ORIGINAL ARTICLE

Protective Role of Vitamin C on Renal Morphology and Functions in Streptozotocin Induced Diabetes in Albino Rats

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ABSTRACT

Aim: To study the renoprotective effect of Vitamin C on the histology of the proximal convoluted tubules in streptozotocin (STZ) induced diabetes in albino rats.
Study design: A prospective experimental study.
Place: Department of Anatomy, Post Graduate Medical Institute, Lahore.
Duration: January to April 2011 for a period of 12 weeks.
Material and methods: Forty five male albino rats were divided into three groups A, B and C respectively. Group A served as the control group, group B and C were administered STZ in a single dose of 45mg/kg whereas group C additionally received Vit C in a dose of 10g/L/day in drinking water three days before the administration of STZ. The rats were sacrificed at the end of the experimental period, their blood samples taken and their kidneys dissected, fixed in alcoholic formalin and processed further for histological examination. Serum glucose, glycated Hemoglobin (HbA1C) and serum creatinine levels were compared in all the three groups.
Results: The microscopic examination of the proximal convoluted tubules revealed marked epithelial, cytoplasmic and nuclear changes in the STZ treated group B and significant reduction in the severity of these changes in Vit C treated group C. There was a considerable increase in serum glucose and glycated Hb levels in both STZ and Vit C treated groups as compared to control. Serum creatinine levels were significantly elevated in group B as compared to control, whereas in group C its level was significantly reduced when compared to group B.
Conclusion: The results of the investigation indicated that Vit C administration suppressed the progression of renal injury in diabetic rats. Our findings conclude that Vit C supplementation has a protective role against deterioration of renal structure and functions brought about by free radical toxicity in diabetes mellitus.
Key words: Streptozotocin, vit C, reactive oxygen species, antioxidant, serum creatinine.

INTRODUCTION

Diabetes Mellitus forms an enormous and still increasing health burden worldwide. Mortality and morbidity are caused by the occurrence of complications. One of the microvascular complications is diabetic nephropathy in both type 1 and type 2 diabetes mellitus, which is the leading cause of renal failure in adults at the moment[1].

Accumulating research suggests that oxidative stress is a significant contributor to the pathogenesis of diabetic nephropathy. The normal kidney generates a substantial amount of oxidative stress because of its high metabolic activity that is balanced by an extensive antioxidant system. However, in pathologic states such as hyperglycemia, oxidant balance shifts towards a pro-oxidant state that accelerates tissue and vascular injury. This oxidative damage progresses concomitant with worsening glucose metabolism, vascular dysfunction and kidney disease[2].

Streptozotocin (STZ) is an alkylating agent antibiotic that experimentally produces diabetes due to B-cell death by the mechanism of DNA damage in rodent islets[3]. During STZ metabolism, various toxic intermediates are produced including Reactive oxygen Species (ROS). ROS can react with proteins, lipids, nucleic acids and carbohydrates, therefore causing inflammation, apoptosis, fibrosis and cell proliferation[4].

The role of antioxidants in the prevention and treatment of diabetic nephropathy has been extensively studied in recent years. In addition to endogenous antioxidants, also dietary antioxidants like Vit C, E and beta carotene may restore the redox balance. Vitamins are a class of nutrients that are essentially required by the body for its various biochemical and physiological processes. Mostly, the human body does not synthesize them. So they must be supplied by the diet in the required amount.

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Vitamin C (Ascorbic Acid) is a diet-derived water soluble anti-oxidant participating in the normal protecting mechanism of the body[5].

It is an important antioxidant in humans capable of scavenging OFR. It is considered the most important anti–oxidant in plasma and forms the first line of defence against plasma lipid peroxidation[6]. In diabetic rats, Vit C levels decrease in plasma and Organs[7]. Deficiency of Vit C leads to the enhancement of Oxidative stress, which might be responsible for mitochondrial dysfunction in diabetes[8]. A positive relation has been demonstrated between high plasma Vit C level and reduction of complications of diabetes[9]. It is one of the major contributors to serum total antioxidant activity. It may therefore be beneficial in preventing the oxidative renal damage and stress by preserving the renal morphology in diabetes mellitus.

MATERIAL AND METHODS

This study was conducted in the Department of Anatomy, Post Graduate Medical Institute, Lahore (PGMI). Forty five healthy male albino rats, 90-120 days old, weighing around 250-300 grams were obtained from the Animal House of PGMI and were kept under observation for 1 week before commencement of study. The animals were divided into three groups. Group A served as control and were injected with a vehicle of 1ml of Citrate buffer. Group B and C received STZ (Sigma Aldrich, USA) intraperitoneally in a single dose of 45 mg / kg[10] at 4 pH dissolved in 1 ml of citrate buffer, whereas Group C additionally received Vit C in a dose of 10 g/L/day[11] in drinking water three days prior to the administration of STZ.

The animals were kept in 3 separate propylene cages with sieved steel cover equipped with drinking water bottle and woodchip floor bedding under natural environment. Food and water were supplied adlibitum.

The Serum Glucose of all the animals was recorded on the first day of the experiment and once weekly. Serum glucose of all the animals in Group B and C was recorded on the fourth day after administration of STZ and only those animals were considered diabetic whose blood glucose was > 300 mg / dl. Blood samples were collected from tail vein and estimated with Accu-chek Active one touch Glucometer (Roche Diagnostics, USA).

The rats of all the three groups were sacrificed at the end of 12th week and blood samples were collected from the cardiac region by intra cardiac puncture immediately after sacrifice to carry out Glycated Hemoglobin test (HbA1c) and serum creatinine by colorimetric method. They were now fixed on a dissecting board, abdomen was opened and both the kidneys were exposed for any gross change in colour, consistency, shape and size with the help of a magnifying glass. After washing with normal saline, the kidneys were fixed in alcoholic formalin for 24 hours. After fixation, they were kept in 70% alcohol overnight. Dehydration was done with ascending strengths of alcohol, with changes of one hour each i.e. 80%, 90%, 95%, absolute I and absolute II alcohol. The tissue was cleared in two changes of xylene for one hour each. It was now infiltrated in two changes of paraffin for one hour each in the laboratory oven at 59^{o} C. Then paraffin blocks of tissue were made and 5 micron thick longitudinal sections were cut with the help of a rotatory microtome. Sections were mounted on labelled glass slides and stained with Periodic Acid Schiff (PAS) Hematoxylin[12] for a detailed morphological examination of the proximal convoluted tubules of the cortex under the light microscope.

Tubular lumen was examined for any epithelial or nuclear debris in 8 x ocular and 40 x objective. The brush border and the basement membrane were studied in detail. Cytoplasmic and nuclear details were observed and the interstitium of the cortex was examined for any signs of inflammation or fibrosis.

Various quantitative changes between the experimental groups and control group were evaluated by student's t-test. The difference was regarded statistically significant if the p-value was less than or equal to 0.05. All calculations were done by utilizing computer software SPSS version 10.

RESULTS

Group A: On gross examination, the kidneys of control group A appeared bean shaped reddish brown in colour and soft in consistency with a smooth shiny surface covered with a delicate fibrous capsule which stripped off with ease. The morphological examination of PAS – Hematoxylin stained sections of Kidneys of control group revealed a normal intact cortical and medullary portions of renal architecture. The proximal tubules were subjected to detailed histological study under the light microscope. They were closely packed and mostly confined to the cortex. Their lining epithelial cells were regularly arranged on an intact and well defined basement membrane. The cells appeared simple cuboidal with a centrally located spherical nucleus. The cytoplasm of the tubular cells appeared fine and granular. The luminal surfaces of these cells presented a distinct and regular brush border. The lumina of the tubules was devoid of any cellular or nuclear debris. The interstitium of the cortical area was normal, with no

signs of inflammation or fibrosis observed (Fig 1). The mean values of Serum Glucose and Glycated Hemoglobin in Control Group A were 88±5 and 6.10±0.30 respectively (Table 1). Comparison to Groups B and C is shown in Table 1. Upon grading of the tubular damage (Table 2) there were no lesions observed in Group A. Comparison of grading of renal pathology to Groups B and C is shown in Table 2.

Group B: On gross examination, the kidneys of STZ treated group B appeared bean-shaped, brownish-black in colour, swollen and hard in consistency. The capsules were not stripped off easily. The morphological examination of PAS-Hematoxylin stained sections of kidneys belonging to STZ treated group revealed a cortical architecture which was distorted with deranged arrangement of cortical labyrinth and medullary rays. Most of the proximal tubules showed dilatation with severe sloughing and degeneration while others with necrotic changes showed shrinkage in size. Many of the cells showed vacuolated appearance obscuring cytoplasmic details. The underlying basement membrane was found highly discontinuous and distorted. The lumina of most of the tubules appeared enlarged due to loss of brush border and flattening of epithelia. They also contained cellular and nuclear debris. In most of the tubular cells, the nuclei appeared pyknotic and in some of the cells, no nuclei were seen due to their loss into the lumen (FIG 2). The interstitium surrounding the distorted tubules showed large areas of mononuclear cell infiltration. The mean values of serum glucose and Glycated Hemoglobin in STZ treated Group B were 392±15 and 15.83±0.25 (Table 1) which were significantly increased as compared to Groups A & C (Table 1). Grading of tubular damage in Group B indicated severe degeneration of tubular epithelium(+++) and distortion of brush border and basement membrane(++), (Table 2). The comparison of its severity to Groups A&C is also shown in Table 2. S/C levels were significantly increased as compared to control (Table 1).

Group C: On gross examination of STZ and Vit C treated group C, the kidneys of the animals appeared bean shaped, dark red in colour and soft in consistency. The capsules were stripped off easily. The morphological examination of PAS-Hematoxylin stained sections revealed a cortical architecture which was not as regular as seen in the control group, but the cortical labyrinth and medullary rays were well defined as compared to STZ treated group. There were vacuoles seen in the cytoplasm of a few cells and epithelial casts were observed in the lumina of a few tubules. Most of the cells contained a centrally located spherical nucleus, though there were pyknotic nuclei observed in a few cells. The basement membrane was found disrupted in a few tubules. The brush border was found indistinct and scanty in a few tubules, but most of the tubules had a well preserved brush border membrane (Fig 3). The interstitium of the cortical areas showed no signs of acute or chronic inflammation or fibrosis. The mean values of Serum Glucose and Glycated Hemoglobin in Vit C treated Group C were 378±10 and 14.97±0.18 (Table 1), which were insignificant as compared to Group B and highly significant as compared to Group A. Comparison is shown in Table 1. Grading of tubular damage in this group revealed a significant improvement and preservation of renal morphology (±) as compared to Group B (Table 2). Comparison of grading of tubular pathology to Groups A & B is shown in Table 2.

Table 1: Mean values of serum glucose, glycated hemoglobin and serum creatinine at 12 weeks duration

Groups	**Treatment received**	**Serum Glucose(gm/dl)**	**Glycated Hemoglobin (HbA1c)mg/gmHb**	**Serum creatinine (mg/dl)**
Group A	Citrate buffer	88± 5	6.10 ± 0.30	0.95± 0.07
Group B	STZ	392**± 15	15.83**± 0.25	2.16±0.24**
Group C	STZ + VIT C	378**± 10	14.97**± 0.18	1.20±0.07*

Each value is Mean S.D. for 15 rats in each group, *Key: P<0.05 Significant, **P<0.001 Highly Significant as compared to control.

Table 2: Grading of clinical signs and renal pathology in all the 3 groups of rats

S. No.	**Kidney Lesions**	**Group A**	**Group B**	**Group C**
1	Degeneration of tubular epithelium	-	+++	±
2	Tubular dilatation	-	++	±
3	Epithelial casts in tubular lumen	-	+++	±
4	Cytoplasmic vacuoles	-	++	±
5	Distortion of Brush Border membrane	-	++	±
6	Distortion of Basement membrane	-	++	±
7	Interstitial Inflammation	-	+	-

Key to scores: No lesions observed - Mild focal lesions± Moderate, Multi focal Lesions+
Moderately severe, diffuse lesions ++ Very severe, diffuse lesions+++

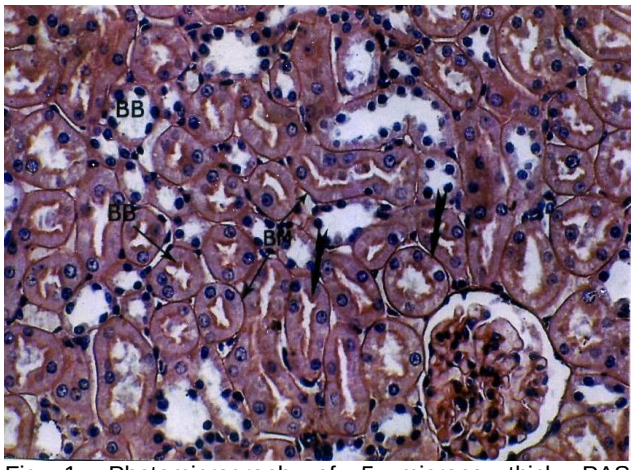

Fig 1: Photomicrograph of 5 microns thick PAS-Hematoxylin stained section from cortex of kidney in Group A (control) rat showing normal architecture of proximal convoluted tubules with intact brush border and basement membrane. X 400.

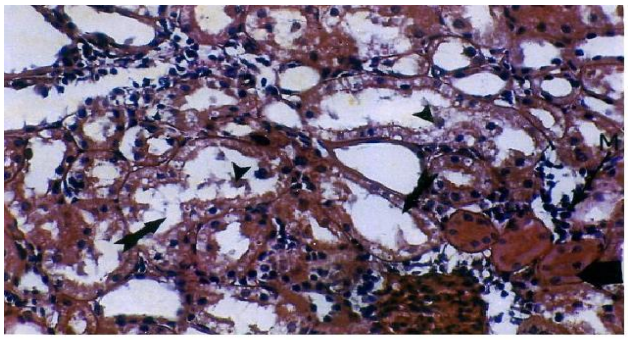

Fig. 2: Photomicrograph of 5 microns thick PAS-Hematoxylin stained section from cortex of kidney in Group B (STZ treated) rat showing distorted architecture, renal tubules with flattened epithelia, nuclear & epithelial debris in the lumina. X 400.

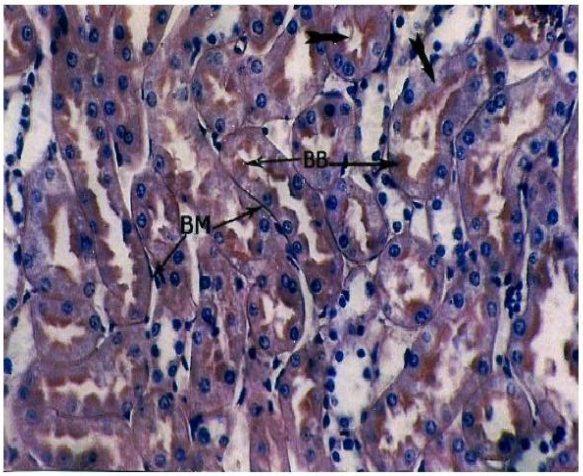

Fig. 3: Photomicrograph of 5 microns thick PAS-Hematoxylin stained section from cortex of kidney in Group C (STZ + Vit C treated) rat showing well preserved proximal tubules with few luminal casts.

DISCUSSION

This study demonstrated that administration of Vit C during diabetes can reduce the severity of renal damage by preserving the morphology of proximal convoluted tubules. Oxidative stress contributes to renal injury and antioxidant supplementation mitigates renal disease in diabetic and non-diabetic rats[13] Plasma levels of Ascorbic Acid are decreased and its primary oxidation product is increased in experimental diabetes[14]. Ascorbic Acid as a naturally occurring major antioxidant is reported to be low in diabetic tissue. It plays a role in many biological processes including free radical scavenging and protection of lipid membrane as well as hormone synthesis and homeostasis[15]. It is conceivable that the enhanced oxidative stress in diabetes and the resulting diminution in Ascorbic Acid leads to further enhancement of free radical reactions and other dysfunctions. Vitamin C is an essential nutrient that functions as a non-enzymatic antioxidant in the cytosol. Various experimental studies indicated that this vitamin is effective in preventing the oxidative renal damage and stress[16]. Ismaila and Olufunsho[17] in 2010 argued that Vit C and E could be a good supplement in the management of renal tissue damage caused by toxic dose of Acetaminophen in albino rats. Kanter et al[18] demonstrated that Vit C treatment alone or with Vit A may prevent endotoxin – induced renal damage. In one study conducted by Basahandey & Alwasel[19] in 2011, administration of Vitamin C prevented carbon tetrachloride induced renal toxicity. Vit C might ameliorate oxidative damage by decreasing lipid peroxidation and altering antioxidant defence system[20], or by donating electrons to free radicals and quench their reactivity[21]. Vit C as an antioxidant protects VLDL from oxidation and may therefore facilitate its uptake by the liver and hence promote its removal from plasma[22]. A study conducted by Ajith and co-workers[23] in 2007 showed that higher doses of vitamins were effective to protect oxidative renal damage and Vit C was a better nephroprotective agent than Vit E. In the present study, the histopathological examination of the proximal convoluted tubules of diabetic group B rats showed distorted and scanty brush border membranes of the cells. These results are in agreement with Zafar et al[10], who in 2009 observed a loss of brush border in most of the kidney sections with progressive wide spread tubular necrosis in the kidneys of STZ induced diabetic rats.

The results of the present study also revealed an insignificant role of Vit C on elevated blood glucose levels. This is in agreement with the study conducted by Mariem and Narongsak[8] in 2011 where

Vit C administered in a dose of 1gm/L/day for 52 weeks did not reduce blood glucose levels in STZ-induced diabetic rats. Similar insignificant results of Vit C on serum glucose were demonstrated by craven et al[24] in 1997 where he administrated 10g/kg/day of Vit C to rats made diabetic by STZ.

CONCLUSION

These results support the potential utility of antioxidant treatment for the prevention of renal injury in diabetes. Dietary supplementation with Vit C could be an easy, inexpensive and useful method of protecting diabetic patients from renal damage, without having a significant effect on serum glucose. Therefore, strategies to reduce oxidative stress in diabetes mellitus may exert favourable effects on the progression of diabetic nephropathy.

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