Chapter 8

Summary and Conclusion
Plants are used by man from time immemorial for their extraordinary healing abilities and pain relieving properties. Presently, the medical fraternity has increasingly started using plants to overcome various illness and sufferings, mainly to obviate the profound side effects encountered in usage of modern drugs. Due to economic reasons, providing modern medicinal healthcare in developing countries such as India is still a far-reaching goal. From the identified higher plants in the world, less than 1% has been screened pharmacologically and very few in regard to diabetes mellitus. Therefore, it is prudent to look for options in herbal medicine for diabetes as well. It is seen that certain resistant cases of diabetes who do not respond well to modern medicines such as chlorpropamide, tolbutamide and glibenclamide respond well when treated with herbal preparations, alone or in combination with other oral hypoglycemic agents.

Hypoglycemic activity has been recorded with numerous plants, many of which are used in traditional herbal treatments of diabetes. Compounds with antidiabetic activities come from a range of chemical classes including polysaccharides and proteins, flavanoids, steroids, terpenoids and alkaloids. Some compounds such as lectins and fiber are negatively correlated with the glycemic index. The plants used as food, food additives and beverages from which hypoglycemic activity or antidiabetic agents have been identified.

High levels of reactive oxygen species (ROS) have been found to play a role in the pathogenesis of NIDDM [Halliwell and Gutteridge, 1985]. Altered antioxidants defenses in diabetes might lead to the development of diabetic induced complications. Increased plasma level glucose is also responsible for the damage to cell membranes through non-enzymatic glycosylation of proteins, auto-oxidation of glucose and increased metabolism of glucose by the sorbitol-polyol path way [Sato et al., 1979].

Natural antioxidants strengthen the endogenous antioxidant defenses from ROS and restore the optimal balance by neutralizing the
reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. Antioxidants, vitamin C and E have been shown to reduce the oxidative stress in experimental diabetes [Madhu et al., 1997].

In this thesis initially we have checked the antidiabetic potency of several plant extracts using alloxan induced diabetic models and compared their antidiabetic activity with their in vitro antioxidant potential for their ability to scavenge superoxide, hydroxyl radical and lipid peroxidation. Hypoglycemic activities of the extracts were also compared with that of insulin, and antioxidant activity was compared with that of Zingiber officinale. Terminalia belerica was the most active as an antioxidant followed by Aegle marmelos. Major ingredients in T. belerica are ellagic and gallic acid, which are known antioxidant. T. belerica and A. marmelos could effectively scavenge the free radicals superoxides and hydroxyl radicals and inhibit lipid peroxidation makes it as the best antioxidant among all the extracts have been tested. Continuous administration of A. marmelos and T. belerica reduced the serum glucose levels maximally. These results indicated a relationship of antioxidant activity with their glucose lowering property.

Since Aegle marmelos and Terminalia belerica extract, maximally reduced the serum glucose level and had significant in vitro antioxidant activity were selected to continue for in vivo study. 75% methanolic extract of A. marmelos and T. belerica (100 mg/kg, body wt.) significantly decreased serum glucose level and oxidative stress in diabetic animals. Alloxan administration produced elevated level of lipid peroxidation, hydroperoxides and conjugated diene that is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system result in tissue damage. In the in vivo study, perturbation of GSH status of a biological system can lead to serious consequences. SOD, CAT and GPx constitute a mutually supportive team of defense against ROS.
In our study, decline in the activities of these antioxidant enzymes in alloxan-induced animals and attainment of near normalcy in *A. marmelos* and *T. belerica* treated rats indicate oxidative stress elicited by alloxan had been reduced significantly by these extracts in vivo.

The study was extended to find out the role of oxidants and antioxidants in the pathogenesis of human diabetes mellitus, especially during secondary complications. In our study, 40 diabetic patients were selected and most of the patients in this group had poor glycemic control. Seventy-five percentages of these patients were suffering from at least one-micro/macrovascular complications. We have found that poor glycaemic control in diabetic patients was also associated with decreased free radical scavenging activity. Elevated levels of lipid peroxidation, hydroperoxide and conjugated cienne seen in these diabetic patients are clear manifestation of excessive formation of free radicals resulting in tissue damage. The activity of superoxide dismutase catalase, glutathione peroxidase and glutathione reductase were found to be lower in diabetic patients when compared to normal. The results of this study suggested that poor glycaemic control is associated with reduced free radical scavenging activity in patients with NIDDM.

TNF-α levels have been shown to be increased significantly in many diabetic patients who had complications. Increased TNF-α levels in patients with diabetes has been demonstrated and determination of TNF-α levels might offer a diagnostic tool to determine patients at high risk. We have also investigated the effect of some plant extracts on the inhibition of nitric oxide (NO) and TNF-α in normal as well as drug treated diabetic mice. *In vitro* TNF-α bioassay using L929 cells were also carried out in the presence of the extracts of *A. marmelos* and *T. belerica*. Addition of the extracts reduced the cytotoxicity to L929 cells by TNF in a concentration dependent manner as seen from the morphology of L929 cells. Similarly the herbal extracts reduced elevated NO and TNF in
macrophage by the administration of lipopolysaccharides. Increased TNF-α levels in alloxan diabetic animals were found to be decreased by the plant extracts indicating that herbal extracts could inhibit TNF-α levels both in vitro and in vivo and this may be another mechanism of the action of herbal extracts in diabetes.

Both animal and human studies have suggested that there is a decrease in insulin-mediated glucose transport in diabetes mellitus. Insulin enhances glucose uptake by muscle cells and fat and suppresses hepatic glucose production. Normally, insulin comes in contact with the cell; glucose transporter protein GLUT-4 gets translocated to the plasma membrane from the cytoplasm to facilitate transport of glucose into the cell [Charron et al., 1989]. Exposure to TNF-α has been shown to decrease GLUT-4 protein in cultured cells producing a decrease in the glucose transport. It has been shown that TNF-α blocks the insulin receptor tyrosin kinase activity [White and Kahn, 1994]. Increased TNF-α production could attribute to the insulin resistance seen in the diabetic patients. We also checked the glucose uptake and levels of glucose transporter protein (GLUT-4) using the isolated rat gastrocnemius muscle and diaphragm to evaluate the roles of selected herbal extracts on glucose transport to the cells. 14C glucose uptake by muscles and diaphragm in presence of insulin was found to be significantly increased when the animals were prior treated with A. marmelos and T. belerica. In another experiment the GLUT-4 protein was estimated by ELISA method using monoclonal rat antibody. We found that the change in insulin responsiveness after extract treatment was accompanied by a parallel change in GLUT-4 protein content after treatment of A. marmelos. In previous studies the treadmill training protocol increased muscle GLUT-4 protein content by only 30% in gastrocnemius muscle. On the other hand, in our study, the rats were administered with 200mg/kg body weight of A. marmelose for 10 days increased GLUT-4 protein in rat
gastrocnemius muscle but not with *T. belerica* extract. As previously reported, total GLUT-4 protein content has been considered to be one of the determining factors of insulin responsiveness in skeletal muscle [Hansen et al., 1995; Henriksen et al., 1990].

The study was extended by carrying out the experiment on a formulation in STZ-induced diabetic rats. A polyherbal formulation was made using five most active plants based on our preliminary study on their antioxidant and antidiabetic property. We also carried out the synergistic effect of these herbs in both *in vitro* and *in vivo* antioxidant activity. Liver function markers like ALP, ALT, AST and bilirubin in serum were elevated in STZ-induced diabetes when compared with normal animals and chronic administration of the herbal formulation reduced the levels. Renal function indicators like creatinine and urea were also elevated in the STZ-induced diabetic rats when compared with normal rats. These levels were reduced by the formulation in a dose dependent manner. One of the mechanisms studied was the inhibition capacity of the serum α-amylase level by the formulation. The drastic changes of antioxidant enzyme levels were turned to normal by the formulation treatment. We have also investigated the effect of the formulation on nitric oxide (NO) and tumour necrosis factor- α (TNF-α) bioassay by *in vitro*. The result showed the synergistic activity of the formulation and which was supported by its significant antioxidant property than individual drugs and its hyperglycaemic activity. Even though at higher dose (1000 mg/kg) the combination does not produce any major toxicity and reverse the organ functions to normal. Some of the drug is acting at low concentrations and it could be proposed that the drugs in this preparation may be acting synergistically to give the desired result.

Ethnomedical approach for diabetes is a practical, cost-effective and logical. The goals of medicine no matter, to which group it belongs,
are the same i.e. the welfare of the patient. One can look towards for a future of integrated medicine and hope that research in alternative medicine will help to identify safe and effective drug not only for diabetes but also for several other diseases for which effective medications are not available.