

# Effect of alphatocopherol on diameter of proximal convoluted tubules of kidney in diabetic mice

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## Abstract

**Objective:** To evaluate the effects of alphatocopherol supplement on proximal convoluted tubular diameter of kidney in diabetic mice.

**Methods:** The randomised controlled trials was conducted partly at the National Institute of Health (NIH), Islamabad, and partly in Army Medical College, Rawalpindi, from November 2009 to November 2010. Thirty adult female mice BALB/C were randomly divided into three equal groups. Group A served as the control group. Group B was made diabetic by the intraperitoneal injection of streptozotocin. Group C received injection streptozotocin and was fed with alphatocopherol (vitamin E) supplemented diet. After 12 weeks, the animals were sacrificed and their kidneys were removed for histomorphological study.

**Results:** Diabetes caused significant changes in the diameter of proximal tubule of Experimental Group B (diabetic) compared to the controls in Group A, but these changes were prevented in alphatocopherol treated Group C. Tubular diameter in Group B was significantly reduced compared to the Control Group A ( $p < 0.05$ ), but there was no statistical difference in tubular diameter of Group C and Group A ( $p > 0.05$ ).

**Conclusion:** Significant difference in proximal tubular diameter of kidneys between diabetic and alphatocopherol treated diabetic mice confirm that vitamin E does extend a protective role in improving diabetic nephropathy.

**Keywords:** Alphatocopherol, Proximal tubular diameter, Army Medical College, Rawalpindi. (JPMA 64: 46; 2014).

## Introduction

Diabetes mellitus (DM) is a complex syndrome which may be due to decrease in the synthesis of insulin (Type-I diabetes) or due to decrease in the secretion of insulin from b-cells of islets of Langerhans of pancreas (Type-II diabetes). Severe insulin deficiency results in gross abnormalities in glucose homeostasis and lipid metabolism.<sup>1</sup> The prevalence of diabetes in the world among adults (aged 20-79 years) was 6.4% affecting 285 million adults in 2010, and it may increase to 7.7% affecting 439 million adults by 2030. Between 2010 and 2030, there may be 69% increase in the number of adults with diabetes in developing countries and 20% increase in the developed countries.<sup>2</sup> Indeed, there is widespread acceptance of the possible role of reactive oxygen species (ROS) generated as a result of hyperglycaemia in causing many of the secondary complications of diabetes such as retinopathy, neuropathy, cardiomyopathy and nephropathy.<sup>3</sup> Diabetics have 20-fold increase in the risk of renal failure.<sup>4</sup> Since numerous studies demonstrated that oxidative stress, mediated mainly by hyperglycaemia-induced generation of free radicals, it has become clear that reducing oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications.<sup>5</sup> Vitamin E has been reported to protect against diabetic renal injury. For this reason, there has been an increased interest in the use of dietary antioxidant supplementation as an intervention to attenuate diabetic complications.<sup>6</sup> Vitamin E is a generic term used for tocopherol and tocotrienols. It is a membrane bound lipid soluble and naturally occurring antioxidant that has been shown to protect

animal tissue against oxidative damage such as lipid peroxidation both in vitro and in vivo.<sup>7</sup> This study was undertaken to evaluate the effects of alpha tocopherol on the proximal convoluted tubules diameter of kidney in diabetic mice.

## **Subjects and Methods**

The study was conducted partly at the National Institute of Health (NIH), Islamabad, and partly at the Army Medical College, (AMC), Rawalpindi. Thirty adult female mice BALB/C (weight 25-40g) were obtained from the animal house of NIH. The mice were kept under standard laboratory conditions and were maintained on palleted form of laboratory diet which was prepared at the animal house and water ad libitum. Animals were divided into 3 equal groups. Group A (Negative Control) was maintained on routine NIH diet for 12 weeks; Group B (Positive Control) was made diabetic by giving single injection of streptozotocin 120mg/kg body weight per animal intraperitoneally. The group was maintained on routine NIH diet for 12 weeks; and Group C which received single injection of streptozotocin and was fed with routine NIH diet enriched with alphatocopherol (500 mg/kg of diet) for 12 weeks. The animals were sacrificed after 12 weeks and their kidneys were removed for histomorphological analysis.

## **Results**

The diabetic Group B mice were sluggish throughout the trial, while Group C mice were sluggish initially but later became apparently healthy and active. No death was observed in any group. Tubular diametres of 20 randomly selected rounded proximal tubules were measured from the basement membrane of cells on one side to the basement membrane of cells on the opposite side in five fields at 40X by using ocular micrometre. Three observations were noted and their average was taken as the final reading.

Proximal and distal convoluted tubules could be easily differentiated in Haematoxylin and Eosin (H & E) stain. Proximal tubules were more in number and of larger diameter having low columnar cells with brush border, while distal tubules were smaller in diameter, less in number, having cuboidal epithelium without brush border. The basement membrane of tubular epithelium was strongly Periodic acid schiff stain (PAS) positive. Brush border were also strongly stained with PAS stain while distal convoluted tubule (DCT) stained lighter than proximal convoluted tubule (PCT) (Figure-1a).

Mean tubular diameter of PCT in kidney of control Group A were  $44 \pm 2.415 \mu\text{m}$  and  $43.375 \pm 2.433 \mu\text{m}$  respectively (Table).

Table-1: Mean values of proximal tubular diameter in kidneys of different groups.

Groups	Tubular Diametre ( $\mu\text{m}$ )	
	Right	Left
Control Group A 43.375 $\pm$ 2.433	44 $\pm$ 2.415	
Experimental Group B 29.625 $\pm$ 2.639	30 $\pm$ 2.635	
Experimental Group C 42.562 $\pm$ 1.762	41.625 $\pm$ 2.129	
P value calculated between group A & B 0.000		0.000
P value calculated between group A & C 0.481		0.061
P value calculated between group B & C 0.000		0.000

Statistical difference of tubular diameter of right kidney between:

Group A and B:  $< 0.05^*$

Group A and C:  $> 0.05$

Group B and C:  $< 0.05^*$

Statistical difference of tubular diameter of left kidney between:

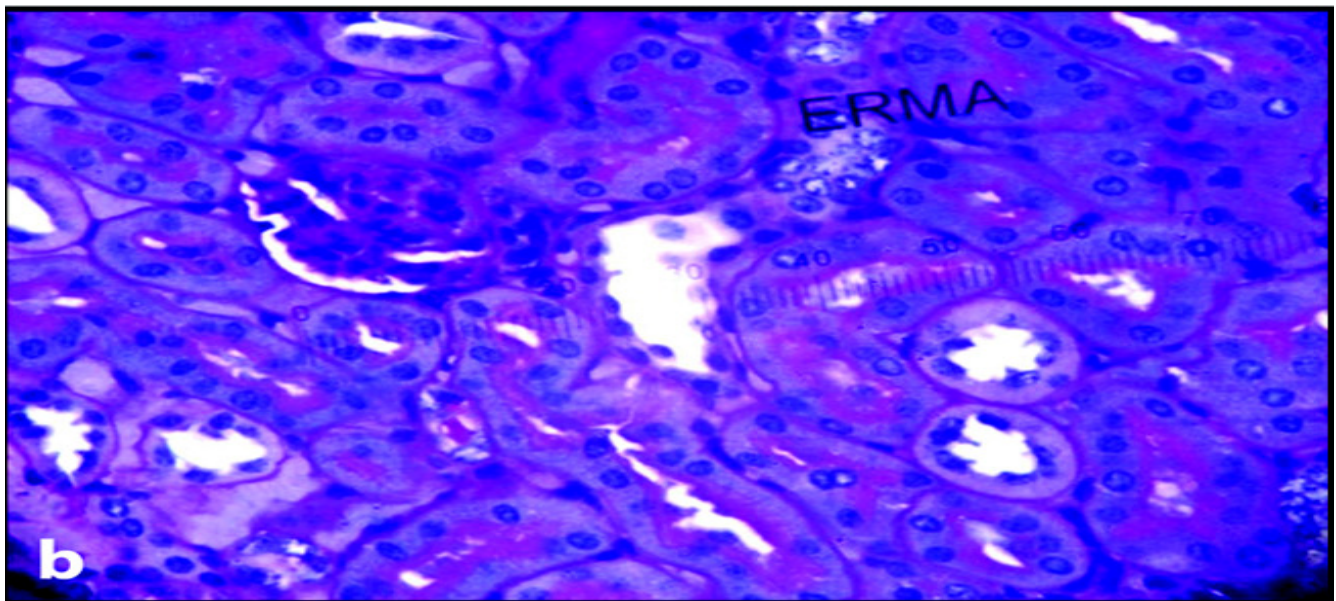
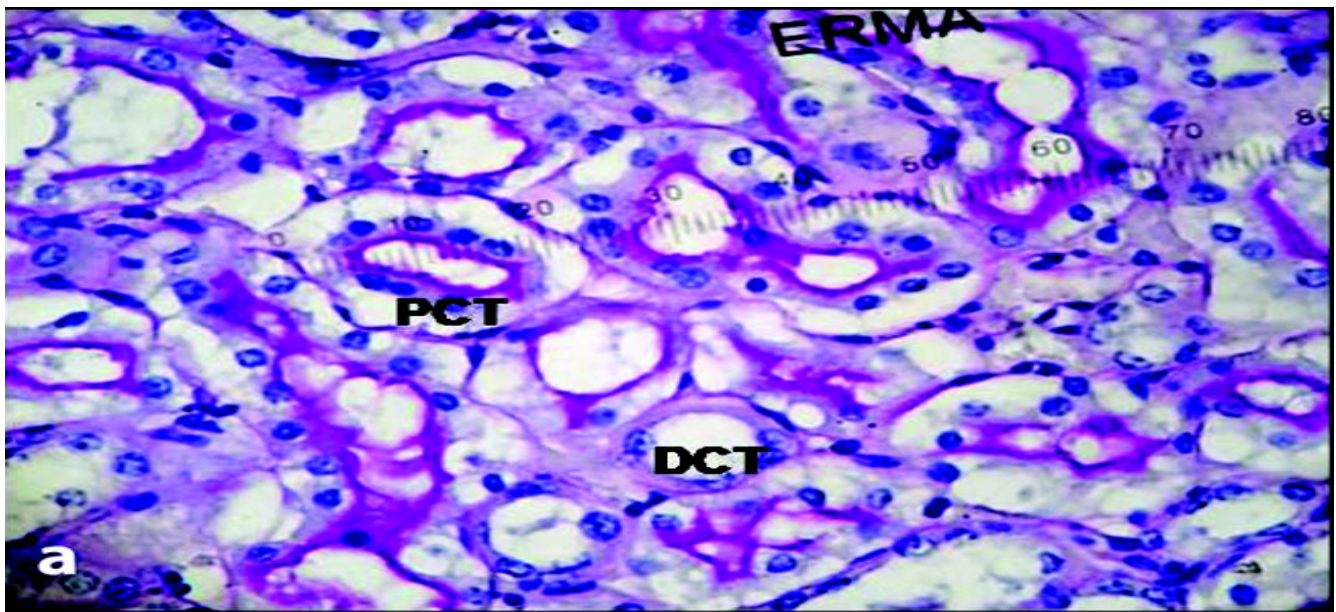
Group A and B:  $< 0.05^*$

Group A and C:  $> 0.05$

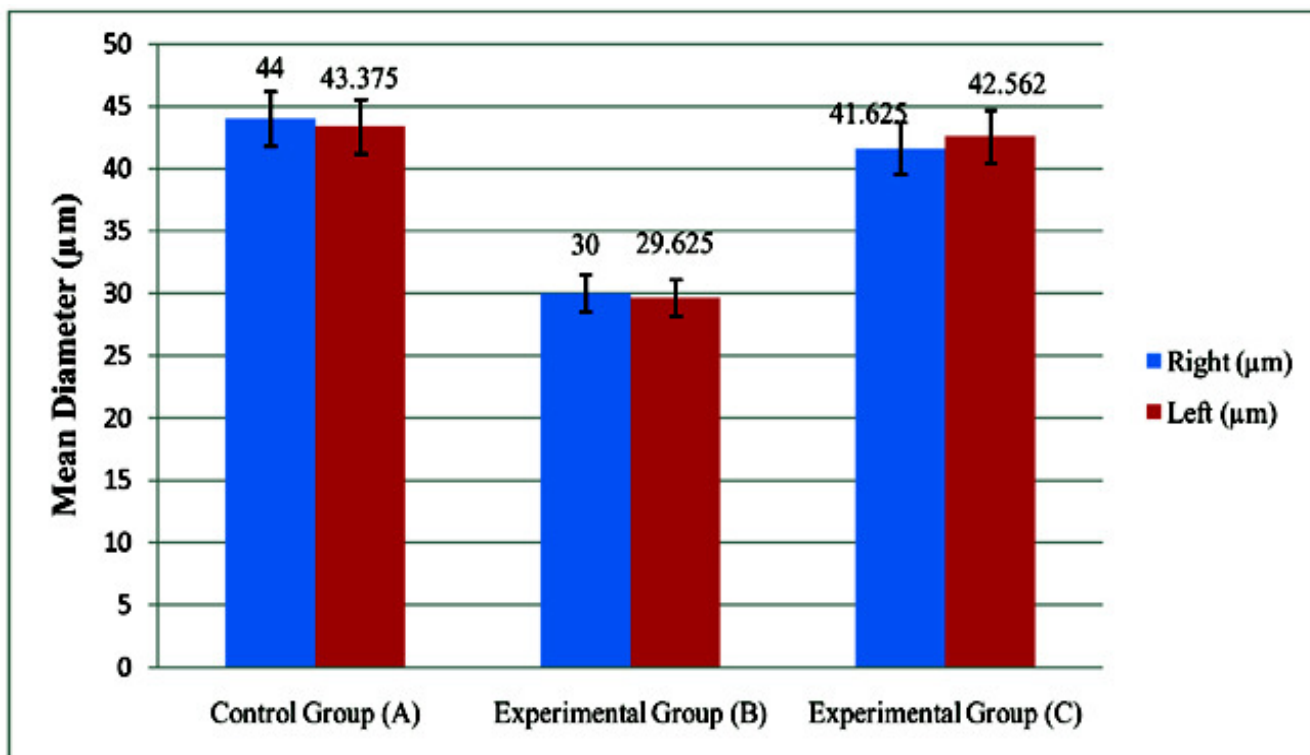
Group B and C:  $< 0.05^*$

\*Statistical difference is significant.

Mean tubular diameter of PCT in kidneys of experimental Group B were 30 $\pm$ 2.635 $\mu\text{m}$  and 29.625 $\pm$ 2.639 $\mu\text{m}$  (Figure-1b)



**Figure-1** : Comparison of the tubular diameter between the Group B and Group C.  
 (a) Photomicrograph of Experimental Group C (diabetic mouse treated with alphatocopherol) showing measurement of tubular diameter of proximal convoluted tubule (PCT). It showed improvement in tubular diameter. DCT (distal convoluted tubule). PAS stain. (b) Photomicrograph of Experimental Group B (diabetic mouse) showing measurement of tubular diameter of proximal convoluted tubule (PCT). It showed tubular atrophy. PAS stain. respectively which was statistically significant from control Group A ( $p < 0.05$ ) (Figure-2).



**Figure-2:** Comparison of mean luminal diameters of proximal convoluted tubule (PCT) in kidneys of different groups.

Mean tubular diameter of right and left kidney in Group C were  $41.625 \pm 2.129 \mu\text{m}$  and  $42.562 \pm 1.762 \mu\text{m}$  respectively which was significantly different from Group B with ( $p < 0.05$ ). There was no statistically significant difference in mean tubular diameter between Group A and Group C ( $p > 0.05$ ).

## Discussion

Diabetic nephropathy is the most common cause of chronic renal disease and is the foremost indication for dialysis and transplantation. Various studies have reported protective effects of antioxidants such as groundnuts oil,<sup>8</sup> soyabean oil<sup>9</sup> and vitamin E<sup>10</sup> against the oxidative damage of diabetes. Prakasam<sup>11</sup> observed that levels of alphatocopherol, chain-breaking antioxidant was significantly decreased in liver and kidney of streptozotocin diabetic rats. There is also evidence that increase in glucose concentration depresses the natural antioxidant defence system of the body. The imbalance between oxygen-free radicals and antioxidants defence system results in DNA damage and, hence, tissue damage.

This suggests that demand for antioxidants like vitamin E is increased due to activation of free radical related metabolism in diabetes. Impaired generation of antioxidants results in increase in oxidative injury due to the failure of protective mechanism. So any compound with rich antioxidant properties might contribute towards partial or complete alleviation of organ damage.<sup>12</sup>

The current study used alphatocopherol as an antioxidant and found that it had blood glucose-lowering effects on streptozotocin-induced diabetic mice. It was further indicated that structural changes in tissue taken from PCT of the kidneys of diabetic group were near to normal in those diabetic animal which were fed with alphatocopherol supplemented diet.<sup>13</sup> Considering our results, significant difference in

blood glucose level as well as other diabetic nephropathic parameters between the diabetic and alphatocopherol-treated diabetic mice confirm that vitamin E does extend a clear protective action against streptozotocin-induced diabetic nephropathy.

Regarding tubular diameter, our study showed that there was a remarkable difference between the luminal diameter of PCT when compared between diabetic and diabetic mice treated with vitamin E ( $p < 0.05$ ). Tubular atrophy in diabetic nephropathy (DN) ultimately leads to end-stage renal disease.<sup>14</sup> Hyperglycaemia can induce deoxyribonucleic acid (DNA) fragmentation and stimulate apoptosis in PCT because of increased oxidative stress. It causes tubular atrophy which may contribute to development of DN. Antioxidants might inhibit the development of DN by suppressing apoptosis.<sup>15</sup> Kumar<sup>16</sup> observed apoptosis of tubular cells of cortex and medulla in streptozotocin induced diabetic rats at 2 and 12 weeks of experimentation and its reversal by insulin. DN is not only a glomerular disease, but is also characterised by impaired tubular function as well. Injury to the proximal tubules has been suggested by the urinary excretion of low molecular weight proteins and tubular enzymes.<sup>17</sup> The finding of tubular diameter in experimental Group C after treatment with vitamin was strongly supported by Anjaneyulu<sup>18</sup> who showed that catechin administration, which is antioxidant, helps in the prevention of tubular atrophy in cyclosporine induced nephrotoxicity. Some researchers have proved that vitamin E improved the glomerular function, but did not have effect on tubular function.<sup>19</sup> In this study the general morphology of PCT of alphatocopherol-treated mice compared with diabetic mice was much improved and seemed quite normal in appearance compared to those in diabetic mice.

## Conclusion

Considering our results, significant difference in proximal tubular diameter of kidney between the diabetic and alphatocopherol treated diabetic mice confirm that vitamin E does extend a clear protective action against streptozotocin induced diabetic nephropathy. However exact mechanism of action of this protective effect is not known.

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