DISCUSSION
1. GENERAL

With explosive increase in its prevalence diabetes mellitus, as a chronic medical disorder is to be reckoned on par with hypertension and arteriosclerosis. A versatile disease, diabetes, in view of its frequent clinical and epidemiological link with the other two, amounts to a health problem of paramount concern for a very large proportion of world population. There is no cure for diabetes but management is possible. Elevated glucose levels play an imperative role in the development of long-term complications of diabetes mellitus. Therefore it is critical to control the glucose levels for prevention and attenuation of these complications. Normally there is a precise balance between the utilization of glucose by peripheral tissues and its production by liver and kidney. This balance is relentlessly affected in Type-1 diabetes because of the deficiency of insulin. Exogenous insulin is quite helpful in ameliorating these conditions. But it fails to produce a well-controlled glycemic condition in association with variable dietary intake and physical activity. Episodes of severe hypoglycemia leading to deleterious cerebral impact are common during insulin administration (Mc Call, 1992).

Consequently there is a need to develop antidiabetic drugs that are effective, safe, produce a well-controlled normoglycemia and prevent the long-term complications.

In searching for effective drugs in diabetes, Heylinger et al (1985) identified vanadium as an orally active insulin-mimetic agent. Although insulin-like actions of vanadium had previously been demonstrated using isolated cells and tissues, the physiologically pertinent effects initiated intense research and an ongoing debate over the mechanism of vanadium actions and its potential to treat diabetes. Despite many unanswered questions regarding the cellular mechanism of vanadium’s effects, numerous reports from animal
and clinical studies have generated considerable enthusiasm for the potential therapeutic worth of vanadium in diabetes.

Vanadium treatment has been shown to alleviate hyperglycemia and improve glucose tolerance, without significantly raising insulin levels (Heyliger et al, 1985 and Brichard et al 1988). Vanadate has been reported to increase glucose uptake in liver, skeletal muscle and brain (Meyerovitch et al, 1987; Mayerovitch et al, 1989). Treatment of STZ-diabetic rats with vanadium restored the expression of GLUT4 to levels near those seen in non-diabetic animals in skeletal and cardiac muscle (Strout et al 1990; Kopp et al, 1987). Vanadate has also been shown to normalize blood glucose levels in diabetic human subjects (Goldfine et al 1995). Thus vanadium emerged as an important oral antidiabetic agent. However, it was soon discovered that vanadium has several toxic effects on the animals and thus seriously undermined its antidiabetic potential. Efforts have been made to reduce the toxic effects of vanadate without compromising with its antidiabetic properties. Different forms of vanadate have been tried. These include metavanadate, vanadyl sulphate and new organic vanadium complex bis-(maltolacto)-oxovanadium(IV)(BMOV). Although these compounds were effective at lower doses nevertheless had several toxic effects on the animals.

Another strategy adopted to reduce the toxic effects of vanadate was to coadminister it with other compounds. Treatment of STZ-rats with oral metavanadate and Tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate) has been reported to reduce plasma glucose levels (Domingo et al, 1992). Similarly vanadate along with ascorbic acid has also been shown to reduce blood glucose levels without causing any major toxic effects (Gomez et al, 1991).
The present study explores the possibility of using low doses of Vanadate (0.2mg/ml) in combination with Trigonella seed powder (TSP) and evaluates their effect on general parameters of animals like Body weight and tissue weights, blood glucose levels, key regulatory enzymes of carbohydrate metabolism including Pyruvate kinase and lactate dehydrogenase in Liver, Kidney, Heart, Muscle and Brain and Pyruvate dehydrogenase and PEPCK in liver and kidney, Hepatocyte Nuclear factor (HNF-4alpha) levels in liver and glucose transporter (GLUT4) distribution in different fractions of skeletal muscle of alloxan-diabetic rats.

The use of TSP and vanadate in combined dose was based on two ideas. First, it has been shown that organic complexes of vanadium are non-toxic and found to have an increased capacity to control hyperglycemia (Sakurai et al, 1990; Yuan et al, 1997). Therefore it was presumed that in the combined treatment sodium orthovanadate might be forming (in vivo) very effective antidiabetic complexes with organic compounds present in Trigonella seeds as suggested earlier in vitro (Ramasarma, 1990). Second, it has been observed that the therapeutic efficiency of vanadate in STZ-diabetic rats is dependent on the severity of diabetes and the residual pancreatic insulin stores and secretory function (Cam et al, 1997; Cam et al 1999). The insulin-like effects of vanadium observed in studies with isolated cells are often demonstrable only at concentrations that are not achieved in vivo and are considered toxic in whole animal studies. Trigonella seed powder like other antidiabetic plant extract such as Momordica charantia and Ficus bengalensis are known to rejuvenate the β-cells in the islets of Langerhans, thus increasing the capacity of insulin secretion in Type 1 diabetes (Ahmed et al, 1998). The insulinotropic property of 4-hydroxyisoleucine, an amino acid extracted from Trigonella is also suggestive of insulin secretion modulation in its therapeutic action (Broca et al, 1999). Therefore trigonella seeds would make the pancreatic
beta-cells to secrete insulin and low doses of vanadate would be more effective in the presence of small amounts of insulin.

2. Effect of antidiabetic compounds on general parameters.

The present study employs the use of alloxan-diabetic rats for the experiments. This experimentally induced diabetes is considered equivalent to type 1 diabetes. Several physiological modifications like change in liver weight and kidney weight and a decrease in body weight are characteristic of experimental diabetes equivalent to type 1. A decrease in liver weight and an increase in kidney weight were observed in diabetic animals. The results obtained in the present study correlate well with the earlier studies. Glucose overutilization in kidney during diabetes causes an increase in glycogen, basement membrane and ribose 5-phosphate formation (Sochor et al., 1979; Steer et al., 1982). An increase in nucleic acid and protein synthesis occurs in the diabetic rat kidney, which correlates with kidney hypertrophy during diabetes (Cortes et al., 1981). However, these changes contrast sharply with the liver in which glycogen accumulation, nucleic acid and protein synthesis and albumin synthesis are all decreased in diabetes (Khandelwal et al., 1979; Jefferson et al., 1980). In the present study, a significant increase in fluid intake was observed in the experimental diabetic rat, which is associated with polydipsia and polyuria, characteristic features of type 1 diabetes.

There was a four-fold increase in blood glucose levels of alloxan-diabetic rats after 21 days of insulin withdrawal. Diabetic animals receiving three weeks of treatment with Insulin showed a marked reduction in hyperglycemia. Vanadate mimicked the effect of insulin and three weeks of treatment with 0.6mg/ml Vanadate lowered elevated blood glucose levels to almost control values. Trigonella treatment partially revived normoglycemia after three weeks of treatment. 0.2mg/ml Vanadate and Trigonella in combination corrected hyperglycemia more effectively than Vanadate and Trigonella
treatment alone. It was worth noting that combined treatment did not result
in weight loss, which has been observed in the vanadate treated diabetic rats
in the present work and as shown in earlier studies also (Venkatesan et al,

Therefore, after showing improvement in the glucose homeostasis of
diabetic rats treated with antidiabetic compounds, the metabolic
consequences of this treatment on key parameters of glucose metabolism
were further examined.

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

3.1. Pyruvate kinase (PK)

Pyruvate kinase, a key regulatory enzyme of glycolysis catalyses the third
rate-limiting reaction of glycolysis. It catalyses the conversion of
Phosphoenolpyruvate to Pyruvate which feeds into a number of metabolic
pathways, thus placing this enzyme at a primary intersection, regulating the
entry of glucose flux into tricarboxylic acid cycle and other routes of
metabolism. The activity of PK, the key enzyme controlling the final step of
the glycolytic pathway, determines the relative amount of glucose that is
channeled into synthetic processes or used for glycolytic energy production
(Mathupala et al 1997 and Mazurek et al 1997). Pyruvate kinase enzyme
exists in different isozymic forms and their distribution in various tissues is
such so as to serve their metabolic functions. The M-type isozymes is present
in tissues with high glycolytic rate and energy demand, like muscle, heart
and brain where as the L-type, which is under allosteric control of a variety
of effectors, is distributed in tissues possessing gluconeogenic machinery
such as liver and kidney.
Pyruvate kinase is an important enzyme of glycolysis and any alteration in the enzyme activity will severely affect the glucose utilization. This is what exactly happens in diabetes mellitus. L-PK mRNA level also shows a decrease in diabetic rats and in fasted rats (Noguchi et al 1983, 1982). Insulin administration to diabetic rats result in an increase in L-PK mRNA levels in liver. Increase in the levels of L-PK mRNA as the result of high glucose diet or by insulin administration to diabetic rats is largely due to stimulation of gene transcription (Munich et al 1987 and Noguchi et al 1982). Insulin has been shown to cause an increase in M2-PK activity also (Traxinger et al 1992).

In agreement with earlier studies, a decrease in the activity of pyruvate kinase was observed in liver, heart and muscle of alloxan-diabetic rats, whereas in kidney and brain there was a small increase in the activity of Pyruvate kinase. The active and total form of PK was also assayed in the liver and kidney of diabetic animals. Active and total PK decreased significantly in the liver of diabetic rats. The mRNA levels of PK-L also showed a similar decrease in the diabetic state.

In kidney active form of PK showed a small increase in the diabetic condition, where as no significant difference was found in the total form of PK.

Treatment of diabetic rats with Insulin and vanadate, trigonella and vanadate and trigonella in combination resulted in the reversal of altered of Pyruvate kinase activity and its expression after 21 days of treatment. Trigonella treatment restored the PK activity and expression partially. The combined treatment of Vanadate and Trigonella was most effective in correcting the alterations in Pyruvate kinase activity and expression.
3.2 Phosphoenolpyruvate carboxykinase (PEPCK)

PEPCK, which catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, plays a crucial role in gluconeogenesis. PEPCK activity is principally controlled at the level of gene expression and is sensitive to a number of hormones (Hanson and Reshef, 1997). PEPCK is expressed primarily in the liver, kidney and small intestine, although low levels of the enzyme have been detected in other tissues (Hanson and Reshef, 1997 and Rajas et al 2000). Of the two known isoforms of PEPCK, the mitochondrial isoform (PEPCK-M) is constitutively expressed, whereas the cytosolic form (PEPCK-C) is tightly regulated by hormonal and dietary factors and is the form of this enzyme that is elevated in diabetes (Hanson and Reshef, 1997; Ballard, Hanson 1969 and Davies, et al 1999). In the liver, PEPCK expression is enhanced by glucagon (acting via cAMP), glucocorticoids, thyroid hormone, and fasting, whereas insulin and high-carbohydrate diet decrease PEPCK-C synthesis (Hanson and Reshef, 1997; Nandan and Beale, 1992). In the kidney, transcription of PEPCK-C gene is stimulated by glucocorticoids, fasting, and metabolic acidosis (Hanson and Reshef, 1997; Nandan and Beale, 1992). Physiological suppressors of basal renal PEPCK expression have not yet been described, and insulin, the main regulator of PEPCK in the liver, has been reported to have no effect on PEPCK in the kidney (Hanson and Reshef, 1997). The rate of change in PEPCK-C gene transcription is rapid (~20 minutes in the liver). PEPCK mRNA and protein have half-lives of about 0.5 hour and 6-8 hours, respectively (Hanson and Reshef, 1997, Friedman et al., 1993). PEPCK being a key enzyme in gluconeogenesis, it is not surprising that PEPCK enzyme activity and mRNA levels are elevated in the liver and kidney of most animal models of diabetes and insulin resistance (Nandan and Beale, 1992, Mapes and Watford, 1989; Mosseri et al, 2000; Lemieux et al., 1984; Pagliassotti et al, 1994).
In agreement with previous reports on animal models of diabetes, PEPCK activity and mRNA levels were significantly higher in the liver of STZ-diabetic rats compared with control rats (Hanson and Reshef, 1997; Nandan and Beale, 1992; Kramer et al, 1999; Wang and Yu, 1997) and were restored after insulin treatment (Nandan and Beale, 1992; Kramer et al, 1999), indicating that the insulin signaling pathway was not impaired. Earlier *in vitro* studies have shown that the inhibitory effects of insulin on PEPCK gene expression in the liver are mediated by the effects of phosphatidylinositol 3-kinase (PI3-K) (Agati et al, 1998; Gabbay et al, 1996) on an insulin-responsive region in the PEPCK gene promoter (Hanson and Reshef, 1997). The links between PI3-K and PEPCK promoter are still undefined. A few studies (Liao et al, 1998), but not all (Agati et al, 1998), support a role for protein kinase B (PKB) in the repression of glucocorticoid and cAMP induction of PEPCK. However, increased levels of plasma glucagon observed in STZ-diabetic rats (Pugazhenthi and Khandelwal, 1990) suggests that, *in vivo*, the inhibitory effects of insulin on PEPCK may also be mediated by suppression of secretion and/or actions of glucagon.

The contribution of kidney to the elevated levels of gluconeogenesis in diabetes is important to consider, in view of the finding that kidney may account for more than 40% of total endogenous glucose production (Foster et al, 1997). As in other studies, PEPCK activity and mRNA levels were significantly higher in the kidney of diabetic rats compared with nondiabetic controls (Nandan and Beale, 1992; Mapes and Watford, 1989; Lemieux et al., 1984). It is believed that diabetes induces renal PEPCK mRNA indirectly by causing acidosis (Nandan and Beale, 1992, Mapes and Watford, 1989; Pollock, 1989) because correction of acidosis prevents the increase in PEPCK gene expression (Mapes and Watford, 1989). Treatment with insulin normalized both PEPCK activity and expression in the kidney. In contrast to liver, metabolic acidosis is the primary regulator of renal PEPCK (Hanson
and Reshef, 1997; Mapes and Watford, 1989; Pollock, 1989). Hence, it appears that the effects of insulin on renal PEPCK observed in this study were due to improvement in the metabolic state.

Treatment of diabetic rats for 21 days with vanadate, trigonella and vanadate in combined dose resulted in the normalization of PEPCK activity and levels of it mRNA. Trigonella treated rats had PEPCK activity and mRNA levels only partially normalized after treatment. The combined dose of vanadate and trigonella was most effective of all the treatments in restoring the activity of PEPCK and its mRNA levels.

3.3 Changes in Pyruvate dehydrogenase complex

Pyruvate dehydrogenase complex is a key regulatory enzyme of glucose metabolism; it is involved in the oxidative decarboxylation of pyruvate to form Acetyl CoA. Acetyl CoA is then oxidized to H₂O and CO₂ by the citric acid cycle. This multienzyme complex occupies a key position in the regulation of carbohydrate metabolism. This enzyme complex has two regulatory components a kinase and a phosphatase, in addition to three catalytic components. The enzyme is regulated by phosphorylation/dephosphorylation as well as the inhibition by its products acetyl CoA and NADH (Garland and Randle, 1964; Siess et al., 1971; Blass and Lewis, 1973; Tsai et al., 1973). The dephosphorylated form (PDHₐ) of the enzyme is the active form whereas phosphorylated form (PDHₗ) is the inactive form. Phosphorylation of the enzyme takes place in the presence of NADH and Acetyl CoA the products of this reaction. On the other hand presence of NAD⁺ activates the phosphatases and thus activates the PDH complex.
In the present study the active form of PDH (PDH<sub>a</sub>) was found to decrease in liver and kidney of alloxan-diabetic rats. There was no significant change in the total form of the enzyme. There are several reasons why there is a decrease in the PDH<sub>a</sub>. First in the diabetic state NADH and Acetyl CoA levels show an increase due to excessive oxidation of fatty acids. NADH and Acetyl CoA inhibit the enzyme by stimulating its phosphorylation. Second pyruvate formed in the glycolytic cycle is used for gluconeogenesis and only a small amount is left for decarboxylation.

Treatment of diabetic animals for 21 days with Insulin, vanadate, Trigonella and vanadate and Trigonella in combined dose restored the altered levels PDH in liver and kidney. We have already shown the restoration of glycolytic and gluconeogenic enzymes in these treatment groups and by restoring enzymes of these pathways.

3.4 Lactate dehydrogenase

Changes in Liver, Muscle and Heart:

The activity of lactate dehydrogenase is significantly altered in liver, skeletal muscle and heart during diabetes as the entry of glucose into these tissues is regulated by insulin. In the present study the activity of LDH was significantly reduced in these tissues. The changes during diabetes in these tissues are in agreement with the earlier published data (Sochor et al 1985). The decreased activity may be due to in vivo inactivation of the enzyme by ketone bodies, creatine phosphate or long chain fatty acids known to affect many glycolytic enzymes. The other possible reason for the observed decrease in diabetes is the non-availability of the substrate itself. The normal ratio of lactate to pyruvate in the cells at usual cellular levels of NAD<sup>+</sup> and NADH is approximately 10:1 and this ratio increase during diabetes, thus depleting the availability of pyruvate (Substrate for lactate dehydrogenase).
Vanadate and trigonella administration separately and in combined dose for 21 days effectively reversed the alterations in LDH activity to normal levels. Orthovanadate and trigonella seed powder treatments thus improved the altered glycolytic flux during diabetes in insulin dependent tissues. This improvement can be by making more NADH available as a result of increased oxidation of glucose through the glycolytic pathway.

Changes in Kidney and Brain:

A significant increase in the activity of LDH was observed in Kidney after 21 days of diabetes. The entry of glucose is independent of insulin in kidney and more and more glucose enters the kidney and is therefore, overutilized through the glycolytic route and other alternative routes. LDH activity increases as a result of the increased availability of its substrate pyruvate that is the end product of glycolytic pathway.

After 21 days of treatment with Insulin, vanadate, trigonella and vanadate and trigonella in combination, the increased LDH activity in the kidney substantially returned to normal values. The combined treatment of vanadate and trigonella was most effective of all treatments in reversing LDH activity to normal levels.

There was no significant difference in the lactate dehydrogenase activity in whole brain after 21 days of diabetes. Treatment with Insulin, Vanadate, Trigonella and Vanadate and Trigonella in combined dose did not change the brain lactate dehydrogenase levels.

4. Effect of antidiabetic compounds on Hepatocyte Nuclear factor (HNF-4α)

Gene expression is controlled by transcriptional regulatory proteins, which bind specific DNA sequences and recruit cofactors and the transcription apparatus to promoter. Transcriptional regulator HNF4α (a nuclear receptor)
plays a crucial role for the normal functioning of liver along with other regulators HNF1α and HNF6 (Costa et al, 2003 and Watt et al, 2003). Mutations in HNF4α are the cause of type 1 form of maturity-onset diabetes of the young (MODY1) a genetic disorder characterized by the onset of diabetes mellitus before 25 years of age and an autosomal dominant pattern of inheritance (Fajans et al, 2001).

Genome-scale location analysis has revealed that HNF4α regulates about 12% genes in hepatocytes (Odom et al, 2004). HNF4α is known to regulate genes of key enzymes of protein, fat and carbohydrate metabolism including L-pyruvate kinase and Phosphoenolpyruvate carboxykinase (PEPCK) (Hall et al, 1995).

In vivo effects of Insulin, Vanadate, Trigonella and the two in combination on the expression of HNF4α protein were examined in the alloxan-diabetic rats. In agreement with earlier studies HNF4α protein levels increased significantly in liver of alloxan diabetic rats in comparison to control rats (Oyadomari et al, 2000). The HNF4α expression is upregulated by Glucagon and repressed by insulin in hepatocytes (Oyadomari et al, 2000). A straightforward interpretation is that HNF-4α mediates up-regulation of blood glucose levels in response to glucocorticoids and glucagon and down-regulation in response to insulin. Therefore in the diabetic condition in the absence of insulin glucagon increases the HNF4α protein levels.

Treatment with Insulin, vanadate, trigonella and the combined dose of vanadate and Trigonella to diabetic rats corrects the altered levels of HNF-4α after three weeks of treatment. The restoration of HNF-4α levels to normal values is very important because HNF-4α regulates the expression of genes of a host of protein including key glycolytic and gluconeogenic enzymes.
4. Changes in the expression and translocation of Glucose transporter (GLUT4) protein

A key role of insulin is to facilitate the uptake of glucose from blood into muscle and adipose tissues [Kahn, 1996; Holman and Sandoval, 2001; Zorzano et al, 1996]. In muscle, two glucose transporter isoforms are expressed, GLUT1 and GLUT4. The latter is quantitatively more abundant in adult rat muscle and is distributed among intracellular compartments in the basal state, from where it is rapidly translocated to the plasma membrane in response to insulin or exercise (Holman and Sandoval, 2001; Zorzano et al, 1996; Pessin et al, 1999; Napoli et al, 1996; Tomas et al, 2001; Coderre et al, 1995). GLUT1 is mainly located at the plasma membrane (Zorzano et al, 1996) and is considered to be responsible for basal glucose transport. GLUT4 expression is down-regulated when there is a relative insulin deficiency, such as in STZ-induced diabetes and chronic fasting (Charron et al, 1999).

Glut4 protein levels were measured by immunoblot analysis in the whole homogenate and membrane fraction of skeletal muscle. In agreement with the previous studies GLUT4 protein significantly decreased in the whole homogenate as well in the total membrane fraction of skeletal muscle of alloxan-diabetic rats. Since glucose transport in skeletal muscle occurs mainly through GLUT4 the reduction in the GLUT4 levels results in decreased uptake of glucose and, therefore, contributes to the increased blood glucose levels in diabetic condition.

Treatment of rats with Insulin, Vanadate, Trigonella and Trigonella and Vanadate in combined dose restored the GLUT4 levels close to the normal values. The combined treatment was more effective in correcting the alteration in GLUT4 protein.
Similar results were obtained by the immunohistochemical analyses of GLUT4 in the skeletal muscle. In the diabetic state GLUT4 content decreased drastically in the cell membrane when compared to control. Treatment with Insulin, Vanadate, Trigonella and the two in combination restored the GLUT4 content in the cell membrane.

The revival of GLUT4 levels is very important because one of the main reasons of hyperglycemia in the diabetic state is the decreased uptake of glucose by the skeletal muscle. Restoration of GLUT4 levels would, therefore, enhance the uptake of glucose in the skeletal muscle and thus help in assuaging the hyperglycemic condition.

5. Effect of Insulin and Vanadate on GLUT4 translocation in vitro

Vanadate has been shown to mimic most of the actions of insulin in vitro as well in vivo (Tolman et al, 1979 and Heyliger et al, 1985). The concentration of vanadate needed to elicit any insulin-mimetic effect in vitro is much higher as compared to that needed in vivo (Cam et al, 2000). Several hypotheses have been put forward to explain this, but one of the plausible explanations is that the presence of traces of residual insulin in diabetic animals makes vanadate to act at much lower doses (Cam et al, 1999). Zero-insulin level diabetic rats have been found to respond to very high doses of vanadate. Therefore, physiologically relevant doses of vanadate are only insulin enhancing rather than insulin mimetic.

In order to see the effect of different doses of vanadate on GLUT4 translocation in the absence and presence of insulin, HeLa cells were transfected with HAGLUT4-GFP and incubated with vanadate and insulin. Our results clearly and convincingly show that vanadate elicits insulin like effects at much lower concentrations in the presence of small amounts of insulin. Our idea of using low doses of vanadate in combination with Trigonella was based on this proposition. Trigonella seeds contain 4-
hydroxylisoleucine and some other insulinotropic compounds which make the residual beta-cells to release small amounts of insulin and low doses of vanadate effectively elicit insulin like effects. Our *in vitro* studies validate our hypothesis. However, extensive studies with other cell types like adipocytes, myotubes and cardiomyocytes are required to further confirm the findings of the present study. Quantitative laser scanning Confocal imaging of the live cells would be helpful in measuring the extent of GLUT4 translocation by different doses of vanadate and insulin.

6. Therapeutic potential of Vanadate and Trigonella

Glucose is the most important fuel used for energy requirement of the body. A tightly regulated network of hormones delicately balances the plasma glucose levels. Insulin counters an array of hormones to regulate the glucose metabolism. Insulin signals a state of energy abundance, and activates glucose uptake, metabolism and storage as glycogen in muscle and fat tissue. These organs make up most of the body's mass. At the same time, insulin restrains processes that release stored energy; lipolysis and ketogenesis, glycogenolysis, proteolysis and gluconeogenesis. Insulin is necessary for uptake of amino acids to tissues and for protein synthesis. Insulin is the central actor in homeostasis; the stabilization of the internal milieu. One of the main acute effects of insulin is to regulate the disposal and storage of dietary glucose by stimulating the uptake of glucose into muscle and fat. Insulin regulates glucose uptake into these cells by recruiting membrane vesicles containing the GLUT4 glucose transporters from the interior of cells to the cell surface, where it allows glucose to enter cells by facilitative diffusion. Thus, glucose uptake by muscle and fat cells is regulated by modulating the number of GLUT4 glucose transporters on the surface of cells. Therefore deficiency, absence or inaction of insulin results in alterations in the normal glucose metabolic pathways that show the way to the onset of hyperglycemia and subsequently diabetes mellitus.
Diabetes Type I is a fatal disease in which insulin secretion completely fails. The total lack of insulin leads to two metabolic crises; a marked increase in the rate of lipolysis in adipose tissue and activation of hepatic gluconeogenesis in spite of high plasma glucose levels. The dramatically increased rate of lipolysis in adipose tissue follows the lack of insulin inhibition of hormone-sensitive lipase. The increase in fatty acid levels leads to a massive synthesis of ketone bodies in the liver. These then exceed the buffer capacity of the blood, leading to ketoacidosis. Excess acid is a potent poison for the brain.

The high levels of glucose seen in diabetes 1 and 2 are toxic. They can lead to activation of alternative pathways for example formation of sorbitol in the lens of the eye, increasing osmotic pressure and disturbing protein synthesis. The major toxic effect of glucose is probably glycation of proteins. It is believed that much of the neurological and circulatory defects that follow diabetes are due to glycation. A number of drugs are used to treat diabetes but there is not a single drug, which is effective as well as without any side effects. Although new drugs are coming up and the management of diabetes is becoming easier, there is another twist to the sorry tale. A number of people particularly in the developing world have little or no access to these new generation antidiabetic drugs. Therefore, there is a need to make a therapy for diabetes, which is potent, safe and cost effective. Keeping this in mind scientists tried to explore the possibility of using trace metal elements and plant extracts as antidiabetic compounds. Trace metal elements like vanadium, tungsten and selenium were used and found to have antihyperglycemic, hypoglycemic and antidiabetic properties. But the outcome was not as effective as it sounded; these metal elements affected other processes in the cell and therefore were quite toxic to the organism.

Several plants have also been screened for a possible antidiabetic potential. A few of them like trigonella foenum graecum, memordica charantia have been
extensively studied. The results have been encouraging and though these natural products only partially reverse hyperglycemia, no side effect has been reported with their use.

The present results show that vanadate and Trigonella separately and in the combined dose successfully revived normoglycemia and corrected the alteration in the biochemical pathways studied in experimental diabetic rats. Vanadate and Trigonella treated diabetic rats had reduced glucose levels as compared to the diabetic untreated diabetic rats. The alterations in the key regulatory enzymes of glucose metabolism like Pyruvate kinase; Phosphoenolpyruvate carboxykinase, Lactate dehydrogenase and pyruvate dehydrogenase were normalized by Vanadate and Trigonella treatment. In addition the elevated levels of important transcriptional regulator Hepatocyte nuclear factor-4 (HNF-4α) in the diabetic liver were also brought back to control values by the treatment with Trigonella and Vanadate. There was a substantial decrease in the GLUT4 protein expression and translocation in the skeletal muscle of diabetic rats. After three weeks of treatment with Vanadate and Trigonella GLUT4 levels were revived to normal values.

The combined treatment of Vanadate and Trigonella was more effective in reversing the alterations in the above-mentioned parameters. Diabetic rats treated with the combined dose of Vanadate and Trigonella gained weight during the course of treatment, this is in contrast to the rats treated with high doses of vanadate that failed to gain weight. The mortality was much lower in the group of rats that were treated with vanadate and Trigonella as compared to the ones that received Vanadate alone.

On the basis of present results and the earlier studies in our laboratory it could be deduced that low doses of vanadate could be effectively used in combination with Trigonella seed powder to counter the diabetic alterations
and prevent the long-term term complications in diabetes. Present results are based on the studies in the animal experimental diabetic model, which is equivalent to type 1 diabetes, and further studies are required to ensure their safe use in human subjects.