HISTOMORPHOLOGICAL EFFECT OF CELECOXIB ON CELLULAR DIAMETER OF PROXIMAL CONVOLUTED TUBULES OF KIDNEY WITH PROTECTIVE EFFECT OF LYCOPENE IN ALBINO RATS; AN EXPERIMENTAL STUDY.

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ABSTRACT… Objectives: To observe the cellular diameter of proximal convoluted tubules of kidney of albino rats on celecoxib induced kidney with protection by lycopene. Study Design: Experimental study. Setting: BMSI (Anatomy Department), JPMC, Karachi. Period: 4th May 2015 to 3rd June 2015. Materials and Methods: Ninety to one twenty days old, forty healthy adult, male Albino rats of 200-220gm were taken for the study. The rats were divided into 4 groups, Group A was control group, Group B receive Celecoxib 50 mg/kg body weight orally, Group C receive Celecoxib 50 mg/kg body weight orally along with lycopene50 mg/kg body weight orally and Group D receive lycopene 50 mg/kg body weight orally for 30 days. At the end of study rats were sacrificed and renal tissue sections were stained with hematoxylin and eosin.

Results: Markedly increased cellular diameter of proximal convoluted tubules was observed in rats taking celecoxib. Slides stained with hematoxylin and eosin showed altered and degenerative changes in the renal parenchyma of cortex and medulla. Ballooning of cells, hemorrhage and moderate edema was seen in celecoxib group. Renal interstitium showed infiltration of mononuclear cells, congested and dilated blood vessels. However, renal architecture was improved and reversed in celecoxib along with lycopene receiving group.

Conclusion: This study concludes that lycopene decreased the cellular diameter of proximal convoluted tubules in celecoxib treated group.

Key words: Celecoxib, Infiltration, Lycopene.
proven to cause nephrotoxicity such as interstitial nephritis, impairment of renal perfusion, increase serum urea, creatinine and Blood urea nitrogen.\textsuperscript{9,10} Celecoxib produces reactive oxygen species (ROS), which can cause acute renal failure and interstitial nephritis by promoting medullary interstitial cellular apoptosis.\textsuperscript{11,12,13} Celecoxib reduces glomerular filtration rate (GFR) and raise blood pressure.\textsuperscript{14,15}

Carotenoids are the pigmented fat soluble dietary antioxidant, present in fruits like watermelon, grape fruit and tomato which we generally use in our diet.\textsuperscript{16,17,18} Lycopene is an acyclic carotenoid, gives red color pigment to the fruits and vegetable. It is made up of an aliphatic hydrocarbon abundantly present in tomato and tomato based products.\textsuperscript{19,20}

It reduces the risk of cardiovascular diseases, glomerulosclerosis, congestion of vessels and diabetes.\textsuperscript{21,22} It is a chemopreventive agent and effective in the prevention of cancer such as mammary tumor and prostatic cancer etc.\textsuperscript{23} It prevents lipid peroxidation and decreases the risk of multiple sclerosis, age-related macular degeneration, and atherosclerosis etc.\textsuperscript{24,25}

Since no morphometric and histological study has been done so far to evaluate the ameliorative role of Lycopene on Celecoxib induced damage to the kidney, so this opportunity has been availed to undertake this research.

Celecoxib causes undesirable histomorphological effects and metabolic disorders on multiple organs like, kidney, liver and gastrointestinal tract etc. It frequently damages morphology and functions of kidney. Lycopene is an acyclic carotenoid having highest oxygen-quenching capacity. That’s why this study was designed to observe the nephroprotective role of lycopene on celecoxib induced kidney and compare the results with previous studies.

**MATERIAL AND METHODS**

This study was conducted for the duration of four weeks (4\textsuperscript{th} May 2015 to 3\textsuperscript{rd} June 2015), in the Department of Anatomy, BMSI, JPMC, Karachi.

Forty healthy adult Albino rats, 90-120 days old, weighing 200-220gm were obtained from the Charles River Breeding Laboratories, Brooklyn, Massachusetts, USA and cross bred at the animal house of BMSI, JPMC, Karachi. They were kept under observation for 7 days to assess their health and dietary habits before the beginning of study. Animals were given Celecoxib 50 mg/kg along with lycopene 50 mg/kg orally, according to the study plan dosage. The animals were divided into four groups A, B and C according to the study plan.

- **Group A**: was served as control.
- **Group B**: was received Celecoxib 50 mg/kg orally.
- **Group C**: was received Celecoxib 50 mg/kg along with lycopene 50 mg/kg orally.
- **Group D**: was received lycopene 50 mg/kg orally.

Each animal was weighed before the beginning of experimental study and kept in cages of animal house BMSI, JPMC prior to administration of drug. During the experimental study animals were kept under observation to note any change in their behavior and general conditions. All the animals were on standard lab diet and water. At the end of study time period they were weighed and sacrificed.

The animals was anesthetized under ether in a glass container and then fixed on a dissecting board with the help of pins. A longitudinal mid line incision from manubrum sterni up to pubic symphysis was given by scalpel. A transverse incision was given which crosses the longitudinal incision in the middle to obtain a proper exposure of abdominal cavity and thoracic cavity. Both kidneys were identified and exposed. Kidneys were carefully examined for any obvious gross change in color, shape, size, contour and consistency. Kidneys were removed from body of animals and absolute weight of each kidney was recorded with the help of sartorious balance. After cleaning with normal saline, each kidney was excised in to two longitudinal halves for separate fixative; half section was fixed in 10 % formalin for routine hematoxylin and eosin stains and other half in alcoholic formalin for periodic acid shift
stain for 24 hours. Renal tissue was processed by dehydrating in ascending grades of alcohol from 70 – 100 percent. Cleared in xylene, infiltrated and embedded in paraffin wax. 4 to 5 microns thick longitudinal sections were cut on rotatory microtome and then mounted on albumenized glass slides. Slides will be fixed on hot plate at 30 – 32 °C and then stained with hematoxylin and eosin.25

Data was analyzed through SPSS version 20.0. The entire continuous variables were presented as Mean ± Standard Deviation. To see the significance in tissue slides under light microscope at 40x were evaluated by one sample t-test. P-value < 0.05 considered to be statistically significant.

RESULTS

Control Group-A
The animals of Group-A remained alive and healthy, their food intake and response to external stimulus were also normal throughout the study till the end of experimental period.

Celecoxib treated Group-B
The animals of Group-B (Celecoxib treated) looked ill. Their food intake decreased and their response to stimuli was sluggish. They gradually became weak and lethargic.

Celecoxib with Lycopene treated Group-C
The animals of Group-C appeared comparatively healthy, active and their response to stimuli was better than Group-B. Their dietary habits were also normal.

MICROSCOPICAL OBSERVATION OF KIDNEY

Microscopic Observations of Group A
The histological examination of Haematoxylin and Eosin (H & E) stained kidney sections in group-A showed absolutely normal architecture of renal parenchyma with well-defined outer cortex and inner medulla. Any sign of degeneration was not observed; interstitium of renal cortex and renal medulla was sparse containing small capillaries filled with blood. The lining epithelium of proximal convoluted tubules showed low columnar cells, having eosinophilic granular cytoplasm and basophilic spherical nuclei (Figure-1a).

The mean cellular diameter of proximal convoluted tubular cells in group A was 10.2 ± 1.09 μm (Table-I, Figure-1).

Microscopic Observations of Group B
The H&E stained kidney sections of group-B animals were showed altered and degenerative changes in the renal parenchyma of cortex and medulla.

The lining epithelial cells of proximal were low cuboidal with ill-defined brush border at the luminal surface and ballooning of cells was observed. Renal interstitium showed inflammatory cells with moderate edema, congested and dilated blood vessels renal medulla showed infiltration of mononuclear cells (Figure-2).

The mean cellular diameter of proximal convoluted tubular cells of group B was 14.83 ± 1.83 μm. There was a significant increase (P < 0.05) was observed in the mean cellular diameter of tubular cells of group B when compared with group A (Table-I & Figure-1).

Microscopic Observations of Group C
The H&E stained sections of group-C showed normal histology of renal parenchyma. The renal cortex and medulla were appeared normal with slight congestion and hemorrhage around glomeruli. Lining epithelium was low columnar with intact basement membrane and brush border of microvilli at the luminal surface of cells were observed (Figure-3).

The mean cellular diameter of proximal convoluted tubular cells of group-C was 9.8 ± 0.83 μm. A moderately significant decrease (P < 0.005) was observed in the mean cellular diameter of proximal convoluted tubules of group-C when it compared with group-B and insignificant decrease (P < 0.05) was observed in the mean cellular diameter of proximal convoluted tubular cells of group-C when compared with group-A (Table-I & Figure-1).
MICROSCOPICAL OBSERVATIONS ON LYCOPENE TREATED GROUP-D
The animals of group D were only treated by lycopene. The purpose was to find out that if lycopene itself caused any alteration in the morphological and histological architecture in rat kidney. The findings in group D were more or less similar to control group-A.

<table>
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<tr>
<th>Groups</th>
<th>Treatment given</th>
<th>Mean value of cellular diameter</th>
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<tbody>
<tr>
<td>A (n=10)</td>
<td>ND</td>
<td>10.2±1.09</td>
</tr>
<tr>
<td>B (n=10)</td>
<td>Celecoxib</td>
<td>14.83±1.83</td>
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<tr>
<td>C (n=10)</td>
<td>Celecoxib + Lycopene</td>
<td>9.8±0.83</td>
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*Mean±SEM
Statistical analysis of the mean cellular diameter of proximal convoluted tubular cells of kidney in different groups of albino rats.

Table-I. *Mean values of cellular diameter of proximal convoluted tubules of kidney (µm) in different groups of albino rats

Key:
Non-significant*
Significant**
Moderately-significant***
Highly-significant****

Microscopic examination showed normal architecture of kidneys, arrangement of tubular epithelial cells (proximal convoluted tubules), brush border of proximal tubules and basal lamina in control group (A) (Figure-1a).

Group B (celecoxib treated) showed tubular necrosis, dilatation of renal tubules and shrinking of glomeruli, mononuclear cell infiltration, apoptosis, hemorrhage and loss of brush border in proximal convoluted tubules (Figure-2a).

Group C (lycopene treated) showed preservation of architecture of kidneys, insignificant dilatation of renal tubules (proximal and distal convoluted tubules), restoration of brush border of proximal tubules and basal lamina (Figure-3a).

Figure-1. Showing mean values of cellular diameter (µm) of proximal convoluted tubule of kidney in different groups of Albino rats.

Figure-1a. Photomicrograph showing normal cytoarchitecture of kidney normal glomerulus, proximal and distal renal tubules in control group-A at 40x

Figure-2a. Photomicrograph showing altered cytoarchitecture of kidney glomerulus has vacuolation, cells disrupted, apoptosis, brush border of proximal is absent and hemorrhagein group-B (celecoxib treated) at 40x
DISCUSSION
Different group of drugs are clinically used for pain management, inflammation and fever such as NSAIDs, glucocorticoids and disease-modifying ant rheumatic drugs (DMARDs). Celecoxib is COX-2 inhibitor, commonly used in patients of arthritis and primary dysmenorrhoea. Histological examination shows mononuclear cell infiltration, tubular damage, and glomerulonephritis. Lycopene is bioflavonoid and related to beta carotene. It is strong antioxidant and protects cell from reactive oxygen species damage. It is present in red pigmented fruits and vegetables, decreases the risk of chronic diseases like glomerulosclerosis, cancer, and congestion of vessels. It enhances immunity by maintaining structural and functional integrity of immune cells.

Microscopic examination of celecoxib treated renal tissues of group-B animals showed disrupted architecture of renal parenchyma. Proximal tubules with ill-defined microvilli at the luminal surface of epithelium, vacuolation and ballooning of cells were noticed. Blood vessels were appeared dilated and congested with slight shrinkage of renal corpuscles. The similar findings was observed in celecoxib induced oxidative injury to the renal tissue in wistar rats and necrosis of tubular cells with shortening of the microvilli in rats when treated by celecoxib, which produces the free radicals due to inhibition of anti-oxidative enzymes and cause the oxidative injury to the renal tissue.

The present study also showed that the lycopene markedly ameliorated the celecoxib induced oxidative damage to the renal tissue and prevented the damaging effect of celecoxib similar to control group. This was due to the anti-oxidative property of the lycopene which prevented the oxidative injury to the kidney and increased the anti-oxidative enzymes which protect the cell from necrotic and apoptotic changes.

CONCLUSION
The above mentioned study concluded that celecoxib treated group showed significant increase in cellular diameter of proximal convoluted tubular cells however celecoxib + lycopene treated group showed decrease in cellular diameter of proximal convoluted tubular cells same as group A. For that reason it is recommended that, avoids frequent use of celecoxib and it is suggested that celecoxib should be used along with lycopene to reduce its side effects.

REFERENCES


## AUTHORSHIP AND CONTRIBUTION DECLARATION

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