SUMMARY
1. General

Diabetes mellitus stands for any stipulation that is characterized by chronic hyperglycemia and turbulence of carbohydrate, protein and fat metabolism. The condition results when there is inadequate production or defective action of insulin, the hormone that is in charge of the regulation of carbohydrate, protein and fat metabolism in the body.

According to the World Health Organization, in 2004, more than 150 million people worldwide suffer from some form of diabetes. Even more startling; by the year 2025 this number is anticipated to double. Unlike many other diseases, such as malaria, diabetes is rampant everywhere but more so in economically successful and highly industrialized countries such as the United States. As of 2002, about 18.2 million United States nationals suffer from diabetes. The rise of the diabetic scourge is no less distressing in India with the number of diabetics projected to be three times its current estimated number by 2025. The impact of the this disease has of late, been so alarming that it finds a place in the top 10 and perhaps in the top 5 most devastating diseases in the developed world, and is becoming rapidly more so.

Long-term diabetes mellitus can have detrimental effects on numerous organs of the body. Prolonged high blood glucose levels can produce the chronic complications of diabetes mellitus. They include:

- Endothelial damage - manifesting as microvascular or macrovascular damage.

- Proliferative retinopathy leading to blindness;

- Peripheral neuropathy can lead to foot ulcers leading to necrosis and infection (gangrene), eventually requiring amputation;
- Nephropathy causes chronic renal failure requiring dialysis or transplantation.

- Ischemic heart disease, stroke and neuropathy are other possible complications -- probably stemming from blood vessel damage.

Consistent with its damaging profile Diabetes mellitus is the most 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 widespread cause of blindness among non-elderly adults.

Diabetes as of today is recognized as a life-long disease with several serious acute or chronic complications possible. It requires multifarious therapy, education and life-style modifications to diminish bad outcomes. At this inscription, there is no cure for either Type 1 or 2 diabetes and treatment is necessarily a long-term incessant effort. The goals of diabetes management are several: not only near-normal glycemic control (and so avoidance of both acute and chronic hyperglycemia), but also prevention of hypoglycemia episodes, thus reducing the risk of long-term complications and preserving quality of life for patients.

Several major studies (involving very large numbers of patients) have revealed, clearly and convincingly and beyond reasonable doubt, that keeping blood glucose levels as close as possible to 'normal', nondiabetic, values really does avert, impede, and delay chronic diabetic complications: diabetic retinopathy, nephropathy, microangiopathic and macroangiopathic damage as well as neuropathy. Close glucose control should be undertaken with care, as keeping blood glucose levels 'normally' low in diabetics leaves less scope for medication / diet / exercise error and so increases the risk of a (possibly dangerous) hypoglycemic episode.
Insulin since its discovery has translated as a life saving therapy for diabetes. The success of insulin therapy in Type1 diabetes has been overwhelming. Exogenous insulin, however, fails to produce a well-controlled hyperglycemia in association with variable dietary intake and variable physical activity. Insulin treatment does not effectively prevent the long-term complications associated with diabetes. In Type2 diabetes insulin is relatively ineffective because of the increased insulin resistance of the responding tissue. Various oral hypoglycemic drugs are also being used for the treatment of diabetes. These drugs have been fairly effective in Type2 diabetes and have helped in the management and prevention of complication associated with diabetes. However, these drugs often cause hypoglycemia and other toxic conditions. As a consequence there is a need to find new drugs that are potent, safe and cost effective.

Renewed concern in finding replacements for such drugs and insulin, that act possibly at the intra or intercellular level and are effective in both types of diabetes, is now resurgent the world over. The insulin mimetic and the antidiabetogenic properties of various chemical and natural agents have thence been studied.

Some plant extracts have also shown hypoglycemic effects. Some of these have been tested in experimental animal models and their hypoglycemic effect has been expounded. These plants include *Alium sativum* (garlic), *Momordica charantia* (bitter gourd) fruit extract, *Ficus bengalagensis* (banyan) bark, *Ficus carica* (fig) leaves and *Trigonella foenum graecum* (fenugreek) whole seed powder. Trigonella seeds have been shown to have potent antihyperglycemic and hypoglycemic properties. Several studies have indicated that treatment of fenugreek seeds lower the elevated blood glucose in experimental diabetic rats and partially correct the disturbances in the metabolic pathways without causing any toxic effects.
A number of trace metals are known to be capable of decreasing the blood sugar. Among metal elements the insulin mimetic effects of vanadium, Tungstate and manganese have been demonstrated along with some other trace metals. Vanadium and tungstate, however, have shown promising insulin-mimetic effects.

Vanadate in particular has been extensively studied for its insulin mimetic and antidiabetic actions. Almost all the actions of insulin on glucose uptake and metabolism as well as lipid metabolism have been found to be stimulated by vanadate in in-vitro and in-vivo eg, glucose transport, glycolysis, glycogen synthesis, lipogenesis and inhibition of lipolysis. However, the toxic effects on the treated animals undermine the antidiabetic prospect of vanadium. The toxicity increases as the dose of vanadium increases. At low doses vanadate shows little or no toxicity but fails to elicit any insulin mimetic or antidiabetic effect. Several studies have been carried out to reduce the toxic effects of vanadium without negotiating with its antidiabetic potential.

The present work investigates the prospect of using low doses of vanadate in combination with Trigonella foenum graecum seed powder and assesses their antidiabetic effect in alloxan-diabetic rats. Alloxan diabetic rats were treated with Insulin, Vanadate (0.6mg/ml), Trigonella and a combined dose of Vanadate (0.2mg/ml) and Trigonella for 21 days.

The parameters that were used to evaluate the antidiabetic effect of vanadate and Trigonella include body weight, tissue weight, blood glucose levels, activity and expression of Key enzymes of carbohydrate metabolism like Pyruvate kinase, and Lactate dehydrogenase in Liver, Kidney, Muscle, Heart and Brain; PEPCK and Pyruvate dehydrogenase in liver and kidney; expression of HNF-4α protein in liver and distribution of Glucose transporter (GLUT4) in subcellular fractions of the skeletal muscle.
2. Effect of antidiabetic compounds on general parameters:

There was a decrease in the body weight of diabetic animals. Liver and heart weight also dropped off drastically whereas kidney weight increased in the diabetic condition. Brain weight of the diabetic animals did not show any changes after 21 days of diabetes.

Treatment of diabetic animals with insulin, trigonella and trigonella and vanadate (0.2g/ml) in combination corrected the alterations in the body and tissue weights after 21 days. Vanadate (0.6mg/ml) treatment to diabetic animals could not improve the weight loss of diabetic animals.

Alloxan-diabetic rats were characterized by four-fold increase in the blood glucose. Diabetic animals after 21 days of treatment with Insulin, vanadate, trigonella and trigonella and vanadate in combination, exhibited normoglycemia. Trigonella treatment, however, only partially normalized glucose levels. The combined dose of vanadate and trigonella was found to be most effective in correcting the elevated levels of plasma glucose levels.

Vanadate (0.2mg/ml) when given to control rats didn’t show any toxic effects like loss in body weight and increased mortality but failed to lower hyperglycemia when given to diabetic rats for three weeks.

3. Effect of antidiabetic compounds on the enzyme activities and expression:

Significant changes were observed in the activities of pyruvate kinase and lactate dehydrogenase in liver, kidney, muscle, heart and brain; Pyruvate dehydrogenase and PEPCK in liver and kidney of diabetic rats.

There was a decline in the activities of LDH and PK in liver, heart and muscle of diabetic rats. L-PK mRNA levels also showed a significant
decrease in the diabetic liver. Kidney and brain PK activity increased in the diabetic condition.

PEPCK activity showed a marked increase in the liver and kidney of diabetic rats. Liver PEPCK mRNA levels also dropped off significantly in the diabetic state.

Activity of PDH (active form) showed a considerable decrease in the liver and kidney of diabetic rats. However, PDH (total form) did not change in the diabetic condition.

Treatment of diabetic rats with insulin, vanadate, trigonella and trigonella and vanadate in combination restored the alterations in the enzyme activity and expression. Trigonella treatment only partially corrected these abnormalities. The combined dose of vanadate and trigonella was the most effective in reversing the aberrations in the enzyme activities and mRNA levels to normal values.

4. Effect of antidiabetic compounds on the HNF-4α protein levels:

HNF-4α protein levels were estimated by immunoblotting. A significant increase in the HNF-4α protein was observed in the liver of alloxan-diabetic rats. Treatment of diabetic rats with insulin, vanadate, trigonella and trigonella and vanadate in combination for 21 days resulted in the normalization of HNF-4α protein levels.

5. Effect of antidiabetic compounds on the modulation of GLUT4 translocation:

GLUT4 distribution was examined in the skeletal muscle by immunoblotting and immunohistochemical analysis. GLUT4 levels were measured in the whole homogenate and membrane fractions of skeletal muscle of control, diabetic and diabetic rats after 21 days of treatment with insulin, vanadate,
trigonella and trigonella and vanadate in combination. There was marked increase in the GLUT4 levels in the whole homogenate and membrane fraction of diabetic muscle. The changes were more pronounced in the membrane fractions. Immunohistochemical analyses of GLUT4 showed similar results. In the diabetic state there was a marked reduction in the GLUT4 content in the membrane. Treatment with antidiabetic compounds for 21 days restored the GLUT4 levels to those of control values. The combined treatment of vanadate and Trigonella was most effective in normalizing GLUT4 protein levels.

6. Effect of Vanadate and insulin on GLUT4 translocation in vitro

The rationale of using low dosages of vanadate in combination with Trigonella in alloxan-diabetic rats was based on the assumption that hydroxyisoleucine and other insulinotropic compounds present in Trigonella seeds help in the release of small amounts of insulin from the left-over beta-cells of alloxan-diabetic rats. The idea was that in the presence of insulin, vanadate would be effective even at very low doses. Our in vivo studies showed convincingly that low doses of vanadate in combination with Trigonella were very effective in correcting the altered metabolic state of alloxan-diabetic rats.

To validate our hypothesis that vanadate elicits insulin-like effects at low doses in the presence of small amounts of insulin. We incubated GLUT4-GFP transfected HeLa cells with different concentrations of vanadate and insulin, separately and in combination and monitored the translocation of GLUT4-GFP from the cytoplasm to the membrane. We found that low doses of vanadate or insulin were unable to translocate GLUT4-GFP, when used separately but were very effective when used in combination. Thus our in vitro observations corroborate our idea of using vanadate in low doses in combination with Trigonella.
Antidiabetic potential of vanadate and trigonella:

Sodium orthovanadate acts optimally at a concentration of 0.6mg/ml in drinking water to act as hypoglycemic agent for experimental diabetic rats. At this dose vanadium is quite toxic to the animals. The chronic response to various vanadium compounds following STZ-diabetes induction in both wistar and Sprague-Dawley rats, was studied earlier. At low doses vanadate is relatively safe but fails to impart any hypoglycemic and antidiabetic activity.

Trigonella foenum graecum (Trigonella) seeds have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems. The components responsible and the mechanism by which Trigonella exerts these effects is not clearly understood. However, several studies have shown the presence of steroid saponins in Trigonella seeds. Saponin compounds diosgenin, alkaloids and trigonelline inhibit intestinal glucose uptake in vitro. 4-Hydroxyisoleucine, a modified amino acid extracted and purified from fenugreek seeds also displayed an insulinotropic property in vitro, stimulated insulin secretion.

The present results divulge that the low doses of vanadate could be successfully used with trigonella seed powder to effectively counter the diabetic aberrations in alloxan-diabetic rats. Low doses of vanadate when given to control rats did not result in weight loss of animals; the blood glucose levels were also not affected. However, when diabetic rats were treated with low doses of vanadate for three weeks, it could not lower hyperglycemia. Thus vanadate shows little toxicity at low doses but fails to elicit any antidiabetic effects.

Low doses of Vanadate in combination with trigonella effectively control plasma glucose levels and correct alterations in the key parameters of
carbohydrate metabolism. Thus toxicity of vanadate can be reduced by using low doses and combining it with trigonella seed powder.

The results obtained in the present study are based on studies using experimentally induced diabetic rats. Extensive investigations are necessary to establish the safety and efficacy of these antidiabetic drugs in human subjects.