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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; Cederberq 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 lypophilic 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)

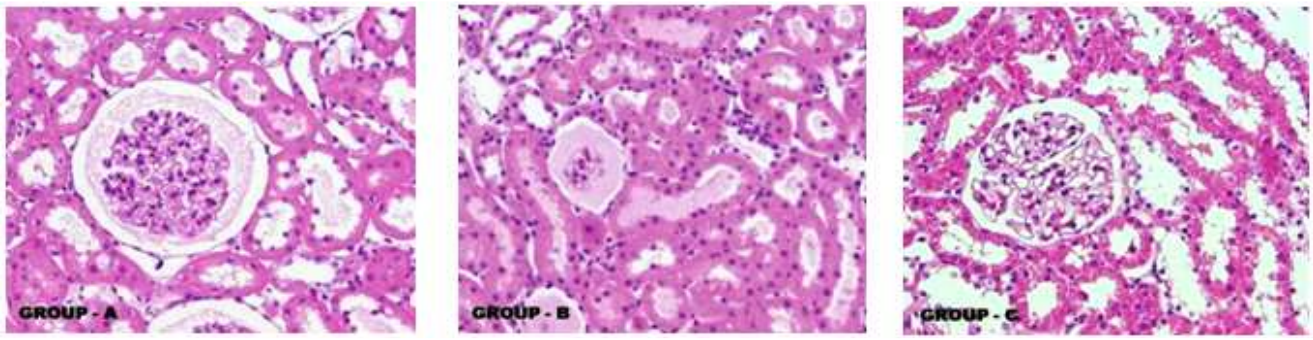

Fig: 1. ***Histopathological changes show the effects of VEC on kidneys of diabetic rats. H&E staining of kidneys was performed. Group-A: Control group (C); Group-B: Diabetic group (D); Group-C: Diabetes + Vitamins – E & C (D + VEC) group rats. (Magnification X 200)***

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)***

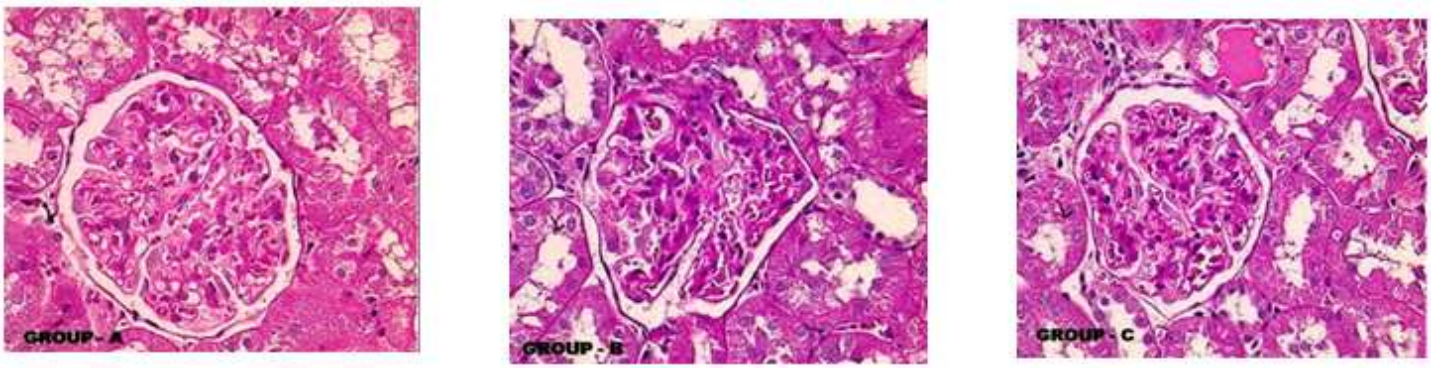

Fig: 2. ***Histopathological changes of Renal Glomeruli show the effects of VEC on kidneys of diabetic rats. H&E staining of kidneys was performed. Group-A: Control group (C); Group-B: Diabetic group (D); Group-C: Diabetes + Vitamin s– E & C (D + VEC) group rats. (Magnification X 200)***

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)

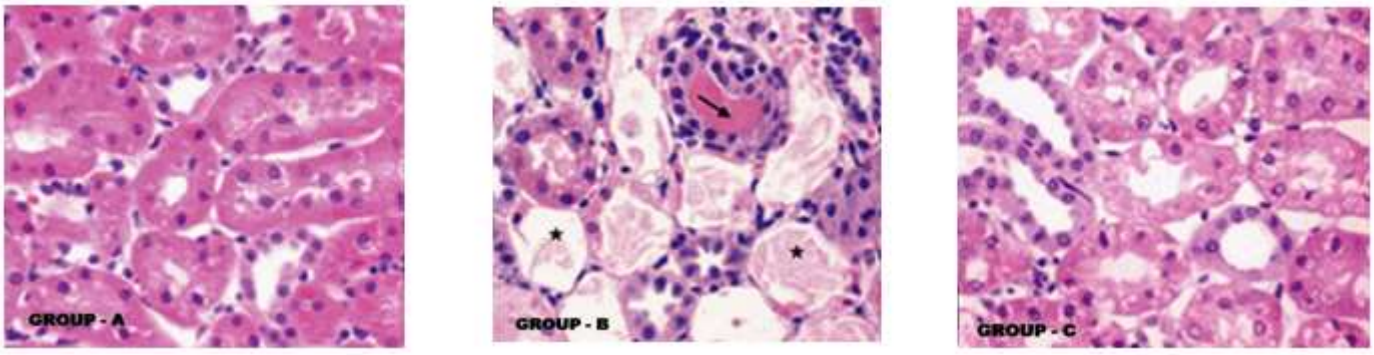

Fig: 3. ***Histopathological changes of Renal Tubules show the effects of VEC on kidneys of diabetic rats. H&E staining of kidneys was performed. Group-A: Control group (C); Group-B: Diabetic group (D); Group-C: Diabetes + Vitamins – E &C (D + VEC) group rats. (Magnification X 200)***

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 mesangilisation 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). Di-tochopherole 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; Rüster 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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