PUBLICATIONS AND ABSTRACTS
Publications:

- **Mohamad S; Taha A; Bamezai R.N.K; Basir S.F. and Baquer N.Z.** Lower doses Of Vanadate in Combination with Trigonella Restore Altered Carbohydrate Metabolism and Antioxidant Status in Alloxan-diabetic Rats. Clinica Chimica Acta; 342/1-2 pp 105-114, 2004

- Ali Abdul Lattif, Nivedita Gupta, Sameer Mohamad, Uma Banerjee, Rajendra Prasad, Najma Zaheer Baquer The interrelationship between the key enzymes of glyoxylate cycle and the site of infection caused by *Candida albicans* in Indian HIV/AIDS, diabetic and burn patients. (Communicated)

- Expression and translocation of GLUT4 in alloxan-diabetic rat skeletal muscle: Modulation by vanadate and Fenugreek treatment. (Manuscript in preparation)

**Poster Presentations in Conferences:**

- “Pyruvate Metabolism in Experimental Diabetes: Effect of Antidiabetic Compounds” Annual Congress of Association of Clinical Biochemists of India (ACBICON) held in Jaipur, India in Feb 2003.

- “Combined doses of Vanadate and Fenugreek correct the altered levels of Pyruvate kinase, Phosphoenolpyruvate carboxykinase and Hepatocyte Nuclear factor – 4alpha in alloxan diabetic rat liver” International Conference on “Emerging Trends in Molecular and
Cellular Biology" held in the School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.

- "In vivo effect of Fenugreek on pyruvate kinase, phosphoenolpyruvate carboxykinase and Hepatocyte Nuclear Factor-4 (HNF-4a) levels in alloxan diabetic rat liver" 18th International Diabetic congress held in Paris, France in August 2003.
Lower doses of vanadate in combination with trigonella restore altered carbohydrate metabolism and antioxidant status in alloxan-diabetic rats

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Abstract

Background: Vanadate treatment to diabetic rats has been reported to correct the altered carbohydrate metabolism and antioxidant status. However, vanadate exerts these effects at relatively high doses and several toxic effects are produced. We used low doses of vanadate in combination with Trigonella foenum graecum seed powder (TSP) and evaluated their effect on the enzyme changes in diabetic rats. Methods: Alloxan-diabetic rats were treated separately with insulin, vanadate (0.6 mg/ml), TSP and a combined dose of Vanadate (0.2 mg/ml) and TSP for 21 days. At the end of the experimental period, blood glucose levels and activities of pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) were measured in cytosolic fraction in the liver and kidney. Results: Blood glucose levels increased markedly in diabetic rats. Treatment with antidiabetic compounds resulted in the reduction of glucose levels. Rats treated with combined dose of vanadate and trigonella had glucose levels comparable to control ones. Similar results were obtained with the activities of PK, PEPCK, SOD, GPx, GR, and CAT in liver and kidney of diabetic rats. Combined dose of vanadate and Trigonella was found to be most effective in correcting these alterations. Conclusions: Lower doses of vanadate could be used in combination with TSP to effectively counter diabetic alterations without any toxic side effects.

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Keywords: Sodium orthovanadate; Trigonella foenum graecum; Alloxan diabetes; Oxidative stress; Antioxidant status

Abbreviations: PEPCK, phosphoenolpyruvate carboxykinase; PK, pyruvate kinase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; TSP, trigonella seed powder.

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1. Introduction

A balance between glucose production and its utilization is necessary to maintain normal blood glucose levels. Diabetes is characterized by elevated production and low utilization of glucose [1]. PK (EC 2.7.1.40) and PEPCK (EC 4.1.1.32) are key regulatory enzymes of glycolytic and gluconeogenic path-
ways, respectively [2,3]. The activity of PK and levels of mRNA show a decrease in the liver of diabetic rats [4]. There is no change in PK-mRNA levels but the activity of PK shows an increase in the kidney of diabetic rats [4]. The gluconeogenic enzyme PEPCK activity and levels of its mRNA show a marked increase both in liver and kidney in the diabetic state [5]. This imbalance results in constant hyperglycemia in the diabetic state.

Oxidative stress is suggested to be a potential contributor to the development of complications in diabetes [6–8]. Oxidative stress may result from overproduction of precursors to oxygen free radicals and/or decreased efficiency of antioxidant system. There is a strong belief that free radical production increases during diabetes [8]. The antioxidant enzymes SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GPx (EC 1.11.1.9) and GR (EC 1.6.4.2) are some of the biological antioxidant enzymes that directly scavenge free radicals or prevent their conversion to toxic products [9]. Diabetes is associated with altered levels of these enzymes that result in increased oxidative stress [10]. This oxidative stress may be caused due to several mechanisms including glucose auto-oxidation and non-enzymatic protein glycation [8,11–13]. Non-enzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Schiff’s bases and more stable Amadori products are formed [14,15]. Both Schiff’s base and Amadori products have been found to generate free radicals, suggesting that glycation of proteins enhances its potential exposure to oxidative damage [8,16,17]. Advanced glycation end products (AGES) are then formed through oxidative reactions and cause irreversible chemical modifications of proteins [14,18].

Sodium orthovanadate administration to experimentally induced diabetic animals elicits a decrease in blood glucose levels and improves the altered lipid and glucose homeostasis including the reversal of key glycolytic, gluconeogenic and lipogenic enzymes [19–21]. However, the major problem with vanadium treatment of diabetic animals has been its potential toxicity [22]. The chronic response to various vanadium compounds, following streptozotocin-diabetic induction in Wistar rats has been studied [23], the most common toxic effects are diarrhea, decreased fluid and food intake, dehydration, and loss in body weight [19,24–27]. Vanadate shows little toxicity at low doses but severe toxic effects are reported at high doses [28].

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 [29,30]. The components responsible and the mechanism by which Trigonella exerts these effects in not clearly understood. However, several studies have shown the presence of steroid saponins in Trigonella seeds [31,32]. Saponin compounds diosgenin, alkaloids and trigonelline inhibit intestinal glucose uptake in vitro [33]. 4-hydroxyisoleucine, a modified amino acid extracted and purified from fenugreek seeds also displayed an insulinitropic property in vitro, stimulated insulin secretion in vivo and improved glucose tolerance in normal rats and dogs and in rat model of type 2 diabetes mellitus [34]. Besides 4-hydroxyisoleucine, arginine and tryptophan are the other amino acids having antidiabetic and hypoglycemic effect. In addition to this many trace elements, which are the components of Trigonella, have been found to possess antidiabetic effects.

The present study explores the possibility of using low doses of vanadate (0.2 mg/ml) in combination with TSP and evaluates their effect on altered carbohydrate metabolism and antioxidant enzymes in liver and kidney of alloxan-diabetic rats.

2. Materials and methods

Alloxan monohydrate, sodium orthovanadate, NADH, NADPH, PEP, ADP, LDH, GR and GSSG were from Sigma (St. Louis, MO). All other reagents were of analytical grade. Female albino rats of the Wistar strain weighing between 200 and 210 g and age of 3–5 months were used throughout this study. Animals were given standard chow and tap water ad libitum until treatment or sacrifice. Diabetes was induced by using alloxan monohydrate following the method of Sochor et al. [35]. A group, of 60–70 rats were starved for 24 h and diabetes was induced by a single subcutaneous injection of alloxan monohydrate dissolved in a freshly prepared 0.154 mol/l sodium acetate buffer (pH 4.5), at a
dose of 15 mg/100 g body weight. Each animal was then given 2 U of insulin for the next 7 days. Control animals were given only the vehicle. The animals were then grouped into control (C), diabetic (D), insulin treated diabetic (D+I), vanadate-treated diabetic (D+V), Trigonella treated diabetic (D+T) and diabetic treated with vanadate and Trigonella (D+T+V). All the animal experiments have been approved by the Institutional Animal Ethics committee (IAEC) of Jawaharlal Nehru University, New Delhi, India.

The insulin treated diabetic group (D+I) received IP injections of 2 units of protamine-zinc insulin for 3 weeks after insulin withdrawal to the diabetic animals and were given the normal pellet diet and tap water ad libitum until the date of the experiment. The vanadate-treated diabetic group (D+V) rats were given 0.6 mg/ml of sodium orthovanadate dissolved in drinking water. In order to minimize the mortality due to vanadate toxicity the vanadate solution was prepared in 0.5% of sodium chloride as suggested by Heyliger et al. [20] and used in our laboratory. The diabetic animals, grouped into diabetic rats treated with TSP (D+T), were given Trigonella seed powder (5% w/w) mixed with their standard pellet food, Trigonella seeds were ground by using a grinder and then mixed with powdered pellet. The treatment was continued until the day of the sacrifice. Tap water was given together with the food ad libitum. D+T+V group animals were given Trigonella seed powder (5% w/w) mixed with their standard food and Vanadate in the drinking water (0.2 mg/ml) containing 0.5% NaCl. Another group included diabetic rats that received vanadate (0.2 mg/ml) alone. A group of control rats was divided into 2 and given (0.2 mg/ml) and (0.6 mg/ml) vanadate for 3 weeks.

Rats were sacrificed by cervical dislocation; tissues were rapidly excised, and washed with chilled normal saline. The tissues were then blotted dry and weighed. A 10% tissue homogenate (w/v) of the tissues (liver and kidney) was prepared by using a Potter Elvejhem homogenizer fitted with a teflon plunger in 0.25 mol/l sucrose, 0.02 mol/l triethanolamine hydrochloride buffer of pH 7.4 containing 0.12 mmol/l dithiothreitol (DTT). Homogenates were then centrifuged at 1000 × g for 10 min to remove nuclei and cell debris. The supernatant was then again subjected to centrifugation at 100,000 × g for 30 min. The supernatants were used for the determination of enzyme activity. All enzymes were assayed in Beckman DU-68 spectrophotometer using coupled enzyme assays with the exception of CAT and SOD. One unit of enzyme activity is defined as 1 μmol of NADH/NADPH oxidized/min/g tissue except for SOD and CAT.

The PK activity was measured by following the method of Gutman et al. [36]. The reaction mixture in a volume of 1 ml contained the following in the final concentration: 67.5 mmol/l Tris–HCl buffer pH 7.4, 95 mmol/l KCl, 6.75 mmol/l MgSO4, 0.4 mmol/l NADH, 3 mmol/l ADP, 2 units of purified LDH and 0.15 mmol/l PEP. Reaction was started by addition of the supernatant fraction containing approximately 20 μg protein. PEPCk activity was measured in 100,000 × g cytosolic fraction by the decarboxylation assay described by Jomain-Baum and Schramm [37]. The decarboxylation assay determines the formation of phosphoenolpyruvate by an equilibrium displacement to generate oxaloacetate from malate by malate dehydrogenase in a 1-ml mixture containing the following in the final concentration. 50 mmol/l Tris, pH 8.0, 0.75 mmol/l MnCl2, 1 mmol/l NAD+, 6 U malate dehydrogenase, 1 mmol/l GTP and 20 μl cytosolic extract containing about 200 μg protein. The reaction was started by adding 100 μl of 10 mmol/l malate and the oxaloacetate formation from malate by malate dehydrogenase was measured spectrophotometrically at 37 °C by measuring NADH formation at 340 nm. A reaction blank was run without malate by following NADH formation for each assay.

The activity of superoxide dismutase (SOD) was measured by the method of Marklund et al. [38] with some modification, which is an assay based on the ability of SOD to inhibit the autoxidation of pyrogallicol by 50%. The assay mixture of 1 ml contained in final concentration, 50 mmol/l sodium phosphate buffer, 0.1 mmol/l EDTA, 0.48 mmol/l pyrogallol and appropriate amount of tissue extracts containing 7–10 μg of protein.

The change in absorbance of the assay mixture at 420 nm was monitored for 3 min at 25 °C against a blank that contained all the reagents except for the tissue homogenate. One unit of enzyme is defined as the amount of enzyme that causes 50% maximal inhibition of pyrogallic autoxidation.
Table 1
General parameters of the control (C), diabetic (D), and diabetic rats treated with Insulin (D+I), Vanadate (D+V), Trigonella (D+T) and combined dose of Vanadate and Trigonella (D+T+V)

<table>
<thead>
<tr>
<th>General parameters C</th>
<th>D</th>
<th>D+I</th>
<th>D+V</th>
<th>D+T</th>
<th>D+T+V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>220±16.7</td>
<td>140±18.5</td>
<td>205±16.3</td>
<td>165±12.4</td>
<td>200±14.6</td>
</tr>
<tr>
<td>Liver wt. (g)</td>
<td>6.2±0.61</td>
<td>4.1±0.72</td>
<td>6.9±0.80</td>
<td>4.8±0.54</td>
<td>6.0±0.67</td>
</tr>
<tr>
<td>Liver wt. g/100g body wt.</td>
<td>2.8±0.27</td>
<td>2.9±0.51</td>
<td>3.3±0.39</td>
<td>2.9±0.32</td>
<td>3.0±0.34</td>
</tr>
<tr>
<td>Kidney wt. (g)</td>
<td>1.1±0.12</td>
<td>1.64±0.16</td>
<td>1.2±0.19</td>
<td>1.3±0.18</td>
<td>1.2±0.11</td>
</tr>
<tr>
<td>Kidney wt. g/100g body wt.</td>
<td>0.5±0.05</td>
<td>1.2±0.11*</td>
<td>0.6±0.09</td>
<td>0.8±0.11</td>
<td>0.6±0.05</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>119±9.5</td>
<td>116±10.7</td>
<td>115±8.3</td>
<td>123±11.7</td>
<td>117±7.7</td>
</tr>
<tr>
<td>Protein (mg/g liver)</td>
<td>92±7.1</td>
<td>88±6.9</td>
<td>95±7.2</td>
<td>90±6.1</td>
<td>88±7.7</td>
</tr>
<tr>
<td>Protein (mg/g kidney)</td>
<td>119±9.5</td>
<td>116±10.7</td>
<td>115±8.3</td>
<td>123±11.7</td>
<td>117±7.7</td>
</tr>
</tbody>
</table>

Each value is the mean±SEM of 5 separate experiments. 
P values are shown as *p<0.01, **p<0.05 vs. control.

The assay of CAT was performed by following the method of Aebi [39]. The assay mixture of 1 ml in the final concentration contained 50 mmol/l sodium phosphate buffer pH 7.0 and 10 mmol/l hydrogen peroxide. The reaction was started by the addition of cytosolic fraction containing 2–3 μg protein. One unit of enzyme activity is defined as the amount of enzyme required to decompose 1 μmol of H₂O₂.

The activity of GPx was measured using a coupled enzyme assay as described by Lawrence and Burk [40]. The assay mixture of 1 ml contained in the final concentration, 10 mmol/l potassium phosphate buffer pH 7.0, 25 mmol/l EDTA, 0.5 mmol/l GSH, 2 mmol/l sodium azide, 1.5 IU GR, 0.1 mmol/l NADPH and the cytosolic fraction containing about 50 μg of protein. The reaction was started by the addition of t-butyl hydroperoxide and the decrease in the absorbance was monitored at 25 °C at 340 nm.

The GR activity was measured in the soluble tissue extracts by the modified method of Erden et al. [41]. The reaction mixture of 1 ml contained the following in the final concentration: 4.1 mmol/l Tris–HCl pH 7.5, 15 mmol/l MgCl₂, 5.7 mmol/l EDTA, 60 mmol/l KCl, 2.6 GSSG and 0.1 mmol/l of NADPH. The reaction was started by the addition of tissue extract containing approximately 100 μg of protein. The decrease in absorbance was monitored at 25 °C at 340 nm.

Blood glucose was estimated using Glucose Enzyme kit from Ranbaxy Laboratories India. This quantitatively estimates D-glucose, the form that is present in blood plasma. Soluble protein was determined by the method of Lowry et al. [42] using BSA as standard.

All values were calculated as means±SEM. The ANOVA test followed by Dunnet Multiple comparison test was employed for statistical comparison.

![Fig. 1. Changes in plasma glucose levels of control, diabetic and diabetic rats after 21 days of treatment. Results are expressed as mean±SEM of five separate experiments. *p<0.05, **p<0.01.](image)

Table 2a
Comparison of body weights of control and different doses of vanadate-treated rats after 21 days of treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control+vanadate</th>
<th>Control+vanadate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>220±16.7</td>
<td>213±12.1</td>
<td>170±7.5*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean±SEM of five separate experiments. 
P values are shown as *p<0.05 vs. control.
Table 2b
Comparison of plasma glucose levels of diabetic and different doses of vanadate-treated diabetic rats after 21 days of treatment

<table>
<thead>
<tr>
<th></th>
<th>Diabetic</th>
<th>Diabetic+vanadate (0.2 mg/ml)</th>
<th>Diabetic+vanadate (0.6 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>440±15.5</td>
<td>380±25.7</td>
<td>111±7.2</td>
</tr>
</tbody>
</table>

Each value is the mean± SEM of 5 separate experiments. P values are shown as: °p<0.01, °p<0.05 vs. control.

between control and various groups. Significance was considered at p < 0.05.

3. Results

The changes in general parameters like body weight, tissue weight, blood glucose and protein as observed in all the experimental groups and controls are summarized in Table 1. Body weights were significantly lower in the diabetic group (p < 0.01). Vanadate treatment could not improve the weight loss, when compared to the control where as insulin, Trigonella and Trigonella and vanadate in combination resulted in significant increase in body weights as compared to the diabetic rats.

Liver weight of the diabetic rats decreased in comparison to the controls; although the same being compared on a functional basis as liver weight/100 g body weight did not show any significant difference between the control, diabetic and various treatment groups. On the other hand there was an increase in kidney weight of diabetics as compared to the control and the difference in the weights was even more significant when expressed as per 100 g body weight (p<0.01). Rats receiving vanadate, TSP and the two in combination showed the reversal to near normal values.

Alloxan diabetic rats showed a five-fold increase in plasma glucose concentration when compared to control rats, the levels of which were brought down in all the treated rats. Trigonella treatment, however, resulted in a partial revival of normoglycemia (p<0.05). As evident from the given table, combined dose of vanadate and trigonella was more effective than vanadate and trigonella alone in lowering hyperglycemia in the diabetic rats (Fig. 1).

There was no significant change in the protein content of control, diabetic and diabetic rats receiving various treatments. All the enzyme activities are expressed as per milligram protein and, therefore, represent true changes under these conditions.

Table 2a shows the changes in the body weight of control rats after 3 weeks of treatment with vanadate (0.2 and 0.6 mg/ml). As is evident there is no significant change in the body weight of vanadate (0.2 mg/ml) treated rats where as 0.6 mg/ml treated rats showed a significant decrease of body weight (p<0.05).

Fig. 2. Change in the activity of PK in liver and kidney of control, diabetic and diabetic rats after 21 days of treatment. Results are expressed as mean ± SEM of five separate experiments. **p<0.01, *p<0.05.
Changes in plasma glucose of diabetic rats after treatments of vanadate (0.2 and 0.6 mg/ml) are shown in Table 2b. The 0.2 mg/ml vanadate treatment failed to correct hyperglycemia whereas treatment with 0.6 mg/ml vanadate significantly decreases hyperglycemia ($p < 0.01$).

4. PK and PEPCK activities

Since vanadate, TSP and combined dose of vanadate and TSP were able to normalize the blood glucose levels when administered to diabetic rats, a possible mechanism to explain this action might involve increase in the glycolytic flux and a concomitant decrease in the gluconeogenesis. To gain an insight into the status of glycolytic and gluconeogenic flux the activities of key regulatory enzymes of the two pathways were assayed in control and various experimental groups. The activity of PK in liver and kidney are presented in Fig. 2. PK activity decreases markedly in the liver ($p < 0.01$) and increases significantly in the kidney of alloxan-diabetic rats ($p < 0.01$). Fig. 3 represents the activity of PEPCK in liver and kidney of control, diabetic and diabetic rats receiving various treatments. PEPCK levels increased
markedly in liver \((p<0.01)\) and kidney \((p<0.01)\) of diabetic rats respectively. \(D+I, D+V, D+T\) and \(D+T+V\) groups showed PK and PEPCK values being restored close to control values. \(D+T+V\) was the most effective of all the treatments.

5. Antioxidant enzymes

In diabetes, the persistence of hyperglycemia has been reported to cause increased production of oxygen free radicals through autooxidation and non-enzymatic glycation. If the diabetic state is associated with a generalized increase in tissue oxidative stress it might well be reflected in the changes in the tissue antioxidant system. Therefore, the activities of some major antioxidant enzymes were measured in control and experimental rats. As is evident from Fig. 4, SOD activity shows a significant reduction in diabetic liver \((p<0.01)\), which agrees well with the earlier published data [10]. Similarly kidney shows a lower activity in the diabetic state \((p<0.01)\) as shown in Fig. 4. Fig. 5 shows CAT activity decreasing in liver \((p<0.01)\) and kidney \((p<0.01)\). GPx activity decreases significantly in liver \((p<0.01)\) and increases in kidney in the diabetic state (Fig. 6). As shown in Fig. 7 GR activity decreases significantly in liver \((p<0.01)\) and shows a small increase in the kidney of diabetic rats.

Treatment with Insulin, vanadate, TSP and vanadate and TSP in combination reversed the status of these enzymes to normal values. The enzyme activi-
ties in the D + T + V group of rats were near to control values for SOD, CAT, GPx and GR.

6. Discussion

The present results showed that 3 weeks of treatment with insulin, vanadate, Trigonella and vanadate and Trigonella in combination result in a marked reduction in hyperglycemia in the diabetic rats. The combined treatment was more effective than the other treatments. It was worth noting that combined treatment did not result in weight loss, which has been observed in the vanadate-treated rats in the present work and in earlier studies [43]. Therefore, after showing the improvement in glucose homeostasis in the treated animals the metabolic consequence of this treatment in liver and kidney were further examined.

Glycolysis and gluconeogenesis are two important pathways of carbohydrate metabolism. Hyperglycemia is the result of a decreased utilization (glycolysis) and increased production of glucose (gluconeogenesis) by liver and kidney [1] indicating that these two pathways are altered in diabetes. PK is a key regulatory enzyme of glycolytic pathway. Liver and kidney are two main sites of glucose production through gluconeogenesis. PEPCK is a key regulatory enzyme of the gluconeogenic pathway. We observed a significant decrease in the activity of PK in liver and a moderate increase in kidney of alloxan diabetic rats. PEPCK levels were markedly elevated in liver and kidney in the diabetic condition. We have therefore used these two enzymes as parameters to evaluate the antidiabetic effect of vanadate and Trigonella, given separately and in combination.

Oxygen derived free radicals are generated in aerobic organisms during physiological or pathophysiological oxidative metabolism of mitochondria. Free radicals may react with a variety of biomolecules including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue thereby interfering with cell function. Under normal physiological condition there is a critical balance in the generation of oxygen free radical and its antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity [44]. Impairment in the oxidant/antioxidant equilibrium in favor of the former provokes a situation of oxidative stress and generally results from hyperproduction of reactive oxygen species. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in wide spectrum of human diseases [45]. In vitro and in vivo studies have shown that in a variety of tissues, hyperglycemia and possibly elevated free fatty acid levels result in the generation of oxygen free radicals and considerably increase oxidative stress [46]. Several reports have shown the alterations of the antioxidant enzymes, in the diabetic condition. [47–49]. Thus various tissues in the diabetic state are more prone to oxidative damage and could result in various complications in long term diabetes implying that the restoration of
antioxidant status is an important parameter to evaluate the effect of an antidiabetic compound. We have selected major antioxidant enzymes CAT, SOD, GPx and GR to evaluate the antioxidant status in liver and kidney using the combined therapy of vanadate and Trigonella. Antioxidant enzymes SOD and CAT showed a significant decrease in liver and kidney whereas GPx and GR decreased in liver and increased in kidney. This is in agreement with the earlier published data [47,49].

Treatment with insulin, vanadate, Trigonella and the combined dose of vanadate and Trigonella corrected the altered levels PK, PEPCK, SOD, CAT, GPx and GR in liver and kidney of diabetic rats. Trigonella treatment partially normalized hyperglycemia 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 treatment was most effective in correcting hyperglycemia and rectifying the disturbed levels of the enzymes studied. Low doses of vanadate (0.2 mg/ml) alone did not result in weight loss when given to control rats but when administered to diabetic rats it was not effective in reviving normoglycemia (Table 2a,b). In the present study, therefore, the dose of vanadate has been reduced (thereby its toxicity) without compromising with its antidiabetic potential by combining it with Trigonella.

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References


In vivo effect of Fenugreek on pyruvate kinase, phosphoenolpyruvate carboxykinase and Hepatocyte Nuclear Factor-4 (HNF-4α) levels in alloxan diabetic rat liver.

School of Life Sciences, Jawaharlal Nehru University, Delhi, India.

Background and Aims: A balance between glucose utilization and its production regulates blood glucose levels. All the tissues utilize glucose but liver is the main site of glucose production. Glucose utilization decreases where as glucose production increases significantly in the liver of alloxan-diabetic rats thereby increasing the blood glucose levels. Pyruvate kinase (PK) and Phosphoenolpyruvate carboxykinase (PEPCK) are key regulatory enzymes of glycolytic and gluconeogenic pathways respectively. Total and active PK shows a marked decrease where as PEPCK increases in liver of diabetic rats. Hepatocyte Nuclear Factor-4 (HNF-4alpha) is an important transcription factor found in liver and other tissues. It has been shown to regulate the expression of a host of enzymes involved in carbohydrate and lipid metabolism including PK and PEPCK. HNF-4 alpha levels go up significantly in the diabetic state. Our aim was to use the above parameters to evaluate the antidiabetic effect of Fenugreek.

Materials and Methods: Female Wistar rats (180-220g) were used for the experiments. Diabetic rats were obtained by injecting a single subcutaneous injection of alloxan monohydrate (15mg per 100g body weight) 0.154M Na acetate, pH 4.5. Alloxan treated rats were given 2 Units of insulin for sevendays. Diabetic rats were treated separately with Insulin and Fenugreek powder (5% in feed) for 21days and one group was left untreated. Rats were sacrificed and liver was excised and frozen in liquid nitrogen. Blood was taken in a separate tube for glucose estimation. 1g of liver was homogenized and centrifuged to collect the cytosolic fraction to estimate the activity of PK and PEPCK. PK and PEPCK were estimated by spectrophotometric method. HNF-4α protein levels were checked by Western transfer.

Results: Fasting Blood Glucose levels in the Alloxan-diabetic rats were 450 ±35mg/dl after one month of alloxan injection as compared to control rats (75± 10 mg/dl). Insulin treated diabetic rats had fasting blood glucose level of 100± 15 mg/dl. The values for the Fenugreek treated diabetic rats were 120± 12 mg/dl. Active PK levels went down by 30% where as total PK levels were decreased by 20% in the liver of diabetic rats. Insulin treatment restored the PK levels to 95% of the control values. The PK levels in the Fenugreek treated diabetic rats were 90% of the control values. PEPCK levels go up by 70% in the liver of diabetic rats. Insulin and Fenugreek treated rats had these values lowered by 50% and 40% respectively. HNF-4α levels show a substantial increase in the diabetic condition. Insulin and Fenugreek treated rats had these levels close to control rats.

Conclusion: Fenugreek seed powder when given to diabetic rats not only controls the elevated blood glucose levels but also corrects the altered levels of important enzymes of the carbohydrate metabolism in the liver. Fenugreek treated rats had PK, PEPCK and HNF-4α levels close to control values. Our observation suggests that Fenugreek seeds have the potential of being used as an antidiabetic therapy. Further studies are needed to purify the compound(s) responsible for the antidiabetic effect and its mechanism of action has to be elucidated.