that the combination, MPHC₂, in addition to its hypoglycemic property may also be a useful drug in preventing weight gain. The hypolipidemic property of the combination MPHC₂ may contribute in preventing atherosclerosis and other complications associated with dislipidemia. Hence this polyherbal combination, MPHC₂ may be considered as an efficient antidiabetic drug which may also effectively treat complications associated with the disease, after further confirmatory studies.
To conclude, the key results from our study focused on:

- **MPHC$_2$** the selected polyherbal combination possess potential $\alpha$-amylase and $\alpha$-glucosidase inhibitory activity. The glucose lowering effect of this combination may be due to the inhibition of these enzymes.

- **MPHC$_2$** exhibits significant hypoglycemic activity as revealed through the studies on STZ-induced diabetic rats.

- The hypoglycemic activity of the combination may be by way of improving glycogenesis and glycolysis process in liver as observed through increase in liver glycogen content and increase in hexokinase activity after treatment with the combination MPHC$_2$.

- The antidiabetogenic action of the combination may also be due to depression of gluconeogenic enzyme activity in liver.

- The *in vivo* study on STZ-induced rats also revealed that MPHC$_2$ possess significant hypolipidemic activity and was better than metformin. Hence this polyherbal combination, MPHC$_2$ not only helps to combat hyperglycemia, but also prevent dyslipidemia- an important risk factor for the micro and macro vascular complications of diabetes.

- Antioxidant potential of MPHC$_2$ as observed in the *in vitro* and *in vivo* studies may be due to the presence of various antioxidant compounds in the combination or by promoting the level of antioxidant molecules which may be directly or indirectly responsible for its hypoglycemic property.

- **MPHC$_2$** is found to possess hepatoprotective activity as seen through the various parameters of liver function observed through *in vivo* studies, and its effect was better when compared to Metformin treatment.
The polyherbal combination MPHC\textsubscript{2} proved to be effective in preserving the kidney function as seen through decrease in Blood urea nitrogen levels after MPHC\textsubscript{2} treatment on STZ-induced rats.

There was a significant down regulation of PPAR\textgamma expression in MPHC\textsubscript{2} treated adipocytes indicating antiadipogenic activity of the combination.

Obesity is a major risk factor for metabolic syndrome and type 2 diabetes. Most antidiabetic drugs promote weight gain. But MPHC\textsubscript{2} promote hypoglycemia without inducing adipogenesis through significant down regulation of PPAR\textgamma expression as observed in the molecular study on adipocytes.

The polyherbal combination MPHC\textsubscript{2} with fruits of \textit{Momordica charantia}, leaves of \textit{Aloe vera}, \textit{Annona squamosa}, \textit{Gymnema sylvestre} and the whole plant of \textit{Scoparia dulcis} in the ratio 8: 7: 10: 10: 7 if proved clinically, would be a safe alternative remedy for a multifactorial disease - Diabetes Mellitus that holds the hope of new generation of antidiabetogenic drugs.

6.6 Scope for future studies

Sub-acute and chronic toxicity studies could be conducted to study the toxicity of the polyherbal combination MPHC\textsubscript{2}, to determine the long term safety of the combination, even though acute toxicity study has been conducted.

A study of human trials could be conducted to elucidate the efficacy of this polyherbal combination in patients with diabetes mellitus.

Molecular studies on other tissues like muscle could be conducted to observe the expression of glucose transporters.

The long term effectiveness of the combination in treating the micro and macro vascular complications of diabetes may also be studied.