Efficacy of lower doses of vanadium in restoring altered glucose metabolism and antioxidant status in diabetic rat lenses

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Vanadium compounds are potent in controlling elevated blood glucose levels in experimentally induced diabetes. However the toxicity associated with vanadium limits its role as therapeutic agent for diabetic treatment. A vanadium compound sodium orthovanadate (SOV) was given to alloxan-induced diabetic Wistar rats in lower doses in combination with *Trigonella foenum graecum*, a well-known hypoglycemic agent used in traditional Indian medicines. The effect of this combination was studied on lens morphology and glucose metabolism in diabetic rats. Lens, an insulin-independent tissue, was found severely affected in diabetes showing visual signs of cataract. Alterations in the activities of glucose metabolizing enzymes (hexokinase, aldose reductase, sorbitol dehydrogenase, glucose-6-phosphate dehydrogenase) and antioxidant enzymes (glutathione peroxidase, glutathione reductase) besides the levels of related metabolites, [sorbitol, fructose, glucose, thiobarbituric acid reactive species (TBARS) and reduced glutathione (GSH)] were observed in the lenses from diabetic rats and diabetic rats treated with insulin (2 IU/day), SOV (0·6 mg/ml), *T. f. graecum* seed powder (TSP, 5%) and TSP (5%) in combination with lowered dose of vanadium SOV (0·2 mg/ml), for a period of 3 weeks. The activity of the enzymes, hexokinase, aldose reductase and sorbitol dehydrogenase was significantly increased whereas the activity of glucose-6-phosphate dehydrogenase, glutathione peroxidase and glutathione reductase decreased significantly in lenses from 3 week diabetic rats. Significant increase in accumulation of metabolites, sorbitol, fructose, glucose was found in diabetic lenses. TBARS measure of peroxidation increased whereas the levels of antioxidant GSH decreased significantly in diabetic condition. Insulin restored the levels of altered enzyme activities and metabolites almost to control levels. Sodium orthovanadate (0·6 mg/ml) and *Trigonella* administered separately to diabetic animals could partially reverse the diabetic changes, metabolic and morphological, while vanadate in lowered dose in combination with *Trigonella* was found to be the most effective in restoring the altered lens metabolism and morphological appearance in diabetes. It may be concluded that vanadate at lowered doses administered in combination with *Trigonella* was the most effective in controlling the altered glucose metabolism and antioxidant status in diabetic lenses, these being significant factors involved in the development of diabetic complications, that reflects in the reduced lens opacity.

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1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion/action, or both. The chronic hyperglycemia of diabetes is associated with long-term dysfunction and damage to various organs, especially the tissues requiring insulin for glucose uptake (The Expert Committee on the Diagnosis and Classification of Diabatetes Mellitus 2003).

Keywords. Antioxidant enzymes; diabetes; glucose metabolism; lens; polyol pathway; *Trigonella foenum graecum*; vanadate

Abbreviations used: AR, Aldose reductase; G6PDH, glucose-6-phosphate dehydrogenase; GR, glutathione reductase; GPx, glutathione peroxidase; GSH, glutathione; SDH, sorbitol dehydrogenase; TBARS, thiobarbituric acid reactive species; SOV, sodium orthovanadate; TSP, *Trigonella foenum graecum* seed powder.

Lens is one such tissue which is worst affected in diabetes. Glucose is transported into the lens via facilitated transport (Kuck 1962; Levari *et al* 1961) independent of insulin hence, in diabetes glucose over utilization occurs in the lens (Gonzalez *et al* 1978). The primary source of energy production in lens is the glycolytic pathway (Chylack and Cheng 1978). However in elevated glucose concentrations excessive glucose is fluxed through different metabolic pathways like polyol pathway and pentose posphate pathway.

The polyol pathway comprising of enzymes aldose reductase and sorbitol dehydrogenase catalyzing the conversion of excessive glucose to sorbitol and sorbitol to fructose respectively is activated in diabetic lens (Lee and Chung 1999). This accumulation of sorbitol and fructose owing to their failed diffusion out of the lenticular cells is held responsible for increased oxidative stress triggering a series of events that finally culminate in the development of cataract in long-term diabetes (Chung *et al* 2003; Varma *et al* 1979). Tissue antioxidant status is also altered in diabetes resulting in increased oxidative damage and tissue injury which is stimulated by the generation of free radicals (Genet *et al* 2002; Saxena *et al* 1993; Lou 2003). Activity of important biological antioxidant enzymes, glutathione peroxidase and glutathione reductase, that directly scavenge free radicals or prevent their conversion to toxic products (Freeman and Crapo 1982), is also altered in diabetic condition (Wohaieb and Godin 1987; Genet *et al* 2002).

To prevent the development of diabetic complications the different metabolic derangements occurring in diabetes needs to be controlled. Many transition elements have been studied and found effective in controlling the altered glucose homeostasis in diabetes (Wolff 1993). Vanadium is one such nutritional trace element that has been found to be very effective in reducing blood glucose thereby generating normoglycemia (Willsky *et al* 2001; Meyerovitch *et al* 1987). It is known to act as a potent insulin mimetic agent by increasing glucose transport and metabolism in skeletal muscle, liver and adipose tissue (Pugazhenthi and Khandelwal 1990; Heylinger *et al* 1985; Shechter 1990). However studies have also shown its adverse effects like rhinitis, pharyngitis, chronic productive cough in human and severe diarrhea, dehydration and death in experimental diabetic rats (Roshchin *et al* 1980; Domingo *et al* 1995). Therefore the toxicity associated with vanadium limits its role as a therapeutic agent for the treatment of diabetes (Domingo 2002). Vanadium at lower doses in combination with *Trigonella foenum graecum*, commonly called fenugreek, an Indian herb has been found to show almost complete reversal of the raised blood glucose levels and altered metabolic changes in experimental diabetes in tissues like kidney (Raju *et al* 2001; Baquer *et al* 1998). The purified hypoglycemic compounds from *Trigonella* has been shown to augment insulin release and modulate key enzymes of glycolysis, gluconeogenesis and lipogenesis, detailed mechanism of action is however still not fully understood (Moorthy *et al* 1989; Puri *et al* 1994).

The powdered seeds of *Trigonella* have been used as a hypoglycemic agent in alloxan diabetic rats and even also in type I and type II diabetic patients earlier (Gupta *et al* 2001; Grover *et al* 2002). *Trigonella* seeds are known to be rich in fibers and saponins, both purported to have beneficial effects in the treatment of diabetes (Puri *et al* 1994; Sauvaire *et al* 1996). So far the use of vanadium and *Trigonella* have been studied in liver and kidney only in diabetes (Raju *et al* 2001; Genet *et al* 2002). In the present study an attempt has been made to study the effect of vanadate and *Trigonella* given alone as well as in a combination to diabetic animals to see its efficacy in restoring the metabolic alterations and morphological changes in lens due to sustained hyperglycemia.

2. Materials and methods

2.1 *Animals*

Adult female albino rats of Wistar strain, aged about three months, weighing between 205–215 g, were used in all the experiments. Rats were kept in climate-controlled room in the animal house facility of Jawaharlal Nehru University at a maintained temperature and relative humidity at 22°C and 55% respectively. The animals were given standard chow (Hindustan Lever Ltd.) and tap water *ad libitum*. All institutional guidelines of the Institutional Animal Ethics Committee of Jawaharlal Nehru University were adhered to throughout the experiments.

2.2 *Induction of diabetes and study design*

In each set of experiment about 80 rats were starved for 24 h and divided into control and experimental groups. Diabetes was induced as described by Sochor *et al* (1988), by injecting alloxan monohydrate subcutaneously (15 mg/100 g body wt.) freshly prepared in 0·154 M sodium acetate buffer (pH 4·5). From next day alloxan-treated experimental rats were given 2 IU of zinc-insulin i.p. daily for the next seven days. This treatment reduces the mortality rate in the diabetic animals. The severity of diabetes was checked in alloxan diabetic rats using urine glucose detection strips (Diastix, Bayer Diagnostic, India).

2.3 *Treatment with antidiabetic compounds*

Insulin was then withdrawn and diabetic rats randomly divided into five groups: Diabetic (D), diabetic treated with insulin (D + I = injected 2 IU/day of insulin), vana-

date (D + V = administrated 0·6 mg/ml sodium orthovanadate in drinking water), *Trigonella* [D + T = fed 5% *Trigonella* seed powder in the standard food pellets *ad libitum* (Raju *et al* 2001)] and with both *Trigonella* and vanadate (D + V + T = given 5% *Trigonella* in the standard food pellets and 0·2 mg/ml sodium orthovanadate in drinking water until sacrifice). To reduce the initial mortality in D + V group rats were administered sodium orthovanadate at a dose of 0·2 mg/ml of 80 mM NaCl for initial 3 days followed by 0·4 mg/ml for next 3 days followed by the dose of 0·6 mg/ml till the day of sacrifice as described earlier (Saxena *et al* 1993). Rats were sacrificed after 3 weeks of treatment.

2.4 *Preparation of homogenates for enzyme and metabolite assays*

The control and treated rats were sacrificed by cervical dislocation and lenses were excised instantaneously, washed with normal saline, blotted dry and weighed. Of the two lenses per animal one was used for enzyme assay and other for metabolites. For metabolites the lenses were frozen immediately in liquid N_2 and kept at 80°C to be used later. For enzyme assays lens homogenates were prepared in 0·25 M sucrose, 0·02 M triethanolamine buffer, pH 7·4 containing 0·12 mM dithiothreitol using a Potter Elvehjem Homogenizer fitted with a Teflon plunger. Homogenates were then centrifuged at 1000 *g* for 10 min to remove nuclei and cell debris. The pellet was discarded and the supernatant was further centrifuged at 12,000 *g* for 20 min at 40°C for 30 min. The supernatants were used for all the enzyme assays.

For metabolite assays the frozen lenses were homogenized in 9 volumes of 1 N perchloric acid. The homogenates were centrifuged at 6000 *g* for 10 min. The supernatants were neutralized with 2 N KOH and centrifuged at 1200 *g* for 10 min to remove KClO4 resulting in clear extracts, these clear extracts were used for all metabolite determination.

2.5 *Estimation of enzyme activities*

Aldose reductase (EC 1·1·1·21) activity in the cytosolic fraction was estimated in a Beckman DU-68 spectrophotometer by the methods of Kinoshita *et al* (1963). The reaction mixture contained the following in the final concentration: 50 mM phosphate buffer (pH 7·4), 300 mM glucose and 0·2 mM NADPH in the final volume of 1·0 ml. The reaction was initiated by adding 0·1 ml of cytosol, containing ~ 0·4 to 0·6 mg protein.

Sorbitol dehydrogenase (EC 1·1·1·14) was measured by the method of Gerlach and Hiby (1974). The reaction mixture contained the following in the final concentration of: 0·107 M Triethanolamine buffer (pH 7·4), 300 mM fructose and 0·2 mM NADH (alkaline pH) in the final volume of 1·5 ml. The reaction was initiated by the addition of 0·2 ml of cytosol, containing ~ 0·8 to 1·0 mg protein.

Hexokinase (EC 2·7·1·1) was measured by the method of Gumaa and McLean (1972). The reaction mixture contained the following in final concentration of: 0·1 M Tris-HCl (pH 7·4), 8 mM $MgCl_2$ (pH 7·0), 0·4 mM $NADP^+$, 8 mM/2 mM of ATP/Mg^{2+} (pH 7·2), 0·5 mM glucose and one unit of purified glucose-6-phosphate dehydrogenase. The reaction was initiated by addition of 0·1 ml of cytosol, containing ~ 0·4 to 0·6 mg protein. The reduction of NADP at 340 nm was followed for 5 min as a measure of enzyme activity.

Glucose-6-phosphate dehydrogenase (EC 1·1·1·49) was assayed by the method of Baquer *et al* (1973). The reaction mixture contained the following in the final concentrations: 10 mM Tris-HCL (pH 7·8), 0·25 mM D-glucose-6-phosphate, 0·25 mM $MgCl_2$ in a total reaction volume of 1·0 ml. The reaction was initiated by the addition of cytosol containing 0·2–0·5 mg of protein. The reduction of NADP at 340 nm was followed for 5 min as a measure of enzyme activity in a Beckman DU68 Spectrophotometer.

Glutathione peroxidase (EC 1·11·1·9) was measured using a coupled enzyme assay system linked with GR as described by Lawrence and Burk (1976). The reaction mixture contained the following in the final concentration of: 10 mM potassium phosphate buffer pH 7·0, 25 mM EDTA, 0·5 mM GSH, 2 mM sodium azide, 1·5 IU GR, 0·1 mM NADPH in the final reaction volume of 1·0 ml 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.

Glutathione reductase (EC 1·6·4·2) was measured by the modified method of Erden and Bor (1984). The reaction mixture contained the following in the final concentration: 4·1 mM Tris-HCl, pH 7·5, 15 mM $MgCl_2$, 5·7 mM EDTA, 60 mM KCl, 2·6 IU GSSG and 0·2 mM of NADPH in final reaction volume of 1 ml. 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.

2.6 *Enzyme units*

One enzyme unit is defined as the oxidation/reduction of one μmol of NADH or NADPH per gram fresh lens weight per minute at 25°C.

2.7 *Determination of metabolites*

Glucose was measured as described by Bergmeyer *et al* (1974) using a coupled assay system linked to hexokinase and glucose-6 phosphate dehydrogenase (G6PDH). The

assay mixture contained the following in final concentration: 100 mM Tris HCl, pH 7·8, 0·26 mM $NADP^+$, 8·0 mm ATP, 5·0 Mm $MgCl_2$ and appropriate sample volume.The reaction was initiated by adding one unit of purified yeast G6PDH.

Fructose were estimated as described by Bergmeyer *et al* (1974) using coupled assay system linked to hexokinase, phosphoglucose isomerase and G6PDH on Beckman DU-68 spectrophotometer. The reaction mixture contained the following in final concentration of 0·25 M triethanolamine, pH 7·5, 2·5 mM $MgSO_4$, 1·1 mM ATP, 0·8 Mm NADP and appropriate sample volume. The reaction was initiated by adding one unit of purified yeast G6PDH.

Sorbitol was measured by modified enzymatic method of Malone *et al* (1980) on Varian Cary (Eclipse) Flourescence spectrophotometer. The reaction mixture contained the following in a final concentration of: 0·05 M glycine buffer, pH 9·4, 0·2 mM NAD^+ and an appropriate amount of sample volume in a final volume of 3 ml. The reaction was initiated by adding 1 U of sorbitol dehydrogenase.

For all the above metabolites measurement the reduction of NAD^+ and $NADP^+$ or oxidation of NADH or NADPH was measured at 340 nm.

Reduced glutathione (GSH) was measured by the method of Griffith (1980). The reaction mixture contained the following in a final concentration of: 0·20 mM NADPH, 0·6 mM 5,5′-dithiobis-(2-nitrobenzoic acid, 0·5 U glutathione reductase in 125 mM sodium phosphate buffer (pH 7·5), 6·3 mM EDTA and appropriate sample volume. The rate of reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) was measured at 412 nm.

Lipid peroxidation was measured as thiobarbituric acid reactive species (TBARS) concentration as a measure for malondialdehyde by the method of Yagi (1984). The reaction mixture contained the following in a final concentration of: 5 mM potassium phosphate buffer, pH 7·4, 1 trichloroacetic acid, 0·1% thiobarbituric acid and appropriate sample volume. Absorbance was measured at 532 nm against blank using a Beckman DU-68 spectrophotometer.

2.8 *Blood glucose and protein estimation*

Blood glucose was estimated in the plasma using glucose measuring kit utilizing glucose oxidase method from Ranbaxy Laboratories Ltd., India. This quantitatively estimates D-glucose, the form that is present in blood plasma. Soluble proteins in lens extracts were determined by the method of Lowry *et al* (1951) using BSA as standard. Glycosylated haemoglobin was measured by the method of Raheja *et al* (1981).

2.9 *Morphological study*

The lenses from the rats of different experimental groups were excised washed in saline and photographed with Minolta Digital camera for the detection of opacity in the lenses.

2.10 *Statistical analysis*

Results are presented as mean ± SEM of four or five values from separate experiments. Statistical difference between control and various groups was determined by one-way analysis of variance (ANOVA) followed by Dunnet multiple comparison tests. *P* values less than 0·05 were considered significant.

2.11 *Chemicals*

All purified enzymes, coenzymes, substrates, standards and buffers were purchased from Sigma Chemicals Company, USA. All other chemicals used were of analytical grade and purchased from SRL and Qualigens, India.

3. Results

3.1 *Changes in physiological parameters*

The experimental animals were monitored for changes in the physiological parameters throughout the study. The alloxan-induced diabetic group showed a significant ($P < 0·01$) 4-fold increase in plasma glucose concentration when compared to control; this increase was nearly normalized in all the treatment group of rats. Combined dose of vanadate and *Trigonella* was more effective than vanadate and *Trigonella* alone in lowering alloxan induced hyperglycemia in the diabetic rats as shown in figure 1. The results are consistent with our previous studies (Raju *et al* 2001).

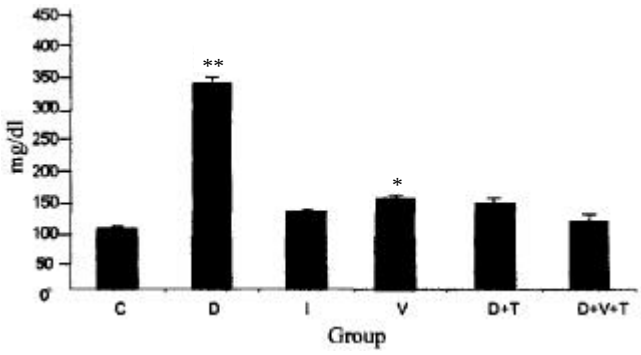

Figure 1. Blood glucose levels of control (C), diabetic (D) and diabetic rats treated for 21 days with insulin (D + I), vanadate (D + V), *Trigonella* (D + T) and *Trigonella* and vanadate (D + V + T) combination after 3 weeks of diabetes induction. Results are expressed as mean ± SEM of values from five separate experiments. *$P < 0·05$; **$P < 0·01$. The significant comparisons of each experimental group shown are with control value.

Changes in the general parameters like body weight, lens weight, glycosylated haemoglobin, fluid and antidiabetic agent intake are summarized in table 1. Body weights of the diabetic group ($P < 0{\cdot}01$) were significantly lowered at the end of the study. Vanadate (0·6 mg/ml) treatment was found to cause a reduction in body weight gain but at a dose 0·2 mg/ml no significant change in body weight of control rats was observed (table 2a) as shown in earlier studies (Saxena *et al* 1993). A significant weight gain in diabetic treated animals was observed when vanadate was administered in combination with *Trigonella*.

The glycosylated haemoglobin which can be taken as marker for the glycosylation of proteins was found significantly increased ($P < 0{\cdot}01$) in diabetic rats. The different treatments given to the diabetic animals prevented the glycosylation of haemoglobin. A decrease in lens weight was observed in the diabetic animals when compared to the controls, which was reversed significantly with vanadate treatment (table 1). Alloxan diabetes induces polydipsia, significantly ($P < 0{\cdot}01$) increasing water consumption by diabetic rats which is reduced significantly on treatment with vanadate and *Trigonella* separately as well as in combination. The total lenticular protein levels were found to be decreased in diabetic group in comparison to control. The absolute intake of *Trigonella* in the *Trigonella* treated groups did not show significant difference (table 2b).

As can be seen from figure 2 the combined treatment of the diabetic animals prevented the development of opacity in the lenses.

3.2 *Changes in the enzyme activity*

To show functional aberrations in the lens changes in glucose metabolizing enzymes of glycolysis, pentose phosphate pathway (PPP) and antioxidant enzymes in the

Table 1. General parameters of control (C), diabetic (D) and diabetic treated with insulin (D + I), vanadate (D + V), *Trigonella* (D + T) and combination of *Trigonella* and vanadate (D + V + T) after 3 weeks of diabetes induction.

Groups	Body weight (g)		Glycosylated Hb	Lens (2) wt. (mg)	Lens proteins (mg/g wet tissue wt.)	Fluid intake ml/day/rat
	Initial	Final				
Control	202 ± 5	217 ± 9	4·5 ± 0·51	88 ± 1·6	357 ± 3·9	35 ± 7·2
Diabetic	198 ± 6	154 ± 4**	7·2 ± 0·65**	66 ± 0·23	320 ± 2·7*	215 ± 21**
Diabetic + insulin	200 ± 9	185 ± 10	5·6 ± 0·19	74 ± 0·28	352 ± 3·0	98 ± 19
Diabetic + vanadate	203 ± 7	175 ± 8*	5·2 ± 0·2*	78 ± 2·06	340 ± 4	60 ± 8·4
Diabetic+ *Trigonella*	202 ± 3	193 ± 11	5·1 ± 0·16	78 ± 1·4	347 ± 3·4	40 ± 3·9
Diabetic + vanadate + *Trigonella*	210 ± 6	202 ± 11	5·4 ± 0·29	86 ± 3·2	351 ± 3·5	39 ± 4·2

Results are expressed as mean ± SEM of five values from separate experiments.
*$P < 0{\cdot}05$; **$P < 0{\cdot}01$. The significant comparisons of each experimental group shown are with the control value.

Table 2. (**a**) Comparison of the body weights of the control and the control treated with different doses of vanadium.

	Control	Control + vanadate (0·2 mg/ml)	Control + vanadate (0·6 mg/ml)
Body weight (g)	220 ± 16·7	213 ± 12·1	170 ± 7·5

Results are expressed as mean ± SEM of five values from separate experiments.

(**b**) Total intake of SOV and *Trogonella* seed powder after 21 days of administration.

	SOV (mg/rat/day)	*Trigonella* seed powder (g/rat/day)
Diabetic + vanadate	29·6 ± 2·3	–
Diabetic + *Trigonella*	–	1·18 ± 0·1
Diabetic + vanadate + *Trigonella*	12·5 ± 1·2	1·26 ± 0·1

Results are expressed as means ± SEM of five values from separate experiments.

different treatment groups of diabetic animals are presented in tables 3 and 4.

3.2a *Hexokinase and G6PDH*: The activity of hexokinase, the rate limiting enzyme of glycolysis was found to be increased significantly ($P < 0{\cdot}05$) by 65% in lenses from diabetic group as compared to the control potentiating increased glucose flux in the absence of insulin through glycolysis. Insulin treatment to diabetic animals lowered the enzyme activity by about 27%. Vanadate at a higher dose of 0·6 mg/ml in drinking water could not effectively lower the enzyme activity but when was given at a lower dose in combination with *Trigonella* partially ($P < 0{\cdot}05$) lowered the increased enzyme activity in the diabetic animals. The activity of G-6-PDH, the key enzyme of pentose phosphate shunt generating redox potential for the cell in the form of NADPH, was reduced in diabetic animals. It was normalized almost near to control levels with the combination therapy of *Trigonella* and lowered vanadium (table 3).

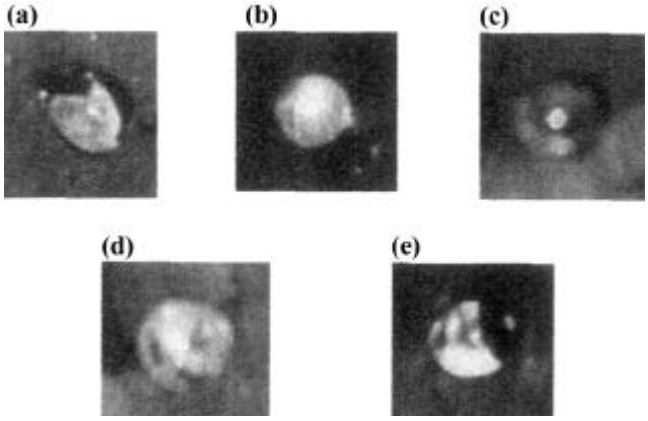

Figure 2. Photomicrographs of lenses from the control (**a**), diabetic (**b**) and diabetic treated with *Trigonella* (**c**), with vanadate (**d**), and with the combination of vanadate and *Trigonella* (**e**).

3.2b *Aldose reductase and sorbitol dehydrogenase*: The activities of polyol pathway enzymes, aldose reductase (AR) and sorbitol dehydrogenase (SDH) showed a significant ($P < 0{\cdot}05$) elevation in the diabetic animals when compared with the controls. Insulin treatment to the diabetic animals lowered the AR activity by 12% in diabetic animals bringing it comparable to control values. Vanadate given at a dose of 0·6 mg/ml caused an insignificant decline in AR activity. *Trigonella* significantly ($P < 0{\cdot}05$) reduced the enzyme activity in diabetic lenses whereas vanadate at a dose of 0·2 mg/ml in combination with *Trigonella* brought down the increased activity significantly ($P < 0{\cdot}01$) when compared with the diabetic animals. The SDH activity was normalized to varying levels with different treatments, results obtained however were not significant, vanadate 0·6 mg/ml ($P < 0{\cdot}05$), *Trigonella* ($P < 0{\cdot}05$) and the combination of *Trigonella* and vanadate by 18% in the diabetic animals when compared with the control (table 4).

3.2c *Glutathione reductase and glutathione peroxidase*: The activity of antioxidant enzyme glutathione reductase

Table 3. Effect of the different doses of vanadate and *Trigonella* on the activities of rate limiting enzymes of the glycolysis and pentose phosphate shunt along with antioxidant enzymes and the related metabolites in the lens of 3 week diabetic rats.

Groups	Control	Diabetic	Diabetic + insulin	Diabetic + vanadate	Diabetic + *Trigonella*	Diabetic + vanadate + *Trigonella*
Enzymes						
Glucose metabolizing						
Hexokinase	0·14 ± 0·015	0·23 ± 0·027*	0·17 ± ·016	0·19 ± 0·015	0·16 ± 0·012	0·15 ± 0·029
G-6PDH	0·46 ± 0·045	0·37 ± 0·015*	0·41 ± 0·031	0·39 ± 0·033	0·38 ± 0·029	0·42 ± 0·044
Antioxidant						
Glutathione reductase	1·1 ± 0·01	0·85 ± 0·017*	0·98 ± 0·027	0·91 ± 0·02*	0·93 ± 0·036	1·0 ± 0·11
Glutathione peroxidase	5·8 ± 0·23	3·8 ± 0·18*	5·1 ± 0·09	4·8 ± 0·1	5·0 ± 0·06	5·3 ± 0·2
Related metabolites						
Glucose (µmol/g)	1·02 ± 0·04	8·11 ± 0·07**	2·1 ± 0·04	3·6 ± 0·07	3·75 ± 0·12	2·12 ± ·11
GSH (µmol/g)	0·15 ± 0·008	0·09 ± 0·007*	0·13 ± 0·013	0·12 ± 0·011	0·12 ± 0·013	0·14 ± 0·09
TBARS (µmol/g)	0·13 ± 0·006	0·25 ± 0·027**	0·16 ± 0·019	0·18 ± 0·018	0·20 ± 0·02	0·15 ± 0·025

Results are expressed as mean ± SEM of five values from separate experiments.
*$P < 0{\cdot}05$; **$P < 0{\cdot}01$. The significant comparisons of each experimental group shown are with the control value. The enzyme activities are expressed as µ/g lens wt.
TBARS, Thiobarbituric acid reactive species; GSH, reduced glutathione.

(GR) decreased significantly ($P < 0{\cdot}01$) in diabetic animals as compared with the control. On the other hand the glutathione peroxidase (GPx) activity was found to be decreased significantly ($P < 0{\cdot}05$) in diabetic animals. The GR activity was increased to normal levels with the different treatments namely insulin, *Trigonella* and vanadate ($P < 0{\cdot}05$). When compared with control animals, a total reversal was observed in a combination of D + V + T group ($P < 0{\cdot}01$) when compared with diabetic rats. The GPx activity was also reversed by the different treatments (table 3).

3.3 *Changes in metabolite levels*

The changes in levels of metabolites sorbitol, fructose, glucose, TBARS and reduced glutathione are shown along with the enzymes in tables 3 and 4. A 40-fold increase in sorbitol levels was observed in the lens of diabetic animals. The different treatments given to the diabetic animals could not completely reverse the sorbitol levels to control values but lowered them significantly ($P < 0{\cdot}01$) in comparison to diabetic animals between the combined treatment of vanadate (0·6 mg/ml) and *Trigonella* then of vanadate and *Trigonella* given alone even at higher dose of 0·6 mg/ml. The fructose accumulation in diabetic lenses was significant ($P < 0{\cdot}01$) being more than 10-fold than that of control animal lenses. The various antidiabetic treatments given to diabetic animals significantly lowered the fructose levels but could not completely reverse them even with vanadate and *Trigonella* combination, this latter treatment showed maximum reversal near to the control group.

Table 3 show changes in the lenticular glucose, TBARS and GSH levels. The levels of glucose increased significantly ($P < 0{\cdot}01$) by 7 fold in the diabetic animal lenses as compared to control. This was reversed to normal values in D + I, D + V, D + T groups and D + V + T groups. The levels of GSH decreased in diabetic lens ($P < 0{\cdot}05$) by 40% in diabetic animals. Treatment with insulin, vanadate, *T. f. graecum* seed powder (TSP) and vanadate and TSP in combination reversed the levels of the metabolite to normal values. TBARS levels were found to be increased significantly ($P < 0{\cdot}01$) by 90% in diabetic animals. The different treatments did lower the TBARS levels but only the D + V + T group showed a near complete reversal of TBARS levels ($P < 0{\cdot}01$) as compared to diabetic animals.

4. Discussion

In this study the effect of sodium orthovanadate (SOV) a vanadium compound was studied on the integration of glycolytic route, polyol pathway and pentose phosphate pathway and its role in the development of opacity in the diabetic rat lenses. SOV is known to control the diabetes-induced hyperglycemia (Brichard *et al* 1988). However, the toxicity associated with SOV limits its therapeutic efficacy (Domingo *et al* 1991; Domingo 2002). To reduce vanadium-associated toxicity and maintain its insulin-mimetic anabolic effects, SOV was combined with TSP (5%) (Raju *et al* 2001; Genet *et al* 2002). The different treatments given to the diabetic rats resulted in marked reduction in hyperglycemia. In contrast to the observed significant loss in body weight of the vanadium-treated rats, the combination-treated rats showed reduction in weight loss which was consistent with previous studies (Raju *et al* 2001; Saxena *et al* 1992). Enhanced glucose flux through polyol pathway was observed in diabetic rats. This enhancement manifested itself in the form of enhanced sorbitol and fructose accumulation this increased sorbitol and fructose accumulation has been implicated in diabetes-induced cataract formation (Gonzalez

Table 4. Changes in the activities of the polyol pathway enzymes and the levels of related metabolites in the cytosolic fraction of lens after 3 weeks of treatment.

Groups	Control	Diabetic	Diabetic + insulin	Diabetic + vanadate	Diabetic + *Trigonella*	Diabetic + vanadate + *Trigonella*
Enzymes						
U/g lens wt.						
Aldose reductase	0·15 ± 0·004	0·18 ± 0·003*	0·16 ± 0·005	0·16 ± 0·007	0·15 ± 0·003	0·14 ± 0·004
Sorbitol dehydrogenase	0·31 ± 0·032	0·45 ± 0·029*	0·37 ± 0·024	0·35 ± 0·026	0·40 ± 0·021	0·32 ± 0·028
Related metabolites						
Sorbitol (μmol/g)	0·33 ± 0·05	16·04 ± 0·21**	0·61 ± ·08	1·8 ± 0·02	2·1 ± 0·13	1·08 ± 0·02
Fructose (μmol/g)	0·59 ± 0·02	7·46 ± 0·21**	1·79 ± ·10	3·11 ± 0·18	3·11 ± 0·13	1·48 ± ·19

Results are expressed as mean ± SEM of five values from separate experiments.
*$P < 0{\cdot}05$; **$P < 0{\cdot}01$. The significant comparisons of each experimental group shown are with the control value.

et al 1987; Williamson *et al* 1993; Ramasamy *et al* 1997). Vanadium enhances fructose metabolism through activating ketohexokinase (Adachi 1995). Glycolysis is inhibited in the diabetic lens due to mainly the competition for availability of NAD, which is channelled into the sorbitol pathway in diabetes instead of into glycolysis where it is required for the forward reaction of glyceraldehydes-3-phosphate dehydrogenase (Williamson 1993). This result is consistent with studies of Pirie (1957) where, hexokinase, considered as a pacemaker of the lens increases significantly in alloxan-induced diabetic lens (Gonzalez *et al* 1978). The increase in sorbitol levels within the cell with concomitant decrease in NADPH held responsible for the development of oxidative stress within the lenicular cells leading to cataract formation due to increased apoptosis of these cells (Takamarg *et al* 2003). The amount of sorbitol and fructose within the lens increases with the duration of diabetes, which agrees well with the increased activity of aldose reductase and a decreased activity of sorbitol dehydrogenase shown after the first two weeks of diabetes induction (Gonzalez *et al* 1983; Efimov *et al* 1984). This correlates with the maximal rate of sorbitol and fructose accumulation in the first two weeks of diabetes induction and may be held responsible for the high levels of these metabolites observed in the present study (Gonzalez *et al* 1983; Efimov *et al* 1984). The decrease in G6PDH activity in diabetic animals is observed here. This decrease may be considered as an adaptive phenomenon by which the supply of NADPH for aldose reductase is cut down, results are in consistence with earlier reports (Siddiqui and Rahman 1980). NADPH is mostly utilized by GR, AR and other reductases. The ratio of G6PDH : GR in respect to the change in the activity was found to be same, and hence the synthesis of reduced GSH from GSSG is also decreased in the same proportion in diabetic rats. A relatively high concentration of GSH, a non-enzymatic antioxidant is known to exist in lens tissue under normal conditions and is known to act as a major scavenger of singlet oxygen (Slaughter *et al* 2003). A depletion of GSH observed here and reported earlier (Lou *et al* 1988) in diabetic lens may lead to a decreased defense system but an increase of GPx observed in diabetic lens may be seen as a compensatory mechanism to detoxify the hyperglycemia – induced hydrogen peroxide formation which also results in generation of excess oxidized glutathione (Lou 2003). The depleted levels of glutathione may also be attributed to osmotic compensation (Mitton *et al* 1999). TBARS levels showed a correlation with the levels of GSH (Altomare *et al* 1995).When seen in terms of TBARS: GSH inverse ratio TBARS level increase was found to be double in comparison to a loss of GSH in diabetic lens. Insulin does not stimulate glucose entry into intact lens (Farkas and Patterson 1957) and act indirectly by controlling the altered blood glucose levels in diabetes (Levari *et al* 1961). The treatment with insulin restored the altered levels of enzymes hexokinase, AR, SDH, GPx, GR and metabolites sorbitol, fructose, glucose in the diabetic lens. Whereas vanadate treatment alone could restore only the polyol pathway enzymes and failed to normalize the decreased antioxidative enzymes GPx and GR, may be because vanadate itself has prooxidative activity (Takamarg *et al* 2003). The combined treatment was able to restore the cellular antioxidant capacity evident in the elevated GSH levels. The antioxidative potential of the combined treatment may be ascribed to *Trigonella* which has been shown in earlier studies to increase antioxidants as GSH and beta-carotene and lower lipid peroxidation (Anuradha and Ravikumar 2001). Some unknown constituents of *Trigonella* may be attributed for this reduced prooxidative activity of vanadate in the combination treatment. The increased clarity of the lens in this treatment group can be suggested because of normalized metabolic and antioxidative milieu within the lens (figure 2). The combined treatment holds potential as an antidiabetic agent effectively preventing the development of diabetic complications. This combination can be explored further to develop a therapy, with reduced toxicity and better therapeutic index which provides a controlled normoglycemic state over sustained diabetes.

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References

Adachi K 1995 Effects of vanadate on glucose metabolism in the lens of rats with streptozotocin-induced diabetes-ketohexokinase and aldolase activity; *Nippon Ganka Gakkai Zasshi*. **99** 34–39

Altomare E, Vendemiale G, Grattagliano I, Angelini P, Micelli-Ferrari T and Cardia L 1995 Human diabetic cataract: role of lipid peroxidation; *Diabetes Metabolism* **21** 173–179

Anuradha C V and Ravikumar P 2001 Restoration on tissue antioxidants by fenugreek seeds (*Trigonella foenum graecum*) inalloxan-diabetic rats; *Indian J. Physiol. Pharmacol.* **45** 408–420

Baquer N Z, Gupta D and Raju J 1998 Regulation of metabolic pathways in liver and kidney during experimental diabetes: Effects of antidiabetic compounds; *Indian J. Clin. Biochem.* **13** 63–80

Baquer N Z, McLean P and Greenbaum A L 1973 Enzymic differentiation in pathways of carbohydrate metabolism in developing brain; *Biochem. Biophys. Res. Commun.* **53** 1282–1288

Bergmeyer H U, Bernt E, Schmidt F and Stork F 1974 *Assay for hexoses* (New York: Academic Press)

Brichard S M, Okitolonda W and Henquin J C 1988 Long term improvement of glucose homeostasis by vanadate treatment in diabetic rats; *Endocrinology* **123** 2048–2053

Chung S S M, Ho E C M, Lam K S L and Chung S K 2003 Contribution of Polyol Pathway to Diabetes-Induced Oxidative Stress; *J. Am. Soc. Nephrol.* **14** S233–S236

Chylack L K and Cheng H M 1978 Sugar metabolism in the crystalline lens; *Surv. Ophthalmol.* **23** 26–34

Domingo J L 2002 Vanadium and tungsten derivatives as antidiabetic agents: a review of their toxic effects; *Biol. Trace. Elem. Res.* **88** 97–112

Domingo J L, Gomez M, Llobet J M, Corbella J and Keen C L 1991 Improvement of glucose homeostasis by oral vanadyl or vanadate treatment in diabetic rats is accompanied by negative side effects; *Pharmacol. Toxicol.* **68** 249–253

Domingo J L, Gomez M, Sanchez D J, Liobet J M and Keen C L 1995 Toxicology of vanadium compounds in diabetic rats: The action of chelating agents on vanadium accumulation; *Mol. Cell Biochem.* **153** 233–240

Efimov A S, Obrosova I G and Velikii N N 1984 Sorbitol pathway of glucose metabolism in streptozotocin diabetes of varying duration and severity (abstract); *Probl. Endokrinol. (Mosk).* **30** 71–76

Erden M and Bor N M 1984 Changes in reduced glutathione, glutathione reductase and glutathione peroxidase after radiation in guinea pigs; *Biochem. Med.* **31** 217–227

Farkas T F and Patterson J W 1957 Insulin and the lens; *Am. J. Ophthalmol.* **44** 341–346

Freeman B A and Crapo J D 1982 Biology of diseases: free radicals and tissue injury; *Lab. Invest.* **47** 412–426

Genet S, Kale R and Baquer N Z 2002 Alterations in antioxidant enzymes and oxidative damage in experimental diabetic rat tissues: Effect of Vanadate and fenugreek (*Trigonella foenum graecum*); *Mol. Cell Biochem.* **236** 7–12

Gerlach U and Hiby W 1974 Assay of sorbitol; in *Methods in enzymatic analysis* (ed.) H U Bermeyer (New York: Academic Press) vol. 3, pp 1323–1330

Gonzalez A M, Sochor M, Hothersall J S and McLean P 1978 Effect of experimental diabetes on the activity of hexokinase in rat lens: an example of glucose overutilization in diabetes; *Biochem. Biophys. Res. Commun.* **84** 858–864

Gonzalez A M, Sochor M and McLean P 1983 The effect of an aldose reductase inhibitor (Sorbinil) on the level of metabolites in lenses of diabetic rats; *Diabetes* **32** 482–485

Gonzalez A M, Sochor M and McLean P 1987 The effect of an aldose reductase inhibitor (sorbinil) on the levels of metabolites in lenses of diabetic rats; *Diabetes* **32** 482–485

Griffith O W 1980 Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine; *Anal. Biochem.* **107** 207–212

Grover J K, Yadav S and Vats 2002 Medicinal plants of India with anti-diabetic potential; *V. J. Ethnopharmacol.* **81** 81–100

Gumaa K and McLean P 1972 The kinetic quantitation of ATP.D-glucose-6-phosphotransferase; *FEBS Lett* **27** 293–297

Gupta A, Gupta R and Lal B 2001 Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study; *J. Assoc. Physicians India* **49** 1057–1061

Heylinger C E, Tahiliani A G and McNeill J H 1985 Effect of Vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats; *Science* **227** 1474–1477

Kinoshita J H, Futterman S, Satoh K and Marola L O 1963 Factors affecting the formation of sugar alcohols in ocular lens; *Biochim. Biophys. Acta* **74** 340–350

Kuck J F 1962 Glucose metabolism and fructose synthesis in the diabetic rat lens; *Invest. Ophthalmol.* **1** 390–395

Lawrence R A and Burk R F 1976 Glutathione peroxidase activity in selenium deficient rat liver; *Biochem. Biophys. Res. Commun.* **71** 952–958

Lee A Y and Chung S S 1999 Contributions of polyol pathway to oxidative stress in diabetic cataract; *FASEB J.* **13** 23–30

Levari R, Kirnblueth W and Wertheimer E 1961 The effect of insulin on the uptake of monosaccharides by the rat lens; *J. Endocrinol.* **22** 361–369

Lou M F 2003 Redox regulation in the lens; *Prog. Retin Eye Res.* **22** 657–682

Lou M F, Dickerson J E, Garadi R and York B M 1988 Glutathione depletion in the lens of galactosemic and diabetic rats; *Exp. Eye Res.* **46** 517–530

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Malone J I, Knox G, Benford S and Tedesco T A 1980 Red cell sorbitol: an indicator of diabetic control; *Diabetes* **29** 861–864

Meyerovitch J, Farfel Z, Sack J and Shechter Y 1987 Oral administration of Vanadate normalizes blood glucose levels in Streptozotocin-treated rats; *J. Biol. Chem.* **262** 6658–6662

Mitton K P, Linklater H A, Dzialoszynski T, Sanford S E, Starkey K and Trevithick J R 1999 Modelling cortical cataractogenesis 21: in diabetic rat lenses taurine supplementation partially reduces damage resulting from osmotic compensation leading to osmolyte loss and antioxidant depletion; *Exp. Eye Res.* **69** 279–289

Moorthy R, Prabhu K, Murthy P S and Bull 1989 Studies on the isolation of hypoglycemic principle from the seeds of fenugreek (*Trigonella foenum graecum*) seeds; *Diabetes* **9** 69–72

Pirie A 1957 The biochemistry of the eye; *Bibl. Ophthalmol.* **49** 287–377

Pugazhenthi S and Khandelwal R L 1990 Insulin like effects of Vanadate on hepatic glycogen metabolism in non-diabetic and Streptozotocin-induced diabetic rats; *Diabetes* **39** 821–827

Puri D, Prabhu K and Murthy P S 1994 Hypocholesterolemic effect of hypoglycemic principle of fenugreek (*Trigonella foenum graecum*) seeds; *Indian J. Clin. Biochem.* **9** 13–16

Raheja B S, Iyer P D, Bhargava D and Krishnaswamy P R 1981 Glycosylated haemoglobin: its value in the management of diabetes mellitus; *J. Assoc. Physicians India* **29** 609–613

Raju J, Gupta D, Rao A R, Yadava P K and Baquer N Z 2001 *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes; *Mol. Cell Biochem.* **224** 45–51

Ramasamy R, Oates P J and Schaefer S 1997 Aldose reductase inhibition protects diabetic and non-diabetic rat hearts from ischemic injury; *Diabetes* **46** 292–300

Roshchin I V, Ordzhonikidze E K and Shalganova I V 1980 Vanadium – toxicity, metabolism, carrier state; *J. Hyg. Epidemiol. Microbiol. Immunol.* **24** 377–383

Sauvaire Y, Baissac Y O, Petit P and Ribes G 1996 Steroid saponins from fenugreek and some of their biological properties; *Adv. Exp. Med. Biol.* **405** 37–46

Saxena A K, Saxena P, Kale R K and Baquer N Z 1992 Effect

of Vanadate administration on polyol pathway in diabetic rat kidney; *Biochem. Int.* **26** 59–68

Saxena A K, Saxena P, Kale R K and Baquer N Z 1993 Impaired antioxidant status in diabetic rat liver: Effect of vanadate; *Biochem. Pharmacol.* **45** 539–542

Shechter Y 1990 Insulin-mimetic effects of vanadate. Possible implications for future treatment of diabetes; *Diabetes* **39** 1–5

Siddiqui M A and Rahman M A 1980 Effect of hyperglycemia on the enzyme activities of lenticular tissues of the rats; *Exp. Eye Res.* **31** 463–469

Slaughter M R, Thakkar H and O'Brien P J 2003 Differential expression of the lenticular antioxidant system in laboratory animals: A determinant of species predilection to oxidative stress-induced ocular toxicity; *Curr. Eye Res.* **26** 15–23

Sochor M, Baquer N Z and McLean P 1988 Effect of aldose reductase inhibiter sorbinil on metabolites in diabetic lens; *Biochem. Pharmacol.* **37** 3349–3356

Takamarg Y, Kubo E, Tsuzuki S and Akagi Y 2003 Apoptotic cell death in the lens of rat sugar cataract; *Exp. Eye Res.* **77** 51–57

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003 Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus; *Diabetes Care* **26** S5–S20

Varma S D, Scocket S S and Richards R D 1979 Implications of aldose reductase in cataracts in human diabetes; *Invest. Ophthalmol. Vis. Sci.* **18** 237–241

Williamson J R, Chang K, Frangos M, Hasan K S, Ido Y, Kawamura T, Nyengaard J R, Van den Enden M, Kilo C and Tilton R G 1993 Hyperglycemic pseudohypoxia and diabetic complications; *Diabetes* **42** 801–813

Willsky G R, A B G, Kostyniak P J, Yang L Q, Khan H R and Crans D C 2001 Effect of vanadium (iv) compounds in the treatment of diabetes: In vivo and in vitro studies with vanadyl sulfate and bis(maltolato)oxovanadium (iv); *J. Inorg. Biochem.* **85** 33–42

Wohaieb S A and Godin D V 1987 Alterations in free radicals tissue-defense mechanisms in Streptozotocin-induced diabetes in rat: effects of insulin treatment; *Diabetes Care* **36** 1014–1018

Wolff S P 1993 Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications; *Br. Med. Bull.* **49** 642–652

Yagi K 1984 *Assay for blood plasma or serum* (New York: Academic press)

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