Conclusion
Type 1 diabetes is commonly diagnosed in children and young adults and occurs due to low production of insulin by pancreas. Diabetes mellitus shows typical symptoms like polyuria, polydipsia, polyphagia, extreme fatigue, blurry vision, slow-healing wounds, recurrent infections, sudden weight loss.

Increased blood glucose level cause imbalance in the generation of reactive oxygen species (ROS) and its utilization that result in oxidative stress. Protective pathways including antioxidant enzyme like, superoxide dismutase, glutathione peroxidase and catalase also get altered in oxidative stress. Oxidative stress also leads to lipid peroxidation, dyslipidemia and DNA damage.

Various oral hypoglycemic drugs and insulin are used in the treatment of diabetes and are effective in controlling blood glucose level. But use of these drugs and insulin is restricted by their side effects, limited action, secondary failure rates and pharmacokinetic properties.

Vanadate is reported to have antidiabetic properties. Vanadate antidiabetic properties are more effective in higher doses which are toxic too. Lower doses are safe but not effective in lowing hyperglycemia. Various side effects of vanadate have been reported like in bones, liver, kidney lesions, weight loss and gastrointestinal discomfort.

*Azadirachta indica* is a natural herb which has shown promising antidiabetic properties. Therefore combined therapy of lower doses of vanadate with *Azadirachta indica* was studied to increase the effectiveness even at lower dose.
Our study investigates the prospect of using low doses of vanadate in combination with *Azadirachta indica* leaf extract and assesses their antioxidant capacity by measuring levels of antioxidant enzyme defense systems mainly consisting of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase/gluthathione reductase in STZ-induced diabetic rat. The efficacy of the treatment was further examined by measuring the level of lipid peroxides, GLUT4 and PKCβ2. DNA damage due to oxidative stress is also studied to check the effectiveness of combined treatment. Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin. Streptozotocin damages pancreatic β-cells and induces clinical type 1 diabetes. Male rats of Wistar strain were used in this study. Diabetic rats were treated with insulin, vanadate (0.6 mg/ml), *Azadirachta indica* and a combined dose of vanadate (0.2 mg/ml) and *Azadirachta indica* for 21 days.

The general parameters include body weight and organs weight. The streptozotocin diabetic rat showed decrease in the body weight. Liver and heart weight also decreased in diabetic animals. However the weight of kidney increased in diabetic rats. Brain weight of diabetic animal did not show any change. The treatment of diabetic rats with insulin, *Azadirachta indica* and lower dose of vanadate with *Azadirachta indica* corrected the change in the body and organs weight after 21 days. However vanadate (0.6 mg/ml) treatment of diabetic animal could not improve the weight loss. The combined treatment was most effective in normalizing body weight, liver weight and heart weight.
Blood parameters like glucose level and glycosylated hemoglobin also show increased values in streptozotocin induced diabetic rats. After 21 days of treatment of diabetic rats with insulin, vanadate, *Azadirachta indica* and lower dose of vanadate with *Azadirachta indica* results in improvement of elevated values. The combined treatment was found to be most effective in normalizing the elevated level of blood glucose level and glycosylated hemoglobin.

Vanadate (0.2 mg/ml) when given to control rats did not show any toxic effect like loss in body weight and increased mortality but failed to lower hyperglycemia when given to diabetic rats for 21 days.

We have found that treatment with insulin, vanadate, *Azadirachta indica* and lower dose of vanadate with *Azadirachta indica* result in improvement of altered lipid level. Total cholesterol (TC) and triglycerides (TG) were significantly reduced in all the treated groups. The level of HDLC increased in the treated groups. The treatment with *A. indica* and vanadate combined is more effective.

Diabetic rats showed decrease in the glycogen content of liver and skeletal muscles as compared to normal rats. The decreased glycogen level in liver and skeletal muscles improved almost in all treated groups. The combined treatment with vanadate and *Azadirachta indica* is more effective.

Superoxide dismutase (SOD) is the first line antioxidant defense system. The activity of SOD was measured in the liver, kidney, heart, muscle and brain of diabetic rats and rats treated with different antidiabetic compounds. The

Catalase is the second important antioxidant enzyme defense system to scavenge free radical. The activity of CAT is measured in the liver, kidney, heart, muscle and brain of diabetic rats and rats treated with different antidiabetic compounds. The activity of CAT decreased in the liver, kidney and brain of diabetic rats. Heart and muscle showed a remarkable increase in CAT levels of diabetic rats.

After catalase, H$_2$O$_2$ catalysis is done by glutathione peroxidase. The activity of GPx was measured in the liver, kidney, heart, muscle and brain of diabetic rats and rats treated with different antidiabetic compounds. Liver and muscle showed lowering in the activity whereas heart, brain and kidney showed increase in the activity.

Gluthathione reductase converts the oxidized glutathione formed by GPx into reduced glutathione so that the intracellular GSH/GSSG ratio is maintained in the cells at an optimum level. The activity of glutathione reductase is measured in the liver, kidney, heart, muscle and brain of diabetic rats and rats treated with different antidiabetic compounds. Liver and muscle show decreased GR activity whereas kidney, heart and brain display increased activity.

Treatment of diabetic rats with insulin, vanadate, Azadirachta indica and combined treatment of vanadate and Azadirachta indica restored the alteration in the enzyme activity. The combined dose of vanadate and Azadirachta
*indica* was the most effective in normalizing the aberrations in the enzyme activities.

In diabetic animals, increase in lipid peroxidation is measured by the formation of MDA. The concentration of malondialdehyde (MDA) formation was measured in liver, kidney, heart, muscle and brain tissue of control, diabetic and diabetic animals treated with antioxidant compounds. Insulin, vanadate and *Azadirachta indica* treatment normalized the MDA level. Vanadate and *Azadirachta indica* combined treatment is more effective in normalizing the MDA levels in the diabetic animals.

Western blot analysis of GLUT4 protein showed decreased level of GLUT4 protein in diabetic rats. The treatment of diabetic rats with insulin, vanadate, *Azadirachta indica* and combined treatment of vanadate and *Azadirachta indica* restored the alteration in the protein level. The combined dose of vanadate and *Azadirachta indica* was the most effective in correcting the aberrations in protein level.

Western blot analysis of SOD protein showed decreased level of SOD protein in diabetic rats. The treatment of diabetic rats with insulin, vanadate, *Azadirachta indica* and combined treatment of vanadate and *Azadirachta indica* restored the alteration in the protein level. The combined dose of vanadate and *Azadirachta indica* was the most effective in normalizing the aberrations in protein level.

Western blot analysis of PKCβ2 protein shows increased level of PKCβ2 protein in diabetic rats. The treatment of diabetic rats with insulin,
vanadate, *Azadirachta indica* and combined treatment of vanadate and *Azadirachta indica* restored the alteration in the protein level. The combined dose of vanadate and *Azadirachta indica* was the most effective in normalizing the change in protein level.

Oxidative stress can lead to apoptotic degradation of DNA which is evaluated by DNA laddering method. Liver genomic DNA was isolated from control rats and different experimental rats. DNA extracted from streptozotocin treated diabetic group of rats showed the smearing pattern which is a feature of DNA fragmentation and apoptosis. However, DNA extracted from control group of rats showed no smearing pattern. Diabetic rat's treatment with insulin, vanadate, *Azadirachta indica* and lower dose of vanadate with *Azadirachta indica* prevent genomic DNA fragmentation.

Correction of altered antioxidant status could mainly be because of the subsequent normalization of hyperglycemia. It is postulated that hyperglycemia leads to imbalance in reactive oxidant species causing oxidative stress.

*Azadirachta indica* is a wonderful tree with lots of medical properties and its different parts like seeds, leaves, bark, flowers and fruits contain various biologically active compounds. These *Azadirachta indica* active compounds are reported to have antioxidant and anti hyperglycemic properties. *Azadirachta indica* is used for the cure of various disorders since many years.

Vanadate is a remarkable antioxidant and it mimics insulin effect by correcting glucose level in blood. Higher doses of vanadate are effective in correcting high blood glucose level but show adverse effects. At lower doses
vanadate does not show toxic effect but is not effective in lowering blood glucose level.

Observations of our study shows that low doses of vanadate could be successfully used with *Azadirachta indica* leaf extract to effectively normalize the diabetic aberrations in streptozotocin induced diabetic rats.