Review

Metabolic and molecular action of *Trigonella foenum-graecum* (fenugreek) and trace metals in experimental diabetic tissues

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Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycaemia resulting in defective insulin secretion, resistance to insulin action or both. The use of biguanides, sulphonylurea and other drugs are valuable in the treatment of diabetes mellitus; their use, however, is restricted by their limited action, pharmaco-kinetic properties, secondary failure rates and side effects. *Trigonella foenum-graecum*, commonly known as fenugreek, is a plant that has been extensively used as a source of antidiabetic compounds from its seeds and leaf extracts. Preliminary human trials and animal experiments suggest possible hypoglycaemic and anti-hyperlipedemic properties of fenugreek seed powder taken orally. Our results show that the action of fenugreek in lowering blood glucose levels is almost comparable to the effect of insulin. Combination with trace metal showed that vanadium had additive effects and manganese had additive effects with insulin on *in vitro* system in control and diabetic animals of young and old ages using adipose tissue. The *Trigonella* and vanadium effects were studied in a number of tissues including liver, kidney, brain peripheral nerve, heart, red blood cells and skeletal muscle. Addition of *Trigonella* to vanadium significantly removed the toxicity of vanadium when used to reduce blood glucose levels. Administration of the various combinations of the antidiabetic compounds to diabetic animals was found to reverse most of the diabetic effects studied at physiological, biochemical, histochemical and molecular levels. Results of the key enzymes of metabolic pathways have been summarized together with glucose transporter, Glut-4 and insulin levels. Our findings illustrate and elucidate the antidiabetic/insulin mimetic effects of *Trigonella*, manganese and vanadium.

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1. Introduction

It is projected that the incidence of diabetes is on the rise. The present number of diabetics worldwide is over 150 million and this is likely to increase to 300 million or more by the year 2025 (King *et al.* 1998; Shaw *et al.* 2010). Reasons for this increase include increase in sedentary lifestyle, consumption of energy-rich diet, obesity and life span. Although biguanides and sulphonylurea are valuable in the treatment of diabetes mellitus, their use is restricted by their limited action, pharmaco-kinetic properties, secondary failure rates and accompanying side effects. Moreover, these therapies only partially compensate for metabolic derangements seen in diabetes and do not necessarily correct the fundamental

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Abbreviations used: AGE, advanced glycation end product; BMOV, bis(maltolato) oxovanadium IV; 4-OH-Ile, 4-hydroxyisoleucine; CAT, catlase; FFA, free fatty acids; Glut-4, glucose transporter-4; GPx, glutathione peroxidase; GR, glutathione reductase; MAO, monoamine oxidase; MDA, Malondialdehyde; NIDDM, non-insulin-dependent diabetes mellitus; PEPCK, phosphoenolpyruvate carboxykinase; PK, pyruvate kinase; SOD, superoxide dismutase; SOV, sodium orthovanadate; TBARS, thiobarbituric-acid-reactive substances; TSP, *Trigonella* seed powder

biochemical lesion (Taylor and Agius 1988; Bailey *et al.* 1989). Nature has been a source of medicinal treatments for thousands of years, and plant-based systems continue to play an essential role in the primary health care of 80% of the world's developing and developed countries (King *et al.* 1998). Biguanides developed from a prototypic plant molecule is an excellent example of plant-based antidiabetic drugs. The current therapeutic agents used for diabetes have been discussed by Moller (2001) with their molecular targets, sites of action and adverse events occurring. Thus, it will be very significant to look for new and if possible more effective and efficacious antidiabetic molecules from the vast reserves of phytotherapy. *Trigonella foenum-graecum* is one such plant that has been extensively used as a source of antidiabetic compounds, from its seeds, leaves and extracts in different model systems (Raju *et al.* 2001; Srinivasan 2006; Khalki *et al.* 2010).

Fenugreek is traditionally used in India, especially in the Ayurveda and Unani systems (Grover *et al.* 2002; Srinivasan 2006). Preliminary animal and human trials suggest possible hypoglycaemic and anti-hyperlipedemic properties of fenugreek seed powder taken orally. Fenugreek seeds contain 50% fibre (30% soluble fibre and 20% insoluble fibre) that can slow the rate of post-parandial glucose absorption. This may be a secondary mechanism for the hypoglycaemic effect.

Broca *et al.* (1999, 2000) reported that 4-hydroxyisoleucine (4-OH-Ile), an amino acid extracted and purified from fenugreek seeds, displays an *in vitro* insulinotropic activity, which is of great interest, and that its stimulating effect is related to the immolation of glucose concentration in the medium as shown in isolated pancreatic beta cells. Such glucose dependency is not shown by sulphonylurea; in fact, hypoglycaemia remains a common undesirable side effect of sulphonylurea treatment in non-insulin-dependent diabetes mellitus (NIDDM) diabetic patients. 4-Hydroxyisoleucine is only found in plants, and owing to its particular insulinotropic action (Broca *et al.* 1999, 2000), it might be considered as a novel secretagogue with potential interest for the treatment of type II diabetes, a disease characterized by defective insulin secretion associated with various degrees of insulin resistance (Baquer *et al.* 2009).

The results of Broca *et al.* (1999, 2000) suggested improvement of the diabetic state, of streptozotocin-treated rats, at least partly from a direct stimulating effect of 4-OH-Ile on beta cell function. These authors demonstrated that 4-OH-Ile is able to stimulate insulin secretion *in vivo* and improve glucose tolerance in normal rats and dogs, suggesting that 4-OH-Ile could now be considered for the treatment of NIDDM.

Sauvaire *et al.* (1998) demonstrated *in vitro* that the amino acid 4-OH-Ile in fenugreek seeds increased glucose-induced insulin release in human and rat pancreatic cells. This amino acid appeared to act only on pancreatic beta cells, as the levels of somatostatin and glucose glucagons were not altered.

In humans, fenugreek seeds exert hypoglycaemic effect by stimulating glucose-dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of α-amylase and sucrose (Amin *et al.* 1987). Fenugreek seeds also lower serum triglycerides, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). These effects may be due to sapogenins, which increase biliary cholesterol excretion in liver, leading to lowered serum cholesterol levels (Yadav *et al.* 2004, 2005). The lipid-lowering effect of fenugreek might also be attributed to its oestrogenic constituent, indirectly increasing the thyroid hormone T4. Thus, dietary supplements that can modulate glucose homeostasis and potentially improve lipid parameters would be desirable. This is especially true for diabetes parameters in patients with metabolic syndrome. These patients already manifest abnormalities of glucose handling and could benefit from a low-risk inexpensive, food-based intervention aimed at normalizing their metabolic milieu. Fenugreek is a dietary supplement that may hold promise in this regard.

Insulin stimulates cellular glucose uptake in muscle and adipose tissues by inducing the translocation of glucose transporter-4 (Glut-4) from an intracellular pool to the plasma membrane. In the diabetic state, because of deficiency of insulin, Glut-4 translocation does not take place efficiently and Glut-4 transporters remain inside, where they are not functional. This results in decreased uptake of glucose by muscle cells, which contribute significantly to the elevated blood glucose levels. Therefore, restoration of Glut-4 will achieve normoglycaemia. The effectiveness of the antidiabetic compounds vanadate and *Trigonella* have been successfully used to reverse the diabetes effect on the Glut-4 transporter to normal levels in experimental diabetes (Mohammad *et al.* 2006b).

2. Metabolic pathways affected: regulation of blood glucose

The interrelationships among alternative routes of glucose metabolism are shown in figure 1A, and the central role of glucose in carbohydrate, fat and protein metabolism have been recently reviewed (Baquer *et al.* 2009). The principal metabolic pathways are shown, as are some key intermediates and the products of the metabolic interconversions that lead to pathological complications (figure 1B). As seen in the figure 1B, glucose overutilization in diabetes shows that the glucose movement into many cells, including those of the kidney, certain nerve tissues, the eye, seminal vesicles, erythrocytes and leucocytes, is not dependent on insulin. In diabetes, the concentration gradient between the extracellular and intracellular compartments is sufficient to drive glucose into these cells. The increased activity of the

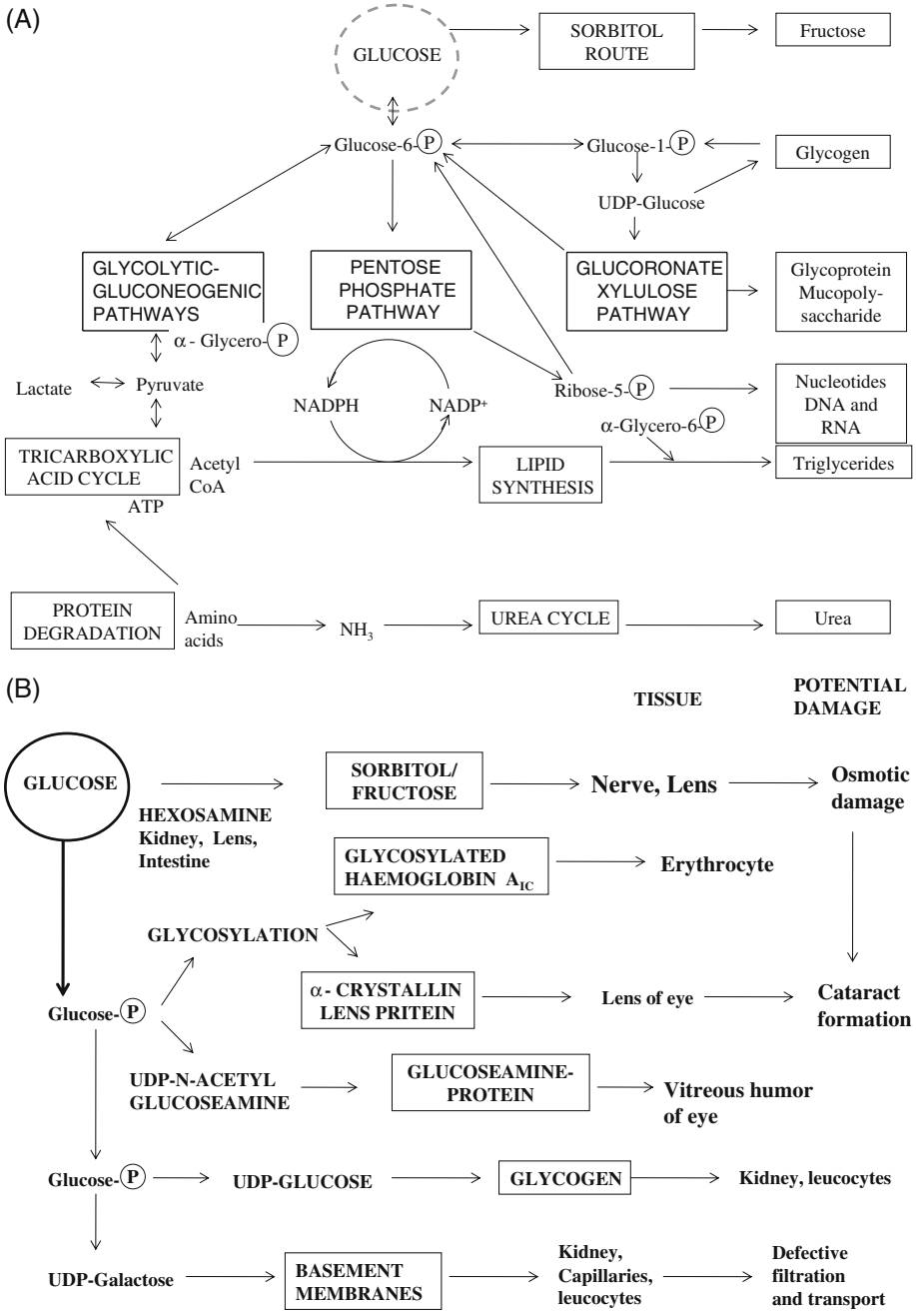

Figure 1. Metabolic pathways effected: glucose over-utilization. Glucose over-utilization and induced pathological changes in tissues resulting from non-insulin-requiring pathways. Inter-relationships among alternative routes of glucose metabolism. The central role of glucose in carbohydrate, fat and protein metabolism is summarized. Derived from Baquer *et al.* (2003).

sorbitol and the glycogenic pathways yields osmotic damage, while glycosylation reaction leads to aberration in the eye and the basement membranes of cells, which in turn affect permeability and transporter mechanisms. These reactions may account for many of the pathological changes observed in severe uncontrolled diabetes. The above phenomenon can be assessed by measuring changes in the activity of a variety of enzymes occurring, for example, in the kidney, which facilitate rates of glucose utilization along specific metabolic routes (Sochor *et al.* 1979, 1985).

The characteristic changes occurring in uncontrolled diabetes are rise in blood glucose and increase in glycogen breakdown, gluoconeogensis, fatty acid oxidation, ketone body production and urea formation. There is depression in

the cells of those tissues that are normally dependent on insulin.

Diabetes has classically been considered to be a disease with glucose overproduction by liver and underutilization by insulin-requiring tissues such as muscle and adipose. The cells of those tissues that have an insulin-dependent glucose transporter system are relatively unaffected by high blood glucose concentration in a diabetic patient, because the specific transporter system for glucose into the cell is not active in the absence of insulin. However, this is not so for the insulin-independent cells in which glucose entry is largely governed by the concentration gradient across the exterior and interior of the cell, for example, in the kidney, nerves and erythrocytes. In consequence, overutilization of glucose can occur in these tissues. Thus, in diabetes there appears to be diversion of glucose from insulin-dependent pathways to those not requiring the hormone, insulin. The facilitation of such process in insulin-independent tissues due to raised glucose levels results in the pathological process leading to diabetic complications. Figure 1B shows glucose overutilization and induced potential damage to the tissues (Sochor *et al.* 1985).

Mohammad *et al.* (2004) and Siddiqui *et al.* (2006) showed changes in general parameters after the vanadate and *Trigonella* treatments in diabetic animals. Body weights were significantly reduced in the diabetic groups: vanadate treatment could not improve the weight loss when compared with the controls, whereas insulin, *Trigonella* and *Trigonella* and vanadate in combination resulted in significant increase in body weights as compared with the diabetic rats. Liver weight of the diabetic rats decreased in comparison with the controls' although the same being compared on a functional basis as liver weight/100 gm body weight did not show any significant difference between the control, diabetic and various treatment groups (Sochor *et al.* 1985). On the other hand, there was an increase in kidney weight of diabetics as compared with the controls. Animals receiving vanadate, *Trigonella* seed powder (TSP) and the two in combination showed reversal to near normal values of most parameters (Mohammad *et al.* 2006a; Siddiqui *et al.* 2005).

3. Insulin mimetic action of manganese, vanadate and *Trigonella*

The link between obesity, insulin resistance, NIDDM and dietary fat has been extensively investigated (Baquer *et al.* 2003). It has been a field of study that has been accelerated by the heightened awareness of the importance of obesity to cardiovascular problems, NIDDM and insulin resistance syndrome. Insulin resistance underlies a constellation of adverse metabolic and physiological changes; insulin resistance syndrome is a strong risk factor for the development of type-2 diabetes and coronary heart disease (Baquer *et al.* 2003). The *in vitro* effect of 1 mM manganese and insulin on the conversion of [1-^{14}C]-glucose (glucose molecule labelled on position 1 with radioactive carbon 14) to $^{14}CO_2$ and [^{14}C] lipids by adipose tissue from control rats is shown in table 1.

Insulin action changes with age, and the clinical importance of these changes and the potential importance of declining insulin sensitivity changes with the trace metal manganese, has been shown in a group of young, old and young diabetic and old diabetic rats as compared with their respective controls (Baquer *et al.* 2003). Manganese may act like a hormone, as shown earlier, in eliciting a change in cyclic nucleotides, which act as a second messenger resulting in the modulation of the metabolic profiles. Thus, it is possible that insulin and dietary Mn^{2+} may have a common mechanism of action in raising the cellular concentration of cGMP, and such a mechanism will be in accord with the number of similarities between enzymes changes induced by Mn^{2+} and by insulin in liver and adipose tissue (Baquer *et al.* 1975; Subasinghe *et al.* 1985).

The importance of manganese in the regulation of protein phosphatases, including pyruvate dehydrogenase phosphatase, on the effectiveness of insulin action is held to be a control regulatory feature of the insulin mimetic action of manganese (Kunjara *et al.* 1999; McLean *et al.* 2008). The insulin-like action of nickel and certain other transition metal ions on lipolysis in rat adipocytes has been shown (Saggerson *et al.* 1976). It is possible that the presence of manganese augment the activity of manganese-dependent enzymes by increasing their stability.

Insulin mimetic action of vanadate has been studied in various normal and diabetic tissue in insulin responsive cells, and the effects have been discussed by Shechter (1990). Vanadate administration to diabetic rats has been shown to mimic insulin in translocation of Glut-4 to the plasma membrane both *in vitro* as well as *in vivo* (Meyerovitch *et al.* 1987); however, vanadyl compounds had been found to be toxic at doses that show the insulin mimetic effects.

Administration of *Trigonella foenum foenum-graecum*, seed powder to diabetic animals has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrates and lipid metabolism to near normal levels in various animal models (Raju *et al.* 2001; Vats *et al.* 2003; Yadav *et al.* 2004, 2005; Mohammad *et al.* 2006b). The components responsible and the mechanism by which *Trigonella* exerts their effects are not clearly understood. However, earlier studies have shown the presence of steroid saponins in *Trigonella* seeds (Petit *et al.* 1995; Basch *et al.* 2003). Saponin compounds diasgenin, alkaloids and trigonelline – inhibit intestinal glucose uptake *in vitro* (Al-Habori *et al.* 2001).

Table 1. *In vitro* effect of manganese (1 mM) and insulin (0.001 mM) on the conversion of [1-^{14}C]-glucose to [^{14}CO2] and [^{14}C]-lipids by adipose tissue from control rats of different ages and diabetic rats

	[1-^{14}C]-glucose		Biodata		
Additions	^{14}CO2	^{14}C-lipids	Body wt. (gm)	wt. 2 fat pads (gm)	Blood glucose (mM)
Controls (young)			168±4	1.52±0.24	4.9±0.20
None	6.95±0.67	5.71±0.55			
+ Insulin	23.5±3.6^{c}	17.6±3.3^{c}			
+ Mn^{2+}	11.5±1.3^{c}	9.72±1.01^{b}			
+ Ins + Mn^{2+}	33.9±6.4^{c}	28.8±5.6^{c}			
Alloxan diabetic (young)			158±6	1.02±0.33	25±5^{c}
None	1.93±0.29	2.10±0.35			
+ Insulin	4.19±0.23^{c}	4.11±0.47^{b}			
+ Mn^{2+}	3.16±0.55^{a}	3.06±0.50^{a}			
+ Ins + Mn^{2+}	4.93±0.65^{a}	4.58±0.68^{a}			
Controls (old)			409±31	6.15±0.47	5.7±1.0
None	2.82±0.49	2.64±0.29			
+ Insulin	3.75±0.58	2.82±0.35			
+ Mn^{2+}	2.95±0.49	2.53±0.17			
+ Ins + Mn^{2+}	3.64±0.22^{a}	4.22±0.58			
Alloxan diabetic (old)			254±19^{b}	1.86±0.20^{c}	29±4^{c}
None	1.43±0.17	1.41±0.17			
+ Insulin	2.34±0.42^{a}	1.72±0.29			
+ Mn^{2+}	1.78±0.20	1.48±0.21			
+ Ins + Mn^{2+}	2.30±0.14^{a}	1.70±0.14			

Values are given as means ± SEM of at least eight values. *P*-values are $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$ compared with the sample without addition; changes in biodata were compared with corresponding controls. The age of young rats was 6–8 weeks and of old rats, 16 weeks; the duration of diabetes was 3 weeks. Derived from Baquer *et al.* (2003).

Extensive reviews have been written of health benefits on physiological effects of *Trigonella foenum-graecum* (fenugreek) and therapeutic applications in animal system as well as on humans, including antidiabetic and related physiological phenomenon (Basch *et al.* 2003; Srinivasan 2006; Khalki *et al.* 2010).

4. Physiological and biochemcial changes

The antidiabetic properties of insulin and vanadium *in vivo* and *in vitro* have been elucidated and reviewed by Ramasarma (1996), Sekar *et al.* (1996) and Baquer *et al.* (1998), including clinical studies (Goldfine *et al.* 1995; Verma *et al.* 1998). Both insulin and vanadium administration elicit a decrease in blood glucose levels and improve the altered lipid and glucose homeostasis in experimental diabetic animals, including the reversal of key glycolytic, gluconeogenic and lipogenic enzymes. Treatment of the diabetic animals with TSP was also able to normalize the blood glucose levels when administrated to diabetic rat (Mohammad *et al.* 2006b).

A possible mechanism to explain this action may involve an increase in the glycolytic flux and a concomitant decrease in gluconeogenesis. The changes in the activities of a few key enzymes of glycolysis, gluconeogenesis and lipogenic pathways in tissues of experimental diabetic animals together with antidiabetic treatments with insulin, vanadate and *Trigonella* have been shown (table 2). Reversal with the antidiabetic compounds administered, namely, vanadium, *Trigonella,* manganese and insulin, showed that most parameters, including blood glucose levels and enzyme changes, reversed to the control levels (Gupta *et al.* 1999; Raju *et al.* 2001; Mohammad *et al.* 2004; Yadav *et al.* 2004; Preet *et al.* 2006).

Oxidative stress is suggested to be a potential contributor to the development of complications in diabetes (Wolff 1987; Baynes 1991; Ceriello *et al.* 1992). Oxidative stress may result from an overproduction of precursors to oxygen free radicals and/or decreased deficiency of antioxidant enzymes systems. There is a strong belief that free radical production increases during diabetes (Baynes 1991). The antioxidant enzymes SOD, CAT, GR and GPx are some of the biological antioxidant enzymes that directly scavenge

Table 2. Effect of *Trigonella foenum-graecum* and sodium orthovanadate on physiological, biochemical and molecular parameters in diabetic rat tissue

Parameters changes	Control	Diabetes	Trigonella treatment	Vanadate treatment	References
Body weights	↔	↓	↑	↓	Siddiqui *et al.* (2006)
Blood glucose levels	↔	↑	↓	↓	Mohammad *et al.* (2004)
Insulin levels	↔	↓	↑	↑	Kumar *et al.* (Unpublished)
Carbohydrate metabolism		L K	L K	L K	
Glycolytic	↔				
Hexokinase isozymes					Yadav *et al.* (2004)
Type I		↓ ↑	↑ ↓	↑ ↓	
Type II		↓ ↑	↑ ↓	↑ ↓	
Type IV		↓	↑	↑	
Phosphofructokinase		↓ ↑	↑ ↓	↑ ↓	Mohammad *et al.* (2004)
Pyruvate kinase		↓ ↑	↑ ↓	↑ ↓	
Lactate dehydrogenase		↓ ↑	↑ ↓	↑ ↓	
Gluconeogensis	↔				
Glucose-6-phosphatase		↑ ↓	↓ ↑	↓ ↑	Preet *et al.* (2006)
Fructose-1,6-bisphosphatase		↑ ↓	↓ ↑	↓ ↑	Raju *et al.* (2001)
PEPCK		↑ ↓	↓ ↑	↓ ↑	
Lipogenic	↔				
G-6-Pdehydrogenase		↓ ↑	↑ ↓	↑ ↓	Gupta *et al.* (1999)
Malicenzyme		↓ ↑	↑ ↓	↑ ↓	
ICDH (NADP)		↓ ↑	↑ ↓	↑ ↓	
ATPCL		↓ ↑	↑ ↓	↑ ↓	
FAS		↓ ↑	↑ ↓	↑ ↓	

Table 2. (continued)

Antioxidant enzymes (Liver, brain, Muscle, Heart, Kidney) (a) Superoxide dismutase (b) Catalse (c) GPx (d) GR	↔	↓	↑	↑	Genet *et al.*, (2002) Siddiqui *et al.* (2005) Mohammad *et al.* (2004)
Lipid profile		L K S	L K S	L K S	Yadav *et al.* (2004)
Total lipids Triglyceride Cholesterol	↔	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	
Lipid peroxidation (Liver, Kidney, Brain) Malondialdehyde (MDA)	↔	L K B ↑ ↑ ↑	L K B ↓ ↓ ↓	L K B ↓ ↓ ↓	Genet *et al.* (2002)
Membrane fluidity	↔	↓ ↓ ↓	↑ ↑ ↑	↑ ↑ ↑	Siddiqui *et al.* (2005)
Membrane bound enzymes		L K B	L K B	L K B	Siddiqui *et al.* 2006
(a) Na^+K^+ATPase	↔	↓ ↑ ↓	↑ ↓ ↑	↑ ↓ ↑	
(b) Ca^{2+}ATPase					
Mitochondrial dehydrogenases					
ICDH-NAD	↔	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	Thakran *et al.* 2003
ICDH-NADP		↓ ↑ ↑	↑ ↓ ↓	↑ ↓ ↓	
MDH		↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	
GLDH		↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	
D-β-HBD		↓ ↑ ↑	↑ ↑ ↓	↓ ↑ ↓	
Membrane fluidity (Liver, brain, Muscle, Heart, Kidney)	↔	↓	↑	↑	Siddiqui *et al.* (2005)
Glucose transporter-4 (Heart, Muscle, Brain)	↔	↓	↑	↑	Mohammad *et al.*(2004)

Changes in biochemical and molecular parameters in experimental diabetes and effect of antidiabetic compounds. No Changes (↔), Increased (↑), Decreased (↓). L, Liver; K, Kidney, B, Brain, S, Serum. Tissues given in brackets were taken and showed similar changes for all.

free radicals or prevent their conversion to toxic products (Freeman and Crapo 1982). Diabetes is associated with altered levels of these enzymes that results in increased oxidative stress. The changes in these enzymes are given in table 2, together with the levels of MDA, a lipid peroxidative product formed in the tissues due to peroxidation of lipids during oxidative stress (Genet *et al.* 2002; Mohammad *et al.* 2004; Siddiqui *et al.* 2005). Advanced glycation end products (AGEs) are formed through oxidative reactions and cause irreversible chemical modifications of protein (Wieland 1983; Mullarky *et al.* 1990). Sodium orthovanadate administration to experimentally induced

diabetic animals elicits a decrease in blood glucose level and alters their lipid and glucose homeostasis, including the reversal of key glycolytic, gluoconeogenic and lipogenic enzymes (Heyliger *et al.* 1985; Meyerovitch *et al.* 1987; Sekar *et al.* 1996). The chronic response to various vanadium compounds in experimental diabetic Wistar rats has been studied earlier. The most common toxic effects are diarrhoea, decreased fluid and food intake, dehydration and loss in body weight (Becker *et al.* 1994; Mohammad *et al.* 2006b).

Thus, various tissues in the diabetic state are more prone to oxidative damage resulting in various complications in long-term diabetes, implying that the restoration of the antioxidant status is an important parameter for evaluating the effects of an antidiabetic compound. The results are presented in table 2. The antioxidant enzymes SOD and CAT showed significant decrease in diabetic liver and kidney, whereas GPx and GR decreased in liver and increased in kidney; this was in agreement with earlier published data (Mak *et al.* 1996; Genet *et al.* 2002).

Treatment with insulin, vanadate, *Trigonella* and the combined dose of vanadate and *Trigonella* corrected the altered levels of PK, PEPCK, SOD, CAT, GPx and GR in liver and kidney of diabetic rats (Genet *et al.* 2002; Mohammad *et al.* 2004). *Trigonella* treatment partially normalized hyperglycaemia and restored the altered enzyme activities. Vanadate, on the other hand, was more effective in amending these parameters, but resulted in a significant weight loss of the treated animals; the combined treatments was most effective in correcting hyperglycaemic and normalizing glucose homeostasis. Low doses of vanadate alone did not result in weight loss when given to control rats, but when administered to diabetic rats, it was not effective in reviving normoglycaemia (Mohammad *et al.* 2004).

5. Lipogenesis and lipid profiles

Plasma lipid level is usually raised during diabetes and presents a risk factor for coronary heart disease. Lowering plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease. Yadav *et al.* (2004, 2005) had earlier shown an increase in serum total lipids, triglycerides and total cholesterol levels. The changes were also observed in the liver and kidney.

The increase in lipid profile may be a result of increased breakdown of lipids and mobilization of free fatty acids (FFA) from the peripheral deposits. Insulin inhibits the hormone-sensitive lipases, and these become active in the absence of insulin. Other hormones such as glucagon and catecholamines, known to increase during diabetes, compound the effect by stimulating lipolysis. A marked prevention in the alteration of lipid profile by a combined treatment with sodium orthovanadate (SOV) and TSP to diabetic animal was shown earlier (Yadav *et al.* 2004). There could be two possibilities for the prevention of alteration in the lipid profile. First, the rate of lipogenesis is normalized by SOV and TSP, in a way similar to the effect of insulin on lipid metabolism, and the results showed that the enzyme activities were maintained at near normal level; Second, the attainment of normoglycaemia in the animals was achieved (Raju *et al.* 2001; Yadav *et al.* 2004; Mohammad *et al.* 2006b). SOV was earlier shown to stimulate fatty acid synthesis in isolated rat haptocytes (Agius and Vaartje 1982). Brichard *et al.* (1994) have shown that SOV compounds activate lipogenesis and inhibit lipolysis in rat adipose tissue. Raju *et al.* (2001) had earlier demonstrated that TSP stimulates hepatic lipogenic enzymes. It has been reported that insulin acts by increasing the phosphorylation of ATP-citrate lyase by c'AMP-dependent protein kinase because SOV is known to mimic insulin action and also inhibit Na^+K^+ATPase (Siddiqui et al. 2006), thereby making more ATP available for phosphorylation; it is possible that SOV acts in a way similar to insulin to increase the activity of ATP citrate lyase. TSP also showed similar enhancement in the enzyme activity (Yadav *et al.* 2004).

6. Modulation of the glucose transporter-4, in muscle, heart and brain

The impairment of heart glucose metabolism in diabetes may contribute to the mechanical dysfunction and cardiomyopathy. Siddiqui *et al.* (2006) showed that Glut-4 protein significantly decreased in the total membrane fractions of cardiac muscle of alloxan diabetic rats (table 2). Because glucose transport in cardiac muscle occurs mainly through Glut-4, the reduction in the Glut-4 level results in decreased uptake of glucose and, therefore, contributes to the increased blood glucose levels in diabetic conditions. Levels of Glut-4 protein expression were also restored after the treatment with different antidiabetic compounds. Results similar to those regarding cardiac muscle during diabetes were also obtained in skeletal muscle and brain (Mohammad *et al.* 2006a).

7. Membrane-linked enzymes, fluidity and membrane structural changes

Several major studies have revealed, clearly and convincingly and beyond reasonable doubt, that keeping blood glucose levels as close as possible to normal, non-diabetic values really does even impede and delay chronic diabetic complications like diabetic retinopathy, nephropathy, microangiopathic and macroangiopathic damage as well as neuropathy (Brownlee 1995; King and Brownlee 1996). Results from our group

presented here showed that treatment with TSP and vanadate could increase glucose utilization and reduce glycosylation of proteins, ROS formation and lipid peroxidation by controlling hyperglycaemia (table 2). TSP is reported to also have antioxidant properties (Genet *et al.* 2002). A reduction in the production of free radicals, lipid peroxides formation can beneficially prevent the decreased activity of the membrane-bound enzyme Na^+K^+ATPase (Siddiqui *et al.* 2006). The beneficial effect of vanadate could be through its insulin mimetic effect: vanadate stimulates phosphorylation of the insulin receptor either directly by activation of the tyrosine kinase present in the beta-subunit of the insulin receptor or through its inhibitory effect on phosphotyrosyl phosphatase (Swarup *et al.* 1982; Baquer *et al.* 2009). Heyliger *et al.* (1985) demonstrated that the addition of vanadate to the drinking water of diabetic rats would prevent the cardiac depression found in the diabetic rats; this has been confirmed by Siddiqui *et al.* (2006).

Diabetic animals elicit tissue-specific alterations in Na^+K^+ATPase activities in liver, brain and heart, showing a decrease and a significant increase in the kidney. As discussed earlier, hyperglycemia has been shown to generate free radicals from auto oxidation of glucose, formation of AGEs and increased polyol pathways with concomitant increase in cellular lipid peroxidation and damage to membranes in diabetes. The formation of thiobarbituric-acid-reactive substances (TBARS; MDA formation) was also increased in the diabetic tissues (table 2). This increased MDA formation disturbs the anatomical integrity of the membranes, leading to inhibition of several membrane-bound enzymes. In contrast to liver and heart the kidney showed significant increase in the Na^+K^+ATPase, and this can be related to the Na^+-dependent solute transport (Fedorak *et al.* 1987).

Significant biochemical and molecular changes occur in mitochondria with experimentally induced diabetes in tissues like liver, kidney and brain (Thakran *et al.* 2003). In pancreatic beta-cells, redox imbalance is reported to potentiate apoptosis (Hamaoka *et al.* 1999).

Apoptosis or programmed cell death has also been implicated in diabetic retinopathy and neuropathy due to abnormalities in mitochondrial function (Barber *et al.* 1998; Srinivasan *et al.* 2000). Mazat *et al.* (2001) have hypothesized that not all tissues are equally affected in case of mitochondrial cytopathies. The rate of mitochondrial ATP synthesis in some tissues is maintained at the expense of changes in metabolite concentrations, which might lead to increased free radical generation. The improvement of mitochondrial metabolic disturbance by SOV and TSP during diabetes reinforces their potential as antidiabetic agents (Thakran *et al.* 2003). Significant changes were observed in the activities of Na^+K^+ATPase and Ca^{2+}ATPase in liver, kidney, heart and brain tissues of diabetic rats. Protein expression of alpha-1 isoform of Na^+K^+ATPase showed significant decrease in the heart. Diabetic kidney showed significant increase in the two ATPases. Administration of combined dose of SOV and *Trigonella* were most effective in reversing the aberrations in the enzyme activities and alpha-1 levels to normal values (Siddiqui et al. 2006). Monoamine oxidase (MAO) showed a significant increase in the membrane fractions in diabetic brain, and the reversal by antidiabetic compounds was achieved. The reversal of MAO in diabetic brains by insulin has been reported, as well as the changes in catecholamine levels in diabetes, the latter acting as counter regulatory hormones (Mayanil *et al.* 1982; Gupta *et al.* 1992).

8. Molecular changes

A few enzymes of metabolic pathways were taken to study their expression at molecular levels and effects of experimental diabetes on these enzymes were assessed together with their reversal by administration of antidiabetic compounds, namely vanadate and *T. foenum-graecum*.

The results obtained from various enzymes of different metabolic pathways confirm and reiterated that the action of the *Triogenella* in these diabetic tissues occurs at the molecular levels. The activities of the glycolytic enzymes PK was measured in liver and kidney together with the gluconeogenic enzyme PEPCK. An almost complete reversal was seen at the mRNA levels of the two enzymes. Glut-4 levels at the mRNA levels also showed a reversal with *Trigonella* administration when using cardiac and skeletal membrane fractions (table 2). Other enzymes studied at molecular levels were liver arginase (Salimuddin *et al.* 1999), peripheral nerve enzyme aldose reductase, gluconeogenic enzyme glucose 6 phosphatase (Gupta *et al.* 1999; Preet *et al.* 2005) and glucose transporter Glut-4 in diabetic brains (Kumar 2010).

9. Proposed mechanism for antidiabetic action of SOV and *Trigonella foenum-graecum*

Diabetes mellitus is a complex metabolic disorder, as outlined earlier, characterized by high glucose levels due to the inability of the body cells to utilize glucose properly. Although insulin treatment and other chemical therapies can control many aspects of diabetes, numerous complications are common in the disease. In the present review, an attempt is made to elucidate the role of *T. foenum-graecum* seed powder in primarily controlling the blood glucose levels in experimentally induced diabetic animals, which is the most important metabolite controlling metabolism, and then to study the various metabolic pathways at biochemical and molecular levels to assess the effectiveness of *T. foenum-graecum* in controlling and preventing diabetic changes. Vanadium is an insulin mimetic trace metal. Its effect is also seen and the toxicity of vanadium was found to be reduced

when administered with *T. foenum-graecum* (Raju *et al.* 2001). Insulin effects were also included to assess whether the reversal of the high glucose levels with antidiabetic compounds was as effective as insulin.

Puri *et al.* (2002) had isolated an active compound from fenugreek that showed hypoglycemic properties in diabetic rabbits. The authors found significant attenuation of the glucose tolerance curve and improvement in the glucose-induced insulin response, suggesting that the hypoglycaemic effect may be mediated through stimulating insulin-producing beta-cells of the Islets of Langerhans (Baquer et al. 2009). Although the treatment lowered fasting blood glucose significantly, it could not elevate the serum insulin levels, suggesting an extrapancreatic mode of action; this effect may also be increase the sensitivity of tissue to the available insulin.

Although extensive work has been undertaken to elucidate the mechanism by which SOV could exert its effects, the same with TSP is not very clear. However, plausible hypothesis that may be involved in the therapeutic action is discussed – TSP may exert its therapeutic effects though modulation of insulin secretion. Data from our laboratory have shown an almost 70% increase in the plasma insulin levels with *Trigonella* treatment of diabetic animals (Kumar 2010). Madder and Thorne (1987) attributed it to dietary fibres present in the fenugreek seeds which help in the management of metabolic abnormalities associated with diabetes such as peripheral insulin resistance and lipid peroxidation abnormalities. Petit *et al.* (1995) and Yoshikawa *et al.* (1997) reported the isolation of furostanol saponins called trigoneoside Ia, Ib, IIa, IIb,IIIa and IIIb, glycoside and trifoenoside A. They claimed that these saponins are the active compounds owing to their hypoglycaemic effects. Srinivasan (2006) has also given the chemical composition of the active antidiabetic ingredient found in *Trigonella*. It has also been demonstrated in some studies that *Triogonella* seeds delayed gastric emptying and caused inhibition of glucose transporter as the seed contain 50% pectin that forms a colloid suspension when hydrated and decreases the rate of gastric emptying and slows carbohydrate absorption (Al-Habori and Raman 1998). Sauvaire *et al.* (1998) and Broca *et al.* (1999) have demonstrated evidence of insulinotropic and antidiabetic properties of 4-OH-Ile isolated from fenugreek seeds in a glucose-dependent manner; 4-OH-Ile is an amino acid found only in plants. These authors suggested that the antidiabetic effect of 4 OH-Ile was at least, in part, from direct pancreatic beta-cells stimulation.

Perspectives involving biochemistry and bioinorganic chemistry of vanadium and its complexes with several types of ligands have been proposed as useful for treating diabetes mellitus in experimental diabetic animals (Sakurai 2002; Nahas and Moher 2009). It can be suggested that there may be some *in vivo* complex formation by SOV with organic compounds made available by *Trigonella* which is responsible for bringing better control of glucose levels and diabetic complications. Shinde *et al.* (2001) showed that chronic treatment with an organic complex of vanadium such as bis(maltolato) oxovanadium IV (BMOV) was effective in improving glucose and lipid homeostasis.

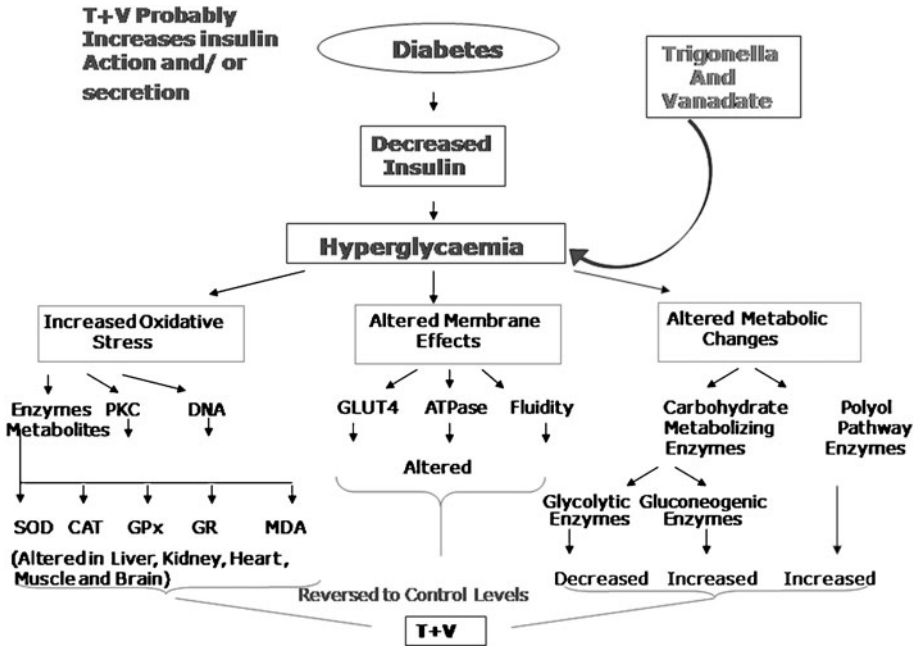

Figure 2. Proposed mechanism of action of *Trigonella foenum-graecum* and sodium orthovanadate in diabetes.

The proposed mechanism of action of *T. foenum-graecum* and SOV are presented in figure 2. The detailed multi-beneficial physiological effects of *Triognella* have been reviewed by Srinivasan (2006). In the present review, the detailed biochemical, physiological and molecular action of fenugreek seed powder given to diabetic animals has been presented. The effects have been seen in various tissues of the rat including brain. The results show that diabetes effects most tissues of the body and these effects, some of them irreversible, can be reversed to near control levels by administration of *Trigonella* in combination with SOV. The parameters used, like enzymes changes, have been shown to be reversed at the molecular levels. Structural changes in membrane structure and changes in membrane-bound and mitochondrial enzymes clearly show the effectiveness of *Trigonella* in the use for the treatment of diabetes and its associated complications. The complications of diabetes have been recently reviewed and discussed in relation to the complications of aging neuronal tissues and many similarities shown in the two physiologically different conditions. Baquer *et al.* (2009) reiterate the phrase that we may refer or consider diabetes as a hastened process of aging.

10. Safety and adverse effects of *Trigonella foenum-graecum*

Basch *et al.* (2003) had reviewed the literature on the safety and adverse effects of *T. foenum-graecum*. Although fenugreek has traditionally been considered safe and well tolerated, some side effects have been associated with its use. Caution in using fenugreek is warranted in patients known to be allergic to it or chickpeas, because of possible cross-reactivity (Patil *et al.* 1997). Other reported side effects include transient diarrhea and flatulence (also mentioned earlier (Sharma 1986; Sharma *et al.* 1996a) and dizziness (Abdel-Barry *et al.* 2000). Hypoglycemia is an expected effect, and therefore, care should be taken to monitor blood glucose levels when beginning fenugreek supplementation (Sharma 1986; Madar *et al.* 1988; Sharma *et al.* 1996b). Decreased body weight has also been reported and attributed to decrease in T3 (Panda *et al.* 1999). The data generated to date on the above in regard to *Trigonella* use in patients are sparse but will hopefully lead to the development of well-designed, adequately powered, randomized clinical trials to evaluate the effect of fenugreek seed powder on measures of insulin resistance, insulin secretion and cholesterol metabolism.

11. Drug interactions

As fenugreek powder is rich in fiber, it can interfere with the absorption of oral medication. Prescription medicines should be taken separately from fenugreek-containing products. Concomitant use of fenugreek with other hypoglycaemic agents might lower serum glucose level more than expected (Basch *et al.* 2003).

Toxicological evaluation of diabetic patients taking fenugreek seed powder at a dose of 25 gm per day for 24 weeks showed no clinical hepatic or renal toxicity and no hematological abnormalities (Sharma *et al.* 1996b). In an animal study, fenugreek powder failed to induce any signs of toxicity or mortality in mice and rats that received acute and subchronic regimes (Muralidhara *et al.* 1999). There was no significant hematological hepatic or histopathological changes in weanling rats that were fed fenugreek seeds for 90 days (Rao *et al.* 1996). The amount of fenugreek used in the diet given to diabetic animals has been discussed in our earlier communication (Raju *et al.* 2001; Mohammad et al. 2004).

This review analyses different metabolic pathways that use biochemical, molecular and histochemical techniques. Studies were conducted in our laboratory on insulin-dependent and insulin-independent tissues including liver, heart, muscle, kidney, peripheral nerve, brain and red blood cells.

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