Effects of sodium-orthovanadate and *Trigonella foenum-graecum* seeds on hepatic and renal lipogenic enzymes and lipid profile during alloxan diabetes

UMESH C S YADAV, K MOORTHY and NAJMA Z BAQUER*

Hormone and Drug Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

**Corresponding author (Fax, 91-11-26104865; Email, nzbaquer@hotmail.com)*

Sodium-orthovanadate (SOV) and seed powder of *Trigonella foenum graecum* Linn. (common name: fenugreek, family: Fabaceae) (TSP) besides being potential hypoglycemic agents have also been shown to ameliorate altered lipid metabolism during diabetes. This study evaluates the short-term effect of oral administration of SOV and TSP separately and in concert (for 21 days) on total lipid profile and lipogenic enzymes in tissues of alloxan diabetic rats. Diabetic rats showed 4-fold increase in blood glucose. The level of total lipids, triglycerides and total cholesterol in blood serum increased significantly during diabetes. During diabetes the level of total lipids increased significantly ($P < 0{\cdot}001$) in liver and in kidney by 48% and 55%, respectively, compared to control. Triglycerides level increased by 32% ($P < 0{\cdot}01$) in liver and by 51% ($P < 0{\cdot}005$) in kidney, respectively, compared to control. Total cholesterol level also increased significantly in both liver and kidney ($P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively). The activities of NADP-linked enzymes; namely glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), isocitrate dehydrogenase (ICDH), and the activities of lipogenic enzymes namely ATP-citrate lyase (ATP-CL) and fatty acid synthase (FAS) were decreased significantly in liver and increased in kidney during diabetes as compared to control. SOV and TSP administration to diabetic animals prevented the development of hyperglycemia and alteration in lipid profile in plasma and tissues and maintained it near normal. Maximum prevention was observed in the combined treatment with lower dose of SOV (0·2%) after 21 days. We are presenting for the first time effectiveness of combined treatment of SOV and TSP in amelioration of altered lipid metabolism during experimental type-I diabetes.

[Yadav U C S, Moorthy K and Baquer N Z 2004 Effects of sodium-orthovanadate and *Trigonella foenum-graecum* seeds on hepatic and renal lipogenic enzymes and lipid profile during alloxan diabetes; *J. Biosci*. **29** 81–91]

1. Introduction

Insulin-dependent diabetes mellitus (IDDM) or type-I diabetes is an autoimmune disorder caused by auto aggressive T-lymphocytes that infiltrate the pancreas and destroy insulin producing *b*-cells. This leads to hypoinsulinaemia and thus a hyperglycemic condition (Bach 1995), which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiac problem (Arky 1982). Most of the metabolic complications associated with type-I diabetes are due to insulin deficiency and related glucose under-utilization of the insulin-depedent tissue, such as liver, and glucose over-utilization in insulin-independent tissue, such as kidney (Sochor *et al* 1985). The insulin deficiency causes excessive break down of lipid in adipose depots, resulting in

Keywords. Alloxan diabetes; lipogenic enzymes; sodium-orthovanadate; total lipid; *Trigonella* seed powder

Abbreviations used: FAA, Free fatty acids; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; ICDH, isocitrate dehydrogenase; SOV, sodium-orthovanadate; TSP, *Trigonella* seed powder.

increased level of free fatty acids (FAA) (Gupta *et al* 1999). Liver plays a central role in the glucose and lipid metabolism and gets severely affected due to insulin deficiency. The liver tissue is involved in the lipid metabolism through uptake, oxidation and metabolic conversion of FAA, synthesis of cholesterol and phospholipids and secretion of plasma lipoproteins. A profound alteration in the concentration and composition of lipid profile in the body occurs during diabetes. The liver cells show a marked increase in the lipid concentration during diabetes (Arky 1982; Sochor *et al* 1987). Further, the accumulation of triglycerides and long chain fatty acyl Co-A in liver leads to reduction in insulin-mediated metabolic activity and can cause type-II diabetes resulting into metabolic syndrome (Moller 2001). There is decrease in the lipogenic enzyme activity in liver and overall rates of hepatic lipogenesis. The decrease in liver weight during diabetes may be attributed to these alterations (lipogenesis and lipolysis) in lipid metabolism (Gupta *et al* 1999).

Diabetes mellitus affects the kidney and is the leading cause of diabetic nephropathy. In addition to prominent roles played by factors such as oxidative stress, advanced glycation end-products and others, abnormal lipid metabolism and renal accumulation of lipids have also been proposed to play a role in the pathogenesis of diabetic nephropathy (Kimmelsteil and Wilson 1936). Several workers have shown the presence of lipid deposits in the kidney of diabetic human and experimental animals and they have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease (Guijarro *et al* 1995; Lee *et al* 1991). Earlier these lipid deposits in kidney were attributed to increased levels of serum lipids. However, Sun *et al* (2002) showed an increased renal lipid synthesis to be responsible for this. They showed the marked increase in SREBP-1 and fatty acid synthase expression in STZ-diabetic rats, resulting in increased renal lipid accumulation and glomerulosclerosis. Other workers have also shown increase in the activities of lipogenic enzymes in kidney during diabetes (Raju *et al* 2001).

The isolation of insulin by Banting and Best was considered as the beginning of a new era for the treatment of diabetes by insulin (Bloom and Ireland 1992), however, existence of NIDDM (type-II diabetes), where long-term insulin therapy becomes ineffective due to insulin resistance, and severe hypoglycemia particularly affecting the brain (Cryer 1992; Reichard *et al* 1993) from insulin therapy in case of type-I diabetes, led the march for the search of an alternative to insulin therapy (Ramasarma 1996). It is well established that complications associated with diabetes can be markedly reduced through good glucose control. Certain metal elements such as vanadium, selenium, molybdenum, tungstate, zinc and manganese (Heyligar *et al* 1985; Baquer *et al* 1998; Ezaki 1990; Goto *et al* 1992; Shisheva *et al* 1992; Baquer *et al* 2003) with potential hypoglycemic activities have been studied earlier. However, vanadium and its various complexes have been particularly favoured for their insulinomimetic effects (Ramasarma 1996). Similarly, extracts from various plant materials have been tested in animal model system and their hypoglycemic effects have been elucidated (Murthy 1995). The absence of toxic effects of plant extracts makes the use of such natural products for their antidiabetic properties favourable. Plant extracts which have been studied so far including *Alium sativum* Linn. (garlic bulbs) (Sheela and Augusti 1992), *Momordica charantia* Linn. (bitter gourd) fruit extract (Shibib *et al* 1993; Ahmed *et al* 1998), *Trigonella foenum-greacum* Linn. seed powder (Moorthy *et al* 1989; Raju *et al* 2001) among others, have been confirmed to possess antidiabetic properties.

The biological potential of sodium-orthovanadate (SOV) as an insulin mimetic and antidiabetic agent is, however, hampered by its toxicity (Dafnis and Sabatini 1994; Domingo *et al* 1995). Several workers have observed short-term toxic responses in animals treated with diabetic compounds such as severe diarrhoea, decrease in weight gain, deaths due to dehydration etc (Ramasarma 1996). In addition, long-term complications such as hematological and biochemical alterations, nephrotoxicity, immunotoxicity, reproductive and developmental toxicity have also been observed (Domingo 2002). To exploit the potential of vanadium compounds and to enhance their bioavailability by reducing their toxicity, attempts are being made to use the complex-forming capability of vanadium compounds with organic compounds (Nandhini *et al* 1993; Srivastava 2000). In the present work, attempts have been made to reduce the toxicity without compromising on their biological effects by reducing the dose of vanadate and combining the treatment with plant product such as *Trigonella* seed powder (TSP). Alteration in lipid metabolism during diabetes, leading to cardio-vascular problems have been studied extensively. However, very few studies have emphasized the role of renal lipid metabolism in this context. Therefore, present work was undertaken to determine alterations in lipid profile and lipogenic enzymes in liver and kidney in diabetic rats, and to compare the effects of these antidiabetic compounds given separately and in combination.

The activities of key lipogenic enzymes such as glucose-6-phosphate dehydrogenase (G6PDH) (EC 1·1·1·49), malic enzyme (EC 1·1·1·40), NADP-linked isocitrate dehydrogenase (ICDH) (EC 1·1·1·42), ATP-citrate lyase (EC 4·1·3·8) and fatty acid synthase (FAS) (EC 2·3·1·85) were assayed in cytosolic fraction of rat liver and kidney in control, diabetic, and treated conditions. Our results demonstrate normalization of blood glucose and marked prevention in alteration of plasma lipid profile and tissue

lipid profile and enzyme activities following the oral treatment with reduced dose (0·2 mg/ml) of SOV in combination with 5% TSP compared to their separate administration.

2. Materials and methods

2.1 *Animals*

Adult female albino Wistar rats, weighing about 200 g, were used in all the experiments. Rats were maintained in the animal house facility of Jawaharlal Nehru University, New Delhi, India at a constant temperature (25°C) and relative humidity (55%). The animals were fed standard chow (Hindustan Lever Ltd., India) and given tap water *ad libitum* until treatment or time of sacrifice. All the animals were cared for according to the rules and regulations of the Institutional Animal Ethics Committee (IAEC) guidelines of Jawaharlal Nehru University, New Delhi.

2.2 *Induction of diabetes and grouping of experimental animals*

Rats were starved for 24 h and divided into control and experimental groups. For the induction of diabetes, each rat in experimental group was injected alloxan monohydrate subcutaneously (15 mg/100 g body weight) freshly prepared in 0·154 M sodium acetate buffer (pH 4·5) according to the method of Sochor *et al* (1985). The control rats received the same volume of the vehicle (sodium acetate buffer) each. From the next day onward each alloxan-treated rat was injected intraperitoneally (i.p.) with 2 IU of protamine-zinc insulin for seven days. The severity of diabetes was checked in the alloxan-treated rats using urine glucose detection strips (Diastix, Bayer Diagnostic India). Insulin was withdrawn on the 8th day and diabetic rats were randomly divided into following groups: diabetic (D), diabetic treated with 2 IU insulin (D + I), diabetic treated with SOV (D + V), diabetic treated with TSP (D + T), and diabetic treated with both SOV and TSP (D + V + T).

2.3 *Treatment with antidiabetic compounds*

Insulin injection (i.p.) was continued for insulin-treated diabetic (D + I) group till the date of sacrifice. They were given normal feed and tap water *ad libitum*. SOV (0·6 mg/ml) was given to SOV-treated diabetic group (D + V) in drinking water containing 0·5% NaCl so as to reduce the toxicity of SOV (Heyligar *et al* 1985). The TSP-treated diabetic group (D + T) animals were given 5% finely powdered *Trigonella* seed (AGMARK brand, purchased from local market) mixed with powdered rat feed (i.e. 5 g of dry TSP in 95 g of powdered rat feed). On an average, rats consumed about 1·25 g of TSP per day as reported by Raju *et al* (2001). The similar amount of powdered TSP and a reduced dose of SOV (0·2 mg/ml along with 0·5% NaCl) in drinking water were given to the diabetic treated with SOV and TSP group (D + V + T). The treatment was continued for 21 days when the animals were sacrificed.

2.4 *Preparation of tissue homogenates*

The control and treated rats were starved overnight and sacrificed by cervical dislocation. Blood was collected immediately and stored at – 80°C until used for glucose and total lipid estimation. The liver and kidneys were rapidly excised, washed with normal saline, blotted dry and weighed. Tissues were homogenized in 9 volumes of freshly prepared cold isotonic sucrose buffer using a Potter Elvehjem tissue homogenizer fitted with a Teflon plunger. The homogenizing buffer contained the following in final concentration: 0·25 M sucrose; 0·02 M triethanolamine hydrochloride, pH 7·4, containing 0·12 mM dithiothreitol (DTT). The whole procedure was carried out at 0–4°C. The homogenates were centrifuged at 1000 g for 10 min to remove the nuclei and the cell debris. The supernatant was further centrifuged at 15,000 *g* for 45 min at 4°C in a refrigerated super speed centrifuge (Sorvall RC-5B). The supernatant thus obtained contained the cytosolic fraction and was used for all the enzyme assays.

2.5 *Estimation of enzyme activities*

The enzymes were estimated spectrophotometrically in the tissue cytosolic fraction at 25°C. All enzymes were assayed by coupled enzymatic reactions using either NAD or NADP and purified enzymes. The reaction was started by addition of the tissue extract and oxidation/reduction of the NAD/NADP was followed at 340 nm using a Beckman DU-68 spectrophotometer. G6PDH and malic enzyme were assayed essentially by the method of Baquer *et al* (1976). NADP-ICDH and FAS were measured according to the methods of Saggerson and Greenbaum (1970). The activity of ATP-citrate lyase was assayed according to the method of Srere (1972).

2.5a *Enzyme unit*: One unit of enzyme is defined as one μmol of NAD/NADH or NADP/NADPH formed per g fresh tissue weight per min at 25°C. For FAS, one unit is defined as one μmol of NADPH oxidized per g tissue per min at 25°C. The activities of all enzymes studied in liver and kidney are presented as total tissue enzyme activity/100 g body weight to depict the biochemical changes in relation to the functional requirements of the animals.

2.6 *Extraction and estimation of lipids*

Total lipids were extracted from the liver and whole kidney according to a modified method of Folch *et al* (1957). The tissues were finely minced and homogenized initially in 2 ml methanol and made-up to 10 ml by adding methanol. Chloroform (20 ml) was added to get the ratio of 2 : 1 chloroform : methanol (v/v). The tissue suspension was stirred for 30 min at 25°C and filtered through Whatman No. 1 filter paper. Extraction was repeated with the residue. To the filtrate 0·2 volume of 0·9% NaCl was mixed with constant stirring to remove the water-soluble impurities. The suspension was taken in a separating funnel and kept for 20 min. The lower phase was collected and concentrated by lyophilization and volume was made-up to 10 ml by adding chloroform : methanol mixture 2 : 1 (v/v) and stored at 0°C until used for the estimation of total lipid. Total lipids, triglycerides and total cholesterol from blood serum, liver and kidney extract were estimated using kits bought from Randox Laboratories Ltd., Crumlin, UK.

2.7 *Estimation of serum glucose*

Glucose was estimated in the blood serum by using glucose Enzokit bought from Ranbaxy-Elily Laboratories Ltd., India, using glucose oxidase method.

2.8 *Statistical analysis*

Results are presented as mean ± SEM of four separate experiments where in one experiment three or more rats were used in each group. The significance of difference between the data pairs was evaluated by analysis of variance (ANOVA) followed by Mann-Whitney *U* test.

2.9 *Chemicals*

All purified enzymes, coenzymes, substrates, standards and buffers were purchased from Sigma Chemicals, St. Louis, MO, USA. Protamine-zinc insulin was purchased from local chemist shop. All other chemicals used were of analytical grade.

3. Results

3.1 *Changes in general parameters during diabetes: Effects of SOV and TSP*

The changes in body weight, tissue weight and blood glucose in control and the experimental groups are summarized in table 1. The body weight of diabetic rats significantly decreased after 21 days of diabetes than the control rats. Treatment with antidiabetic compounds showed marked gain in weight and the combined treatment was found most effective. The whole liver weight showed significant decrease ($P < 0.005$) while whole kidney weight increased significantly. To make a functional comparison of weight gain or loss, tissue weight per 100 g body weight was calculated. While liver showed no significant weight difference per 100 g body weight among various experimental groups, kidney weight per 100 g body weight was significantly increased after 21 days of diabetes. Treatment, especially with combined doses prevented this increase. The diabetic rats showed 4-fold ($P < 0.001$) increase in the blood glucose. Treatment with SOV and TSP separately maintained the blood glucose level to near normal values and is consistent with earlier reports (Gupta *et al* 1999; Raju *et al* 2001). We here report that the treatment with SOV and TSP in combination was more effective than their separate treatments, showing the effectiveness of combined treatment in controlling the blood glucose level.

Table 1. Effects of SOV and TSP on general body parameters (body weight and tissue weight) and blood glucose levels in diabetic rats 21 days after induction of diabetes.

	Control	Diabetic	Diabetic + insulin	Diabetic + vanadate	Diabetic + *Trigonella*	Diabetic + vanadate + *Trigonella*
Body wt. (g)	216 ± 4·0	137 ± 5·7[a]	205 ± 2·3	198 ± 7·8[b]	203 ± 3·5	210 ± 3·0
Liver wt.	5·9 ± 0·7	3·2 ± 0·3[b]	5·6 ± 0·4	5·2 ± 0·6	5·3 ± 0·6	5·8 ± 0·56
Liver wt./100 g body wt.	2·74 ± 0·2	2·3 ± 0·2	2·7 ± 0·2	2·63 ± 0·2	2·6 ± 0·1	2·76 ± 0·14
Kidney wt. (g)	1·3 ± 0·1	1·8 ± 0·06	1·35 ± 0·09	1·36 ± 0·05	1·4 ± 0·09	1·32 ± 0·08
Kidney wt./100 g body wt.	0·6 ± 0·04	1·31 ± 0·06[b]	0·66 ± 0·04	0·68 ± 0·06	0·69 ± 0·04	0·63 ± 0·05
Blood glucose mM/l	5·8 ± 0·42	22·3 ± 0·83[a]	6·05 ± 0·45	6·6 ± 0·23[c]	6·83 ± 0·42[b]	5·9 ± 0·38

Each value is a mean ± SEM of 4 or more separate experiments. The significant comparisons of each experimental group shown are with the control value. [a]$P < 0.001$, [b]$P < 0.005$, [c]$P < 0.01$ are the fisher's P values.

3.2 *Changes in lipid profile during diabetes: Effects of SOV and TSP*

Effect of administering SOV and TSP to diabetic rats on the lipid profile in the blood plasma and tissue extracts are shown in tables 2 and 3 respectively. The total lipids, triglycerides and total cholesterol levels in blood serum showed significant increase of 39% ($P < 0{\cdot}005$), 45% ($P < 0{\cdot}001$) and 53% ($P < 0{\cdot}001$) by 21 days, respectively during diabetes as compared to control values. The total lipids, triglycerides and total cholesterol levels in the liver and kidney are expressed as mg in whole tissue per 100 g body weight in order to have a better understanding of biochemical changes in relation to the functional requirements of the animals. In liver the total lipids and triglycerides showed an increase of 24% ($P < 0{\cdot}001$) and 11% ($P < 0{\cdot}01$) by 21 days, respectively. Total cholesterol level increased by 31% ($P < 0{\cdot}001$). The renal total lipids and triglycerides showed an increase of over 3-fold ($P < 0{\cdot}001$) by 21 days, respectively, while total cholesterol level increased by 3·8-fold ($P < 0{\cdot}001$). The combined treatment of SOV and TSP was most effective in preventing alteration of lipid profile. The maximum prevention in the alteration of the lipid profile was observed in the combined treatment of SOV and TSP by 21 days (figure 1).

3.3 *Changes in hepatic and renal lipogenic enzymes during diabetes: Effects of SOV and TSP*

Effects of antidiabetic compounds on activities of lipogenic enzymes in cytosolic fraction of liver and kidney in different experimental groups after 21 days of treatment, calculated for whole tissue per 100 g body weight are presented in table 4. The hepatic lipogenic enzymes showed significant decrease in activities during diabetes as compared to control. The activity of hepatic G6PDH decreased significantly ($P < 0{\cdot}001$) in diabetic liver by 21 day. The activities of malic enzyme and NADP-isocitrate dehydrogenase in liver cytosol also decreased significantly ($P < 0{\cdot}01$ and $P < 0{\cdot}001$, respectively). The activities of ATP-citrate lyase and fatty acid synthase also decreased significantly ($P < 0{\cdot}001$) during diabetes as compared to respective values in control animals. Treatment with SOV and TSP maintained the lipogenic enzyme activities to near control values by 21 days. The combined treatment

Table 2. Effects of SOV and TSP on total lipids, triglycerides and total cholesterol levels (mg/dl) in blood plasma of diabetic rats 21 days after induction of diabetes.

	Control	Diabetic	Diabetic + insulin	Diabetic + vanadate	Diabetic + *Trigonella*	Diabetic + vanadate + *Trigonella*
Total lipid	457·3 ± 19·6	637·4 ± 21·5[b]	580·4 ± 20·4	577·7 ± 19·8	589·2 ± 18·2	545·3 ± 20·5
Triglyceride	93·9 ± 4·5	136·4 ± 6·1[a]	106·2 ± 5·4	109·6 ± 6·5	106·3 ± 6·7	101·4 ± 7·6
Cholesterol	64·4 ± 5·12	98·6 ± 4·47[a]	73·8 ± 5·15	77·8 ± 3·75	74·5 ± 3·83	71·8 ± 4·53

Each value is a mean ± SEM of 4 or more separate determinations. The significant comparisons of each experimental group shown are with the control value. [a]$P < 0{\cdot}001$, [b]$P < 0{\cdot}005$, are Fisher's P values.

Table 3. Effects of SOV and TSP on total lipids, triglycerides and total cholesterol levels in tissue extract (mg in whole tissue/100 g body weight) of diabetic rats 21 days after induction of diabetes.

	Control	Diabetic	Diabetic + insulin	Diabetic + vanadate	Diabetic + *Trigonella*	Diabetic + vanadate + *Trigonella*
Liver						
Total lipids	276·5 ± 33	343·6 ± 29[a]	314·8 ± 31	325 ± 27	317·7 ± 31	306·9 ± 28
Triglyceride	28·7 ± 1·45	31·9 ± 1·01[c]	30·2 ± 1·61	29·5 ± 1·23	29·3 ± 1·74	28·7 ± 1·19
Cholesterol	10·3 ± 0·41	13·5 ± 0·39[a]	10·4 ± 0·40	10·5 ± 0·49	10·3 ± 0·39	10·2 ± 0·44
Kidney						
Total lipids	55·9 ± 6·8	189·6 ± 16[a]	67·1 ± 6·9	85·1 ± 8·1	77·8 ± 8·5	68·3 ± 6·7
Triglyceride	3·9 ± 0·31	12·8 ± 0·75[b]	4·7 ± 0·3	5·0 ± 0·31	4·8 ± 0·29	4·1 ± 0·27
Cholesterol	2·76 ± 0·07	10·7 ± 0·18[a]	3·07 ± 0·09	3·7 ± 0·14	3·6 ± 0·10	2·88 ± 0·10

Each value is a mean ± SEM of 4 or more separate experiments· The significant comparisons of each experimental group shown are with the control value· [a]$P < 0{\cdot}001$, [b]$P < 0{\cdot}005$, and [c]$P < 0{\cdot}01$ are Fisher's P values.

of SOV and TSP was found to be most effective in preventing the changes after 21 days of diabetes induction.

The enzyme activities in whole kidney per 100 g body weight were higher in the diabetic rats at different significant levels when compared with the activities in the kidney of control rats. The diabetic rats showed significantly higher activity of G6PDH and malic-enzyme ($P < 0{\cdot}01$) in kidney when compared to control rats. The activities of ICDH, ATP-citrate lyase and FAS were higher at significance levels of $P < 0{\cdot}001$, $P < 0{\cdot}01$ and $P < 0{\cdot}005$ respectively when compared to control value. Although enzyme activities were found to be lower in D + T and D + V groups of treated rats, it was much similar to control value in the groups treated with insulin and a combined treatment of SOV and TSP. Thus, as in the liver the activities of lipogenic enzymes in kidney during diabetes

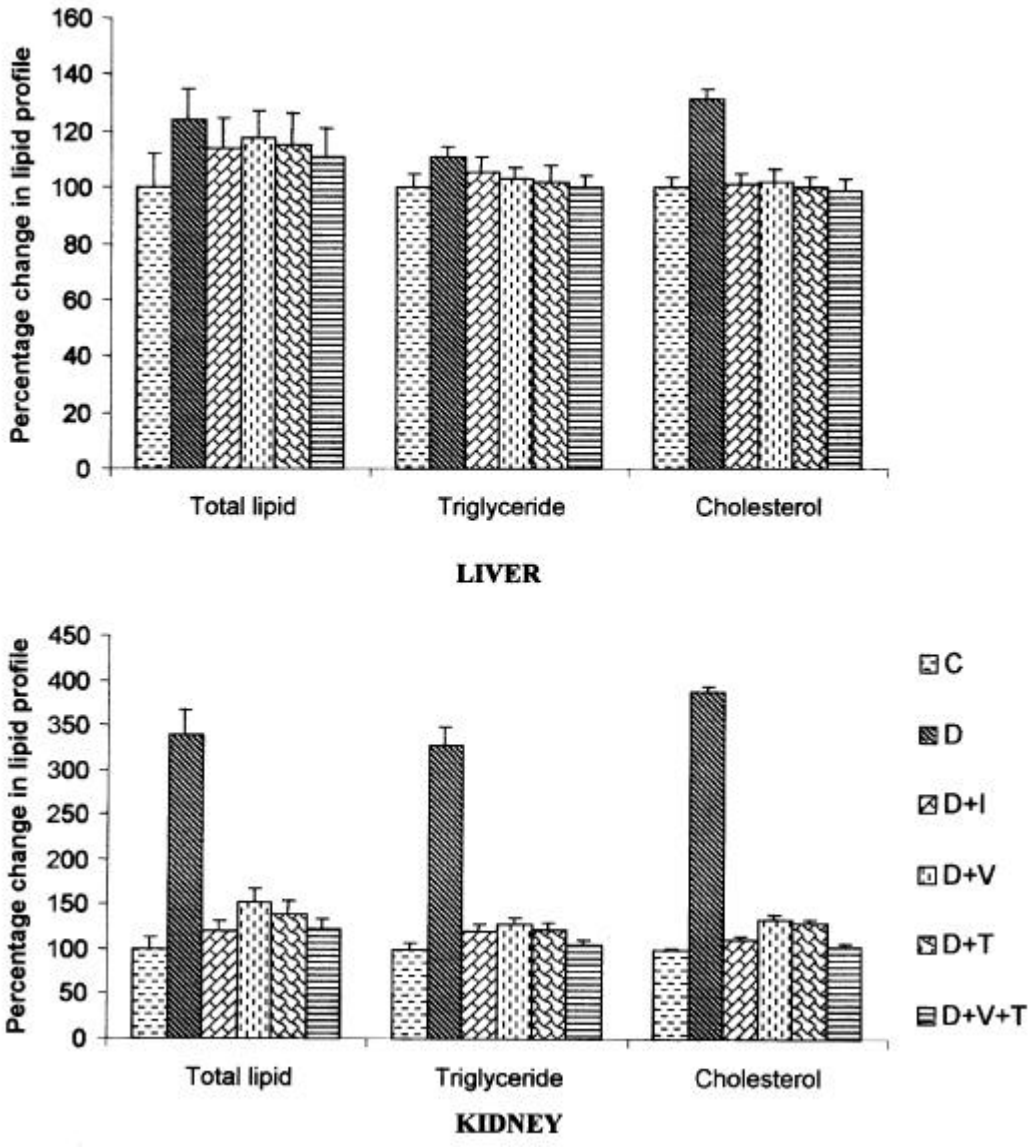

Figure 1. Percentage change in the levels of total lipid, triglyceride and total cholesterol in liver and kidney extract after 21 day of diabetes induction. The control values of total lipid, triglyceride and total cholesterol are $276{\cdot}5 \pm 33{\cdot}2$, $28{\cdot}7 \pm 1{\cdot}45$ and $10{\cdot}3 \pm 0{\cdot}41$, respectively in liver; and $55{\cdot}9 \pm 6{\cdot}8$, $3{\cdot}9 \pm 0{\cdot}31$ and $2{\cdot}76 \pm 0{\cdot}07$ in kidney. The values are expressed as mg in whole tissue/100 g body weight. Each value is a mean ± SEM of 4 or more separate experiments. Details of treatment with different antidiabetic compounds are given in §2. C, Control; D, diabetic; D + I, diabetec + insulin; D + V, diabetic + vanadate; D + T, diabetic + *Trigonella*; D + V + T, diabetic + vanadate + *Trigonela*.

were also maintained near control values by combined oral treatment of SOV and TSP by 21 days.

4. Discussion

Diabetes is known to affect large number of metabolic pathways, including lipid metabolism, by altering the activities of various enzymes involved in these pathways. Insulin deficiency (type-I diabetes) or decreased insulin action (type-II diabetes) results in decrease in glucose utilization by insulin requiring tissues like liver and an increase in glucose production through an increased rate of gluconeogenesis; both resulting in hyperglycemia. As a consequence of increased glucose and decreased insulin level in blood plasma, hepatic regulation of lipid metabolism is greatly altered. As insulin is an important regulator of many enzymes involved in lipolysis and lipogenesis, its deficiency causes major changes in the activity of these enzymes thereby affecting overall lipid metabolism and lipid profiles of various tissues. Kidney on the other hand is an insulin-independent tissue involved in the transport of glucose in the cells and thus gets severely affected due to increased blood glucose. Renal glucose uptake is markedly increased during diabetes and is inversely correlated with renal FFA uptake, which is reduced during diabetes (Meyer *et al* 1998). Thus, the major assumption that kidney lipid deposits, which plays an important role in the pathogenesis of diabetic kidney disease (Guijarro *et al* 1995; Lee *et al* 1991), originate from increased levels of serum lipids has to be further investigated with the possibility of an increase in renal lipid synthesis resulting in renal lipid accumulation.

SOV and TSP are known hypoglycemic compounds and have been shown to control blood glucose level during diabetes (Brichard *et al* 1988; Khosla *et al* 1995; Gupta *et al* 1999). Apart from their hypoglycemic effects, SOV and TSP have also been shown to have an effect on various altered metabolic pathways during diabetes in a way similar to that of insulin (Ramasarma 1996; Sharma *et al* 1990; Sekar *et al* 1996). However, a few toxic side effects of SOV have also been reported (Heyliger *et al* 1985; Domingo *et al* 1991; Domingo 2002). On the other hand TSP has shown many positive and encouraging results in the management of diabetes (Moorthy *et al* 1989; Sharma *et al* 1990). Since SOV toxicity is known to get reduced when it forms complexes with organic compounds with no decrease in its insulin mimetic properties (Nandhini *et al* 1993; Srivastava 2000), the use of SOV-TSP in combination can be suggested as an effective alternative for the amelioration of diabetes.

In the present work, we report the effect of SOV and TSP administration, separately as well as in combination, on the lipid profile of blood plasma and tissues; namely, liver and kidney. Also the lipogenic enzymes; namely, G6PDH, malic enzyme, NADP-isocitrate dehydrogenase, ATP-citrate lyase, and FAS have been studied in these tissues during alloxan-induced diabetes. The experimental approach to treat alloxan-induced diabetic rats with combined doses of reduced amount of SOV (0·2 mg/ml along with 5% NaCl in drinking water) and TSP (5% mixed with rat feed) was to study biochemical effects of whole seed

Table 4. Effects of SOV and TSP on lipogenic enzyme's activities in liver and kidney cytosolic fractions of diabetic rats 21 days after induction of diabetes.

Enzyme	Control	Diabetic	Diabetic + insulin	Diabetic + vanadate	Diabetic + *Trigonella*	Diabetic + vanadate + *Trigonella*
Liver						
G6PDH	2·24 ± 0·14	0·97 ± 0·13[a]	2·19 ± 0·12	2·08 ± 0·16	2·05 ± 0·11	2·24 ± 0·13
ME	2·56 ± 0·07	1·26 ± 0·17[a]	2·53 ± 0·13	2·24 ± 0·09	2·18 ± 0·13	2·59 ± 0·12
ICDH	27·84 ± 1·09	18·05 ± 0·71[c]	27·76 ± 1·89	26·31 ± 0·67	25·8 ± 0·82	27·81 ± 0·98
ATPCL	0·76 ± 0·02	0·25 ± 0·01[a]	0·70 ± 0·01	0·65 ± 0·01	0·62 ± 0·014	0·78 ± 0·01
FAS	1·16 ± 0·04	0·35 ± 0·02[a]	0·97 ± 0·04	0·99 ± 0·02	0·88 ± 0·03	1·14 ± 0·02
Kidney						
G6PDH	1·06 ± 0·14	1·38 ± 0·13[b]	1·05 ± 0·20	1·14 ± 0·19	1·15 ± 0·11	1·05 ± 0·13
ME	1·13 ± 0·08	1·36 ± 0·10[b]	1·22 ± 0·15	1·22 ± 0·11	1·24 ± 0·11	1·15 ± 0·14
ICDH	6·06 ± 0·49	10·18 ± 0·74[a]	6·48 ± 0·89	6·53 ± 0·67	6·71 ± 0·62	6·26 ± 0·93
ATPCL	0·11 ± 0·01	0·16 ± 0·014[b]	0·113 ± 0·01	0·115 ± 0·01	0·12 ± 0·01	0·110 ± 0·01
FAS	0·156 ± 0·03	0·25 ± 0·02[c]	0·16 ± 0·03	0·163 ± 0·01	0·17 ± 0·01	0·157 ± 0·04

Each value is a mean ± SEM of 4 or more separate experiments. The significant comparisons of each experimental group shown are with the control value. Enzyme activities are units in whole tissue/100 g body weight. [a]$P < 0·001$, [b]$P < 0·01$, and [c]$P < 0·05$ are the Fisher's P values. ME, malic enzyme; ATPCL, ATP-citrate lyase.

before identical studies could be carried out with isolated fractions of the TSP in concert with SOV.

The results show that combined treatment for 21 days effectively controls hyperglycemia (table 1). Maintenance of normoglycemia prevents the onset of microvascular complications and also delays progression of complications in diabetes. The SOV and TSP maintain the blood glucose to normoglycemia during diabetes, which acts as an essential trigger for both liver and kidney to revert to their normal metabolic homeostasis. During diabetes liver shows decrease in weight due to enhanced catabolic processes such as glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin and/or cellular glucose in liver cells. There is, however, an increase in kidney weight due to glucose over-utilization and subsequent enhancement in glycogen synthesis (Meyer *et al* 1998), lipogenesis and protein synthesis. These changes may lead to serious microvascular renal complications, which involve a series of metabolic changes in the pathogenesis of diabetic nephropathy (Raju *et al* 2001). Treatment of diabetic rats with SOV and TSP significantly prevented the alteration in liver and kidney weight. The maximum prevention was visible in the combined treatment.

The plasma lipid level is usually raised during diabetes and presents a risk factor for the coronary heart disease (Chatterjea and Shinde 1994). Lowering the plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease (Scott and Grundy 1999). We have shown here an increase in the plasma total lipids, triglycerides and total cholesterol in alloxan diabetic rats. This increase may be a result of increased breakdown of lipids and mobilization of FAA from the peripheral depots. Since insulin inhibits the hormone-sensitive lipases, the latter becomes active in the absence of insulin. Other hormones such as glucagon and catacholamines, known to increase during diabetes, compound the effect by stimulating lipolysis. The lipid profile in liver and kidney also showed an increase. The increase in kidney lipid level during diabetes appears to be due to increased glucose flux and reducing equivalents leading to enhanced over all biosynthetic pathways. However, the increase in hepatic lipid level is not due to *de novo* synthesis and may be due to increased uptake from the portal system as shown earlier (Gupta *et al* 1999; Sun *et al* 2002) and in the present results, that is the activities of most of the lipogenic enzymes in liver decrease during diabetes. The ability of SOV and TSP treatment causing a reduced blood plasma levels and tissue lipid levels including total lipids, triglycerides and total cholesterol have not been reported in earlier studies. We report here the marked prevention in the alteration of lipid profile by a combined treatment of SOV and TSP to diabetic animals after 21 days of diabetes induction.

There could be two possibilities for the prevention of alteration of lipid profile. Firstly, that the rate of lipogenesis is normalized by SOV and TSP in a way similar to the effect of insulin on the lipid metabolism. This can be substantiated by our present results showing the enzyme activities, which were maintained near normal during treatment. Secondly, it could be due to achievement of normoglycemia where there was no further degradation of already accumulated lipid for otherwise glucose starved cells. SOV has been shown to stimulate fatty acid synthesis in isolated rat hepatocytes (Agius and Vaartjes 1982). Brichard *et al* (1994) have shown that SOV compounds activate lipogenesis and inhibit lipolysis in rat adipose tissue. Earlier from our laboratory TSP has also been shown to stimulate the hepatic lipogenic enzymes (Raju *et al* 2001).

During diabetes, lipogenesis is decreased while lipolysis is increased in the hepatic tissue (West 1982), which is the outcome of underutilization of glucose resulting in increased lipolysis and stimulation in the activities of gluconeogenic enzymes (Gupta *et al* 1999; Raju *et al* 2001). In kidney, an over-utilization of cellular glucose occurs through elevated activities of glycolytic and NADP-linked lipogenic enzymes (Sochor *et al* 1985; Raju *et al* 2001). The present results support the possibility that TSP treatment along with SOV to diabetic rats affect NADP-linked lipogenic enzymes, namely G6PDH, malic enzyme and ICDH. As shown in the present results the increased activity of these enzymes by treatments with SOV and TSP separately as well as in combination may suggest restoration of the redox state of hydrogen shuttle system, resulting in controlled NADPH formation by a feedback mechanism.

It has been reported that insulin acts by increasing the phosphorylation of ATP-citrate lyase by c-AMP-dependent protein kinase (Ramakrishna *et al* 1989). Sahng-Wook *et al* (1994) reported that administration of insulin to diabetic rats substantially increased the amount of ATP-citrate lyase in liver cytosol. Since SOV is known to mimic insulin action, and also to inhibit Na^+-K^+-ATPase, thereby making more ATP available for phosphorylation, it could be 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. The additive effect of the two as reported in this study, however, was more pronounced after 21 days of treatment. It has also been shown earlier that in diabetic animals the hepatic FAS is markedly decreased (Sochor *et al* 1987) and this decrease is caused by diminution in the synthesis of the enzyme, and insulin administration corrects this deficit as shown by Lakshmanan *et al* (1972). In kidney, the activity of FAS enzyme increases during diabetes, which is the result of enhanced expression of sterol regulatory element-binding proteins (SREBPs) and

FAS proteins (Sun *et al* 2002). Our results show that SOV and TSP were able to maintain the activity of FAS to control level most effectively by 21 days, when given in combination.

Though, extensive work has been undertaken to work out the mechanism by which SOV could be exerting its effects, the same with TSP is still not very clear. However, plausible hypothesis that may be involved in the therapeutic action of TSP can be considered here. TSP may exert its therapeutic effect through modulation of insulin secretion. Madar 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 abnormalities. Petite *et al* (1995) and Yoshikawa *et al* (1997) reported the isolation of furostanol saponins called trigoneoside Ia, Ib, IIa, IIb, IIIa, IIIb; glycoside and trifoenoside A. They claimed that these saponins are the active principles owing to their hypoglycemic effects. It has also been demonstrated in some studies that *Trigonella* seed delayed gastric emptying and caused inhibition of glucose transport as the seed contain around 50% pectin that forms a colloid suspension when hydrated and can decrease rate of gastric emptying and slow carbohydrate absorption (Al-Habori and Raman 1998). Sauvaire *et al* (1998) and Broca *et al* (1999) have demonstrated evidences of insulinotropic and antidiabetic properties of 4-hydroxyisoleucine isolated from fenugreek seeds in glucose-dependent manner. They suggested that antidiabetic effect of 4-hydroxyisoleucine was, at least in part, from direct pancreatic beta cell stimulation. However, no detailed study has been undertaken to understand whether TSP and other plant extracts follow similar signalling biochemical pathways as taken by insulin. We suggest here that the mode of action of TSP is mainly by reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes involving all the mechanisms discussed and mentioned above.

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). It can be suggested that there may be some *in vivo* complex formation by SOV with organic compounds made available by *Trigonella* that is responsible for bringing the better control of glucose level and the diabetic complications. Recently, it has been shown by Shinde *et al* (2001) that chronic treatment with an organic complex of vanadium such as bis(maltolato)oxovanadium (IV) (BMOV) was effective in improving glucose and lipid homeostasis. Hence, the attempt and emphasis on the use of vanadium complexes in treatment of diabetes mellitus is emerging as a new concept (Sakurai 2002). The present study substantiates this new concept and suggests the effectiveness of combined therapy of SOV and TSP on the control of glucose homeostasis and lipid metabolism during experimental diabetes and can be considered as a better alternative for further investigation for the amelioration of diabetes.

Acknowledgements

UCSY is a recipient of Senior Research Fellowship from the University Grants Commission (UGC), New Delhi. Research grants from UGC and Department of Biotechnology, New Delhi are also acknowledged thankfully.

References

Agius L and Vaartjes W J 1982 The effects of orthovanadate on fatty acid synthesis in isolated rat hepatocytes; *Biochem. J.* **202** 791–794

Ahmed I, Adeghate E, Sharma A K, Pallot D J and Singh J 1998 Effects of *Momordica charantia* fruit juice on islet morphology in the pancreas of the Streptozotocin diabetic rat; *Diabetes Res. Clin. Pract.* **40** 145–151

Al-Habori M and Raman A 1998 Antidiabetic and hypocholesterolaemic effect of fenugreek; *Phytother. Res.* **12** 233–242

Arky R A 1982 Clinical correlates of metabolic derrangements of diabetes mellitus; in *Complications of diabetes mellitus* (ed.) G P Kozak (Philadelphia: W B Saunders) pp 16–20

Bach J F 1995 Insulin-dependent diabetes mellitus as a *b*-cell targeted disease of immunoregulation; *J. Autoimmun.* **8** 439–463

Baquer N Z, Cascales M, McLean P and Greenbaum A L 1976 Effects of thyroid deficiency on the distribution of hepatic metabolism and control of pathways of carbohydrate metabolism in liver and adipose tissue of the rat; *Eur. J. Biochem.* **68** 403–413

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

Baquer N Z, Sinclair M, Kunjara S, Yadav U C and McLean P 2003 Regulation of glucose utilization and lipogenesis in adipose tissue of diabetic and fat fed animals: Effects of insulin and manganese; *J. Biosci.* **28** 215–221

Bloom A J and Ireland A 1992 *Color atlas of diabetes* 2nd edition (London: Wolfe Publication) pp 10–38

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

Brichard S M, Ongemba L N, Girard J and Henquin J C 1994 Tissue-specific correction of lipogenic enzyme gene expression in diabetic rats given vanadate; *Diabetologia* **37** 1065–1072

Broca C, Gross R, Petit P, Sauvaire Y, Manteghetti M, Tournier M, Masiello P, Gomis R and Ribes G 1999 4-Hydroxyisoleucine: experimental evidence of its insulinotropic and antidiabetic properties; *Am. J. Physiol.* **277** E617–E623

Chaterjea M N and Shinde R 1994 Metabolism of carbohydrate Part II; in *Text book of medical biochemistry* 1st edition (New Delhi: Jay Pee Brothers Medical publishers) p. 421

Cryer P 1992 Iatrogenic hypoglycemia as a cause of hypoglycemia associated autonomic failure in IDDM: a vicious cycle; *Diabetes* **41** 255–260

Dafnis E and Sabatini S 1994 Biochemistry and pathology of vanadium; *Nephron* **67** 133–143

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 and Sanchez D J 1995 Toxicology of vanadium compounds in diabetic rats: The action of chelating agents on vanadium accumulation; *Mol. Cell. Biochem.* **153** 233–240

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 rat is accompanied by negative side effects; *Pharmacol. Toxicol.* **68** 249–253

Ezaki O 1990 Insulin like effects of selenate in rat adipocytes; *J. Biol. Chem.* **265** 1124–1128

Folch J, Lees M and Stanley G H S 1957 A simple method for the isolation and purification of total lipids from animal tissues; *J. Biol. Chem.* **226** 497–509

Goto Y, Kida K and Ikeachi M 1992 Synergism in insulin like effects of molybdate plus hydrogen peroxide or tungstate plus hydrogen peroxide on glucose transport by isolated rat adipocytes; *Biochem. Pharmacol.* **44** 174–177

Guijarro C, Kasiske B L, Kim Y, O'Donnell M P, Lee H S and Keane W F 1995 Early glomerular changes in rats with dietary-induced hypercholesterolemia; *Am. J. Kidney Dis.* **26** 152–161

Gupta D, Raju J, Prakash J and Baquer N Z 1999 Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats: effect of insulin and vanadate; *Diabetes Res. Clin. Pract.* **46** 1–7

Heyliger C E, Tahiliani A J and McNeill J H 1985 Effects of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats; *Science* **227** 1474–1476

Khosla P, Gupta D D and Nagpal R K 1995 Effect of *Trigonella foenum-graecum* (Fenugreek) on blood glucose in normal and diabetic rats; *Indian J. Physiol. Pharmacol.* **39** 173–174

Kimmelsteil P and Wilson C 1936 Intercapilary lesion in the glomeruli of the kidney; *Am. J. Pathol.* **12** 83–105

Lakshmanan M R, Nepokroeff C M and Porter J W 1972 Control of the synthesis of fatty acid synthetase in rat liver by insulin, glucagon and adenosine-3′5′cyclic monophosphate; *Proc. Natl. Acad. Sci. USA* **69** 3516–3519

Lee H S, Lee J S, Koh H I and Ko K W 1991 Intraglomerular lipid deposition in routine biopsies; *Clin. Nephrol.* **36** 67–75

Madar Z L and Thorne R 1987 Dietary fiber; *Prog. Food Nutr. Sci.* **11** 153–174

Meyer C, Stumvoll M, Nadkarni V, Dostou J, Mitrakou A and Gerich J 1998 Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus; *J. Clin. Invest.* **102** 619–624

Moller D E 2001 New drug targets for type 2 diabetes and the metabolic syndrome – a review; *Nature* (*London*) **414** 821–827

Moorthy R, Prabhu K M and Murthy P S 1989 Studies on the isolation and effect of orally active hypoglycemic principle from the seed of fenugreek (*Trigonella Foenum-graecum*); *Diabetes Bull.* **9** 69–72

Murthy P S 1995 Potential of medicinal plants for the treatment of diabetes mellitus and other diseases; *Indian J. Clin. Biochem.* **10** 52–53

Nandhini D, Maneemegalai S, Elangovan V, Sekar N and Govindasamy S 1993 Insulin-like effects of bis-glycinato oxovanadium (IV) complex on experimental diabetic rats; *Indian J. Biochem. Biophys.* **30** 73–76

Petit P R, Sauvaire Y D, Hillaire-Buys D M, Leconte O M, Baissac Y G, Ponsin G R and Ribes G R 1995 Steroid saponins from fenugreek seeds: extraction, purification, and pharmacological investigation on feeding behaviour and plasma cholesterol; *Steroids* **60** 674–680

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

Ramakrishna S, Murthy K S and Benzamin W B 1989 Effect of insulin on ATP-citrate lyase phosphorylation. Regulation of peptide A and peptide B phosphorylation; *Biochemistry* **28** 856–860

Ramasarma T 1996 Vanadium complexes with insulin-mimetic actions – A second line of protection against diabetes; *Indian J. Clin. Biochem.* **11** 92–107

Reichard P, Nilsson B Y and Rosenquist U 1993 The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus; *N. Engl. J. Med.* **329** 304–309

Saggerson E D and Greenbaum A L 1970 The regulation of triglyceride synthesis and fatty acid synthesis in rat epididymal adipose tissue; *Biochem. J.* **119** 221–242

Sahng-Wook P, Kyung-Sup K, Suk-Kuy W, Jin-Soo K and Yoon-Soo K 1994 Induction of hepatic ATP-citrate lyase by insulin in diabetic rats; *Yonsei Med. J.* **35** 25–33

Sakurai H 2002 A new concept: The use of vanadium complexes in the treatment of diabetes mellitus; *Chem. Rec.* **2** 237–248

Sauvaire Y, Petit P, Broca C, Manteghetti M, Baissac Y, Fernandez-Alvarez J, Gross R, Roye M, Leconte A, Gomis R and Ribes G 1998 4-Hydroxyisoleucine: a novel amino acid potentiator of insulin secretion; *Diabetes* **47** 206–210

Scott M and Grundy 1999 Diabetes and cardiovascular disease; *Circulation* **100** 1134–1146

Sekar N, Li J and Shechter Y 1996 Vanadium salts as insulin substitute: Mechanism of action, a scientific and therapeutic tool in diabetes mellitus research; *Crit. Rev. Biochem. Mol. Biol.* **31** 339–359

Sharma R D, Raghuram T C and Rao N S 1990 Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes; *Eur. J. Clin. Nutr.* **44** 301–306

Sheela C G and Augusti K T 1992 Antidiabetic effects of S-allyl cyteine sulphoxide isolated from garlic, *Allium sativum* Linn; *Indian J. Exp. Biol.* **30** 523–526

Shibib B A, Khan L A and Rahman R 1993 Hypoglycemic activity of *Coccinia indica* and *Momordica charantia* in diabetic rats: Depression of the hepatic gluconeogenic enzymes glucose-6-phosphate and fructose-1,6-bisphosphatase and elevation of both liver and red cell shunt glucose-6-phosphate dehydrogenase; *Biochem. J.* **292** 267–270

Shinde U A, Mehta A A and Goyal R K 2001 Effect of chronic treatment with Bis(maltolato)oxovanadium (IV) in rat model of non-insulin depedent diabetes; *Indian J. Exp. Biol.* **39** 864–870

Shisheva A, Gefel D and Shechter Y 1992 Insulin like effects of zinc ion *in vitro* and *in vivo*: preferential effects on desensitized adipocytes and induction of normoglycemia in Streptozotocin induced rats; *Diabetes* **41** 982–988

Sochor M, Baquer N Z and McLean P 1985 Glucose over and underutilization in diabetes: Comparative studies on the

changes in activities of enzymes of glucose metabolism in rat kidney and liver; *Mol. Physiol.* **7** 51–68

Sochor S, Baquer N Z, Ball M R and McLean P 1987 Regulation of enzymes of glucose metabolism and lipogenesis in diabetic rat liver by thyroid hormones; *Biochem. Int.* **15** 619–627

Srere P A 1972 The citrate enzymes: their structures, mechanisms, and biological functions – a review; *Curr. Top. Cell. Regul.* **5** 229–283

Srivastava A K 2000 Antidiabetic and toxic effects of vanadium compounds; *Mol. Cell. Biochem.* **206** 177–182

Sun L, Halaihel N, Zhang W, Rogers T and Levi M 2002 Role of sterol regulatory element-binding protein 1 in regulation of renal lipid metabolism and glomerulosclerosis in diabetes mellitus; *J. Biol. Chem.* **277** 18919–18927

West K M 1982 Hyperglycemia as a cause of long-term complications; in *Complications of diabetes* 2nd edition (ed.) H Keen (London: Jarret Edward Arnold) pp 3–18

Yoshikawa M, Murakami T, Komatsu H, Murakami N, Yamahara J and Matsuda H 1997 Medicinal foodstuffs IV Fenugreek seed (1): structures of trigoneosides Ia, Ib, IIa, IIb, IIIa, and IIIb, new furostanol saponins from the seeds of Indian *Trigonella foenum-graecum* L.; *Chem. Pharm. Bull.* (*Tokyo*) **45** 81–87

MS received 6 September 2003; accepted 22 January 2004

Corresponding editor: DESIRAZU N RAO