

Protective Effect of Vitamin E (α Tocopherol Acetate) on Diclofenac-Induced Nephrotoxicity in Young Albino Rats

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ABSTRACT

Objective: To determine the preventive role of Vitamin E on renal parenchyma after given of Diclofenac Sodium in young albino rats.

Study Design: Experimental study,

Place and Duration of Study: This study was carried out in the Department of Anatomy Baqai Medical University and Muhammad Medical College, Mirpurkhas from June 2011 to November 2011.

Methods and Material: 30 young albino rats were taken. They were divided into three groups; A, B and C. The animals in group-A given normal saline 10 ml/kg per day. Group-B received diclofenac sodium 2 mg/kg per day and group-C receives diclofenac sodium 2mg/kg/day dissolved in distilled water with vitamin-E 2 mg/kg/day dissolved in olive oil administered half an hour before the diclofenac sodium by feeding tube per day for 2 weeks. On day 15 all animals were sacrificed with deep ether anesthesia. Their kidneys were removed, fixed in 10 % formalin. Representative blocks were taken and embedded in liquid paraffin. For routine histological examination 5 μ m thick section cut by microtome and stained with H&E, PAS and silver methenamine. Renal histology was done under light microscope to see the proximal and distal tubular diameter and count.

Results: No significant ($P>0.05$) changes were observed in the histopathology of kidney tissues of the groups A and C rats. The group B significantly ($P<0.001$) affected the histopathology of kidney.

Conclusion: It may be concluded that diclofenac sodium produces changes in kidney, which may be attributed to ischaemia induced by inhibition of prostaglandin synthesis resulting in tubular necrosis in young albino rats simultaneous administration of vitamin-E partially protect the morphological and histological changes induced by diclofenac sodium.

Key Words: Diclofenac sodium, Vitamin-E, Young albino rats, Kidneys.

INTRODUCTION

Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) having analgesic, antipyretic and anti-inflammatory activities¹. Diclofenac Sodium completely absorbed after oral administration reaches a peak concentration in plasma within 2-3 hours after single dose. The elimination half life ($t_{1/2}$) of diclofenac sodium is about 3 times longer in synovial fluid compared to plasma².

The diclofenac sodium is metabolized in the liver by cytochrome P450 isozyme of the CYP2C sub family and undergoes hydroxylation during phase 1 and conjugation with glucuronic acid to form metabolites either as conjugates or sulfates during phase 2. Up to 60-70% of metabolites are excreted in the urine and up to 30% excreted in the bile.

Evidence from clinical and pharmacological studies imply that diclofenac sodium exerts its actions by inhibiting cyclooxygenase (COX) enzyme³.

Diclofenac sodium cause deleterious effects on kidney function, especially with respect to solute homeostasis and maintenance of renal perfusion and glomerular filtration. It acts by reducing prostaglandin biosynthesis through inhibition of cyclooxygenase (COX)⁴.

The scarring of the small blood vessels, called capillary

sclerosis due to ischemia is the initial lesion of analgesic nephropathy⁵. Capillary sclerosis is leads to renal papillary necrosis and in turn, chronic nephritis⁶.

Vitamin E is the collective name for a set of 4 related α -, β -, γ -, and δ -tocopherols and the corresponding four tocotrienols α -, β -, γ -, and δ - which are fat-soluble vitamins with antioxidant properties^{7,8}. The major sources of Vitamin-E are avocado, nuts, such as almonds or hazelnuts, red palm oil, seeds, spinach, green leafy vegetables, vegetable oils (canola), corn, sunflower, soybean, cottonseed, olive oil, wheat germ, wholegrain foods, milk and asparagus⁹. The administration of vitamin E (antioxidant) has been shown to be beneficial in prevention and attenuation of renal scarring in numerous animal models of kidney diseases¹⁰. Antioxidative (tocopherol) therapies have been shown to prevent acute decrease in renal function induced by ischemia, contrast media and drugs like diclofenac sodium (NSAID)¹¹.

MATERIALS AND METHODS

This study was carried out during the period from June 2011 to November 2011, in the Department of Anatomy Baqai Medical University and Muhammad Medical College, Mirpurkhas. For this experimental study 30 young albino rats aged 2 weeks, weighing

ranging from 20gm to 30gm were used. They were originally obtained from Charles River breeding laboratories, Brooklyn, Massachusetts, USA, and were cross bred at the animal house of Muhammad Medical College, Mirpurkhas. The animals were kept in the animal house on a balanced diet. They were put under observation for one week prior to the experimental procedure for assessment of their state of health on basis of weight gain or loss.

The animals used in this study were divided into 3 groups: A, B and C. the animals in each group were kept in a separate cage and labeled. Each animal was weighed period to treatment.

Group-A (10 Animals): In this group each animal received normal saline 10 ml/kg body weight orally once daily for 2 weeks.

Group-B (10 Animals): In this group each animal received diclofenac sodium by feeding tube at a dose of 2 mg/kg/day dissolved in distilled water, once daily for 2 weeks.

Group-C (10 Animals): In this group each animal received diclofenac sodium 2mg/kg/day dissolved in distilled water by feeding tube once daily for 2 weeks. These animals also received vitamin-E (α -tocopherol acetate) 2 mg/kg/day dissolved in olive oil administered half an hour before the diclofenac sodium by feeding tube once daily for 2 weeks.

On day 15 the animals were sacrificed kidneys were removed, bisected in two halves, one half fixed in 10% formalin and second in alcoholic formalin. The tissues were sectioned and mounted on slides. They were stained by Haematoxylin & Eosin, silver methamine and periodic acid Schiff stain.

The morphological changes in renal parenchyma were observed under light microscope. Five observations for each parameter were recorded in each animal. Proximal and distal tubular counts were made under 8x ocular and 40x objective with counting reticule in randomly selected five fields in the cortex of the kidney and proximal and distal tubular diameter was measured with the help of ocular micrometer. The data was subjected to statistical analysis Student 't' test was employed to see the significance of the results.

RESULTS

Observations in Group-A (Control): In H&E stained sections the histological structure in the cortical and medullary portion appeared absolutely normal without any change in either glomeruli or tubules as shown in Figure 1.

In PAS stained sections the brush border on the apical surface of proximal tubular epithelial cells stained magenta in colour and almost filled the tubule. The glycogen content of the cytoplasm of proximal tubular cells was quite normal. The basement membrane of proximal and distal tubules also stained magenta, which was distinct and regular.

Silver methenamine stained sections revealed basement membrane of glomeruli, Bowman's capsule and proximal and distal tubules which was faint in outline, and unmeasurable by light microscopy.

The mean values of number of proximal convoluted tubules per unit area as noted in group-A was 24.0 ± 0.49 . when group-A compared with group-B highly significant increase ($P < 0.001$) was noted in group-A, however, when group-A compared with group-C statistically non-significant difference ($P > 0.05$) was observed.

The mean values of diameter of proximal tubules measured in unit area in group-A was $50.9 \pm 0.74 \mu\text{m}$, which when compared with group-B, statistically significant decrease ($P < 0.05$) was noted in group-A, however, when compared with group-C, no significant difference ($P > 0.05$) was observed.

The mean values of number of distal tubules per unit area, as observed in Group-A was 22.7 ± 0.56 , which when compared with that in group-B, a highly significant increase ($P < 0.001$) was observed in group-A, however, when compared with group-C, no significant change was noticed.

The mean values of diameter of distal tubules per unit area in group-A was $38.4 \pm 0.37 \mu\text{m}$, which when compared with group-B, a highly significant decrease ($P < 0.001$) was noted in group-A, however, when compared with group-C, no significant change occurred.

Observations in Group-B: In H&E stained sections the interstitium of renal cortical area was sparse with few inflammatory cells but no marked oedema, many dilated and congested blood vessels were observed as shown in Figure 2.

In PAS stained sections the brush border at the luminal surface appeared scanty and indistinct and at some places it was completely absent. The intracellular glycogen content of the proximal as well as distal tubules was moderately depleted. However, the basement membrane of proximal and distal tubules was intact.

In silver methenamine stained sections the basement membrane was visible as intensely stained black line around proximal and distal tubules which was quite thickened in some tubules but still not measurable by light microscopy.

The mean values of number of proximal convoluted tubule per unit area observed in group-B was 16.1 ± 0.66 , which when compared with that in group-C, a highly significant decrease ($P < 0.001$) was noted in group-B.

The mean values of diameter of proximal tubules per unit area in group-B was $54.3 \pm 0.97 \mu\text{m}$, which when compared with group-C, highly significant increase ($P < 0.001$) occurred in group-B.

Mean values of distal tubular count per unit area as observed under high magnification in group-B was 14.5 ± 0.34 , which when compared with group-C, highly

significant decrease ($P < 0.001$) was observed in group-B.

Mean values of diameter of distal tubules per unit area in group-B was $54.5 \pm 0.59 \mu\text{m}$, which when compared with that in group-C, highly significant increase ($P < 0.001$) was noticed in group-B.

Observation in Group-C: In H&E stained sections the histological structure in the cortical and medullary portion appeared absolutely normal without any change in either glomeruli or tubules as shown in Figure 3.

In PAS stained sections showing normal brush borders at the apical surface of proximal tubules cells. It was well defined and almost filled the lumen of proximal tubules. The intracellular cytoplasm had normal glycogen content, basement membrane also appeared a regular outline.

The basement membrane of proximal and distal tubules was observed in silver methenamine stained sections. These sections showed uniformly continuous black stained basement membrane in both tubules.

The mean values of the number of proximal convoluted tubules per unit area as observed under high magnification in group-C was 22.9 ± 0.66 , which when

compared with that in group-A, no significant change was observed. However, when compared with group-B statistically highly significant increase ($P < 0.001$) was noted in group-C.

The mean values of diameter of proximal tubules per unit area in group-C was $51.6 \pm 0.90 \mu\text{m}$, which when compared with that in group-A, statistically no change was noticed. However when compared with group-B statistically significant decrease ($P < 0.01$) was noted in Group-C.

The mean values of the number of distal tubules per unit area as observed under high magnification in group-C was 20.7 ± 0.67 , which when compared with that in group-A, no significant change was observed. However when compared with group-B statistically highly significant increase ($P < 0.001$) was noted in Group-C.

The mean values of diameter of distal tubules per unit area in group-C was $39.8 \pm 0.32 \mu\text{m}$, which when compared with that in group-A, statistically no change was noticed. However when compared with group-B statistically highly significant decrease ($P < 0.001$) was noted in Group-C.

Table No.1: Comparison of Proximal and distal tubular counts and diameters per unit area between groups A and B.

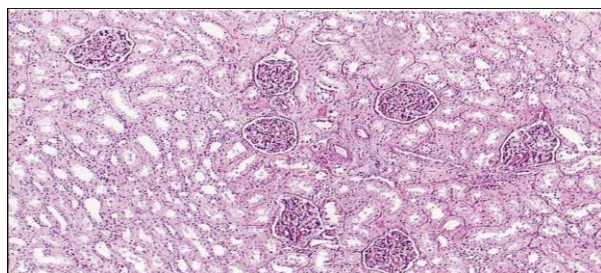
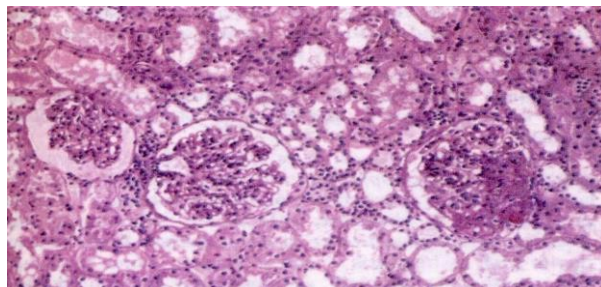
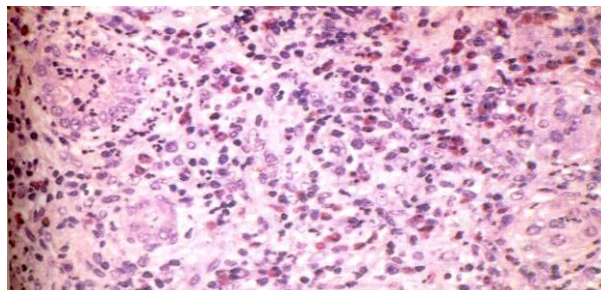
	Group A Controls (n=10)	Group B Diclofenac Sodium (n=10)	P-value
	Mean $\pm$ S.D $\pm$ SEM	Mean $\pm$ S.D $\pm$ SEM	
Proximal Tubular Count per unit area (Under Reticule)	$24.0 \pm 1.56 \pm 0.49$	$16.1 \pm 2.08 \pm 0.66^{**}$	0.001
Mean Diameter of Proximal Tubules (Under Ocular Micrometer)	$50.9 \pm 2.33 \pm 0.74$	$54.3 \pm 3.07 \pm 0.97^{*}$	0.030
Mean Distal Tubular Count (Under Reticule)	$22.7 \pm 1.77 \pm 0.56$	$14.5 \pm 1.08 \pm 0.34^{**}$	0.001
Mean Diameter of Distal Tubules (under Ocular Micrometer)	$38.4 \pm 1.16 \pm 0.37$	$54.5 \pm 1.85 \pm 0.59^{**}$	0.001

Table No.2: Comparison of Proximal and distal tubular counts and diameters per unit area between groups A and C.

	Group A Controls (n=10)	Group C Diclofenac Sodium with Vitamin E (n=10)	P-value
	Mean $\pm$ S.D $\pm$ SEM	Mean $\pm$ S.D $\pm$ SEM	
Proximal Tubular Count per unit area (Under Reticule)	$24.0 \pm 1.56 \pm 0.49$	$22.9 \pm 2.08 \pm 0.66$	0.419
Mean Diameter of Proximal Tubules (Under Ocular Micrometer)	$50.9 \pm 2.33 \pm 0.74$	$51.6 \pm 2.85 \pm 0.90$	0.856
Mean Distal Tubular Count (Under Reticule)	$22.7 \pm 1.77 \pm 0.56$	$20.7 \pm 2.11 \pm 0.67$	0.067
Mean Diameter of Distal Tubules (under Ocular Micrometer)	$38.4 \pm 1.16 \pm 0.37$	$39.8 \pm 1.01 \pm 0.32$	0.079

Table No.3: Comparison of Proximal and distal tubular counts and diameters per unit area between groups B and C.

	Group B Diclofenac Sodium (n=10)	Group C Diclofenac Sodium with Vitamin E (n=10)	P-value
	Mean $\pm$ S.D $\pm$ SEM	Mean $\pm$ S.D $\pm$ SEM	
Proximal Tubular Count per unit area (Under Reticule)	16.1 $\pm$ 2.08 $\pm$ 0.66 **	22.9 $\pm$ 2.08 $\pm$ 0.66	0.001
Mean Diameter of Proximal Tubules (Under Ocular Micrometer)	54.3 $\pm$ 3.07 $\pm$ 0.97 *	51.6 $\pm$ 2.85 $\pm$ 0.90	0.01
Mean Distal Tubular Count (Under Reticule)	14.5 $\pm$ 1.08 $\pm$ 0.34 **	20.7 $\pm$ 2.11 $\pm$ 0.67	0.001
Mean Diameter of Distal Tubules (under Ocular Micrometer)	54.5 $\pm$ 1.85 $\pm$ 0.59 **	39.8 $\pm$ 1.01 $\pm$ 0.32	0.001

**Figure No.1: Photomicrograph of 5 μ m thick H&E stained paraffin section of rat kidney from group-A (control), showing normal architecture of renal cortex under low magnification. x101****Figure No.2: Photomicrograph of 5 μ m thick H&E stained paraffin section of rat kidney from group-B treated with diclofenac sodium, showing dilated blood vessels with marked infiltration of inflammatory cells and damaged tubules. x205.****Figure No.3: Photomicrograph of 5 μ m thick H&E stained paraffin section of rat kidney from group-C treated with diclofenac sodium, and vitamin-E, showing almost normal proximal convoluted tubules (PT) and distal convoluted tubules (DT) x416.**

DISCUSSION

Non-steroidal anti-inflammatory drug (NSAID), are the known nephrotoxic drugs, even in therapeutic doses. Vitamin-E, an antioxidant is known to be a potent scavenger of free radicals which have been implicated in over hundred conditions in humans including ischaemia of many organs¹².

Studies on the diclofenac sodium have shown that prolonged administration of this drug should be considered as a risk for nephrotoxicity¹³. In the present study three groups of animals were used group-A acted as control, group-B received diclofenac sodium while group-C received diclofenac sodium and vitamin-E. The effect of both these drugs were observed including number and diameter of proximal and distal convoluted tubules.

The proximal tubular count was not changed significantly in group-C, when compared with control group-A, whereas a significant decrease in number of tubules per unit area in group-B occurred which may be attributed to damage to the tubular epithelial cells by ischaemia produced by inhibition of prostaglandin in renal arterioles resulting in constriction¹⁴.

The highly significant increase observed in the diameter of proximal tubules in group-B as compared to groups A and C, may be attributed to degeneration of cells in proximal tubules resulting in apparent increase in their diameter¹⁵.

The total number of distal tubules in group-B was significantly lower when compared with group A and C. The decrease in number of tubules may be attributed to focal ischaemic necrosis of some of the tubules resulting in their numbers¹⁶.

The diameter of distal tubules in group-B showed highly significant increase as compared to that in groups A and C, which may be attributed to vacuolar degeneration of cells which fill the lumen of damaged tubules resulting in increase in diameter¹⁶.

CONCLUSION

It may be concluded that diclofenac sodium produces changes in kidney, which may be attributed to ischaemia induced by inhibition of prostaglandin synthesis resulting in tubular necrosis in young albino rats and simultaneous administration of vitamin E partially protect the morphological and histological changes induced by diclofenac sodium.

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