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Beta Cell Protective Effects of Sodium Tungstate in Streptozotocin-Induced Diabetic Rats: Glycemic Control, Blockage of Oxidative Stress and Beta Cell Histochemistry

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ABSTRACT

Background: Diabetes is a major public health problem. The development of new therapies that are able to improve glycemia management and even to cure diabetes is of great interest. In this study, protective effects of sodium tungstate against STZ-induced beta-cell damages were investigated. **Methods:** Sixty rats were divided into six groups: control, diabetic, sodium tungstate treated diabetic rats from one week before STZ injection (TDB), food-restricted diabetic (FRD), tungstate treated control, sodium tungstate treated diabetic rats from one week after STZ administration (TDA). We evaluated serum insulin, glucose and glucose tolerance; liver glycogen content, glucokinase (GK) activity; blood and pancreas antioxidant power, lipid peroxidation; and fuchsin-aldehyde histochemical staining of beta-cells. **Results:** Blood glucose levels of TDB group were lower than other diabetic groups ($P<0.01$). Blood insulin levels of all diabetic groups were lower than controls ($P<0.01$). Glucose intolerance improved in TDB animals. Blood and pancreas antioxidant power, liver glycogen contents and GK activities and granulated beta cells increased in TDB rats in comparison with other diabetic groups ($P<0.01$). Likewise, lipid peroxidation decreased significantly in TDB rats ($P<0.01$). **Conclusions:** Results suggested that sodium tungstate if administrated before STZ injection improves glycemic state by a direct effect on pancreatic beta-cells and preserves them by reducing the activity of these cells at the time of STZ injection, reducing STZ-induced oxidative stress, reducing insulin secretion, or all of the above mentioned. *Iran. Biomed. J. 12 (3): 143-152, 2008*

Keywords: Diabetes mellitus, Sodium tungstate, Beta cells

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders of carbohydrate characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia was found to increase the production of free radicals that is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Evidences indicate that free oxygen radicals and membrane lipid peroxidation are significantly increased in diabetic patients and in experimental diabetic animals [2]. The increased production and/or ineffective scavenging of reactive oxygen species (ROS), resulting in tissue damage that in most instances is assessed by the measurement of lipid peroxides [3, 4].

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [5].

In spite of the introduction of hypoglycemic drugs,

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[#]Dr. Mehdi Harati unexpectedly passed away on 15 March 2007 due to a car accident. His demise has been a painful loss to us.

Abbreviations: Streptozotocin (STZ), Tungstate treated Diabetic Before STZ induction (TDB), Tungstate treated Diabetic After STZ induction (TDA), Food-restricted diabetic (FRD), Tungstate treated control (TC), Glucokinase (GK), malondialdehyde (MDA), Oral glucose tolerance test (OGTT), aspartate aminotransfrase (AST), alanine aminotransferase (ALT), Ferric reducing/antioxidant power (FRAP)

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diabetes and related complications continue to be a major medical problem. Therefore, new agents that are able to control and even to treat diabetes are of great interest. In recent years, several inorganic elements have been described that mimic the effects of insulin or increase insulin action. These include derivatives of vanadium [6, 7], chromium [8], molybdenum [9], cobalt [10] and Zinc [11]. Recent studies have also shown that sodium tungstate possesses anti-diabetic activity in several diabetic animal models [12-14]. This element increases the effects of insulin both *in vivo* and in isolated cells and tissues [15, 16]. Report also indicated that tungstste regenerates pancreatic beta-cells population in neonatal STZ rats, a type 2 diabetes model [17].

Type I diabetes mellitus resulting from selective destruction of the insulin producing beta cells in the pancreatic islets [18]. Hence, search for new agents that protect beta cells from destruction and thereby prevent type I diabetes is needed. The protective effect of sodium tungstate on beta-cells damage induced by streptozotocin in type I diabetic animal model has not been previously investigated. Previous studies indicated that sodium tungstate improves diabetes complications [12-18] and here we evaluate the glycemic control and pancreatic beta-cells protective effects of sodium tungstate in streptozotocin-induced type I diabetic rat.

MATERIALS AND METHODS

Animals and preparations. The study was performed on mature normoglycemic male Wistar rats, weighing 200-220 g, which were separately housed in cages (one rat per cage). Animals were maintained in a room at 23°C ± 2, humidity 45% to 55% with a fixed 12-h artificial light period and allowed to eat and drink *ad libitium*. All animals were fed with standard rodent diet and received human care, as outlined in the guide for the care and use of laboratory animals. This study was approved by Ethical Committee of Zahedan University of Medical Sciences (Zahedan, Iran).

Induction of diabetes in animals. Type I diabetes was induced by a single i.p. administration of streptozotocin (50 mg/kg of body weight) in 0.15 M NaCl with 100 mM sodium citrate buffer (pH 4.5).

Experimental design. Sixty rats were divided into six following groups (n = 10): (i) Control group received standard rodent diet and tap water. After one week they received i.p. vehicle (0.15 M NaCl with 100 mM sodium citrate buffer). (ii) Tungstate treated control (TC) group received standard rodent diet, and tap water supplemented with 1-1.75 mg/ml sodium tungstate during the experiment. (iii) Diabetic group received standard rodent diet and tap water. Diabetes was induced by i.p. injection of STZ as mentioned. (iv) sodium tungstate treated one week before induction of diabetes (TDB). This group received standard rodent diet, and tap water supplemented with 1 mg/ml sodium tungstate at one week before STZ injection. In an effort to increase the normoglycemic response, the concentration of tungstate was gradually raised by increment of 0.25 mg/ml to a maximum concentration of 1.75 by 5 weeks. (v) Food-restricted diabetic group (FRD) received tap water and same amount of food as that consumed by TDB group. Because tungstate treatment was accompanied by a reduced weight gain, which may possible influence glucose homeostasis [14], a group of untreated diabetic rats received a restricted amount of food (FRD) to ensure a body weight gain similar to that of tungstate-treated animals (TDB). This amount was experimentally adjusted daily and was given them in two rations (one-third at 9.00 h and two thirds at 18.00 h). (vi) Sodium tungstate treated one week after induction of diabetes (TDA). This group received standard rodent diet, and tap water supplemented with 1-1.75 mg/ml sodium tungstate at one week after administration of STZ and continued by 5 weeks. Food and fluid intake of all groups were measured daily, and body weight, blood glucose and insulin were measured every week.

The experiment was carried out for 5 weeks after STZ administration in diabetic groups. At the end of the experiment and after overnight fast, all animals were sacrificed under light ether anesthesia. Immediately, blood samples were collected from tail vein. A fraction of blood sample was collected in EDTA for preparation of plasma, and another fraction was immediately placed on ice and centrifuged within 15 min after blood collection at 2500 × g for 15 min. Plasma and serum were stored at -20°C for less than 2 weeks before subsequent analysis.

Isolation of liver and pancreas tissues. After sacrificing the animals, their livers were perfused with cold saline to remove blood. Then, they were quickly sliced, and fragments used to measure enzyme activity, and glycogen content. The

pancreases were quickly removed, placed in cold saline solution and trimmed of adipose tissue. The pancreases were used for determination of antioxidant power and histological study.

Preparation of tissues homogenize for evaluation of oxidative stress. Fractions of pancreases were homogenized (1:10, w/v) in 0.05 M sodium phosphate buffer, pH 7.4 and were directly used for determination of malondialdehyde (MDA) and total antioxidant power.

Oral glucose tolerance test (OGTT). After an overnight fast (at 5 weeks post STZ administration), rats of all groups were received 1 g/kg of a 40% glucose solution by oral gavage. Glucose concentrations were measured before and at 15, 30, 60, and 120 min after glucose administration. OGTT was performed on animals that were anesthetized with i.p. injection of ketamine hydrochloride (60 mg/kg body weight).

Assay. Serum aspartate aminotransfrase (AST), alanine aminotransferase (ALT), and glucose levels were measured by standard methods adapted for a RA 1000 analyzer (Technicon, USA). Serum insulin levels were determined by ultra sensitive rat insulin kit (DRG, France), using double-antibody ELISA. Liver glycogen content was determined as described [19]. Glucokinase (GK) activity in liver homogenates was measured by continuous method as described [20]. Lipid peroxidation product MDA was measured according to the method described by Jain and Levine [21]. Thiobarbitiuric acid reactive substances (TBARS) were expressed per milligram of tissue protein. Botylated hydroxyl toluene was used to prevent an *in vitro* increase of MDA during the experiment. Antioxidant power of blood and pancreas was measured by ferric reducing/ antioxidant power (FRAP). FRAP assay was performed according to the method as described [22]. The results were expressed as μmol/l Fe^{II} per milligram of protein. Protein concentration was determined by folin-ciocalteu method using bovine serum albumin as standard [23].

Pancreas histology. A portion of pancreas was fixed in modified Lillie's solution for one week, processed and embedded in paraplast. Thick sections (4 μm) were stained for granulated beta cells by modified aldehyde fuchsin histochemical method [24]. By this procedure, beta and alpha cells in islets of pancreas were stained purple-violet and yellow, respectively.

Statistical data analysis. Results were expressed as mean ± SE. To confirm normal distribution, data were analyzed by one-sample Kolmogrov-Smirnov test and then by levene's test. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons was used to compare differences among experimental groups. Significant level was set at $P<0.05$. All statistical analyses were performed using SPSS 11 for Windows software system.

RESULTS

Physiological parameters. Table 1 shows body weight, food and fluid intake, urine volume in control and diabetic animal models. Diabetic and TDA groups showed significant ($P<0.01$) weight loss, polyphagia, polydipsia and polyuria, when compared with control rats (control and TC) at the end of treatment period. In TDB and FRD groups polyphagia, polydipsia, and polyuria reduced significantly ($P<0.01$) when compared with diabetic and TDA groups. Also, the body weight of TDB and FRD groups significantly reduced ($P<0.01$) when compared with control animals.

Table 1. Changes in body weight, food and fluid intake, and urine volume in each experimental group (n = 10), at the end of the experiment.

Groups	C	D	FRD	TDB	TC	TDA
Body weight (g)	378.25 ± 11.01	314.45 ± 13.88[a]	283.16 ± 7.29[b]	270.48 ± 9.21[b]	350.48 ± 8.91[b]	321.98 ± 7.11[a]
Food intake (g/day)	35.48 ± 1.49	62.79 ± 2.87[a]	21.74 ± 2.51[b]	23.43 ± 2.54[b]	23.75 ± 1.06[b]	54.79 ± 1.18[a]
Fluid intake (ml/day)	50.38 ± 2.16	378.56 ± 8.73[a]	158.82 ± 8.72[b]	79.73 ± 2.69[b]	41.25 ± 1.65[b]	377.45 ± 8.42[a]
Urine volume (ml/day)	46.66 ± 2.36	351.32 ± 11.27[a]	141.39 ± 8.02[b]	68.45 ± 3.12[b]	38.76 ± 0.42[b]	337.98 ± 1.71[a]

The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction. [a]$P<0.01$ compared to control group and [b]$P<0.01$ compared to diabetic groups.

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Table 2. Change in blood glucose levels in each experimental group (n = 10) during the experiment.

Groups	Blood glucose (mg/dl)						
	-1 week	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
C	78.25 ± 1.3	83.78 ± 0.94	86.45 ± 1.5	82.49 ± 1.7	88.75 ± 1.18	75.42 ± 0.87	82.55 ± 1.61
D	80.51 ± 1.8	82.48 ± 1.35	271.21 ± 3.05[a]	283.32 ± 3.34[a]	291.78 ± 2.97[a]	298.33 ± 3.55[a]	306.89 ± 3.91[a]
FRD	75.32 ± 1.48	79.51 ± 1.03	263.42 ± 3.45[a]	281.13 ± 3.21[a]	289.35 ± 3.83[a]	296.57 ± 2.72[a]	290.44 ± 3.55[a]
TDB	80.19 ± 1.22	83.42 ± 1.32	131.46 ± 1.74[b]	95.45 ± 1.7[b]	87.39 ± 1.98[b]	81.86 ± 2.01[b]	85.39 ± 2.29[b]
TC	83.9 ± 1.28	85.29 ± 1.84	82.78 ± 1.09	87.63 ± 1.24	84.25 ± 2.1	84.74 ± 0.88	84.70 ± 1.68
TDA	83.56 ± 1.32	84.28 ± 1.71	256.03 ± 3.74[a]	262.64 ± 3.8[a]	268.47 ± 3.9[a]	275.56 ± 2.8[a]	280.91 ± 3.33[a]

The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction. -1 week, one week before injection of STZ, and initial time of administration of sodium tungstate in TDB rats; 0 week, initial time of STZ injection in diabetic rats; [a]$P<0.01$ compared to control group and [b]$P<0.01$ compared to diabetic, and FRD groups.

Blood glucose levels. Table 2 shows change in blood glucose levels of control and diabetic rats during the experimental period. After STZ injection, the blood glucose levels of diabetic, TDA and FRD groups significantly increased ($P<0.01$) when compared with controls. In contrast, treatment with sodium tungstate one week before administration of STZ in TDB rats significantly ($P<0.01$) protected rats from elevation of blood glucose levels compared to corresponding diabetic, TDA and FRD rats at any time point of experiment.

Blood insulin levels. Blood insulin level of control and diabetic rats during the experiment is shown in Figure 1. Blood insulin levels in all STZ-induced diabetic groups significantly reduced when compared with control groups over 5 weeks ($P<0.01$). However, sodium tungstate treatment in TDB group lowered blood insulin to greater extent than FRD, diabetic and TDA groups.

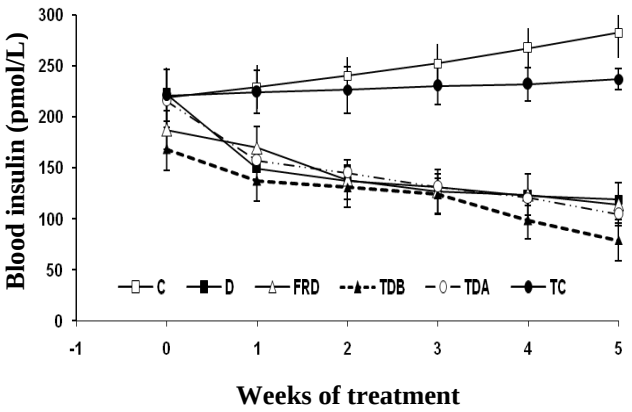

Fig. 1. Change in blood insulin levels in control and diabetic rats during the experiment. The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction (-1 week, One week before injection of STZ, and initial time of administration of sodium tungstate in TDB rats; 0 week, initial time of STZ injection in diabetic rats).

OGTT. After an overnight fast, blood glucose levels (time 0 of the OGTT) in TDB rats were lower ($P<0.01$) than in diabetic, TDA and FRD rats, but were not different from those in control (control and TC) rats (Fig. 2A). During OGTT, mean blood glucose levels remained less than 150 mg/dl in control rats, but rose to more than 500 mg/dl in diabetic, TDA and FRD rats and did not thereafter return to basal levels (Fig. 2A). In sodium tungstate pre-treated rats (TDB), glucose levels were consistently lower ($P<0.01$) than in diabetic, TDA or FRD rats and were normalized at 120 min. In other words, FRD rats unlike TDB group, continued to demonstrate marked glucose intolerance, similar to diabetic and TDA rats. Insulin secretory response in all diabetic groups markedly reduced relative to controls (Fig. 2B).

Liver glucokinase activity. Liver GK activities, which abolished in diabetic, TDA and FRD rats, markedly corrected by sodium tungstate in TDB rats and reached to those of control animals (Fig. 3).

Liver glycogen concentration. Liver glycogen concentration decreased by ~ 75% in diabetic, TDA and FRD when compared with control rats ($P<0.01$). Also, liver glycogen reserves markedly increased by ~92% ($P<0.01$) in TDB rats when compared to those in diabetic, TDA or FRD rats. In diabetic rats, sodium tungstate protected hyperglycemia by an increase in liver glycogen concentration (Fig. 4).

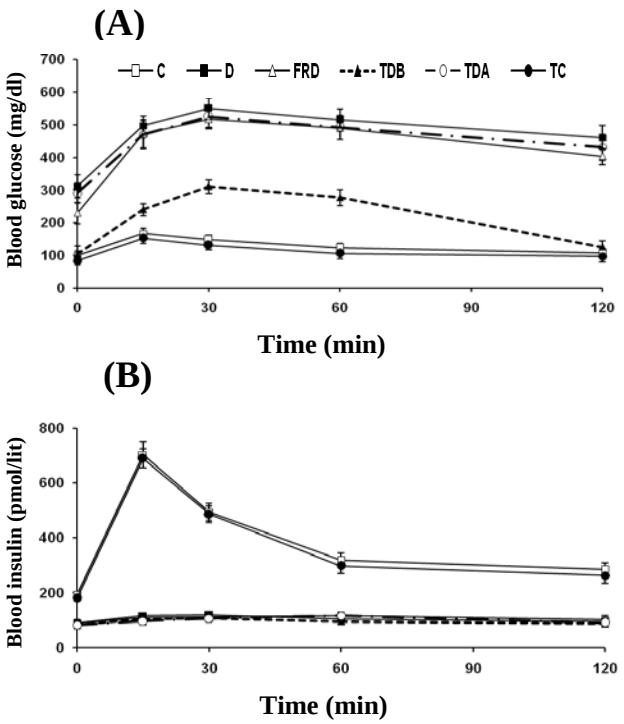

Fig. 2. Change in **(A)** blood glucose and **(B)** blood insulin levels during OGTT in control and diabetic rats. The test performed after 5 weeks of STZ injection. The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction.

Oxidative stress parameters. Diabetes led to increase of TBARS concomitant with decrease FRAP in blood and pancreas whereas sodium tungstate pretreatment counteracted these abnormalities (Table 3).

Hepatotoxicity. Blood aminotransferase activities significantly increased ($P<0.01$) in diabetic, TDA and FRD rats when compared with control animals. Treatment with sodium tungstate before STZ injection significantly decreased ($P<0.01$) blood AST and ALT activity in TDB group as compared to those of diabetic, TDA and FRD groups (Fig. 5).

Islets histology. Figure 6 shows photo-micrographs of aldehyde fucshin histochemical staining of pancreas in control and diabetic rats. Pancreas in control animals possessed normal islets with clusters of purple granulated beta cells Fig. 6A). STZ in diabetic rats led to atrophy of the islets and degenerative changes of beta cells (Fig. 6B). In contrast, pretreatment with tungstate protected marked cellular degeneration of beta cells (of islets in TDB rats (Fig. 6C). The section of FRD pancreas showed atrophy of islet and degenerative changes of beta cells (Fig. 6D). Pancreas in TC animals possessed normal islets (Fig. 6E). STZ in TDA rats led to atrophy of islets and degenerative changes of beta cells (Fig. 6F).

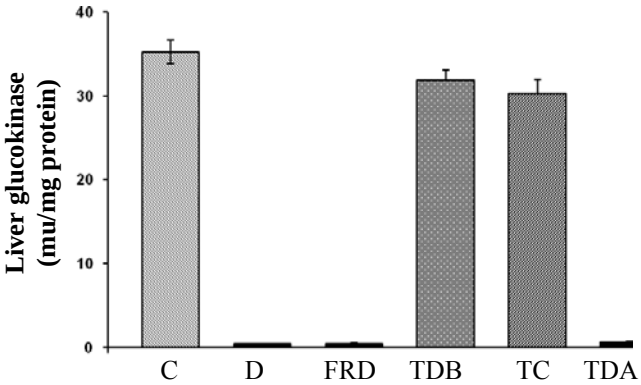

Fig. 3. Change in liver glucokinase activity of control and diabetic rats at the end of the experiment. The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction.

Table 3. Change in Concentration of TBARS and FRAP of blood and pancreas in control and diabetic animals.

Groups	TBARS (nmol/mg protein)		FRAP (μmol Fe^{II}/mg protein)	
	Blood	Pancreas	Blood	Pancreas
C	0.76 ± 0.04	0.41 ± 0.06	0.26 ± 0.04	0.98 ± 0.07
D	1.03 ± 0.09[a]	1.08 ± 0.07[a]	0.11 ± 0.03[a]	0.37 ± 0.04[a]
FRD	0.96 ± 0.08[a]	1.04 ± 0.05[a]	0.13 ± 0.02[a]	0.46 ± 0.03[a]
TDB	0.79 ± 3.85[b]	0.48 ± 0.03[b]	0.22 ± 0.02[b]	0.82 ± 0.06[b]
TC	0.63 ± 0.07	0.35 ± 0.05	031 ± 0.05	0.95 ± 0.04
TDA	0.97 ± 0.04[a]	1.01 ± 0.03[a]	0.14 ± 0.02[a]	0.42 ± 0.03[a]

The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction. [a]$P<0.01$ compared to control group and [b]$P<0.01$ compared to diabetic, and FRD groups.

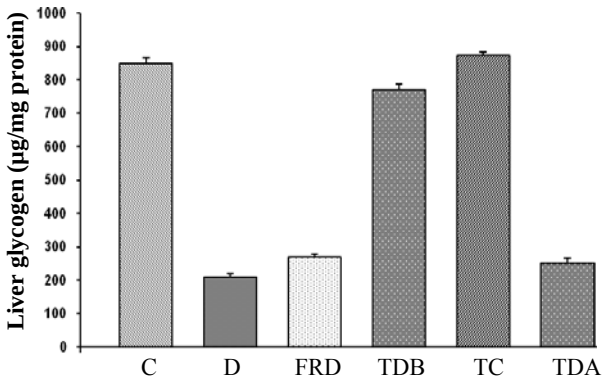

Fig. 4. Changes in liver glycogen concentration of control and diabetic rats at the end of the experiment. The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction.

DISCUSSION

Our study showed that pretreatment with sodium tungstate ameliorates diabetic state after STZ injection. It ameliorated hyperglycemia and impaired hepatic glucose metabolism and, protected beta cells from oxidative stress and degeneration by STZ. STZ was found to generate ROS, which contribute to DNA fragmentation and evoke other deleterious changes in beta cells [25].

Pharmacological interventions, that effectively stabilize a functional beta cell mass at the onset of type I diabetes, have been shown to induce a chronic amelioration of the diabetic state [26]. In rats, pretreatment with exogenous insulin for 5 days offsets the diabetogenic action of STZ by preserving 30% of the pancreatic insulin content, an effect attributed to a reduced activity of beta-cells at the time of STZ injection [27]. In contrast, increased metabolic activity by incubating at high glucose STZ [28]. It has been shown that sodium tungstate has insulin like effects [12], thus in our opinion, this compound may reduce activity of beta cells at the time of STZ injection and prevent them from degeneration. When beta-cell death occurred (in TDA group), in a short time, and with a low dose of sodium tungstate, injuries could not repair completely, and it seems that hyperglycemia and oxidative stress induced by STZ would be hazardous for pancreas, liver, kidneys and other organs.

Our study showed that pretreatment with sodium tungstate ameliorates hyperglycemia and impaired hepatic glucose metabolism seen in diabetic rats. These advantages may attribute to insulin-like properties of tungstate [12] and restoration of B-cells function [13].

Tungstate also affected hepatic glucose metabolism parameters in healthy control (TC) rats. Modifications were similar to those observed in diabetic animals, but the effects were in general much weaker in TC rats. However, glycemia in these animals rose slightly by tungstate. As it was reported by Barbera *et al.* [12], tungstate acts by modulating some mechanisms that are functional in healthy animals, but strongly restricted to diabetes.

Other studies also showed that tungstate could normalize glycemia in diabetic rat. They administered higher doses of sodium tungstate (up to 2 mg/ml) and for longer time (at least 60 days) in Zucker diabetic fatty obese or neonatal STZ-induced diabetic rats [12, 13, 16]. In agreement with our finding, other studies have reported that food restriction has no beneficial effect on hepatic

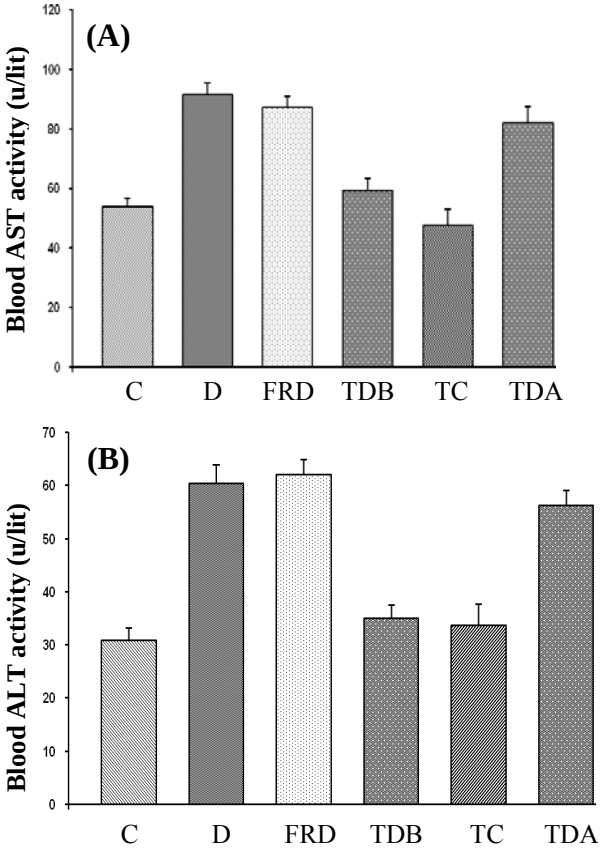

Fig. 5. Changes in **(A)** blood aspartate aminotransferase and **(B)** blood alanine aminotransferase activities of control and diabetic rats at the end of treatment period. The values represent the mean ± SE. C, control; D, diabetic; FRD, food-restricted diabetic; TDB, tungstate treated one week before diabetes induction by STZ, and continued for 5 weeks; TC, healthy rats receiving tungstate; TDA, tungstate treatment began one week after diabetes induction.

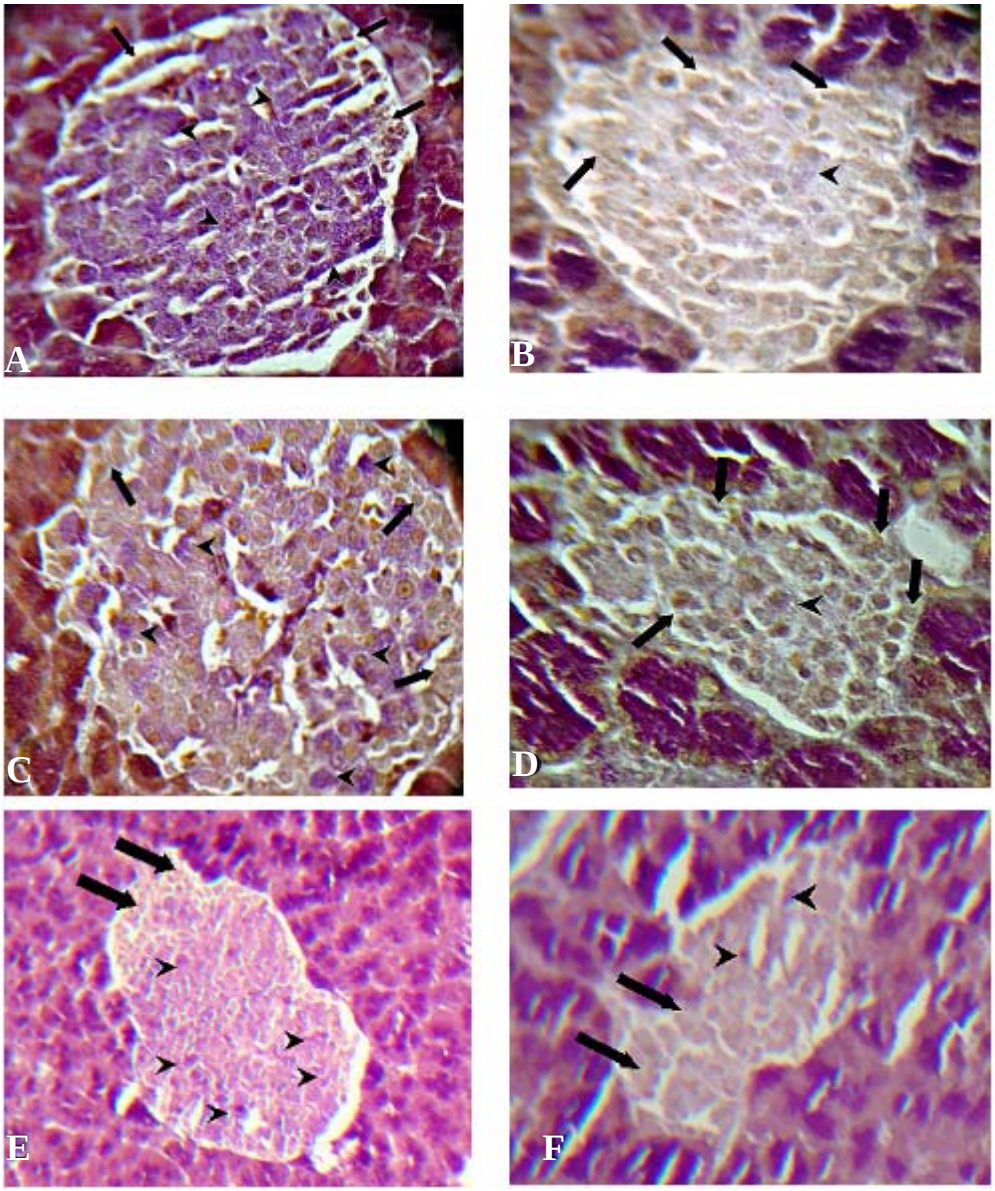

Fig. 6. Demonstrate the histological sections of pancreas in control and diabetic rats. **(A)** C group; **(B)** D group; **(C)** TDB group; **(D)** FRD group; **(E)** TC group; and **(F)** TDA group. Modified aldehyde fuchsin histochemical staining, 400×. Beta cells in pancreatic islets stained purple-violet (arrow heads) and A-cells stained yellow (arrow).

glucose metabolism in STZ-diabetic rats [29]. Thus, our results confirmed that the amelioration of hyperglycemia by sodium tungstate was separate from that of a reduced food intake. Blood insulin levels of TDB, diabetic, TDA and FRD rats were comparable and significantly lower than control groups (control and TC). In TDB group, restriction effectively abolished temporal insulin hypersecretory phase following STZ, and resulted in significantly improved insulin stores. Hence, it appears that tungstate may have preserved the residual beta cell mass by preventing its gradual exhaustion [29].

Tungstate has insulin-like effect and there is agreement between our view and that of other authors [12, 15, 16]. In TDB group, it showed that normoglycemic effect of tungstate was not due to an increase in the serum insulin levels. This suggests that tungstate can exert a direct inhibitory effect on insulin secretion in pancreatic beta cells. Aside from a sustained inhibitory effect on insulin release, it is also possible that normalization of glucose level by tungstate may reduce functional demand and result in partial protection of beta cells. Interestingly, several elements that were found to have glucose-lowering properties in STZ-diabetic rats, such as

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molybdenum, selenium, vanadium and tungsten were similarly shown to induce slight to significant increase in insulin store or glucose-stimulated insulin secretion. Furthermore, tungsten has a direct trophic effect on islet cell line [29].

Barbera *et al.* [12] showed that insulin-like effects of tungstate in the absence of insulin was considerable. Thus, in TC rats, with a normal insulin level, no insulin-like effect was detected.

Tungstate can improve hepatic glucose metabolism by increasing the capacity of the liver to utilize glycolysis and glycogenesis, and to decrease its potential glucose output; increase of liver GK activity, G6PD activity and glycogen content were seen in TDB rats. Also, glucose intolerance improved in TDB animals.

Our results indicated that pretreatment with sodium tungstate protects blood and pancreas of diabetic rats from oxidative stress. In uncontrolled or poorly controlled diabetic patients and STZ-induced diabetic animals, there are an increased glycemia and oxidative stress [29, 30]. Chronic hyperglycemia induces carbonyl stress which in turn can lead to increased lipid peroxidation [31], and increased concentration of lipid peroxidation induces oxidative damage by increasing peroxy radicals and hydroxyl radicals. Thus, lipid peroxidation is one of the characteristic features of chronic uncontrolled diabetes. The most commonly used indicator of lipid peroxidation is TBARS [32]. In our study, significant elevations of TBARS levels were observed in the blood and pancreas homogenate of diabetic rats compared to the corresponding control rats. Administration of sodium tungstate significantly decreased TBARS in pretreated diabetic (TDB) when compared with diabetic, FRD and TDA rats. Oxidative stress in diabetes coexists with a reduction in the antioxidant power [33].

The present work showed a significant reduction in blood and pancreas homogenate FRAP in diabetic rats compared with controls. Similarly, Cakatay and Kayaki [34], indicated that total antioxidant capacity (FRAP) levels in plasma of chronic diabetic animals were decreased significantly as compared to those of controls. Pretreatment with sodium tungstate in TDB rats improved significantly antioxidant power when compared with diabetic, TDA and FRD groups. Likewise, data of this study indicate that pretreatment with sodium tungstate can either increase antioxidant power or reduce the oxidative stress or both in blood and pancreas of diabetic rats. Thus, tungstate can prevent STZ-induced damage of beta cells in this way. It has been shown that sodium tungstate exerted radioprotetive effects on hematopoietic injury caused by exposure to ^{60}cobalt gamma rays in rats [35].

In a study on STZ- induced diabetic rats, sodium tungstate (2 mg/ml in drinking water) during 8 months, decreased serum glucose levels, prevented diabetic induced morphological changes in the kidney and ocular lenz, and reduced mortality. These results supported the possible use of tungstate as a long-term treatment of diabetes [16]. In our study, short time pretreatment with a low dose tungstate could exert this protective effect.

In the present study, blood aminotransferase activities significantly increased in diabetic rats when compared with control animals. Pretreatment with sodium tungstate significantly decreased blood AST and ALT activity in TDB rats as compared to those of diabetic, TDA and FRD groups. Therefore, tungstate pretreatment may prevent STZ-induced hepatotoxicity in diabetic animals. There is an agreement with previous works in this point of view [12, 13, 16].

Histopathological evaluation revealed an increase in the number of existing beta cells in sodium tungstate pretreated rats than untreated diabetic rats. Thus, it can be concluded that sodium tungstate similar to vanadium complexes relatively can conserve beta cells from STZ-induced damages [29, 36]. Accordingly, we found that a 1-week tungstate pretreatment prevents the onset of diabetes. Thus, the protection of beta cells by tungstate can be due to its effects at the time of STZ administration and continue following the induction of diabetes. It has been shown that the reversion of diabetic phenotype observed in tungstate treated nSTZ rats (type II diabetes) is due to an increase in the beta-cell mass through neogenesis and thus lengthening the regenerative period [17].

In conclusion, the present study provided evidence that short-term pretreatment with sodium tungstate leads to amelioration of diabetic complication in animal models. Sodium tungstate preserves pancreatic beta cells in part by reducing STZ-induced oxidative stress, decreases metabolic activity of pancreatic beta cells at the time of STZ injection, reduces insulin secretion and improves hepatic glucose metabolism. Hence, the combined effects of sodium tungstate in eliciting normoglycemia at remarkably low insulin reserves and in preserving residual insulin stores suggest that it may prevent the progressive deterioration of beta cells in prediabetic and newly diagnosed insulin dependent diabetes mellitus. Further studies will be

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required on the beneficial effects of treatment with tungstate in diabetic patients.

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