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Title of the Thesis: **"Role of *Azadirachta indica* extracts on reversal of antioxidant status in experimental diabetes"**

ABSTRACT:

Key words: *Alloxan Diabetes, Hyperglycemia, Oxidative stress, Antioxidant enzymes, Azadirachta indica*

Diabetes mellitus is a group of metabolic disorders characterized by persistent 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 leading to cardiovascular diseases, renal and ocular diseases as well as peripheral neuropathies. 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 lipid peroxides formation. The diabetes management has several roles: 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.

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 level of antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes. The antioxidant potential of *A. indica* was investigated in different tissues by measuring the levels of enzymatic cellular defense systems. 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.

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. Treatment with AILE, AIBE and AISO for 21 days significantly revived the altered index of body weight and organ indices. 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 indicating that the diabetic state was well regulated after the treatment of diabetic animals with *A. indica* extracts. Treatment with AILE, AIBE and AISO were effective in controlling the altered lipids levels. The treatment with *A. indica* extracts

were found more effective than insulin treatment in diabetic rats. 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. 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 AISO significantly controlled the change in enzyme activities in all the tissues and keep the values close to control ones. 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 AISO significantly inhibit the lipid peroxide formation in all the tissues studied.

GLUT 4 protein levels were measured in the membrane fraction of skeletal muscle by immunoblot analysis. The present study has shown 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 $\beta 2$ 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 $\beta 2$ 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 tissues. Oxidative degradation of genomic DNA 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.

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, 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.