5. SUMMARY AND CONCLUSION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis. The disease is progressive and is associated with high risk of complications. Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides.

Defense against the reactive oxidants produced during aerobic metabolism is a complex process and is provided by a system of enzymes and antioxidant compounds capable of preventing excess radical production, neutralizing free radicals and repairing the damage caused by them. A sophisticated enzymatic and nonenzymatic antioxidant defense system including catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) regulates overall ROS levels to maintain physiological homeostasis. The level of these antioxidant enzymes critically influence the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes.

The projected incidence of diabetes is on rise. Present number of diabetes worldwide is around 150 million and this is likely to increase to 300 million or more by 2025. While the symptoms of diabetes can be controlled by insulin replacement, diet and exercise; the long term complications associated with diabetes can lead to cardiovascular diseases, renal and ocular diseases as well as peripheral neuropathies. Consistent with its damaging profile, diabetes mellitus is the frequent cause of adult renal failure worldwide. It is one of the most common non-accidental causes of amputation in the world. It is the most common widespread cause of blindness among non-elderly adults. Till date there is no cure for diabetes and the treatment is necessarily a long term incessant effort. The goal of diabetes management is several: not only normal glycemic control (and so avoidance of both acute and chronic hyperglycemia), but also prevention of hypoglycemic episodes, thus reducing the risk of long-term complications and preserving the quality of life for patients.

Although oral hypoglycemic drugs and insulin are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, their use is restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side
effects. In addition to its ineffectiveness in preventing long term complications, exogenous insulin also fails to produce a well controlled glycemia in association with variable dietary intake and physical activity. Increased episodes of severe hypoglycemia leading to a deleterious cerebral impact are common during insulin administration. Insulin therapy is also ineffective in reversing the long-term complications associated with diabetes.

As the knowledge of heterogeneity of diabetes increases, there is need to look for more efficacious agents with lesser or no side effects. Nature has been a source of medicinal treatment for thousands of years and plant based systems continue to play an essential role in the primary healthcare. Thus, it is prudent in the current context to look for the new efficacious formulations from the vast reserves of phytotherapy for the treatment of diabetes. *Azadirachta indica* has been shown to possess hypoglycemic activity in experimental animals. Each part of *A. indica* has some medicinal property and it has been commonly used to treat diabetes in Indian system of medicine from time immemorial.

Tissues under prolonged hyperglycemic conditions are likely to be subjected to significant oxidative stress and this mechanism may be partly or completely responsible for the organ/system dysfunction and their associated complications. The present study investigated the prospects of using *A. indica* extracts as an antidiabetic agent to combat the hyperglycemic and hypolipidemic conditions in experimental diabetes. The antioxidant potential of *A. indica* was investigated in different tissues by measuring the levels of enzymatic cellular defense systems consisting of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase/Glutathione reductase systems in diabetic rat models. Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway that supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). Any change in G6PD activity can alter the NADPH level in intra-cellular environment and can make cells very sensitive to oxidant damage. Therefore, effect on G6PD was also investigated in different tissues. The efficacy of treatment was further examined by assessing the diabetes induced alterations in glucose transporter protein and protein kinase C-β2 levels, the key determinants of glucose regulation and oxidative stress. DNA damage due to oxidative stress was also studied to monitor the effect of therapy.

Diabetogenic doses of alloxan selectively destroy the β-cells. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in experimental animals, with characteristics similar to type1 diabetes in humans. The present study was carried on
experimentally induced type 1 diabetes in female rats of Wistar strain. Diabetic rats were treated with Insulin, *A. indica* leaf extract (AILE), *A. indica* bark extract (AIBE) and *A. indica* seed oil (AISO) for 21 days.

The experimental diabetic animals showed characteristic symptoms of diabetes including hyperglycemia, glucosuria, polydipsia, polyurea and loss of body weight despite polyphagia. Reduced liver weight was observed in diabetic rats whereas weight of kidney increased in diabetic condition. Treatment with AILE, AIBE and AISO for 21 days significantly revived the altered index of body weight and organ indices.

The blood glucose level was increased to three-fold in alloxan diabetic rats. Diabetic animals receiving three weeks of treatment with insulin showed a marked reduction in hyperglycemia. Treatment with AILE, AIBE and AISO for 21 days corrected the altered glycemia indicating the potential hypoglycemic activity of *A. indica* extracts. The elevation in HbA1c (glycosylated hemoglobin) level was observed in diabetic rats which is a characteristic marker of persistent hyperglycemia in diabetic condition. Treatment with *A. indica* extracts prevented the increase of HbA1c and maintained its level close to the control values. It is a clear indication that the diabetic state was well regulated after the treatment of diabetic animals with *A. indica* extracts.

We have found that treatment with AILE, AIBE and AISO were effective in controlling the altered lipids levels. Total cholesterol (TC) and triglycerides (TG) were significantly reduced in all the treated groups. The treatment with *A. indica* was found more effective than insulin treatment in diabetic rats. A noticeable feature is that the treatment of diabetic animals with AILE, AIBE and AISO for 21 days increased the HDLC level which is considered as good cholesterol, however no considerable improvement was observed in insulin treated group. Improvement in lipid profile is suggestive of the action of *A. indica* on enzymes of lipid metabolism.

In the present study, altered antioxidant enzyme activities were observed in different tissues during diabetes. The enzyme superoxide dismutase (SOD) is the cell’s first line of defense against the toxicity of superoxide radical and the subsequent radical derivatives. While liver, kidney, muscle and brain show significantly lowered activity of SOD, heart shows an increase in SOD activity. In diabetic state, decrease in the levels of CU/Zn SOD was also observed in liver cytosolic fraction by immunoblotting and the results clearly indicate that the expression of SOD at protein level is altered during diabetes.
The enzyme catalase (CAT) is the main regulator of hydrogen peroxide metabolism. In diabetic state, the activity of CAT reduced significantly in liver, kidney and brain and increased in skeletal muscle and cardiac tissue. In heart tissue, activity of SOD and CAT are synergistically increased.

The principal means of disposal of H$_2$O$_2$ after catalase is NADPH dependent Glutathione reductase/Glutathione peroxidase pathway. In the present study, liver and muscle exhibit decreased activity of Glutathione peroxidase (GPx) in diabetic state while a remarkable increase of GPx activity was observed in kidney, heart and brain tissues. Glutathione reductase (GR) converts the oxidized glutathione (GSSG) formed by GPx into reduced glutathione (GSH) so that intra-cellular GSH/GSSG ratio is maintained to physiological limits. A decrease in the activity of GR was observed in liver and muscle tissue of diabetic rats. Kidney, heart and brain display increased activity of GR in alloxan diabetic rats. The observed pattern was in accordance with the GPx activity.

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme of the pentose phosphate pathway that supplies reducing energy to cells by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the supply of reduced glutathione (GSH) in the cells that is used to scavenge free radicals which can cause oxidative damage. In the present study, G6PD activity decreased in all the diabetic tissues studied.

We have found that antioxidant enzymes in different tissues showed different susceptibility to diabetes induced oxidative stress. Insulin supplementation to diabetic rats partially controlled the alterations in enzyme activities in all the tissues studied. Treatment with AILE, AIBE and AIS0 significantly controlled the change in enzyme activities in all the tissues and keep the values close to control ones.

In this study, oxidative modification of lipids were found in microsomal membranes of alloxan diabetic animals in which MDA levels were significantly higher than those of healthy age-matched controls, thereby showing them to be under oxidative stress. Treatment of diabetic animals with AILE, AIBE and AIS0 significantly inhibit the lipid peroxide formation. The present findings show the increased lipid peroxidation in tissues of the diabetic rats, and treatment with AILE, AIBE and AIS0 decreased the level of lipid peroxidation in all the tissues of diabetic rats studied.
GLUT 4 protein levels were measured in the membrane fraction of skeletal muscle by immunoblot analysis. The normalization of GLUT 4 level is an important parameter to evaluate the antidiabetic properties of AILE, AIBE and AISO, as one of the main reasons of hyperglycemia in diabetes mellitus is the decreased uptake of glucose by the insulin dependent tissues. We observed that after 21 days of diabetic induction, the GLUT4 level reduced in both cytosolic and membrane fraction of skeletal muscle indicating that the deficiency of insulin in diabetic state decreased both expression and translocation of GLUT 4 in skeletal muscle tissues. Treatment with AILE, AIBE and AISO partially revived the altered distribution and expression of GLUT4. Restoration of GLUT 4 levels would, therefore, enhance the uptake of glucose in skeletal muscle and thus helps in alleviating the hyperglycemic condition which in turn arrests all diabetic complications.

The protein kinase C (PKC) family of enzymes transduces a myriad of signals promoting lipid hydrolysis. The increased level of PKC β2 in skeletal muscle and cardiac muscle was observed in the present study. The exogenous supplementation of insulin to diabetic rats considerably ameliorated the augmented levels of PKC β2 in the diabetic heart and muscle in experimental diabetic rats. Treatment with AILE, AIBE and AISO restored the levels back to control condition as demonstrated by immunoblots of heart and muscle.

Oxide radicals potentially interact with cellular components including DNA bases or the deoxyribosyl backbone of DNA to produce strand breaks and damaged bases. Pathological condition such as diabetes, which increase the rate of H$_2$O$_2$ production with decreased antioxidant system will lead to the accumulation of H$_2$O$_2$ in tissues and cause DNA degradation. Oxidative DNA degradation was observed in the liver tissue of diabetic animals. However, treatment with A. indica extracts prevented any such damage.

Being a unique source of various types of compounds with diverse chemical structure, A. indica can be exploited for its plausible medicinal applications. Azadirachtin is one compound that exhibits concentration-dependent anti-radical scavenging activity. In present study, considerable amount of azadirachtin has been found in AILE, AIBE and AISO. However, a detailed study is required to ascertain its possible role in controlling diabetes induced oxidative stress.

The present study concludes the potential hypoglycemic, hypolipidemic and antioxidant activity of A. indica. Diabetes induced metabolic derangements and clinical complications can be prevented or reversed with an effective control of hyperglycemia. A
number of drugs and insulin are used to treat diabetes, but none of them is completely effective and without any side effects. The present study showed that AILE, AIBE and AISO successfully attained euglycemia and corrected the alteration in the metabolic pathways studied in the diabetic rats. Results from the present study also suggested an insulin secretion modulation in *A. indica* therapeutic action. Being a natural product with multitude of antidiabetic effects, *A. indica* can possibly be used as insulin replacement or an adjuvant in the management of both Type1 and Type 2 diabetes.

*A. indica* may exhibit its therapeutic effects through modulation of insulin secretion. The present study showed the hypoglycemic and antioxidant properties of *A. indica*. A reduction in the production of free radicals and lipid peroxides formation by restoring the antioxidant enzymes was observed in the present study, which can beneficially prevent the diabetes associated tissue damage. The present results are based on the studies in the animal experimental diabetic model. Further studies are required for their safe use. *A. indica* can be considered a better alternative and further be explored as a means of diabetic control.