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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.

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INTRODUCTION

NSAIDs are most often used therapeutic drugs as antipyretic, for pain management, acute and chronic inflammation worldwide; they are effective in the treatment of different inflammatory conditions e.g osteoarthritis, rheumatoid arthritis, alzheimer's disease, dysmennorhoea and cancer. It plays an integral role in the management of rheumatologic disorders.[1]

Prostaglandin is the main mediator, responsible for pain and inflammation. NSAIDs prevent production of prostaglandin (PGs) from arachidonic acid by inhibiting Cyclooxygenase (COX) enzyme.[2] Cyclooxygenase enzyme has two isoforms, COX-1 and COX-2. NSAIDS inhibits both COX-1 and COX-2 enzymes.[3] COX-1enzyme is present in normal healthy tissues and generates prostaglandins and thromboxanes to maintain integrity and basic cellular functions. It plays a key role in aggregation of platelets and homeostasis of kidneys.[4] COX-2 enzyme is pro-inflammatory in nature and activates in response to mitogens, cytokines, growth factors and carcinogens. COX-2 is induced by physiological and pathophysiological stressors.[4,5]

Selective COX-2inhibitors are more preferred for the therapeutic purpose over COX-1 inhibitors as they exhibit lesser side-effects for example sparing gastrointestinal side effects.[6,7] Celecoxib is a selective COX-2 inhibitor, most commonly used analgesic in long-term therapy for pain and inflammation. It is a chemopreventive and chemotherapeutic drug and associated with angiogenesis and cell proliferation.[8] Celecoxib is

proven to cause nephrotoxicity such as interstitial nephritis, impairment of renal perfusion, increase serum urea, creatinine and Blood urea nitrogen.[9,10] Celecoxib produces reactive oxygen species (ROS), which can cause acute renal failure and interstitial nephritis by promoting medullary interstitial cellular apoptosis.[11,12,13] Celecoxib reduces glomerular filtration rate (GFR) and raise blood pressure.[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.[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.[19,20]

It reduces the risk of cardiovascular diseases, glomerulosclerosis, congestion of vessels and diabetes.[21,22] It is a chemopreventive agent and effective in the prevention of cancer such as mammary tumor and prostatic cancer etc.[23] It prevents lipid peroxidation and decreases the risk of multiple sclerosis, age-related macular degeneration, and atherosclerosis etc.[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 nephroprotectve 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 (4th May 2015 to 3rd 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, JMPC, 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 ^{0} 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-define 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±1.09μm (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.

Groups	Treatment given	Mean value of cellular diameter
A (n=10)	ND	10.2±1.09
B (n=10)	Celecoxib	14.83±1.83
C (n=10)	Celecoxib + Lycopene	9.8±0.83

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

*Mean±SEM

Statistical analysis of the mean cellular diameter of proximal convoluted tubular cells of kidney in different groups of albino rats.

Statistical Comparison	P-Value
B vs. A	P<0.05**
C vs. A	P>0.05*
C vs. B	P<0.005***

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

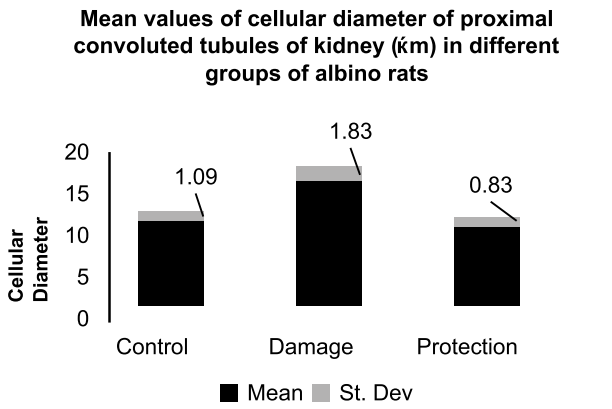

Figure-1. Showing mean values of cellular diameter (μm) of proximal convoluted tubule of kidney in different groups of Albino rats.

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).

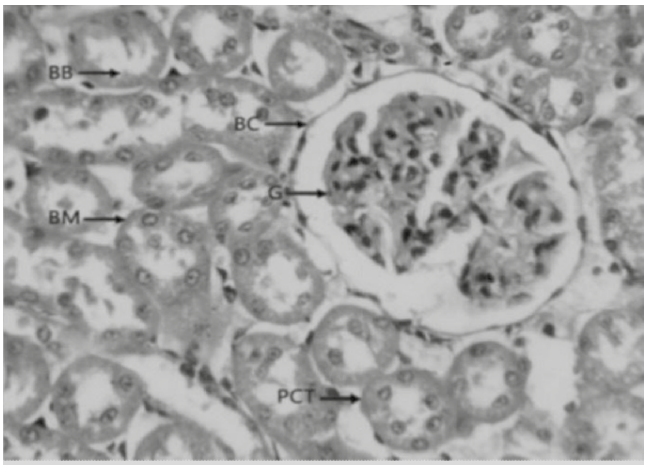

Figure-1a. Photomicrograph showing normal cytoarchitecture of kidney normal glomerulus, proximal and distal renal tubules in control group-A at 40x

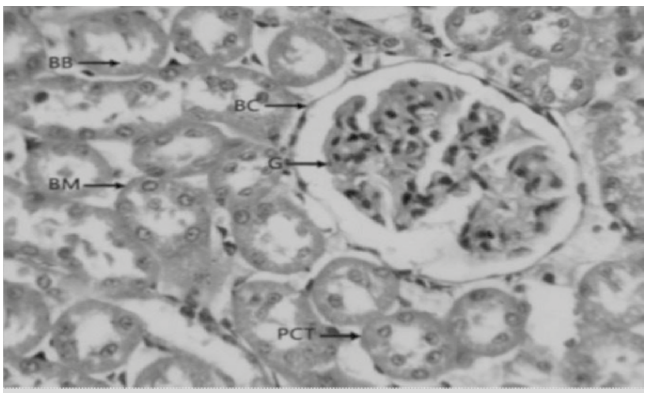

Figure-2a. Photomicrograph showing altered cytoarchitecture of kidney glomerulus has vacuolation, cells disrupted, apoptosis, brush border of proximal is absent and hemorrhagein group-B (celecoxib treated) at 40x

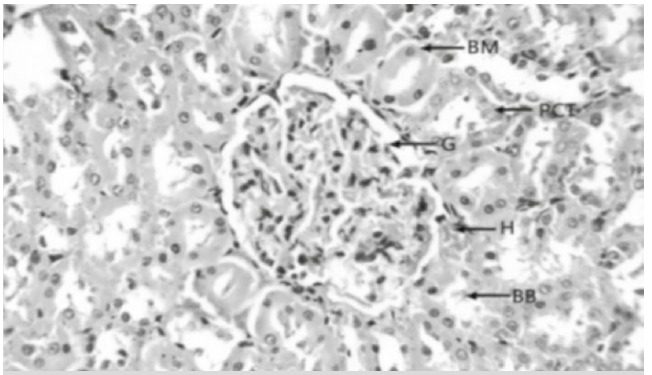

Figure-3a. Photomicrograph showing preserved cytoarchitecture of kidney glomeruli has less vacuolation, brush border of proximal is restored and not as much of hemorrhage in group-C (lycopene treated) at 40x.

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).[1] Celecoxib is COX-2 inhibitor, commonly used in patients of arthritis and primary dysmennorhoea. Histological examination shows mononuclear cell infiltration, tubular damage, and glomerulonephritis.[6]

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.[15]

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.[4] 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.[15,26]

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.[20,22,27]

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

1. Fukte SR, Bhakre DR. **Superiority of natural products in the treatment of arthritis.** International Journal of Pharmaceutical, Biological and Chemical Sciences. 2014; 3(2): 01-09.

2. Almaghour HG, Sherif FM. **Effect of non-steroidal anti-inflammatory drugs on behavioral actions of diazepam in mice.** Neurosciences. 2010; 15 (2):84-9.

3. Kose F, Besen A, Paydas S, Balal M, Gonlusen G, Inal T, Dogan A, Kibar M. **Effects of selective Cox-2 inhibitor, rofecoxib, alone or combination with furosemide on renal functions and renal Cox-2 expression in rats.** Clinical and experimental nephrology. 2010; 14(1): 22-7.

4. Mahmoud FY, Abou- Elghait AT, Rateb A and salah E. **Comparative study on the effect of long-term administration of non-steroidal anti-inflammatory drugs Meloxicam and Ketoprofen on the structure of kidney and gastric mucosa in adult albino rats.** Egypt. J. Histol. 2010; vol (33): 722-734.

5. Jones DA, Carlton DP, McIntyre TM, Zimmerman GA, Prescott SM. **Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines.** Journal of Biological Chemistry. 1993; 268(12): 9049-54.

6. Rossat J, Maillard M, Nussberger J, Brunner HR, Burnier M. **Renal effects of selective cyclooxygenase-2 inhibition in normotensive salt-depleted subjects.** Clinical pharmacology and therapeutics. 1999; 66(1): 76-84.

7. Nasrallah R, Robertson SJ, Karsh J and Hebert RL. **Celecoxib modifies glomerular basement membrane, mesangium and podocytes in OVE26 mice, but ibuprofen is more detrimental.** Clinical Science. 2013; 124(11): 685–694.

8. Prescott SM, Fitzpatrick FA. **Cyclooxygenase-2 and carcinogenesis.** Biochimica et BiophysicaActa (BBA)-Reviews on Cancer. 2000; 1470(2): 69-78.

9. Mohy El-Din MM, Senbel AM, Bistawroos AA, El-Mallah A, Nour El-Din NA, Bekhit AA and Abd El Razik HA. **A novel COX-2 inhibitor Pyrazol derivative has proven effective as an anti-inflammatory and analgesic drug.** Basic and Clinical Pharmacology and Toxicology. 2011; 108(4): 263-273.

10. Woywodt AL, Schwarz AN, Mengel MI, Haller HE, Zeidler HE, Köhler LA. **Nephrotoxicity of selective COX-2 inhibitors.** The Journal of rheumatology. 2001; 28(9): 2133-5.

11. Henrich WL. **Nephrotoxicity of several newer agents.** Kidney International. 2005 Apr 1; 67: 107-9.

12. Katzung BG, Trevor AJ: **Basic and clinical pharmacology.** 11th ed., New York, McGraw Hill, 2015; 769-778.

13. Davies NM, McLachlan AJ, Day RO, Williams KM. **Clinical pharmacokinetics and pharmacodynamics of celecoxib.** Clinical pharmacokinetics. 2000; 38(3):225-42.

14. Olliges A, Wimmer S and Nusing RM. **Defects in mouse nephrogenesis induced by selective and non-selective cyclooxygenase-2 inhibitors.** British Journal of Pharmacology. 2011; 163(5): 927–93.

15. Cooper DL, Murrell DE, Conder CM, Palau VE, Campbell GE, Lynch SP, Denham JW, Hanley AV, Bullins KW, Panus PC, Singh K. **Exacerbation of celecoxib-induced renal injury by concomitant administration of misoprostol in rats.** PloS one. 2014; 9(2): 890-897.

16. Oguz E, Kocarslan S, Tabur S, Sezen H, Yilmaz Z, Aksoy N. **Effects of lycopene alone or combined with melatonin on methotrexate-induced nephrotoxicity in rats.** Asian Pacific journal of cancer prevention. 2014; 16(14): 6061-6066.

17. HaiBo YA, ZhaoFa XU, Wei LI, Yu DE, Bin XU. **The protective role of procyanidins and lycopene against mercuric chloride renal damage in rats. Biomedical and Environmental Sciences.** 2011 Oct 31; 24(5): 550-9.

18. El-Gerbed MS. **Protective effect of lycopene on deltamethrin-induced histological and ultrastructural changes in kidney tissue of rats. Toxicology and industrial health.** 2012 Aug 3:0748233712448115.

19. Atessahin A, Ceriba AO and Yılmaz S. **Lycopene, a carotenoid, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rats. Basic & Clinical Pharmacology & Toxicology.** 2007 Jun 1; 100 (6): 372–376.

20. Mansour HH, El Azeem MG, Ismael NE. **Role of lycopene against hepatorenal oxidative stress induced by sodium fluoride and gamma rays.** Journal of Radiation Research and Applied Sciences. 2011; 4(2A): 435-60.

21. Li W, Wang G, Lu X, Jiang Y, Xu L, Zhao X. **Lycopene ameliorates renal function in rats with streptozotocin-induced diabetes.** International journal of clinical and experimental pathology. 2014; 7(8): 5008.

22. Koul A, Shubrant S and Gupta P. **Phytomodulatory potential of lycopene from lycopersicmesculentum against doxorubicin induced nephrotoxicity.** Indian Journal of Experimental Biology. 2013; (51): 635-645.

23. Gado AM, Adam ANI and Aldahmash BA. **Protective effect of lycopene against nephrotoxicity induced by cyclosporine in rats.** Life Science Journal. 2013; vol (10): 1850-1856.

24. Ural MS. **Chyloropyrifos-induced changes in oxidant/anti-oxidant status and haematological parameters of cyprinuscorpio: Ameliorative effect of lycopene.** Chemosphere, 2013; 90, 2059-2064.

25. Bancroft JD, Floyd AD, Suvarna SK. **Bancroft\'s theory and practice of histological techniques and their diagnostic application, 7th ed.** Edinburg: Elsevier Churchill Livingstone; 2013; 27: 43-44, 135-136,177-178.

26. Gupta S, Sarotra P, Aggarwal R, Dutta N, Agnihotri N. **Role of oxidative stress in celecoxib-induced renal damage in wistar rats.** Digestive diseases and sciences. 2007 Nov 1; 52(11):3092-8.

27. Pal S and Gowda SKP. **Effect of lycopene on lithium induced nephrotoxicity in rats.** World Journal of Pharmaceutical Research. 2014; vol (3): 1026-1036.

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