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ORIGINAL ARTICLES

The Effect of Glutathione Treatment on the Biochemical and Immunohistochemical Profile in Streptozotocin-Induced Diabetic Rats

Fatmagül Yur · Semiha Dede · Turan Karaca · Sevim Çiftçi Yegin · Yeter Değer · Hülya Özdemir

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Abstract This study investigated the possible role of glutathione (GSH) in diabetic complications and its biochemical safety in experimental diabetic rats. Serum biochemical parameters and the histology of the pancreas were investigated. Seven rats were separated as controls. To create the diabetes in rats, 45 mg/kg single-dose streptozotocin (STZ) was administered i.p. The treatment was continued for 1 month. STZ was administered to the diabetes + GSH group, then reduced GSH, dissolved in isotonic salt solution (200 mg/kg), was applied i.p. two times a week. The GSH group received i.p. GSH. Serum biochemical parameters were determined by autoanalyzer. Immunohistochemical procedures were used to determine the percentage of the insulin-immunoreactive β-cell area in the islets of Langerhans. The biochemical parameters changed to different degrees or did not change. Pancreatic cells of the control and GSH groups were healthy, but in the diabetic and GSH-treated diabetic groups we found damage in different numbers. The results from these analyses show that GSH supplementation can exert beneficial effects on pancreatic cells in STZ-induced diabetic rats and can safely be used for therapy in and protection from diabetes and complications of diabetes.

Keywords Biochemical parameter · Diabetes · Glutathione · Immunohistochemistry · Rat

F. Yur · S. Dede (✉) · Y. Değer
Biochemistry Department, Faculty of Veterinary Medicine, Yuzuncu Yil University, Van, Turkey
e-mail: ssdede@hotmail.com; sdede@yyu.edu.tr

T. Karaca
Histology and Embryology Department, Faculty of Medicine, Trakya University, Edirne, Turkey

S. Çiftçi Yegin
Health High School, Giresun University, Giresun, Turkey

H. Özdemir
Pharmacology and Toxicology Department, Faculty of Medicine, Yuzuncu Yil University, Van, Turkey

Introduction

Diabetes mellitus is a common illness with high morbidity and an early mortality rate, causing vascular, renal, retinal and neuropathic disorders in the long term as well as acute metabolic complications. Research has shown that long-term hyperglycemia causes adverse changes in blood vessels. Hyperglycemia, which occurs long before diagnosis, may cause organ damage. Therefore, early diagnosis is important for the treatment of patients with diabetes mellitus (Molitch 1990; Borodaco 2007; ADA 2011).

Glutathione (GSH) plays a protective role as an antioxidant defense system against reactive oxygen species (ROS)-mediated tissue damage. Diabetes causes a number of alterations in the antioxidant defense system (Lim et al. 2010). Alterations in its concentration have also been demonstrated to be a common feature of many pathological conditions including diabetes, cancer, AIDS and neurodegenerative and liver diseases. Additionally, GSH catabolism has been recently reported to modulate redox-sensitive components of signal-transduction cascades (Franco et al. 2007).

GSH is a dietary supplement used as an antioxidant to help protect the body from many diseases and conditions. It is also used to treat infertility (difficulty getting pregnant), cancer, cataracts and human immunodeficiency virus (HIV). GSH is used to detoxify the body of various chemicals. In carrying out several of the above functions,

GSH plays very important roles in maintaining mitochondrial function and integrity, regulating cell proliferation and supporting the immune system (Van Konynenburg 2004).

Experimental animal models are widely used to understand the pathogenesis and prevention of various diseases and to determine treatment possibilities. Experimental models of diabetes have an important role in the investigation of complications caused by diabetes and the determination of treatment approaches. This study investigated the possible role of GSH on the biochemical parameters of blood serum and histological changes in the pancreas in rats with experimental diabetes induced experimentally with streptozotocin (STZ).

Material and Methods

Materials

For this study, 28 Wistar-Albino rats 7–8 weeks old and weighing 180–210 g were used. Rats were housed in cages with ad libitum food and fresh water, a 12-h dark/light cycle and temperature set to 22 ± 2 °C during the 4-week trial.

Methods

Experimental Design

Rats were divided into the following groups.

Control Group: Selected randomly, seven rats were separated for the control group. A single dose of sterile, cold 0.9 % saline was injected i.p.

Diabetes Group (D): Single-dose STZ (45 mg/kg) was applied to seven rats; 72 h later glucose levels in blood samples taken from the tail vein were determined using PlusMED Accuro brand glucometer (Plusmed Trad., Estonia) equipment and its strips. Those rats with blood glucose 270 mg/dl and above were regarded as diabetic and included in the study. Treatment was started on the second day after STZ injection, and this was considered the first day of treatment. Treatments were continued for 1 month.

Diabetes + GSH Group (D + GSH): STZ was administered to the group of seven rats i.p.; 72 h later, glucose levels in blood samples taken from the tail vein were determined by the PlusMED Accuro brand biosensor glucometer. Reduced GSH, dissolved in isotonic salt solution, was applied i.p. at 200 mg/kg two times a week for 1 month to those rats with blood glucose levels of 270 mg/dl and above.

GSH Group (G): Reduced GSH, dissolved in isotonic salt solution, was applied i.p. (Chen et al. 2000) at 200 mg/kg two times a week for 1 month.

Sample Collection

Blood samples were taken from the animals, under ether anesthesia, from the left ventricle of their hearts and placed in tubes with gel. Glucose levels were determined in blood samples taken from the tail vein.

Biochemical Analysis

Glucose, total bilirubin levels, enzyme activity (alanine aminotransferase [ALT], amylase), protein (albumin, total protein, BUN, creatinine, globulin) and macromineral (Ca, P, Na, K) metabolism were determined by autoanalyzer (VetScan; Abaxis, USA).

Immunohistochemical Procedures

Pancreatic tissues were fixed in 10 % neutral buffered formalin, embedded in paraffin and sectioned at 5 μm thickness. Immunocytochemical reactions were performed by S-ABC (streptavidin-biotinylated horseradish peroxidase; DakoCytomation, Glostrup, Denmark) (Kanter et al. 2006). The procedure involved the following steps: (1) endogenous peroxidase activity was inhibited by 3 % H_2O_2 in distilled water for 30 min; (2) sections were washed in tap water for 30 min and in distilled water for 10 min; (3) nonspecific binding of antibodies was blocked by incubation with normal goat serum (X 0907; Dako, Glostrup, Denmark) with PBS, diluted 1:4; (4) sections were incubated with monoclonal mouse antisera against human insulin protein (18-0066; Zymed, San Francisco, CA), diluted 1:50 for 3 h, and then at room temperature; (5) sections were washed in PBS three times for 3 min each; (6) sections were incubated with biotinylated anti-mouse IgG (Dako LSAB 2 Kit); (7) sections were washed in PBS three times for 3 min each; (8) sections were incubated with ABC complex (Dako LSAB 2 Kit); (9) sections were washed in PBS three times for 3 min each; (10) peroxidase was detected with an aminoethylcarbazole substrate kit (AEC kit, Zymed); (11) sections were washed in tap water for 10 min; (12) nuclei were stained with hematoxylin; and (13) sections were mounted in glycerin–gelatin.

Eight islets of Langerhans from each rat (60 islets for each group) were chosen randomly. All experimental groups were scored for intensity of staining with anti-insulin antibodies of β-cells in pancreatic islets (compared with control) as + (weak), ++ (moderate), +++ (strong) or ++++ (very strong). The percentage of the insulin-immunoreactive β-cell area in the islets of Langerhans (80 islets for each group) was then estimated, and the total percentage of insulin-immunoreactive β-cells was calculated from these results (Karaca et al. 2010).

Preparations were evaluated by means of a bright-field microscope and photographed (Optiphot 2; Nikon, Tokyo, Japan).

Statistical Analysis

Data are expressed as means ± SEM. Data from control and experimental groups were analyzed with a one-way analysis of variance, and the Duncan test was applied for multiple comparisons. Differences were considered significant when $p < 0.05$.

Results

Biochemical Results

The highest blood glucose, BUN and creatinine concentrations were detected in the experimental diabetic group (D) ($p < 0.05$). Blood glucose concentrations were same levels in the control and GSH groups, and, high glucose levels of both of diabetic groups (D and D + GSH) were not significantly different, but BUN levels of the GSH group were not different from those of the control groups. Total bilirubin levels did not change.

Albumin and total protein levels of the control and diabetes groups were higher ($p < 0.05$) than those of the other groups. Globulin levels decreased ($p < 0.05$) in all experimental groups compared to the control group, but the D group had higher ($p < 0.05$) globulin levels than the D + GSH and GSH groups. The *A*/*G* ratio of the D and D + GSH groups was increased ($p < 0.05$) compared to the control and GSH groups (Table 1).

ALT activity was increased in the D and D + GSH groups ($p < 0.05$). The highest amylase activity was detected in the control group, and the lowest activity was found in the D + GSH group (Table 1).

The P concentrations in the D + GSH and GSH groups were significantly lower than those of the control and D groups ($p < 0.05$). The P concentrations did not affect experimental diabetes. However, Na levels in GSH-treated groups were higher than those of the control and D groups ($p < 0.05$). GSH treatment decreased K concentrations in the D group ($p < 0.05$) (Table 1).

Histological Results

Immunohistochemical quantification showed that the total number of insulin-positive cells was increased in the pancreas of GSH-treated rats compared with untreated diabetic rats (control) (Table 2; Fig. 1).

All cells of rats in the control and GSH groups were very strongly positive for insulin. The cells of 48 and 12 rats in the diabetic group were weakly and moderately positive, respectively; but a strongly or very strongly positive

Table 2 Semiquantitative analysis of immunohistochemical staining of insulin in β-cells in pancreatic islets of Langerhans in control, GSH, D and D + GSH groups

Group	*n*	+ (weak)	++ (moderate)	+++ (strong)	++++ (very strong)
Control	60	–	–	–	60
G	60	–	–	–	60
D	60	48	12	–	–
D + GSH	60	20	22	11	7

Table 1 Some biochemical parameters

Parameters	Control group ($n = 7$)	GSH group ($n = 7$)	Diabetes (D) group ($n = 7$)	Diabetes + GSH (D + GSH) group ($n = 7$)
Glucose (mmol/l)	5.76 ± 0.43^{a}	4.32 ± 0.95^{a}	19.54 ± 4.64^{b}	16.71 ± 3.85^{b}
Total bilirubin (μmol/l)	6.00 ± 0.44	6.40 ± 0.74	6.13 ± 0.39	5.85 ± 0.14
BUN (mmol/l)	4.22 ± 0.17^{a}	$7.36 \pm 0.71^{a,b}$	10.26 ± 1.94^{b}	11.08 ± 1.93^{b}
Creatine (μmol/l)	28.80 ± 4.07^{a}	26.00 ± 2.58^{a}	45.00 ± 4.85^{b}	26.14 ± 2.71^{a}
Albumin (g/l)	46.20 ± 1.79^{a}	34.80 ± 3.22^{b}	51.80 ± 2.59^{a}	38.28 ± 4.11^{b}
Total protein (g/l)	68.20 ± 1.11^{a}	48.60 ± 3.42^{b}	72.88 ± 2.58^{a}	49.57 ± 2.03^{b}
Globulin (g/l)	22.40 ± 1.03^{a}	13.80 ± 2.01^{c}	17.20 ± 1.46^{b}	11.43 ± 1.13^{c}
A/*G* ratio	2.06^{a}	2.52^{b}	3.01^{b}	3.35^{b}
ALT (U/l)	25.20 ± 5.85^{a}	20.20 ± 2.65^{a}	34.62 ± 4.07^{b}	40.71 ± 8.68^{b}
Amylase (U/l)	763.80 ± 43.21^{a}	589.60 ± 61.80^{c}	640.20 ± 35.11^{b}	422.14 ± 69.17^{c}
Phosphorus (mmol/l)	1.74 ± 0.21^{a}	0.33 ± 0.02^{b}	1.34 ± 0.20^{a}	0.29 ± 0.06^{b}
Sodium (mmol/l)	136.40 ± 0.81^{a}	139.40 ± 1.20^{b}	135.20 ± 2.76^{a}	138.00 ± 1.75^{b}
Potassium (mmol/l)	3.84 ± 0.16^{a}	3.33 ± 1.51^{a}	4.83 ± 0.19^{a}	1.07 ± 0.67^{b}

Within rows, between control and treated animals, means with different superscript letters differ significantly ($p < 0.05$)

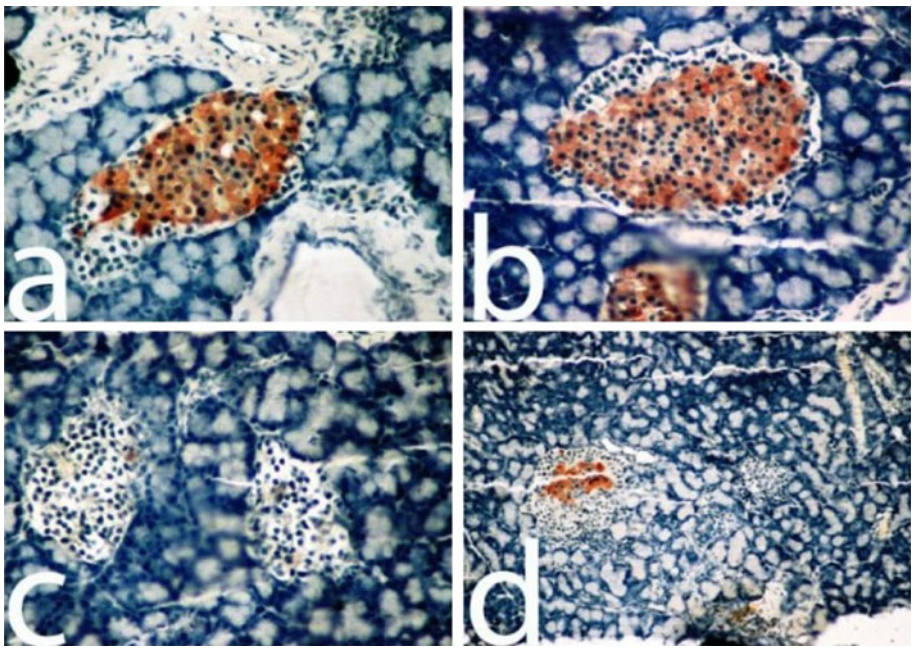

Fig. 1 Semiquantitative analysis of immunohistochemical staining of insulin in β-cells in pancreatic islets of Langerhans in control (**a**), GSH (**b**), D (**c**) and D + GSH (**d**) groups

reaction for insulin in β-cells was not detected in diabetic group. The cells of 18 rats in the GSH-treated diabetic group were strongly or very strongly positive for insulin (Table 2; Fig. 1).

Discussion

Diabetes mellitus is characterized by disturbances of carbohydrate, lipid and protein metabolism. GSH is a ubiquitous intracellular peptide with diverse functions that include detoxification, antioxidant defense, maintenance of thiol status and modulation of cell proliferation. Manipulation of the GSH synthetic capacity is an important target in the treatment of many of these disorders (Lu 2008).

There is considerable interest in the contribution of oxidative stress to diabetes mellitus. An increase in the generation of ROS can occur by nonenzymatic glycation and glucose autoxidation. The working hypothesis is that the loss of glucose homeostasis reduces the capacity to respond to oxidative damage (Stoppa et al. 2006). ROS are elevated by metabolic changes in diabetes, including autoxidation and increased advanced glycation. Antioxidant treatment corrects the blood flow deficit and promotes normal endoneurial oxygenation (Cameron and Cotter 1999).

Some clinical studies have reported that diabetic patients had lower GSH content in erythrocytes or plasma. Moreover, alloxan-induced diabetes significantly decreased GSH levels in blood, kidney, liver and testis compared to controls (Sheng et al. 2005). The plasma GSH/GSSG ratio seems to play a major role in the modulation of glucose homeostasis mainly in diabetics (Paolisso et al. 1992). Abnormal intracellular GSH redox status plays an important role in reducing insulin sensitivity in non-insulin-dependent diabetes mellitus patients (De Mattia et al. 1998). In diabetic rats, decreased levels of GSH in the liver and kidney were observed (Shabeer et al. 2009). The hyperglycemia in diabetes causes free radicals to be formed and the antioxidant system to become insufficient, thus increasing oxidative stress (Karasu 1999; Shabeer et al. 2009).

Dietary antioxidant compounds may offer some protection against early-stage diabetes mellitus and its complications. Endogenous protection by the GSH redox cycle is also compromised by the competing NADPH requirement of elevated polyol pathway flux (Cameron and Cotter 1999). Oral GSH can increase GSH concentrations in several tissues following GSH depletion (Aw et al. 1991). For example, oral GSH treatment restored the hepatic GSH concentration to normal levels in STZ-induced diabetes (Loven et al. 1986), and it can increase myocardial GSH content and antioxidant defense capacity, thereby protecting the intact heart against oxidative damage and functional retardation (Ramires and Ji 2001). The effects of reduced GSH were evaluated in the response of the renal vasculature and aortic rings ex vivo of 4-week alloxan-diabetic rabbits; reduced GSH directly improved the endothelium-dependent response of renal arterioles and aortic rings of diabetic rabbits (Nascimento et al. 2003). Treatment with GSH, a free radical scavenger, is partially effective in the prevention of diabetic neuropathy in STZ-induced diabetic rats (Bravenboer et al. 1992).

In the present study, the glucose levels of the diabetic group were significantly higher, and GSH treatment

slightly decreased glucose levels; but this level was still higher than that in the control group.

Oxidative stress may play a key role in the pathogenesis of diabetic nephropathy. It also decreased serum urea concentrations, attenuated the diabetes-evoked decline in the GSH/GSSG ratio and abolished hydroxyl free radical accumulation in the serum, liver and kidney cortex (Winiarska et al. 2009). Some researchers reported that diabetic rats had higher creatinine and urinary albumin (Abo-Salem et al. 2009; Thorp 2005). Propolis and taurine were used as antioxidants, which can ameliorate oxidative stress and delay the occurrence of diabetic nephropathy in diabetes mellitus (Abo-Salem et al. 2009; Thorp 2005; Winiarska et al. 2009).

The level of urea was increased (significantly) in plasma of diabetic rats compared to the control group (El-Demerdash et al. 2005; Abo-Salem et al. 2009). BUN accumulates in the blood when the kidney malfunctions, indicating that dehydration is present. Serum BUN levels in diabetic control rats increased above normal reference values (Lim et al. 2010). There was a significant elevation in serum urea, while the serum insulin level significantly decreased in the diabetic rats (Eidi et al. 2006).

Diabetic nephropathy presents in its earliest stage with low levels of albumin (microalbuminuria) in the urine. A strong association has been noted between acute increases in serum creatinine levels and diabetic nephropathy (Thorp 2005). Administration of taurine to alloxan-diabetic rabbits effectively improved renal function, as concluded from decreased serum urea and creatinine concentrations as well as diminished albuminuria (Winiarska et al. 2009).

Creatinine accumulates in the blood when the kidney malfunctions, indicating that dehydration is present (Lim et al. 2010). Some researchers have reported that diabetic hyperglycemia induces elevation of plasma levels of urea and creatinine, which are considered to be significant markers of renal dysfunction (El-Demerdash et al. 2005; Eidi et al. 2006; Lim et al. 2010). In this study, BUN increased in the D and D + GSH groups, but no difference was observed in the other groups. Creatinine levels of the D group were higher than those in the other groups. GSH treatment decreased creatinine to near control levels.

The levels of bilirubin were significantly increased in plasma of alloxan-diabetic rats compared to the control group (El-Demerdash et al. 2005). However, in this study, total bilirubin levels did not change.

Albumin is the most abundant plasma protein and a powerful extracellular antioxidant (Hu 1994; Bourdon et al. 1999). Total protein and albumin of the D group were lower than those of the control group (Yüksek 2012). But, in this study, albumin and total protein levels of the D and control groups were similar to but higher than those of the G and D + GSH groups.

The urinary albumin level was three times higher in diabetic animals compared to controls (Winiarska et al. 2009). Diabetic animals presented no alterations in the concentrations of total protein and serum albumin; no protein degradative metabolism was observed in the diabetic dams (Damasceno et al. 2002). HbA_{1C} levels were significantly increased, whereas serum albumin levels were significantly decreased compared to those of controls. Glucose and HbA_{1C} levels were significantly increased, whereas serum albumin was decreased compared to controls (Memisoğullari et al. 2003). Diabetes mellitus leads to metabolic disorders in which proteins and lipids are among the prime targets for oxidative stress (Ramakrishna and Jailkhani 2008). Oxidative stress may play a key role in the pathogenesis of diabetic nephropathy. Compared to control rats, diabetic rats had higher blood urinary albumin (Abo-Salem et al. 2009).

Globulin fractions (α1, α2 and β) of the D group were lower than those of the control group (Yüksek 2012). In this study, globulin levels of all experimental groups were decreased compared to the control group. But the D group had higher globulin levels than those of the G and D + GSH groups. This situation may cause elevation of gamma-globulin levels and reflect immune system activation of diabetes.

In this study, *A*/*G* ratios were increased in all experimental groups compared to the control group. Similarly, Yüksek (2012) reported that the highest *A*/*G* ratio was observed in the diabetic group.

Some researchers have found that ALT activity increased in experimental diabetic rats, and this elevation indicates the protective effect of rutin against the hepatic and cardiac toxicity caused by STZ (El-Demerdash et al. 2005; Eidi et al. 2006; Shabeer et al. 2009; Fernandes et al. 2010). But there is information that diabetes mellitus alone did not alter ALT (Fadillioglu et al. 2008). In this study, the ALT activity increased in the D group but GSH treatment did not affect it.

Liu et al. (2008) reported that for pancreatic exocrine function in alloxan-induced diabetic rats, amylase in the serum was measured and amylase activity decreased in the diabetic group. As one of the pancreatic exocrine enzymes, amylase exhibited reduced activity in the diabetic group. Reduced serum amylase activity has been regarded as one of the factors impairing pancreatic exocrine function in diabetes. Similarly, in this study the amylase activity of the D group decreased compared to the control group. The G and D + GSH groups had low amylase activities.

But some researchers have reported that the activity of amylase was elevated in poorly controlled diabetes and related complications. It has also been reported that amylase activity increases according to the degree of hyperglycemia (Quiros et al. 2008; Abou-Seif and Youssef 2004; Rizvi 2003).

Metal ions are known to play an essential role in living systems, both in growth and in metabolism. It has been reported that the urinary excretion of calcium is increased in two types of diabetes mellitus, causing a decrease in blood levels of these elements from these patients (Abou-Seif and Youssef 2004). The P levels of the G and D + GSH groups decreased significantly in this study, and the Na levels of these groups increased.

The K levels of the G group were lowest and did not change in the diabetic groups in this study. Low serum K is strongly related to glucose intolerance. Reduced serum K increases the risk of lethal ventricular arrhythmias. In uncontrolled diabetes mellitus, as a result of glucose osmosis, both hypertonicity and insulin deficiency impede the entry of K into cells (He and MacGregor 2008). Acidosis increases K levels, and glucose administered with insulin lowers them (Trachtenbarg 2005). Serum K concentration and abnormal glucose metabolism are closely related to cardiac dysfunction (Takagi et al. 2009). There is a depletion of body stores of K, but hypokalemia is usually absent. A few small studies have shown that intermittent hyperkalemia occurs frequently in diabetes.

Semiquantitative analysis of immunohistochemical staining of insulin in β-cells in pancreatic islets of Langerhans in the control group and the G group was ++++ (very strong), showing no damage, as expected. The cells of the D group were + (weak) = 48 and ++ (moderate) = 12. Most of the cells damaged after STZ treatment and in type 1 diabetes recovered, as expected. Actually, it is interesting that the cells of the D + GSH group were stronger than those of the D group (+ = 20, ++ = 22, +++ = 11, ++++ = 7) and the number of damaged cells and the level of damage decreased. This could indicate that GSH treatment can protect the β-cells in pancreatic islets of Langerhans from the harmful effects of STZ.

Positive results have been obtained from clinical research of antioxidant treatment in diabetic complications. For example, vitamin E lowers blood sugar levels in type 1 diabetic rats with an unknown mechanism. Alpha-lipoic acid is licensed for use in diabetic patients in Germany. Research has indicated that GSH could be the most important tissue antioxidant. Blood glucose levels and oxidative stress effects have been controlled using antioxidant therapies (Vincent et al. 2004). Veno et al. (2002) reported that a sufficient supply of GSH may prevent or delay renal and neural dysfunctions in diabetes by providing protection against oxidative stress.

In conclusion, our present results obtained from biochemical and immunohistochemical analyses show that GSH supplementation can exert beneficial effects on pancreatic cells in STZ-induced diabetic rats. GSH may be used safely for therapy and protection in diabetes and related complication of diabetes. The optimal dose and therapy procedure of GSH treatment in diabetes need further and detailed studies.

References

Abo-Salem OM, El-Edel RH, Harisa GE, El-Halawany N, Ghonaim MM (2009) Experimental diabetic nephropathy can be prevented by propolis: effect on metabolic disturbances and renal oxidative parameters. Pak J Pharm Sci 22(2):205–210

Abou-Seif MA, Youssef A (2004) Evaluation of some biochemical changes in diabetic patients. Clin Chim Acta 346(2):161–170

ADA (American Diabetes Association) (2011) Diagnosis and classification of diabetes mellitus. Diabetes Care 34(Suppl 1):62–69

Aw TY, Wierzbicka G, Jones DP (1991) Oral glutathione increases tissue glutathione in vivo. Chem Biol Interact 80(1):89–97

Borodaco A (2007) Classification and diagnosis of diabetes. Terapia 15:6–11

Bourdon E, Loreau N, Blache D (1999) Glucose and free radicals impair the antioxidant properties of serum albumin. FASEB J 13:233–244

Bravenboer B, Kappelle AC, Hamers FPT, van Buren T, Erkelens DW, Gispen WH (1992) Potential use of glutathione for the prevention and treatment of diabetic neuropathy in the streptozotocin-induced diabetic rat. Diabetologia 35:813–817

Cameron NE, Cotter MA (1999) Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. Diabetes Res Clin Pract 45(2–3):137–146

Chen TS, Richie JP, Nagasawa HT, Lang CA (2000) Glutathione monoethyl ester protects against glutathione deficiencies due to aging and acetaminophen in mice. Mech Ageing Dev 120(1–3):127–139

Damasceno DC, Volpato GT, de Mattos Paranhos Calderon I, Cunha Rudge MV (2002) Oxidative stress and diabetes in pregnant rats. Anim Reprod Sci 72(3–4):235–244

De Mattia G, Bravi MC, Laurenti O, Cassone-Faldetta M, Armiento A, Ferri C, Balsano F (1998) Influence of reduced glutathione infusion on glucose metabolism in patients with non-insulin-dependent diabetes mellitus. Metabolism 47(8):993–997

Eidi A, Eidi M, Esmaeili E (2006) Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine 13(9–10):624–629

El-Demerdash FM, Yousef MI, El-Naga NI (2005) Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 43(1):57–63

Fadillioglu E, Kurcer Z, Parlakpinar H, Iraz M, Gursu C (2008) Melatonin treatment against remote organ injury induced by renal ischemia reperfusion injury in diabetes mellitus. Arch Pharm Res 31(6):705–712

Fernandes AA, Novelli EL, Okoshi K, Okoshi MP, Di Muzio BP, Guimarães JF, Fernandes JA (2010) Influence of rutin treatment on biochemical alterations in experimental diabetes. Biomed Pharmacother 64(3):214–219

Franco R, Schoneveld OJ, Pappa A, Panayiotidis MI (2007) The central role of glutathione in the pathophysiology of human diseases. Arch Physiol Biochem 113(4–5):234–258

He FJ, MacGregor GA (2008) Beneficial effects of potassium on human health. Physiol Plant 133(4):725–735

Hu ML (1994) Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 233:381–385

Kanter M, Uysal H, Karaca T, Ozdemir-Sagmanligil H (2006) Depression of glucose levels and partial restoration of pancreatic

β-cell damage by melatonin in streptozotocin-induced diabetic rats. Arch Toxicol 80:362–369

Karaca T, Yörük M, Yörük İH, Uslu S (2010) Effects of extract of green tea and ginseng on pancreatic beta cells and levels of serum glucose, insulin, cholesterol and triglycerides in rats with experimentally streptozotocin-induced diabetes: a histochemical and immunohistochemical study. J Anim Vet Adv 9(1):102–107

Karasu Ç (1999) Increased activity of H_2O_2 in aorta isolated from chronically streptozotocin diabetic rats: effects of antioxidant enzymes and enzyme inhibitors. Free Radic Biol Med 27:16–27

Lim J, Sanders RA, Snyder AC, Eells JT, Henshel DS, Watkins JB 3rd (2010) Effects of low-level light therapy on streptozotocin-induced diabetic kidney. J Photochem Photobiol B 99(2): 105–110

Liu Z, Li J, Zeng Z, Liu M, Wang M (2008) The antidiabetic effects of cysteinyl metformin, a newly synthesized agent, in alloxan- and streptozocin-induced diabetic rats. Chem Biol Interact 173(1):68–75

Loven D, Schedl H, Wilson H, Daabees TT, Stegink LD, Diekus M, Oberley L (1986) Effect of insulin and oral glutathione on glutathione levels and superoxide dismutase activities in organs of rats with streptozocin-induced diabetes. Diabetes 35(5):503–507

Lu SC (2008) Regulation of glutathione synthesis. Mol Aspects Med 30(1–2):42–59

Memisoğullari R, Taysi S, Bakan E, Capoglu I (2003) Antioxidant status and lipid peroxidation in type II diabetes mellitus. Cell Biochem Funct 21(3):291–296

Molitch ME (1990) Diabetes mellitus. In: Walker HK, Hall WD, Hurst JW (eds) Clinical methods: the history, physical, and laboratory examinations, 3rd edn. Butterworths, Boston

Nascimento NR, Costa-e-Forti A, Peter AA, Fonteles MC (2003) Free radical scavengers improve the impaired endothelium-dependent responses in aorta and kidneys of diabetic rabbits. Diabetes Res Clin Pract 61(3):145–153

Paolisso G, Di Maro G, Pizza G, D'Amore A, Sgambato S, Tesauro P, Varricchio M, D'Onofrio F (1992) Plasma GSH/GSSG affects glucose homeostasis in healthy subjects and non-insulin-dependent diabetics. Am J Physiol Endocrinol Metab 263(3 Pt 1): E435–E440

Quiros JA, Marcin JP, Kuppermann N, Nasrollahzadeh F, Rewers A, Di Carlo J, Neely EK, Glaser N (2008) Elevated serum amylase and lipase in pediatric diabetic ketoacidosis. Pediatr Crit Care Med 9(4):418–422

Ramakrishna V, Jailkhani R (2008) Oxidative stress in non-insulin-dependent diabetes mellitus (NIDDM) patients. Acta Diabetol 45(1):41–46

Ramires PR, Ji LL (2001) Glutathione supplementation and training increases myocardial resistance to ischemia–reperfusion in vivo. Am J Physiol Heart Circ Physiol 281:H679–H688

Rizvi AA (2003) Amylase and lipase in diabetic ketoacidosis. Diabetes Care 26(11):3193–3194

Shabeer J, Srivastava RS, Kumar Singh S (2009) Antidiabetic and antioxidant effect of various fractions of *Phyllanthus simplex* in alloxan diabetic rats. J Ethnopharmacol 124(1):34–38

Sheng XQ, Huang KX, Xu HB (2005) Influence of alloxan-induced diabetes and selenite treatment on blood glucose and glutathione levels in mice. J Trace Elem Med Biol 18(3):261–267

Stoppa GR, Cesquini M, Roman EA, Ogo SH, Torsoni MA (2006) Aminoguanidine prevented impairment of blood antioxidant system in insulin-dependent diabetic rats. Life Sci 78(12):1352–1361

Takagi S, Tanabe A, Tsuiki M, Naruse M, Takano K (2009) Age, HbA_{1c}, and prevalence of diabetes mellitus were positively correlated and serum potassium levels were negatively correlated with the severity of cardiac dysfunction. Endocr J 56(8):1009–1018

Thorp ML (2005) Diabetic nephropathy: common questions. Am Fam Physician 72(1):96–99

Trachtenbarg DE (2005) Diabetic ketoacidosis. Am Fam Physician 71(9):1705–1714

Van Konynenburg RA (2004) Is glutathione depletion an important part of the pathogenesis of chronic fatigue syndrome? Presented at the AACFS Seventh International Conference Madison, Wisconsin, 8–10 Oct 2004. http://www.aboutmecfs.org/Rsrch/GluAACFS04.aspx

Veno Y, Kizaki M, Nakagiri R, Kamiya T, Sumi H, Osawa T (2002) Dietary glutathione protects rats from diabetic nephropathy and neuropathy. J Nutr 132(5):897–900

Vincent AM, Russel SW, Low P, Feldman EL (2004) Oxidative stress in the pathogenesis of diabetic neuropaths. Endocr Rev 25(4): 612–628

Winiarska K, Szymanski K, Gorniak P, Dudziak M, Bryla J (2009) Hypoglycaemic, antioxidative and nephroprotective effects of taurine in alloxan diabetic rabbits. Biochimie 91(2):261–270

Yüksek V (2012) The electrophoretical determination of serum protein fraction of lycopene treated experimental diabetic rats. MSc thesis, Yüzüncü Yıl University, Van