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Modulation of some gluconeogenic enzyme activities in diabetic rat liver and kidney: Effect of antidiabetic compounds

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The effects of insulin, sodium orthovanadate and a hypoglycemic plant material, *Trigonella foenum graecum* (fenugreek) seed powder were studied on the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase in diabetic liver and kidney. The significantly increased activities of the two enzymes during diabetes in liver and kidney were found to be lowered to almost control values by the use of the antidiabetic compounds. Diabetic liver exhibited a much greater increase in the activities of the two enzymes than diabetic kidney. The highest percentage of reversal to normal values was seen using the combination of vanadate and *Trigonella* seed powder. The lowered rate of growth of the animals as well as the increased blood sugar were reversed almost to the control levels by the *Trigonella* seed powder and vanadate treatment. The inclusion of the *Trigonella* seed powder overcame the toxicity of vanadium encountered when it was given alone as insulin mimetic agent. Much lower levels of vanadate were needed when it was given in combination with *Trigonella* seed powder. Their combined effects were better at restoring the above parameters than those induced by insulin administration.

Glucose-6-phosphatase [G-6-Pase, EC 3.1.3.9], one of the key enzymes in the homeostatic regulation of blood glucose levels, catalyzes the terminal step in both gluconeogenesis and glycogenolysis[1-3]. The enzyme is mainly found in the gluconeogenic tissues, liver and kidney, where it plays a major role in glucose production[4]. Fructose-1,6-bisphosphatase [F-1,6-BisPase, EC 3.1.3.11] catalyzes one of the irreversible step in gluconeogenesis and serves as a site for the regulation of the process[5]. This enzyme has been extensively studied in liver and kidney of herbivorous animals[6]. Fasting, hyperglycemia, the hallmark of diabetes mellitus is largely the result of glucose over production[7]. Hepatic glucose production is the balance between the flux through glucokinase and glucose-6-phosphatase. Activity of glucokinase is markedly decreased[8,9] and the activity of G-6-Pase plays an important role in glucose homeostasis in liver and in kidney[10]. It was recently shown that the control of G-6-Pase takes place in gluconeogenic tissues, liver and kidney at pretranslational level[11].

Alternatives to insulin therapy in diabetes mellitus are important because of the long-term complications, associated with insulin resistance. Vanadium compounds are insulin mimetic agents and their antidiabetic properties are well established. Several reports however have shown toxic responses in animals that were treated with vanadate compounds[12-14]. Attempts have been made to reduce the chances of toxicity and vanadium accumulation problem by the use of chelating agents e.g. tiron[15,16]. Simultaneously various plant materials are being investigated for their antidiabetic properties[17-19] and *Trigonella foenum graecum* is one among these which is also used in the Indian subcontinent as a vegetable and the seeds as a spice.

In this study vanadate is administered to experimental diabetic rats at a lower than toxic level together with *Trigonella* whole seed powder. Measurement of changes of physiological parameters: whole body, liver and kidney weight, blood glucose concentration and the activities of the two key gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase show a significant shift towards normal values using the combined therapy.

Animals — Female albino rats of the Wistar strain, weighing around 200g were used. They were maintained at constant temperature of about 25°C and were fed *ad libitum* with rat feed (Hindustan Lever Ltd, India) and tap water.

Induction of diabetes — The rats were starved for 24 hr and divided into control and experimental groups, the latter were made diabetic according to the method of Sochor *et al.*[20]. Each rat received a subcutaneous injection of alloxan monohydrate (200mg/kg body weight) freshly prepared in 0.154*M* sodium acetate buffer (*p*H 4.5). Control rats received the same volume of the vehicle. From the next day each diabetic rat was given 2 IU of protamine zinc insulin i.p. daily for the next 7 days. After insulin withdrawal the experimental rats were divided into five groups: diabetic (D), diabetic treated with insulin (D+I), diabetic treated with sodium orthovanadate (D+V), diabetic treated with *Trigonella* seed powder (D+T) and diabetic treated with sodium orthovanadate and *Trigonella* seed powder (D+V+T).

Treatment with antidiabetic compounds — The diabetic treated with insulin (D+I) group animals were administered with 2 IU of protamine zinc insulin suspension i.p., each day for 14 days. The diabetic treated with vanadate (D+V) group animals were given sodium orthovanadate at a dose of 0.6mg/ml in drinking water (freshly prepared) each day for 14 days[12]. The diabetic treated with *Trigonella* (D+T) group animals were given 5% *Trigonella* seed powder

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(Agmark brand) in powdered rat feed (i.e. 5g of powdered *Trigonella* seeds in 95g of powdered rat feed) each day for 14 days. The diabetic treated with vanadate and *Trigonella* (D+V+T) group animals were given 0.2mg/ml of sodium orthovanadate in tap water and 5% finely powdered *Trigonella* seeds in powdered rat feed each day for 14 days.

Preparation of tissue extracts and subcellular fractions — Animals were starved overnight and sacrificed by cervical dislocation. Tissues were dissected out, washed in normal saline and weighed. The tissues were homogenised in 9 volumes of homogenizing buffer (0.25M sucrose and 0.02M triethanolamine, *p*H 7.4, containing 0.12mM dithiothreitol) using a Potter Elvehjam homogenizer fitted with a teflon plunger. The cytosolic fractions were prepared by differential centrifugation of the homogenate[20]. The supernatant fraction was used for the assays.

Determination of G-6-Pase and F-1,6-BisPase activity — The activity of G-6-Pase and F-1,6-BisPase was estimated in liver and kidney supernatant fractions of 14 days experimental rats by the method of Baginsky *et al.*[21] and Tashima and Yoshimura[22] respectively as used earlier in our laboratory[23]. About 1-2 mg of protein was used for both assays. One enzyme unit is defined as the amount of inorganic phosphate, Pi liberated per gram fresh weight per minute at 37°C for both the phosphatases. Phosphate estimation was by the method of Fiske and Subbarow[24].

Blood glucose and protein estimations — Blood glucose was estimated using Glucose Enzokit bought from Ranbaxy Elily Laboratories Ltd., India. This kit quantitatively estimates D-glucose, the form present in the blood plasma. Protein concentration in liver and kidney supernatant fractions was determined by the method of Lowry *et al.*[25] using bovine serum albumin (BSA) as standard.

Statistical analysis — The significance of difference between the data pairs was evaluated by analysis of variance (ANOVA) followed by Mann-Whitney rank sum test.

Chemicals — All substrates, buffers, cofactors, bovine serum albumin, alloxan and sodium orthovanadate were purchased from Sigma Chemicals, St Louis, USA. Protophine zinc insulin IP was purchased from Boots India Ltd., India. All other chemicals used were of analytical grade.

The results of the changes in the general parameters, including changes in body weight, tissue weight, blood glucose and protein content of the soluble fractions are shown in Table 1. A significant decrease in the body weight of the diabetic animals was seen which was improved upon administration of the *Trigonella* (fenugreek) seed powder and a combination of *Trigonella* seed powder and sodium orthovanadate. The blood glucose values shifted towards control levels with vanadate and *Trigonella* seed powder treatments as reported earlier [12,18], and the combined dose of vanadate and *Trigonella* seed powder (Table 2). A three fold lower level of vanadate was enough to decrease the blood glucose level to almost control values when it was given in combination with the *Trigonella* seed powder, inclusion of the latter with vanadate probably lowered the toxicity of vanadium.

The changes in the activities of the two gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase from the liver and kidney of diabetic rats after various treatments are presented in Table 2. The activity of G-6-Pase was almost the same in liver and kidney of the control animals and the increase in the activities during diabetes was much higher (nearly 3-fold) in liver than in kidney. All the treatments with antidiabetic compounds namely, insulin, *Trigonella* seed powder, vanadate and a combination of vanadate with *Trigonella* seed powder were found to restore the enzyme levels to their normal control values, the optimum being the vanadate and *Trigonella* seed powder combination in both liver and kidney. The activity of fructose-1,6-bisphosphatase was also almost the same in liver and

Table 1 — General parameters: Changes in body weight, tissue weight, blood glucose and tissue soluble protein content in rat liver and kidney of different experimental groups.

[Values are mean+SE of 4 or more separate determinations.]

	Control	Diabetic	Diabetic + Insulin	Diabetic + Vanadate	Diabetic + *Trigonella*	Diabetic + Vanadate + *Trigonella*
General Parameters						
Body weight (g)	216 ± 9.0	145+7.6[a]	186+2.3[b]	183+1.5[b]	200+2.9	203+3.3
Liver weight (g)	5.5 ± 0.1	4.9+0.1[d]	5.4+0.1	5.5+0.1	5.8+0.1[d]	5.8+0.1[d]
Liver weight / 100g Body weight	2.5 ± 0.5	3.3 ± 0.7[d]	2.9 ± 0.9	3.3 ± 0.3	2.9 ± 0.1[d]	2.8 ± 0.6[d]
Kidney weight (g)	1.1 ± 0.04	1.5 ± 0.04[b]	1.4 ± 0.04	1.2 ± 0.05	1.4 ± 0.07	1.3 ± 0.05
Kidney weight / 100g Body weight	0.5 ± 0.01	1.0 ± 0.03[b]	0.7 ± 0.04	0.6 ± 0.06	0.7 ± 0.07	0.6 ± 0.04
Blood glucose (mg/dl)	102.6+5	317.6+16[a]	111.6 +4.0	104 +0.8	106 +3.3	105 +4.1
Protein, soluble (mg/g)						
Liver	121+1.1	106 +2.3[b]	108+1.4[b]	117+1.5[b]	123+1.2	124+1.5
Kidney	92.2 ± 0.8	88.7 ± 1.0[c]	91.5 ± 1.0	85.1 ± 0.9[a]	86.2 ± 0.8[c]	88.9 ± 1.0[c]

Fisher's *P* values: [a]<0.005, [b]<0.05, [c]<0.001 and [d]<0.5.

Table 2 — Changes in the activity of glucose-6-phosphatase (G-6-Pase) and fructose-1,6-bispase (F-1,6-BisPase) in rat liver and kidney of different experimental groups. Enzyme units are expressed as units/g/min

[Values are mean+SE of 4 or more separate determinations.]

Experimental conditions	Liver		Kidney	
	G-6-Pase	F-1,6-BisPase	G-6-Pase	F-1,6-BisPase
Control	11.0 ± 0.31	3.80 ± 0.24	12.50 ± 0.24	4.15 ± 0.10
Diabetic	29.24 ± 1.64[a]	11.61 ± 2.05[b]	18.29 ± 0.59[a]	10.90 ± 0.65[a]
Diabetic + Insulin	13.15 ± 0.87[b]	5.68 ± 0.23[b]	12.23 ± 0.25	5.05 ± 0.03[c]
Diabetic + Vanadate	13.92 ± 2.05[b]	6.35 ± 0.28[b]	15.35 ± 1.03[c]	5.68 ± 0.21[a]
Diabetic + Trigonella	14.30 ± 0.39[b]	6.70 ± 0.41[a]	13.17 ± 0.56	6.68 ± 0.43[a]
Diabetic + Vanadate + Trigonella	11.5 ± 0.55	6.20 ± 0.30	11.20 ± 0.25[d]	6.75 ± 0.05[a]

Fisher's *P* values: [a]<0.005, [b]<0.05, [c]<0.001 and [d]<0.5.

kidney but the levels of this enzyme was lower than that of glucose-6-phosphatase from both liver and kidney (almost 3-fold), as shown in Table 2. The diabetic liver and kidney exhibited an approximately 2.5-fold increased level of activity. The restoration of F-1,6-BisPase from liver was most extensive with insulin administration. In the case of kidney, insulin and vanadate separately had the same reversal effects.

In an attempt to gain an insight into the underlying biochemical mechanism of the action of some hypoglycemic agents, like vanadate and *Trigonella* seed powder, we assayed the key gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase from liver and kidney of diabetic rats, liver being the main organ responsible for maintaining homeostasis of the blood glucose and kidney being the organ most affected in diabetes showing glucose overutilization.

Studies by Mitheux *et al.*[11] reported the regulation of mRNA of liver G-6-Pase during experimental diabetes. Further studies also showed the reversal of the increased activity of glucose-6-phosphatase by ethanolic extracts of *Cocinia indica* in both fasted and diabetic rats[17]. *Coccinia* leaf extracts significantly depressed G-6-Pase and F-1,6-BisPase activities in both control and streptozotocin diabetic rats, the hepatic urea cycle enzyme arginase was also found to decrease. Shibib *et al.*[17] and others[26] have concluded from these data that the blood sugar lowering effects of these compounds may be ascribed to peripheral glucose utilization rather than a direct effect on liver gluconeogensis.

The data on the increased level of the two gluconeogenic enzymes from liver and kidney may be due to the activation or increased synthesis of the enzymes contributing to the increased glucose production in diabetes by liver and kidney and the effects of vanadate, *Trigonella* seed powder and the combined treatment may be primarily modulating and regulating the activities of the two gluconeogenic enzymes, either through regulation by c'AMP and any other metabolite activation or inhibition of glyolysis and gluconeogenesis. The redox state of the liver cell is highly reduced in diabetes together with the changes in the energy state[27]. ATP levels are lowered in the cytosol, the mitochondrial ATP, however is not much affected and could be the energy source for the higher levels of gluconeogenesis in diabetes in both liver and kidney. Other metabolites especially the substrates for the G-6-Pase and F-1,6-BisPase, glucose-6-phosphate and fructose-1,6-bisphosphate are known to increase in diabetes in the liver due to the inhibition of hexokinase and phosphofructo-kinase, the two regulatory glycolytic enzymes[20]. All these physiological and metabolic effects during diabetes could be normalized by the administration of vanadium, *Trigonella* seed powder and a combined treatment. The effect of these antidiabetic compounds on the mRNA levels of G-6-Pase and F-1,6-BisPase from both liver and kidney in diabetes and antidiabetic compounds treated animals and also in some other conditions like starvation in which two enzymes have been shown to increase, is under study in our laboratory.

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