

Effect of Supplementation of Diet with Vitamin C on Induction and Consequences of Streptozotocin -Diabetes in Rats

Seyed Reza Fatemi Tabatabaei¹, Ahmad Ali Papahn¹, Mohammad Razi Jalali², Lida Jalilian³

1. Department of Physiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahwaz, Ahwaz, Iran

2. Department of Clinical Pathology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahwaz, Ahwaz, Iran

3. Graduated student of Veterinary Medicine in Shahid Chamran University of Ahwaz, Ahwaz, Iran

Received: 9 November 2010 - **Accepted:** 3 February 2011

ABSTRACT

OBJECTIVE: Streptozotocin (STZ) destroys the beta cells of pancreas by generation of reactive oxygen species and vitamin C has documented antioxidant properties. This study was designed to evaluate the preventive effect of supplementation of diet by vitamin C on induction of STZ-diabetes and its effect on carbohydrate and lipid metabolism of diabetic rats.

MATERIALS AND METHODS: Fifty male Wistar rats were divided randomly into non-diabetic (ND), diabetic (D), C₁, C₂ and C₃ groups. The diet of C₁, C₂ and C₃ groups were supplemented with 2.5, 5 and 10 g/kg of vitamin C, respectively. Four days later all groups except ND, were made diabetic by IP injection of STZ and blood glucose was measured 72 h later to determine the severity of blood glucose elevation. Weight gain was measured weekly. 21 days after induction of diabetes Glycosylated hemoglobin (HbA_{1c}), triglyceride (TG), total cholesterol (TC), HDL-c and LDL-c were measured or calculated in plasma of 6 diabetic rats in each groups that their glucose was more than 200 mg/dl after STZ injection.

RESULTS: Vitamin C significantly prevented blood glucose elevation after STZ injection in group C₂. Weight gain decreased in all diabetic groups. Increase of HbA_{1c} could not be prevented by vitamin C in any groups. TC and LDL-c decreased and HDL% increased in group C₃.

CONCLUSION: We suggest that the amount of vitamin C consumption may have an important effect on STZ-diabetes induction and it may be in agreement with opposite effects of free radicals on insulin receptor signaling. Furthermore, vitamin C may have some beneficial effects on lipid metabolism disorders of diabetes.

KEY WORDS: Diabetes, Streptozotocin, Ascorbic acid, Prevention, Diet, HDL-c, LDL-c

INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia and its complications have been attributed to long duration of this

condition due to the abnormality of glucose metabolism. On the other hand hyperlipidemia has been frequently observed in diabetes mellitus (1,2).

*Correspondence: Seyed Reza Fatemi Tabatabaei, Department of Physiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran. **Tel:** (+98) 611 3330073. **Email:** fatemi_r@scu.ac.ir

Streptozotocin (STZ) is used to induce experimental diabetes by selectively destroying pancreatic beta cells (3). STZ is taken up by pancreatic beta cells via glucose transporter GLUT2 (4). STZ was found to generate reactive oxygen species (ROS), which also contribute to DNA fragmentation and evoke other deleterious changes in the beta cells (5). The inhibition of xanthin oxidase by allopurinol restricts the cytotoxic effect of STZ in vitro. Pre-treatment of beta cells with this inhibitor prevented the STZ-induced decrease of insulin secretion (6). Therefore, intracellular antioxidants attenuate STZ toxicity (3). All tissues in the body contain adequate amounts of antioxidants to protect them against the toxic actions of free radicals. A deficiency of these antioxidants can result in tissue and organ damage (7). It has been showed that pancreatic antioxidant enzymes such as SOD, glutathione peroxidase, and catalase are low in normoglycemic, diabetic-prone BB rats compared with the low-risk group (8). This idea is supported by observation that antioxidants such as probucol can prevent alloxan-induced diabetes in rats, and superoxide dismutase and catalase protected beta cells of isolated pancreatic islets against alloxan cytotoxicity, as did the hydroxyl radical scavenger dimethyl sulfoxide (DMSO) and butanol (9,10). Furthermore, administration of the antioxidant vitamin E to rats, prior to administration of either STZ or alloxan, provided protection against the diabetogenic effect of both these agents (11). Diabetes produces myocardial dysfunction that accelerates cardiovascular morbidity and mortality (12). Hyperglycemia and dyslipidemia have been shown to affect physiologic changes in the vasculature leading to atherosclerosis (13). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby depleting the activity of the antioxidative defense system and promoting the generation of free radicals (14). There is evidence suggesting that antioxidants, especially vitamin E, have potential benefits with respect to cardiovascular disease (15).

Vitamin E is a lipid soluble antioxidant and protects LDL particles from oxidative attack. Vitamin C is required for regeneration of α -tocopherol, may thus preventing LDL oxidation (16). Vitamin C, an aqueous phase antioxidant has been reported to improve whole body glucose disposal in healthy subjects and in diabetic patients (17) and animals (18).

However, we do not know if supplementation of diet with vitamins, especially vitamin C, has any effect on diabetes mellitus by STZ. On the other hand, the effect of vitamin C supplement on the carbohydrate and lipid metabolism of diabetics is controversial. We designed this study to evaluate the effects of different amounts of vitamin C on induction of blood glucose elevation after STZ injection and to assess the effect of vitamin C on some lipid parameters important in cardiovascular diseases in STZ-induced diabetic rats.

MATERIALS AND METHODS

Animals were purchased from the laboratory of Animal House of Veterinary School in Shahid Chamran University. The animal room temperature was 23 ± 1 °C and rats were subjected to 12-h dark/12-h light cycle and had ad libitum access to food and water. All animal manipulations and sample collection were done from 9-12 am.

Fifty male Wistar rats (approximately 195 g) were randomly divided into five groups, non-diabetic (ND), diabetic (D), C₁, C₂ and C₃. The ND and D groups were fed with commercial rat diet without any supplementation, and the diet of group C₁, C₂ and C₃ were supplemented with 2.5, 5, and 10 g/Kg of vitamin C, respectively. Groups D, C₁, C₂ and C₃ were made diabetic by IP injection of 45 mg/kg STZ, four days after vitamin supplementation in the diet of groups C₁, C₂ and C₃. Similar volumes of saline were injected to ND group. All rats in each group were weighed at STZ injection and 1, 2, and 3 weeks after STZ injection. To compare the amount of blood glucose elevation in each group, the blood glucose was measured by glucometer (Glucomen, Italy) in tail vein blood 72 h after

injection of STZ (n = 10 in each group). Six rats with blood glucose greater than 200 mg/dl were maintained in each group and others omitted from the remaining parts of the study. Three weeks after STZ injection, the rats were euthanized by anesthesia using chloroform vapor. Blood was collected from heart by cardiac puncture, and transferred into EDTA treated tubes immediately. Blood was then centrifuged at 3000 rpm for 10 minutes, to separate blood cells and plasma. The plasma tubes were frozen at -20°C until biochemical analysis. RBCs were washed immediately three times with normal saline and glycosylated hemoglobin (HbA1c) measured by a commercial kit (Mahsayaran) by cyanomethhemoglobin method one day later. The total cholesterol (TC), HDL-cholesterol (HDL-c) and triglycerides (TG) were measured by enzymatic assay commercial kits (Zyst-chimi). LDL-c was calculated by the Friedewald formula (19):

$$LDL-c = TC - HDL-c - TG/5$$

Results obtained were expressed as the means ± SEM. Data were analyzed by one-way ANOVA using the Sigmastat software followed by Tukey post hoc test. Differences between groups were considered significant at $P < 0.05$.

RESULTS

Blood glucose of group C₂ did not increased significantly in comparison with group ND (Figure 1). However, the glucose level of all other groups were significantly greater than group ND. Between diabetic groups, blood glucose of group C₂ was also lower than group C₁ ($P < 0.05$). As there were not enough diabetic rats with blood glucose greater than 200 mg/dl in group C₂, this group was completely discarded from the remaining parts of the experiment.

As illustrated in Figure 2, weight gain in diabetic groups was stopped after induction of diabetes, and supplementation of their diets with vitamin C did not have any effect on it. HbA1c has been increased by diabetes

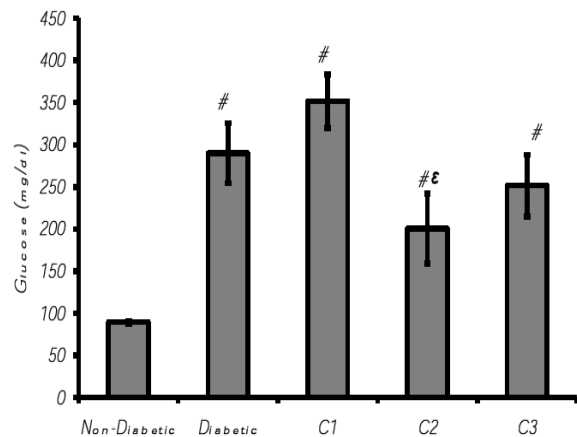


Figure 1- Blood Glucose 72h after STZ injection
#: Significant differences with nondiabetic group.
ε: Significant differences with C1

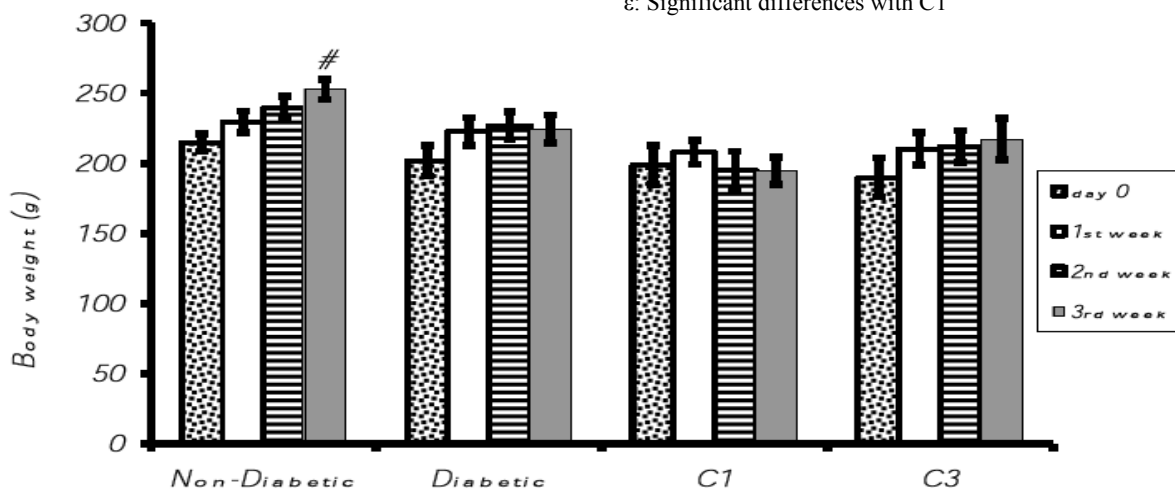


Figure 2- Weight Gain After STZ Injection.
#: Significant difference with weight at STZ injection

Table 1- HbA1c, Triglyceride, Total Cholesterol, HDL-C, LDL-C and VLDL-C in Non-Diabetic, Diabetic, C1 and C3 Groups (N=6 in Each Group)

| Group | HbA1C (mg/dl) | TG (mg/dl) | TC (mg/dl) | HDL-c (mg/dl) | HDL-c% | LDL-c (mg/dl) | LDL-c% |
|----------------|------------------------|-------------|---------------------------|------------------------|------------------------|---------------------------|------------|
| Non-Diabetic | 6±0.35 | 69.33±16.91 | 213.5±22.49 | 7.28±0.45 | 3.41±0.24 | 192.35±21.73 | 90.1±1.9 |
| Diabetic | 7.95±0.45 [#] | 94.33±14.07 | 256±12.45 | 8.83±0.36 [#] | 3.45±0.29 | 228.3±12.49 | 89.18±0.92 |
| C ₁ | 8.28±0.41 [#] | 62.33±14.04 | 212.67±4.34 | 8.4±0.28 | 3.95±0.15 | 191.8±6.56 | 90.19±1.45 |
| C ₃ | 9.02±0.6 [#] | 52.33±5.32 | 155.33±16.16 [*] | 7.73±0.36 | 4.98±0.42 [*] | 137.13±16.23 [*] | 88.28±1.7 |

#: significant difference with non-diabetic group.

*: significant difference with diabetic group

induction in all diabetic groups in comparison with group ND ($P < 0.05$) (Table 1).

TC, HDL-c and LDL-c increased by diabetes induction, but only HDL-c was significantly greater in group D ($P < 0.05$). However, the percentage of HDL-c did not change by diabetes in group D, whereas it was increased by high level of vitamin C in the diet. The minimum level of TG, TC and LDL-c were seen in group C₃, although only TC and LDL-c of this group was significantly lower than group D ($P < 0.05$).

DISCUSSION

Diabetes mellitus is a metabolic disease associated with impaired glucose metabolism which in effect adversely alters intermediary metabolism of lipids and proteins. Formation of protein glycation products releases free radicals; subsequently causing oxidative stresses (20). Most of the complications of the diabetic state are initiated by the generation of free radicals, for instance LDL oxidative modification, leading to atherosclerosis (21).

In the recent study, supplementation of diet with 5 g/kg vitamin C reduced the severity of glucose elevation after STZ injection. Tsujinaka et al. (22) showed that diet high in lipid hydroperoxide by vitamin E deficiency accelerates glucose intolerance through impairments of both sensitivity and secretion of insulin as a result of increased lipid peroxidation. On the other hand, pre-treatment of pancreatic beta cells with allopurinol (xanthin oxidase inhibitor) prevented the STZ-induced decrease of insulin secretion in vitro (6). Prior administration of SOD, catalase,

monomethyl, dimethyl, or monoethyl urea could block alloxan-induced cytotoxic action on pancreatic beta cells (9,10). Pre-treatment with tert-butylhydroquinone, a synthetic antioxidant and vitamin E reduced the severity of STZ-diabetes in rats (11). All these agents can quench either a superoxide anion or a hydroxyl radical, thus may be able to protect against alloxan-induced damage to the pancreatic beta cells both in vitro and in vivo. Therefore, inhibition of diabetes induction by supplementation of diet with 5 g/kg vitamin C may be related to the antioxidative properties of the vitamin.

Beside the results obtained from this study, supplementation of diet with 5 g/kg vitamin C could decrease the severity of STZ diabetes but lower and greater doses did not have any effects on the induction of STZ diabetes. It is possible that lower dose of vitamin C (2.5 g/kg diet) have not been sufficient to protect beta cells from oxidative damage. If vitamin C has reduced the diabetogenic effects of streptozotocin by reduction of oxidative stress, why similar effect did not occur by supplementation of diet with 10 g/kg vitamin C?

Signaling by insulin requires autophosphorylation of the insulin receptor (23). In intact cells, high concentrations of hydrogen peroxide and thiol-reactive agents induce insulin-like effects in the absence of insulin (24). Lower and physiologically relevant concentrations (<0.1 mM) of hydrogen peroxide are not sufficient to trigger the autophosphorylation of the insulin receptor in the absence of insulin, but do enhance the

response to 100 nM insulin (25), indicating that the redox signal has a coregulatory function in insulin receptor activation under physiologically relevant conditions (26). Hydrogen peroxide directly interacts with insulin receptor kinase domain and enhances its autophosphorylation and kinase activity in the presence of ADP (27). On the other hand, autophosphorylation of insulin receptor can be down-regulated by a redox-sensitive protein-tyrosine phosphatase (28). Thus it is possible that high dose of vitamin C in the present study have reduced hydrogen peroxide and thiol-reactive agent by increasing the anti oxidative potency of the organism. Therefore, the ability of hydrogen peroxide to trigger the autophosphorylation of the insulin receptor in the presence of low level of insulin (25) may be reduced by 10 g/kg of vitamin C in group C₃.

At the present study vitamin C not only could not inhibit decrease of weight gain, but also increased glycosylated hemoglobin in diabetic rats. Sridulyakul et al. (29) showed that weight and plasma vitamin C reduction and glycosylated hemoglobin elevation in STZ-diabetic rats were prevented by supplementation of 1 g/l vitamin C in drinking water but the same dose and route of vitamin C administration could not prevent weight loss in STZ diabetic rats in Amatyakul et al. study (30). The effect of vitamin C on glycozylated hemoglobin is controversial. Some investigator reported that vitamin C decreases HbA1c in diabetic patients (31,32) and animal models (18,29), but in some other studies it did not have beneficial effect on HbA1c (33,34). Glucose and vitamin C might occupy the same membrane transport system (35). Taking of the ascorbic acid in the dosage of 1000 mg/per day for seven days (36), and 2000 mg/day for 14 days intensifies the level of glycemia during OGGT in normoglycemic individual, perhaps by competitively inhibiting glucose uptake by pancreatic beta cells (37). The severity and duration of diabetes, dose of vitamin, period and route of vitamin consumption, and different methodologies may be an explanation for these controversies.

During the course of type 1 diabetes, there was a serum lipid profile disturbance characterized by an increase of blood cholesterol (15). This is one of the significant factors in development of cardiovascular diseases (18). Also oxidative modification of LDL is an important step in the development of atherosclerosis (21). This oxidation is initiated and propagated by free radicals where antioxidants become depleted (38). There are reports that show the ability of vitamin C to scavenge superoxide, hydrogen peroxide, hydroxyl radicals etc, in DM (39). In this study vitamin C decreased TC and LDL-c of diabetic rats, dose dependently. Many reports accentuate that vitamin C improves lipid profile in diabetes (18,31,32,33 and 40) but some did not show any benefit (34,41 and 42). As shown in the recent study, it seems that higher amounts of vitamin C may be more effective in lipid profile correction of diabetes (31).

The possible explanation for the hypocholesterolaemic effect of vitamin C is that vitamin C prevents LDL-cholesterol from oxidative damage and aids in degradation of cholesterol (18). Furthermore, this vitamin is needed by the enzyme in the first step of bile acid synthesis (cholesterol 7 α -hydroxylase) thus directing cholesterol towards bile acid synthesis and reduces its level in serum (43). Kaviarasan et al. (35) reported that the level of total cholesterol, triglyceride, lipid peroxidation and glucose increased in hyperlipidemic patients with or without diabetes mellitus, whereas there was decreased plasma concentration of vitamin C and other antioxidants. Taking the above evidence together suggest that vitamin C supplementation dose dependently improves the lipid profile in diabetic rats by acting through cholesterol 7 α -hydroxylase to direct cholesterol into bile synthesis. Furthermore, by scavenging free radicals, it decreases oxidative damage to oxidized LDL-cholesterol.

CONCLUSION

We suggest that supplementation of diet with different amounts of vitamin C have different effects on glucose elevation in STZ diabetic

rats, and this is in agreement with opposite effects of oxidative stress on insulin receptor signaling. Furthermore, administration of vitamin C in diabetics may be beneficial for the improvement of lipid profile. This may, at least in part, reduce the risk of cardiovascular events seen in diabetes mellitus.

REFERENCES

- Battisi WP, Palmisano J, Keane WE. Dyslipidemia in patients with type 2 diabetes: Relation between lipids, kidney disease and cardiovascular disease. *Clin Chem Lab Med* 2003; 41(9): 881-91.
- Hirano T, Mamo JC, Takeuchi H, Nagano S, Takahashi T. Correlation of insulin deficiency and hypertriglyceridemia in diabetic rats. *Diabetes Res Clin Pract* 1991; 12(3): 173-80.
- Szkudelski T. The mechanism of alloxan and STZ action in beta cells of the rat pancreas. *Physiol Res* 2001; 50(6): 537-46.
- Schundl WJ, Ferber S, Johnson JH, Newgard CB. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes* 1994; 43(11): 1326-33.
- Takasu N, komiyada I, Asawa T, Nagasawa Y, Yamada T. STZ- and alloxan-induced H₂O₂ generation and DNA fragmentation in pancreatic islets. H₂O₂ as mediator for DNA fragmentation. *Diabetes* 1991; 40(9): 1141-5.
- Nukatsuka M, Yoshimura Y, Nishiada M, Kawada J. Allopurinol protects pancreatic beta cells from the cytotoxic effect of STZ: in vitro study. *J Pharmacobiodyn* 1990; 13(4): 259 - 62.
- Krishna Mohan I, Das UN. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. *Nutrition* 2001; 17(2): 126 - 51.
- Roza AM, Pieper GM, Johnson CP, Adams MB. Pancreatic antioxidant enzyme activity in normoglycemic diabetic prone BB rats. *Pancreas* 1995; 10(1): 53-8.
- Jörns A, Tiedge M, Lenzen S, Munday R. Effect of superoxide dismutase, catalase, chelating agents, and free radical scavengers on the toxicity of alloxan to isolated pancreatic islets in vitro. *Free Radic Biol Med* 1999; 26(9-10): 1300-4.
- Tibaldi J, Benjamin J, Cabbat FS, Heikkila RE. Protection against alloxan-induced diabetes by various urea derivatives: relationship between protective effects and reactivity with the hydroxyl radical. *J Pharmacol Exp Ther* 1979; 211(2): 415-8
- Slonim AE, Surber ML, Page DL, Sharp RA, Burr IM. Modification of chemically-induced diabetes in rats by vitamin E. Supplementation minimizes and depletion enhances development of diabetes. *J Clin Invest* 1983; 71(5): 1282-8.
- Wold LE, Relling DP, Colligan PB, Scott GI, Hintz KK, Ren BH, Epstein PN, Ren J. Characterization of contractile function in diabetic hypertensive cardiomyopathy in adult rat ventricular myocytes. *J Mol Cell Cardiol* 2001; 33(9): 1719- 26.
- Kaur J, Singh P, Sowers JR. Diabetes and cardiovascular diseases. *Am J Ther* 2002; 9(6): 510 -15.
- Hong JH , MJ Kim, Park MR, Kwag OG, Lee IS, Byun BH, Lee SC, Lee KB, Rhee SJ. Effects of vitamin E on oxidative stress and membrane fluidity in brain of STZ-induced diabetic rats. *Clin Chim Acta* 2004; 340(1-2): 107- 15.
- Harris A, Devaraj S, Jialal I. Oxidative stress, alpha-tocopherol therapy, and atherosclerosis. *Curr Atheroscler Rep* 2002; 4(5): 373-80.
- Mullan BA, Young IS, Fee H, McCance DR. Ascorbic acid reduces blood pressure and arterial stiffness in type 2 diabetes. *Hypertension* 2002 40(6): 804-9.
- Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, Sgambato S, Varricchio M, D'Onofrio F. Plasma Vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics. *Am J Physiol* 1994; 266(2 pt-1) E261-E268.
- Owu DU, Antai AB, Udofia KH, Obembe AO, Obasi KO, Eteng MU. Vitamin C improves basal metabolic rate and lipid profile in alloxan-induced diabetes mellitus in rats *J Biosci* 2006; 31(5): 575-9.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18(6): 499-502.
- Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol* 2004; 24(5) 816-23.
- Bhakdi S, Lackner K K, Han SR, Torzewski M, Husmann M. Beyond cholesterol: The enigma of atherosclerosis revisited. *Thromb Haemost* 2004; 91(4): 639-45.
- Tsujinaka K, Nakamura T, Meagawa H, Fujimiya M, Nishio Y, Kudo M, Kashiwagi A. Diet high in lipid hydroperoxide by vitamin E deficiency induces insulin

ACKNOWLEDGMENT

The authors wish to express their gratitude to the Research Council of Shahid Chamran University for their financial supports of the origin of this article, thesis No. 8458552.

- resistance and impaired insulin secretion in normal rats. *Diabetes Res Clin Pract* 2005; 67(2): 99 - 109.
23. Hubbard SR. Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. *EMBO J* 1997; 16(18): 5572-81.
24. Heffetz D, Bushkin I, Dror R, Zick Y. The insulinomimetic agents H₂O₂ and vanadate stimulate protein tyrosine phosphorylation in intact cells. *J Biol Chem* 1990; 265(5): 2896-2902.
25. Schmid E, Hotz-Wagenblatt A, Hacj V, Dröge W. Phosphorylation of the insulin receptor kinase by phosphocreatine in combination with hydrogen peroxide. The structural basis of redox priming. *FASEB J* 1999; 13(12): 1491-1500.
26. Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; 82(1): 47-95.
27. Schmitt T, Hotz-Wagenblatt A, Klein H, Dröge W. Interdependent Regulation of Insulin Receptor Kinase Activity by ADP and Hydrogen Peroxide. *J Biol Chem* 2005; 280(5): 3795-3801.
28. Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, Barford D. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenylamide intermediate. *Nature* 2003; 423(6941):769-773.
29. Sridulyakul P, Chakraphan D, Patumraj S. Vitamin C supplementation could reverse diabetes-induced endothelial cell dysfunction in mesenteric microcirculation in STZ-rats. *Clin Hemorheol Microcirc* 2006; 34(1-2): 315-21.
30. Amatyakul S, Chakraphan D, Chotpaibulpan S, Patumraj S. The effect of long-term supplementation of vitamin C on pupal blood flow in STZ induced diabetic rats. *Clin. Hemorheol Microcirc* 2003; 29(3-4): 313-9.
31. Afkhami-Ardekani M and Shojaoddiny-Ardekani A. Effect of vitamin C on blood glucose, serum lipids and serum insulin in type 2 diabetes patients. *Indian J Med Res* 2007; 126(5): 471-4.
32. Naziroglu M, Simsek M, Simsek H, Aydilek N, Ozcan Z, Atilgan R. The effects of hormone replacement therapy combined with vitamins C and E on antioxidants levels and lipid profiles in post menopausal women with type 2 diabetes. *Clin Chem Acta* 2004; 344 (1-2): 63-71.
33. Qian P, Cheng S, Guo J, Niu Y. [Effects of vitamin E and vitamin C on nonenzymatic glycation and peroxidation in experimental diabetic rats]. *Wei Sheng Yang Jiu* 2000; 29(4): 226-8.
34. Bishop N, Schorah CJ and Wales JK. The effect of vitamin C supplementation on diabetic hyperlipidaemia: a double blind, crossover study. *Diabet Med* 1985; 2(2): 121-4.
35. Mann GV. The impairment of transport of ascorbic acid. *Ann NY Acad Sci* 1974; 258: 243-52.
36. Pavlovic V, Pavlovic Z 2004 The effect of ascorbic acid on membrane transport of glucose. *Acta Medica Medianae* 43 (1): 39-41.
37. Johnston CS and Yen MF 1994 Megadose of vitamin C delays insulin response to a glucose challenge in normoglycemic adults. *Am. J. Clin. Nutr.* 60(5): 735-8.
38. Kaviarasan K, Arjunan MM, Pugalendi KV. Lipid profile, oxidant-antioxidant status and glycoprotein components in hyperlipidemic patients with/without diabetes *Clin Chim Acta* 2005; 362(1-2):49-56.
39. Young IS, Woodside JV. Antioxidants in health and disease; *J. Clin Pathol* 2001; 54(3): 176-86.
40. Haidara MA, Khloussy H, Ammar H, Aal kassem LA. Impact of alpha-tocopherol and vitamin C on endothelial markers in rats with STZ-induced diabetes. *Med Sci Monit* 2004; 10(2): BR41-6.
41. Evans M, Anderson RA, Smith JC, Khan N, Graham JM, Thomas AW, et al. Effects of insulin lispro and chronic vitamin C therapy on postprandial lipaemia, oxidative stress and endothelial function in patients with type 2 diabetes mellitus. *Eur J Clin Invest* 2003; 33(3): 231-8.
42. McAuliffe AV, Brooks BA, Fisher EJ, Molyneaux LM, yue DK. Administration of ascorbic acid and an aldose reductase inhibitor (tolerestat) in diabetes: effect on urinary albumin excretion. *Nephron* 1998; 80(3): 277-84.
43. White A, Handler P, Smith EL, Hill RL, Lehman IR. Principles of biochemistry 7th ed. Tokyo: McGrawHill Kogakusha Ltd 1994; 619-30.