



MICROSCOPICAL OBSERVATION OF KIDNEY FOR AMELIORATIVE EFFECTS OF VITAMIN - E & C IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Oxidative stress has been suggested as a contributory factor in development and complication of diabetes. Diabetic nephropathy is a serious complication of diabetes mellitus. Oxidative stress has been suggested to play a key role in the pathogenesis of diabetic nephropathy. The aim of the study is to evaluate the effect of Vitamins – E & C (VEC) roles in the antioxidant defense system. It is likely that both Vitamins act in a synergistic manner, with Vitamin E primarily being oxidized to the tocopheroxyl radical and then reduced back to tocopherol by Vitamin C. The purpose of this study was to determine the effects of supplementation of Vitamins – E & C (VEC) on diabetic rat kidney. Adult albino rats were used in the study. The animals were divided into three groups. Group-A: Control group (C); Group-B: Diabetic group (D), streptozotocin (60mg/kg body weight for 3 consecutive days) was administered group; Group-C: Diabetes+Vitamins–E&C (D + VEC) group, received a diet containing a combination of ascorbic acid and di-tocopheryl acetate per kg of feed. Rats were sacrificed on day 21 and renal tissues were taken for Microscopical examination. When compared with the control group, congestion of the glomerular capillaries, increased mesangial cells and distinct mesangium, shortened podocyte processes and disappearance of filtration slit pore of diabetic rat kidney were observed. In the group treated with VEC, glomerular changes were less distinct than the diabetic group. Lengths of the pedicles were similar to the control group. In conclusion, VEC reduced the changes in the glomerular structures due to diabetes.

Key words: *Oxidative stress, kidney, Diabetes, Vitamin-E, Vitamin-C, Antioxidant.*

1. INTRODUCTION

Diabetic nephropathy is one of the most common causes of chronic renal failure. Renal injury is observed in some 35% of patients with Type I and Type II diabetes. Renal disorders observed in Type I and Type II diabetes are similar. Long-term glycemia, genetic factors, race, sex and hypertension have been implicated in the development of diabetic nephropathy (Monhart, 2008; Rychlik, 2008). Diabetic nephropathy presents itself with ischemic nephropathy, nodular glomeruli-sclerosis and renal failure. Clinically, 30 -

300 mg/day or 20 - 200 µg/min micro-albuminurias indicate diabetic nephropathy (Ritz, 2006). Diabetes is an important etiopathological factor in oxidative stress (Punithavatki et al., 2008). As a result of lipid and protein oxidation, the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) increase in kidneys (Prakasam et al., 2005; Je et al., 2001; Yıldırım and Büyükbingöl, 2003). Various studies have reported protective effects of antioxidants such as melatonin (Ökten et al., 2006), ginkgobiloba (Welt et al.,

2007), Circumin (Murugan and Pari, 2006), Groundnut oil (Ramesh et al., 2006), taurin (Wang et al., 2008), herbal medications (Yokozawa et al., 2008), soybean oil (Sena et al., 2008), naringin, a flavonoid glycoside which gives the bitter taste of grapefruit juice (Punithavatki et al., 2008), Vitamin-E (Minamiyama et al., 2008; Hamdy et al., 2008; Ruperez et al., 2008) and Vitamin-C (Ruperez et al., 2008; Wu et al., 2007; Fadugpin et al., 2007) against oxidative damage of diabetes.

The levels of Vitamin E and C in plasma and renal tissues are significantly reduced in diabetic patients (Wu et al., 2007; Kashiba et al., 2002; Peerapatdit et al., 2006). A decrease in Vitamin-C causes hyperlipidemia and hypertension (Wu et al., 2007; Chen et al., 2005). Some studies showed that certain fruits and vegetables (Villegas et al., 2008) and that Vitamins-E and C (VEC) (Harding et al., 2008) are important to prevent or alleviate the complications of diabetes mellitus and also have complication reducing effects. Vitamins-E and C (VEC) not only reduce the risk of thrombo-embolism in patients with diabetes-related hypertension (Haidara et al., 2004) but also exert favorable effects on wound healing (Musalmah et al., 2005). Vitamins-E and C (VEC) has prevention on teratogenic effects in diabetic rats and also auto-immune responses in some cells in babies (Uusitalo et al., 2008; Cederberg et al., 2005). Vitamins-E and C (VEC) can be used as antioxidants separately or in combination, both Vitamins act synergistically (Naziroglu et al., 2004; Kutlu et al., 2005). In the present study, the effects of Vitamin-E, a lipophilic antioxidant and Vitamin-C, a hydrophilic antioxidant, on structural changes in renal tissue were investigated by feeding the experimental diabetes induced rats with a combination of these Vitamins.

2. MATERIALS AND METHODS

2.1 Chemical Agents:

Streptozotocin was purchased from Sigma-Aldrich Chemical Company. It was freshly dissolved in ice-cold 0.05 M citrate buffer (pH 4) and given i.p. in a dose of 60mg/kg body weight for 3 consecutive days for induction of diabetes (Abdel-Wahab MH and Abd-Allah AR, 2000; Yavuz O et al., 2003).

The VEC-supplemented diet contained a combination of di-tocopheryl acetate (600 mg) and ascorbic acid (1 g) per kg of feed. VCE supplemented and un-supplemented food compositions were homogenized using a mixer and pellets were prepared in laboratory by heating below 45°C for 2 days. The VEC supplemented diet contained a combination of 600 mg Vitamin-E (di-tocopheryl acetate) per kg of feed (Naziroglu et al., 2004) and 1 g Vitamin-C (ascorbic acid).

2.2 Animals

The experiments were performed on Albino rats (approx 200 - 250 g) obtained from Animal House, SRM University, Tamilnadu, India. All aspects of animal care complied with the ethical guidelines and technical requirements approved by the Institutional Animal Ethics Committee. Animals were housed individually in cages in an environmentally controlled animal facility (room temperature, 12 h light: 12 h dark cycle) with free access to diet and water ad libitum.

All animals were fed on normal diet for seven days of acclimatization. Diabetes was induced by an intraperitoneal (IP) injection of freshly prepared dissolved mixture of Streptozotocin with ice-cold 0.05 M citrate buffer (pH 4) and given in a dose of 60mg/kg body weight for three consecutive days.

2.3 Experimental Design

The animals were randomly divided into three groups (n = 6) as follows:

Group-A: Control group (C) with normal diet (Saline solution, i.p.);

Group-B: Diabetic group (D) with Streptozotocin, (60mg/kg body weight for 3 consecutive days) was administered;

Group-C: Diabetes+Vitamins-E & C (D + VEC) group, Rats were fed with Vitamins-E and C (VEC) supplemented diet for 15 days prior to induction of diabetes.

2.4 Methods of Analysis

Animals were starved for 16 h before blood collection. Fasting blood glucose was estimated by glucoseoxidase method. Urine was collected in cage urine separator

bottle containing 1 mL of 10% thymol to analysis the glucose level in urine. At the end of the experiment, the animals were sacrificed; they were made unconscious with carbon tetrachloride before sacrificed by euthanasia. The renal tissues were taken for Histopathological study. Histochemical analysis was done according to Bancroft and Stevens (John D. Bancroft and Alan. Stevens, 1983).

3. RESULTS

The kidneys, compare to control group, modest glomerular lesion were noted in diabetic group. Glomerular capillaries were irregular, widened and attached to the Bowman's capsule. Furthermore, mesangial cell number was slightly higher in diabetic group. The degree of tubulo-interstitial damage was modest. There were only few widened tubuli with incipient atrophy of the epithelial cells. In addition, slight focal interstitial fibrosis was observed. Intrarenal arterial vessels showed modest thickening of the walls (Fig: 1, 2 & 3 (Group -B)). The above changes were decreased significantly in VEC treated group (Fig: 1, 2 & 3 (Group -C)).

Fig: 1. Microscopic observation of Kidney, Glomeruli and Tubules (magnification X 200)

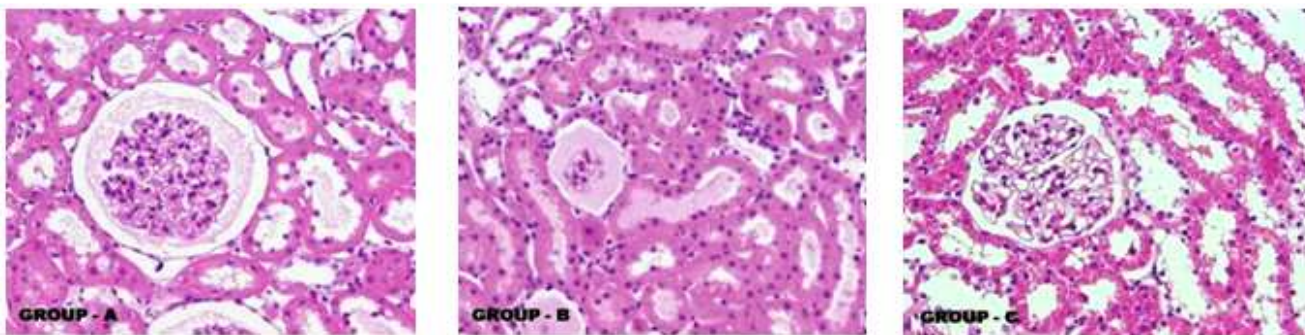


Fig: 1. Histopathological changes show the effects of VEC on kidneys of diabetic rats. H&E staining of kidneys was performed. Group-A: Control group (C); Group-B: Diabetic group (D); Group-C: Diabetes + Vitamins – E & C (D + VEC) group rats. (Magnification X 200)

Thin sections of the renal proximal tubules and glomeruli of the control group looked normal (Fig: 1, 2 & 3 (Group -A)). Podocytes and cytoplasmic extensions, infiltration slits were evenly distributed. Capillary congestion, increase in the number of mesangial cells and shortened podocyte processes were observed in the renal glomeruli secondary to STZ. Basement membrane was thickened in certain

regions. The invaginations on the basal regions of the proximal tubules were irregular collagen fibers were clustered in the areas between the tubules. Compared to the control group, mesangial accumulation, shortened podocyte processes and obscured filtration slits could clearly be identified in the diabetic group. Obscured of the infiltration slits, were not diffuse (Fig: 1, 2 & 3 (Group -B)).

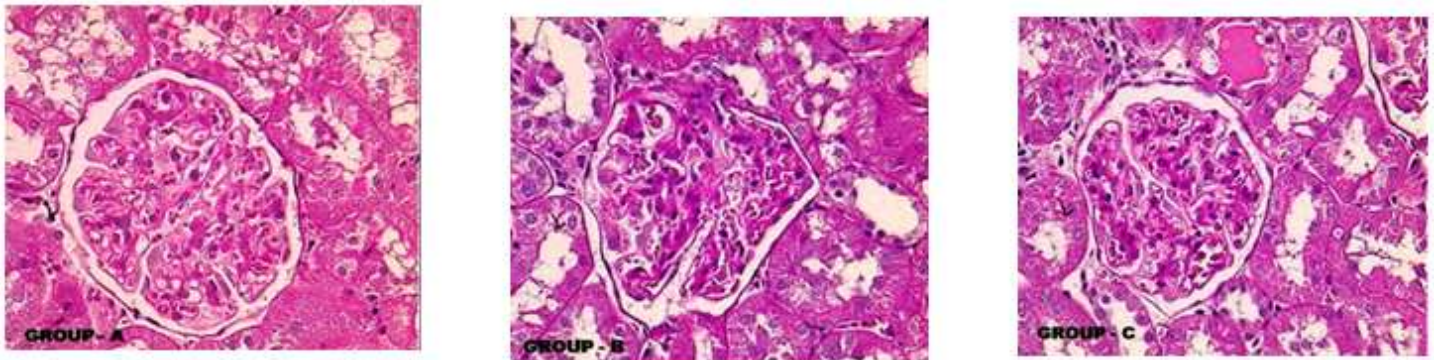
Fig: 2. Microscopic observation of Kidney, Glomeruli (magnificaionX200)

Fig: 2. Histopathological changes of Renal Glomeruli show the effects of VEC on kidneys of diabetic rats. H&E staining of kidneys was performed. Group-A: Control group (C); Group-B: Diabetic group (D); Group-C: Diabetes + Vitamins – E & C (D + VEC) group rats. (Magnification X 200)

degeneration of the glomerular endothelia, diffuse or nodular glomeruli-sclerosis, apoptosis in the podocytes and hyalinization beneath the basal lamina were not observed in this group (Fig: 1, 2 & 3 (Group -B)). Structural changes in the glomeruli

of the diabetic rats fed with VEC were milder than the diabetes group. Increase in mesangial cells was not observed. Podocyte processes and filtration slits generally appeared normal (Fig: 1, 2 & 3 (Group -C)).

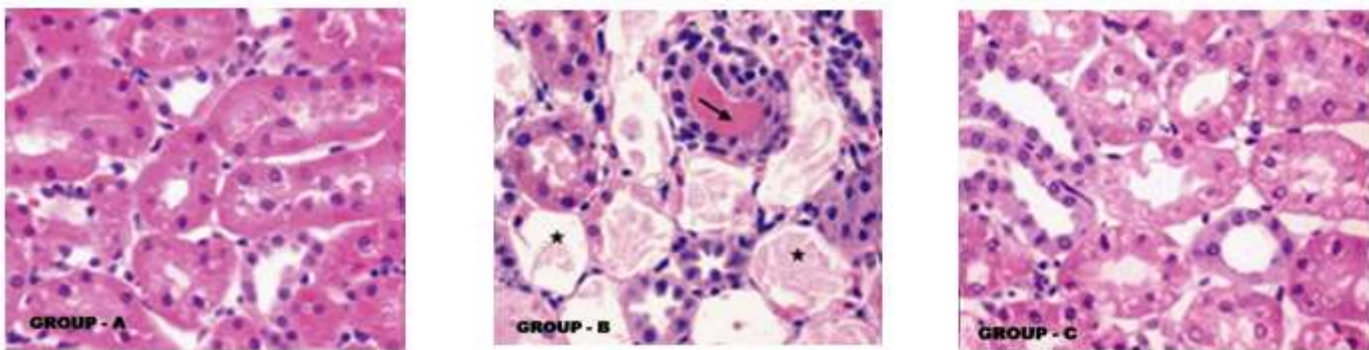
Fig: 3. Microscopic observation of Kidney, Tubules (magnificaionX200)

Fig: 3. Histopathological changes of Renal Tubules show the effects of VEC on kidneys of diabetic rats. H&E staining of kidneys was performed. Group-A: Control group (C); Group-B: Diabetic group (D); Group-C: Diabetes + Vitamins – E & C (D + VEC) group rats. (Magnification X 200)

4. DISCUSSION

Diabetic nephropathy is the most common cause of chronic renal disease and the foremost indication for dialysis and renal transplantation (Estacio and Schrier, 2001). Diffuse or nodular glomeruli-sclerosis, arteriosclerosis, tubulo-interstitial fibrosis

and atrophy occur, proteinuria gradually increases and so does the blood pressure (Alsaad et al., 2007). Glomerulo-sclerosis develops as a result of injury to the podocytes which plays the key role in glomerular filtration (Lee et al., 2007).

Podocyte injury can present itself in two forms: metabolic (biochemical) and hemodynamic (associated with hyperfiltration and hyperperfusion)

(Hostetter et al., 2003; Wolf et al., 2003). Glomerular hyperfiltration and hyperperfusion are essential in mesangialisation and changes in the glomerular basal membrane (Wolf et al., 2003; Menini et al., 2007). In diabetic nephropathy, prostaglandin E2 (PGE2) synthesis in the glomeruli is significantly increased. This is as a result of an increase in mesangial cells (Iino et al., 2005). Diltiazem inhibits PGE2 (Peerapatdit et al., 2006; Wu et al., 2007). Oxidative stress and free oxygen radicals, which develop during nephropathy, trigger apoptosis of the tubular epithelial cells and podocytes of the glomeruli (Blauwkamp et al., 2008; Jung et al., 2008; Ruster et al., 2008; Susztak et al., 2006).

Various agents used to inhibit apoptosis have been tried in the treatment of nephropathy (Isermann et al., 2007). In the present study was observed an increase in mesangial cell in the glomeruli of diabetic kidneys and mesangial accumulation but not apoptosis of podocytes and tubular epithelial cells. Antioxidants are frequently used for diabetes and its complications. Plasma Vitamin C and E concentrations are reduced in diabetes (Murugan et al., 2006; Ramesh et al., 2006; Peerapatdit et al., 2006; Wu et al., 2007; Lee et al., 2007). A positive relation has been demonstrated between high plasma Vitamin C level and reduction in complications of diabetes (Harding et al., 2008). Vitamin C plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells (Sadi et al., 2008; Afkhami-Ardekani et al., 2007; Al-Shamsi et al., 2006).

Furthermore, Vitamin-C regenerates the oxidized Vitamin-E (Chen et al., 2005). Vitamin-E, on the other hand, acts as a non-enzymatic antioxidant and reduces lipid peroxidation and glutathione (Punithavatki et al., 2008; Minamiyama et al., 2008; Lee et al., 2007). Vitamin-E is very effective in glycemic control, lowering the HbA1c levels (Ihara et al., 2000) and preventing the hypertrophic effects of hyperglycemia (Nascimento et al., 2005). However, this is in contrast to the results of some studies, which showed that Vitamin-E was not beneficial in glycemic control and lipid metabolism in Type II diabetes (Ble-Castillo et al., 2005). In another study, the researchers

demonstrated that a combination of Vitamins-E and C (VEC) improved the glomerular functions but did not have any effect on the tubular functions (Farvid et al., 2006).

When exercise is given to rats with STZ-induced diabetes in addition to Vitamins-E and C (VEC), it was observed that lipid peroxidation was significantly reduced, glutathione peroxidase (GSH-Px) was increased and reduced glutathione (GSH) level was decreased (Kutlu et al., 2005). The earliest structural changes in diabetic nephropathy are the increase in mesangial cells and mesangial dilatation. Diffuse thickening of the glomerular basement membrane depends on the severity of the disease.

Vitamins-E and C (VEC) reduce the thickness of the basement membrane (Kedziara-Kornatowski et al., 2003; Davila- Esqueda et al., 2005). It has been argued that glomerular changes can occur in diabetic nephropathy without arterial or tubulo-interstitial changes (Fioretto and Mauer, 2007). Vacuolization within the podocytes, myelin figures and blebs were noted (Farvid et al., 2006). In our results, thickening of the glomerular basement membrane was not diffuse but they were in certain regions. Occasional irregularities of the podocyte processes, shortened podocyte processes and obscured of the infiltration slits were observed.

5. CONCLUSION

In conclusion, alterations were not observed in the renal tubules of rats with STZ induced-diabetes while increased mesangial cells, increased capillary permeability, and obscured of the filtration slits were noted in the glomeruli. Neither apoptosis of the podocytes nor thickening of the basement membrane was observed. These alterations were less pronounced in the diabetic rats treated with Vitamins-E and C (VEC). Vitamins-E and C (VEC) helped alleviation of the renal degeneration by protecting the glomerular structures from oxidative injury. Concomitant administration of Vitamins-E and C (VEC) would be more effective in preventing the complications of diabetes.

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7. REFERENCES

1. Abdel-Wahab MH and Abd-Allah AR. Possible protective effect of melatonin and/or desferrioxamine against streptozotocin-induced hyperglycaemia in mice. *Pharmacol. Res.* 41(5), 2000, 533-537.
2. Afkhami-Ardekani M, Shojaodding-Ardekani A. Effect of Vitamin C on blood glucose, serum lipids and serum insulin type 2 diabetes patients. *Indian J. Med. Res.* 126: 2007, 471-4.
3. Alsaad KO, Herzenberg AM. Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: An update. *J. Clin. Pathol.* 60: 2007, 18-26.
4. Al-Shamsi M, Amin A, Adeqhate E. Effect of Vitamin C on liver and kidney functions in normal and diabetic rats. *Ann. NY Acad. Sci.* 1084: 2006, 371-90.
5. Blauwkamp MN, Yu J, Schin MA, Burke KA, Berry MJ, Carlson BA, Brosius FC, Koenig RJ. Podocyte specific knock out of Selene proteins does not enhance nephropathy in streptozotocin diabetic C57BL/6 mice. *BMC Nephrol.* 9: 2008, 7.
6. Ble-Castillo JL, Carmona-Diaz E, Mendez JD, Larios-Medina FJ, Medina-Santillan R, Cleva-Villanueva G, Diaz-Zagoya JC. Effect of alpha-tocopherol on the metabolic control and oxidative stress in female type 2 diabetics. *Biomed. Pharmacother.* 59: 2005, 290-5.
7. Cederberg J, Erikssan UJ. Antioxidative treatment of pregnant diabetic rats diminishes embryonic dysmorphogenesis. *Birth Defects Res. A. Clin. Mol. Teratol.* 73: 2005, 498-505.
8. Chen L, Jia RH, Qiu CJ, Ding G. Hyperglycemia inhibits the uptake of dehydro-ascorbate in tubular epithelial cell. *Am. J. Nephrol.* 25: 2005, 459-465.
9. Davila-Esqueda ME, Vertiz-Hernandez AA, Martinez-Morales F. Comparative analysis of the reno-protective effects of pentoxifylline and Vitamin E on streptozotocin-induced diabetes mellitus. *Ren. Fail.* 27: 2005, 195-207.
10. Estacio RO, Schrier RW. Diabetic nephropathy; pathogenesis, diagnosis and prevention of progression. *Adv. Int. Med.* 46: 2001, 359-408.
11. Fadupin GT, Akpoqhar AU, Okunade KA. Comparative study of serum ascorbic acid level in people with and without type 2 diabetes in Ibadan Nigeria. *Afr. J. Med. Med. Sci.* 36: 2007, 335-339.
12. Farvid MS, Jalali M, Siassi F, Hosseini M. Comparison of the effects of Vitamins and/or mineral supplementation on glomerular and tubular dysfunction in type 2 diabetes. *Diabetes Care* 29: 2006, 747-8.
13. Fioretto P, Mauer M. Histopathology of diabetic nephropathy. *Semin. Nephrol.* 27: 2007, 195-207.
14. Haidara MA, Khloussy H, Ammar H, Aal Kasem LA. Impact of alpha-tocopherol and Vitamin C on endothelial markers in rats with streptozotocin-induced diabetes. *Med. Sci. Monit.* 10: 2004, 41-6.
15. Hamdy NM, Suwailem SM, El-Mesallamy HO. Influence of Vitamin E supplementation on endothelial complications in type 2 diabetes mellitus patients who underwent coronary artery bypass graft. *J. Diab. Complications* 23: 2008, 167-73.
16. Harding AH, Wareham NJ, Bingham SA, Khaw K, Luben R, Welch A, Forouhi NG. Plasma Vitamin C level, fruit and vegetable consumption and the risk of new-onset type 2

- diabetes mellitus: The European prospective investigation of Cancer - Norfolk prospective study. *Arch. Inter. Med.* 168: 2008, 1485-1486.
17. Hostetter TH. Hyperfiltration and glomerulosclerosis. *Semin. Nephrol.* 23: 2003, 194-9.
 18. Iino K, Iwase M, Sonoki K, Yoshinari M, Iida M. Combination treatment of Vitamin C and desferrioxamine suppresses glomerular superoxide and prostaglandin E production in diabetic rats. *Diab. Obes. Metab.* 7: 2005, 106-9.
 19. Ihara Y, Yamada Y, Toyokuni S, Miyowaki K, Ban N, Adachi T, Kurae A, Iwakuru T, Kubota A, Hiai H, Seino Y. Antioxidant alphatocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. *FEBS Lett.* 473: 2000, 24-6.
 20. Isermann B, Vinnikov IA, Madhusudhan T, Herzog S, Kashif M, Blautzik J. Activated protein C protects against diabetic nephropathy by inhibiting endothelial and podocyte apoptosis. *Nat. Med.* 13: 2007, 1349- 58.
 21. Je HD, Shin CY, Park HS, Huh IH, Sohn UD. The comparison of Vitamin C and Vitamin E on the protein oxidation of diabetic rats. *J. Auton. Pharmacol.* 21: 2001, 231-236.
 22. John D. Bancroft, Alan. Stevens. Histochemical techniques: Theory and practice of histological techniques. Churchill Livingstone (Edinburgh and New York), 1982.
 23. Jung DS, Li JJ, Kwak SJ, Lee SH, Park J, Song YS, Yoo TH, Han SH, Lee JE, Kim DK, Moon SJ, Kim YS, Han DS, Kang SW. FR167653 inhibits fibronectin expression and apoptosis in diabetic glomeruli and in high-glucose-stimulated mesangial cells. *Am. J. Physiol. Renal Physiol.* 295: 2008, 595-604.
 24. Kashiba M, Oka J, Ichikawa R, Kasahara E, Inayama T. Impaired ascorbic acid metabolism in streptozotocin-induced diabetic rats. *Free Radic. Biol. Med.* 33: 2002, 1221-1230.
 25. Kedziara-Kornatowski K, Szram S, Karnatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. Effect of Vitamin E and Vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. *Nephron Exp. Kukner et al.* 035 *Nephrol.* 95: 2003, 134-43.
 26. Kutlu M, Nazioğlu M, Simsek H, Yılmaz T, Kukner AH. Moderate exercise combined with dietary Vitamins C and E counteracts oxidative stress in the kidney and lens of streptozotocin-induced diabetic rat. *Int. J. Vitam. Nutr. Res.* 75: 2005, 71-80.
 27. Lee EY, Lee MY, Honq SW, Chunq CH, Honq SY. Blockade of oxidative stress by Vitamin C ameliorates albuminuria and renal sclerosis in experimental diabetic rats. *Yonsei Med. J.* 48: 2007, 847-55.
 28. Menini S, Iacobini C, Oddi G, Ricci C, Simonelli P, Fallucca S, Grattarola M, Peqliese F, Pesce C, Peqliese G. Increased glomerular cell (podocyte) apoptosis in rats with streptozotocin induced diabetes mellitus: role in the development of diabetic glomerular disease. *Diabetologia* 50: 2007, 2591-9.
 29. Minamiyama Y, Takemura S, Bito Y, Shinkawa H, Tsukioka T, Nakahira A, Suehiro S, Okada S. Supplementation of alphatocopherol improves cardiovascular risk factors via the insulin signaling pathway and reduction of mitochondrial reactive oxygen species in type II diabetic rats. *Free Radic. Res.* 42: 2008, 261-271
 30. Monhart V. Diabetes mellitus, hypertension and kidney. *Vnitr. Lek.* 54: 2008, 499-504.
 31. Murugan P, Pari L. Antioxidant effect of tetrahydrocurcumin in streptozotocin-nicotinamide induced diabetic rats. *Life Sci.* 79: 2006, 1720- 1728.
 32. Musalmah M, Nizrana MY, Fairuz AH, Noor Aini AH, Azian AL, Gapor MT, Wan Ngah WZ. Comparative effects of Vitamin E and alpha-tocopherol on healing and wound tissue antioxidant enzyme levels in diabetic rats. *Lipids* 40: 2005, 575-80.
 33. Nascimento Gomes G, Barbosa FT, Radaeli RF, Cavalar MF, Mello Aires M, Zaladek Gil F. Effect of D-alpha-tocopherol on tubular nephron acidification by rats with induced diabetes mellitus. *Braz. J. Med. Biol. Res.* 38: 2005, 1043-51.
 34. Naziroğlu M, Simsek M, Simsek H, Aydilek N, Özcan Z, Atılğan R. The effects of hormone replacement therapy combined with Vitamins C and E on antioxidants levels and lipid

- profiles in postmenopausal women with type 2 diabetes. *Clin. Chem. Acta.* 344: 2004, 63-71.
35. Ökten F, Özgüner F, Yılmaz HR, Uz E, Dündar B. Melatonin reduces urinary excretion of N-acetyl-beta-D-glucosaminidase, albumin and renal oxidative markers in diabetic rats. *Clin. Exp. Pharmacol. Physiol.* 33: 2006, 95-101.
36. Peerapatdit T, Patchanans N, Likidlilid A, Poldee S, Sriratanasathavorn C. Plasma lipid peroxidation and antioxidant nutrients in type 2 diabetic patients. *J. Med. Assoc. Thai.* 89 Suppl. 5: 2006, 5147-55.
37. Prakasam A, Sethupathy S, Puqalendi KV. Anti-peroxidative and antioxidant effects of casearia esculenta root extract in streptozotocin-induced diabetic rats. *Yale J. Biol. Med.* 78: 2005, 15-23.
38. Punithavatki VR, Anuthama R, Prince PS. Combined treatment with naringin and Vitamin C ameliorates streptozotocin-induced diabetes in male Wistar rats. *J. Appl. Toxicol.* 28: 2008, 806-813.
39. Ramesh B, Saravanan R, Pugalendi KV. Effect of dietary substitution of Groundnut oil on blood glucose, lipid profile, and redox status in streptozotocin-diabetic rats. *Yale J. Biol. Med.* 79: 2006, 9-17.
40. Ritz E. Diabetic nephropathy. *Saudi J. Kidney Dis. Transpl.* 17: 2006, 481-490.
41. Ruperez FJ, Garcia-Martinez D, Baera B, Maeso N, Cifuentes A, Barbas C, Herrera E. Evolution of oxidative stress parameters and response to oral Vitamins E and C in streptozotocin-induced diabetic rats. *J. Pharm. Pharmacol.* 60: 2008, 871-878.
42. Ruster C, Bondeva T, Franke S, Förster M, Wolf G. Advanced glycation end-products induce cell cycle arrest and hypertrophy in podocytes. *Nephrol. Dial. Transplant.* 23: 2008, 2179-91.
43. Rychlik I. Epidemiology of diabetic nephropathy. *Vnitr Lek.* 54: 2008, 488-493.
44. Sadi G, Yılmaz O, Güray T. Effect of Vitamin C and lipoic acid on streptozotocin-induced diabetes gene expression: mRNA and protein expressions of Cu-Zn SOD and catalase. *Mol. Cell Biochem.* 309: 2008, 109-16.
45. Sena CM, Proenca T, Nunes E, Santos MS, Seica RM. The effect of soybean oil on glycaemic control in Goto-Kakizaki rats, an animal model of type 2 diabetes. *Med. Chem.* 4: 2008, 293-297.
46. Susztak K, Raff AC, Schilffer M, Bottinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes* 55: 2006, 225-233.
47. Uusitalo L, Nevalainen J, Niinistö S, Alfthan G, Sandvall J. Serum alpha and gamma-tocopherol concentrations and risk of advanced beta cell autoimmunity in children with HLA-conferred susceptibility to type 1 diabetes mellitus. *Diabetologia* 51: 2008, 773-80.
48. Villegas R, Shu XO, Gao YT, Yang G, Elasy T, Li H, Zhenq W. Vegetable but not fruit consumption reduces the risk of type 2 diabetes in Chinese women. *J. Nutr.* 138: 2008, 574-80.
49. Wang L, Zhang L, Yu Y, Wang Y, Niu N. The protective effects of taurine against early renal injury in STZ-induced diabetic rats, correlated with inhibition of renal Lox-1 mediated ICAM-1 expression. *Ren. Fail.* 30: 2008, 763-771.
50. Welt K, Weiss J, Martin R, Hermsdorf T, Drews S, Fitz G. Ginkgobiloba extract protects rat kidney from diabetic and hypoxic damage. *Phytomedicine* 14: 2007, 196-203.
51. Wolf G, Butzmann U, Wenzel UO. The renin-angiotensin system and progression of renal disease: from hemodynamics to cell biology. *Nephron. Physiol.* 93: 2003, 3-13.
52. Wu JH, Ward NC, Indrawan AP, Almeida CA, Hodgson JM, Porudfoot JM, Puddey IB, Croft KD. Effect of α -tocopherol and mixed tocopherol supplementation on markers of oxidative stress and inflammation in type 2 diabetes. *Clin. Chem.* 53: 2007, 511-519.
53. Wu X, Iguchi T, Hiranoj J, Fujita I, Ueda H, Itoh N, Tanaka K, Nakahishi T. Upregulation of sodium-dependent Vitamin C transporter 2 expression in adrenals increases nor-epinephrine production and aggravates hyperlipidemia in mice with streptozotocin-induced diabetes. *Biochem. Pharmacol.* 74: 2007, 1020-1028.
54. Yavuz O, Cam M, Bukan N, Guven A and

- Silan F. Protective effect of melatonin on beta-cell damage in streptozotocin-induced diabetes in rats. *Acta Histochem*, 105, 2003, 261-266.
55. Yıldırım O, Büyükbingöl Z. In-vivo effect of Vitamin C with cobalt on oxidative stress in experimental diabetic rat kidney. *Diab. Nutr. Metab.* 16: 2003, 208-213.
56. Yokozawa T, Yamabe N, Kim HY, Kang KS, Hur JM, Park CH, Tanaka T. Protective effects of Morroniside isolated from Corni Fructus against renal damage in streptozotocin-induced diabetic rats. *Biol. Pharm. Bull.* 31: 2008, 1422-1428.