Original Article

Proximal Convoluted Tubular Cells of Kidney In Impaired Kidney

# Histomorphological Effect of Celecoxib on Nuclear Diameter of Proximal Convoluted Tubular Cells of Kidney with Ameliorative Effect of Lycopene in Albino Rats; An Experimental Study

**Sadia Sundus[1], Sarwath Fatimee[5], Nadira Hameed[1], Jamil Ahmed Siddiqui[2], Imran Bakar[3] and Shah Jabeen[4]**

## ABSTRACT

**Objective:** Toevaluate the nuclear diameter of proximal convoluted tubular cells in celecoxib impaired kidney with amelioration by lycopene.

**Study design:** Experimental study.

**Place and Duration of study:** This study was conducted in Animal House of BMSI, JPMC, Karachi from May 2015 to June 2015.

**Materials and Methods:** In this study we took 90-120 days old, forty healthy adult male Albino rats of 200-220gm weight. Rats were divided into 4 groups, control group was nominated as Group A, rats of Group B were given Celecoxib 50 mg/kg orally, rats of Group C were given Celecoxib 50 mg/kg with lycopene50 mg/kg and Group D were givenonly lycopene 50 mg/kg orally for 30 days. After the completion of experimental study, rats were dissected and renal tissue sections were stained with hematoxylin and eosin.

**Results:** The nuclei of renal cells became pyknotic and the cells showed apoptotic changes in rats of Group B. Hematoxylin and eosin stained sections showed apoptosis, hemorrhage, necrosis and vacuolation in Group B of albino rats, however renal structure were improved in Group Crats which were given celecoxib with lycopene.

The proximal convoluted tubules of kidney became dilated due to apoptotic changes in renal cells and pyknosis of their nuclei. Renal interstitium showed inflammation, edemaand congestion.

**Conclusion:** This experimental study accomplishes that lycopene improved the pyknotic changes of Group B.

**Key Words:** Apoptosis, reactive oxygen species (ROS), acute kidney injury (AKI), prostaglandins (PGs), interstitial nephritis (AIN), and glomerular filtration rate (GFR).

**Citation of article: Sundus S, Fatimee S, Hameed N, Siddiqui JA, Bakar I, Jabeen S. Histomorphological Effect of Celecoxib on Nuclear Diameter of Proximal Convoluted Tubular Cells of Kidney with Ameliorative Effect of Lycopene in Albino Rats; An Experimental Study. Med Forum 2021;32(2):156-160.**

## INTRODUCTION

Celecoxib is4-[5-(4-methylphenyl)–3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesul- fonamide,is asubclass of NSAIDs,used for pain, fever, redness and edema. [1,2]It selectively inhibits COX-2 enzyme activity because COX-2 enzyme stimulates NADPH oxidase activity,[3] which causes oxidative stress, resulting in excessive production of ROS that leads to tissue injury and metabolic disturbance.[4] COX-2-selective inhibitors were associated with less Gastrointestinal symptoms but increased risks of cardiovascular events via vasoconstriction and platelet aggregation and 2.7 times increase risk of acute Kidney Injury by sodium retention estimated glomerular filtration rate (GFR) alteration, hyperkalemia and raise the blood pressure.[5,6,7]vascular injury and multiple organ failure in sepsis. [8]

NSAIDs reduces renal blood flow which results in tubular obstruction through crystal deposition, which causes acute kidney injury (AKI) and interstitial nephritis (AIN). [9] Raised levels of cyclooxygenase derived oxylipins causes cystic kidney diseases.[10] There is strong association between acute kidney injury (AKI) and usage of NSAIDs. [11]NSAIDs inhibits prostaglandins (PGs) and enzyme cyclooxygenase (COX) production, which converts arachidonic acid into PGs, thromboxanes, and prostacyclins.COX-2 is

[1.] Department of Anatomy / Biochemistry[2] / Pathology[3] / Physiology[4], Fazaia Ruth Pfau Medical College, Karachi.
[5.] Department of Anatomy, Fatima Jinnah Dental College, Karachi.

Correspondence: Dr. Sadia Sundus, Assistant Professor of Anatomy, Fazaia Ruth Pfau Medical College, Karachi.
Contact No: 0300-2850489
Email: usadsun_dr@yahoo.com

Received: June, 2020
Accepted: December, 2020
Printed: February, 2021

always activated when there is any injury occur in the body and release of inflammatory mediators, leading to the stimulation of PGs synthesis.[12]

Celecoxib decreases the synthesis of different inflammatory "prostanoids" byCOX-2 enzyme inhibition[13] because COX-2 enzyme is mainly activated due to inflammation and cell transformation processes[14] and produces prostaglandin E2 (PGE2), which boosts the resistance to apoptosis, probability for invasion, angiogenesis, cell-proliferation and metastasis.[15] It treats different clinical conditions like musculoskeletal disorders, pain, dysmenorrhea and colorectal polyps. Gastrointestinal and bleeding risks are lower with celecoxib, so it is also used for the treatment of OsteoArthritis but it did not improve endothelial.[16] Celecoxib radiosensitize cancer cells leads to suppression of cancer cell proliferation, which shows its strong chemopreventiveaction[17,18]Itinduces apoptosis in tumor cells due to activation of the anti-apoptotic kinase, which promotes apoptosis in cells.[15] Celecoxib is primarily metabolized in the liver and excreted in urine and feces. [12]

Lycopene (LPN) is a member of red-colored carotenoid, found in tomato,[19] red fruits like watermelon, Momordica cochinchinensis, Spreng fruit, papayas, pink grapefruit, vegetables.It possesses a strong antioxidant activity caused by free radicals in human tissues, thusinhibit cellular damage due to reactive oxygen species.[20,21,22,23] It is a lipid-soluble antioxidant, inversely associated with lipid peroxidation and reduced oxidative stress and inflammation. It has several biological activities like aging prevention, anti-cancerous, and anti-inflammation. [24,25]Lycopene played a vital role in the activation of Nuclear factor erythroid 2-related factor 2 (Nrf2) which causes regulation of cellular oxidative stress response.[26]

The intake of tomatoes and its products are associated with raised blood levels of lycopene. It gives protection to cellular damage caused by (ROS) and against oxidation of lipids, proteins, and DNA, so it have substantial roles in preserving tissue and cellular integrity. [27,28]

Meanwhile, No experimental study bring in observation up till now regarding morphometric and histo-pathological changes occur due to Celecoxib along with ameliorative effect of lycopene, therefore this opportunity has been availed to commence this research. As we know that Celecoxib hasadverse effects on kidney and altered its morphology as well as functions while lycopeneis a bioflavonoid and have highest oxygen-quenching capacity. This is the reason we plannedthe study to seelycopene protective effect on celecoxib persuaded renal tissue.

# MATERIALS AND METHODS

It is a four weeks study, which was conducted in the Animal House of BMSI, JPMC, Karachi. 90-120 days old, forty healthy adult Albino rats of 200-220gm weight were brought from the Charles River Breeding Laboratories, Brooklyn, Massachusetts, USA. We observe their well-being and nutritional behaviors one week before the commencement of experimental study. According to the study plan dosage of Celecoxib and lycopene were given to animals.

We divided the animals into four groups.

- Group 1: as standard group.
- Group 2: were given Celecoxib 50 mg/kg orally.
- Group 3: were given Celecoxib 50 mg/kg + lycopene 50 mg/kg orally.
- Group 4: were given lycopene 50 mg/kg orally.

Prior the commencement of experimental study every animal was weighed and retained in animal house cages before drug administration. To note behavioral changes and general conditions all the animals were kept under observation. They were weighed and sacrificed at the end of study.

The animals was sedated under ether and then fixed on a dissecting board. A sagittal incision were given from manubrumsterni to pelvic bone by scalpel. Another incision were given at right angle to the previous incision to get appropriate exposure of thoracic and abdominal cavity. Kidneys were recognized and exposed. They were inspected for noticeable change in pigmentation, contour, and substance. They were detached and absolute weight was recorded with sartorious balance. Both kidneys were excised into longitudinal halves for separate fixative after cleaning with normal saline. For H & E staining one half was retained in 10% formalin and the other section for PAS staining in alcoholic formalin for a day. It was treated in ascending grades of alcohol (70% – 100%) for dehydration and cleared in xylene. Paraffin wax was used for infiltration and embedding and with the help of rotatory microtome, 4 to 5 microns thick longitudinal sections were taken and they were fixed on albumenized glass slides.

By SPSS version 20.0 data was evaluated. One sample t-test is used for the significance of tissue slides at 40x.P-value $< 0.05$ was anticipate as statistically significant.

# RESULTS

**Group1:** Group 1rats were alive and healthy and their dietary habits as well as response to external stimulus was usual during the study. The mean value of nuclear diameter of Group 1 proximal tubular cells was 6.2±0.84µm (Figure-1a) (Table-A).

**Group 2:** Group 2 animals were looking lethargic and their food consumption was reduced as well as their response to stimuli was sluggish. The mean value of nuclear diameter of Group 2tubularcells was 4.2±0.83µm. A remarkable decrease in Group 2($P<0.05$) in the mean value of nuclear diameter of

tubular cells was observed as compare to Group 1(Figure-2a& 3a) (Table-A).

**Group 3:** Group 3 animals seemed relatively healthy and active. Their response to stimuli was improved as compared to Group 2 animals as well as food intake was normal. The mean value of nuclear diameter of Group-3 proximal tubular cells were 6.0±0.70µm. Group 3 showed a remarkable raise (P<0.05) in nuclear diameter of proximal tubular cells in comparison with Group 2 and an unremarkable shrinkage (P>0.05) in nuclear diameter of proximal tubular cells ofGroup3was observed as equated to Group 1(Figure-4a) (Table-1).

**Group 4:** The Group 4 animals were taken to determine that rather lycopene can produce any changes in the architecture of renal tissue of rat. The outcomes of Group 4 was same as Group 1.

**Table No.1: Mean values of nuclear diameter of proximalconvoluted tubular cells of kidney (µm) in different groups of albino rats**

| Groups | Treatment given | Mean value of nuclear diameter of proximal tubules |
|---|---|---|
| 1 (n=10) | ND | 6.2±0.84 |
| 2 (n=10) | Celecoxib | 4.2±0.83 |
| 3 (n=10) | Celecoxib +Lycopene | 6.0±0.70 |

*Mean±SEM

Analysis of data in the mean nuclear diameter of proximal convoluted tubular cells in various Albino Rats groups.

| Data comparison | P-value |
|---|---|
| 2 vs.1 | P<0.05** |
| 3 vs. 1 | P>0.05* |
| 3 vs. 2 | P<0.05** |

Key:

Non-significant*
Significant**
Moderately significant***
Highly significant****

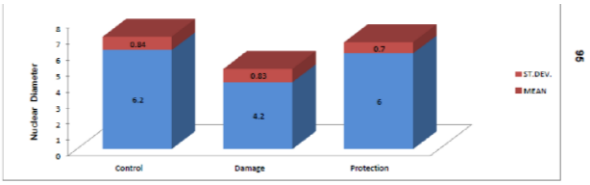


**Figure No.1: Mean values of nuclear diameter of proximal convoluted tubular cells of kidney (um) in different groups of albino rates**

Group 1animals on microscopic examination exhibited usual renal architecture. Proximal convoluted tubular epithelial cells was shown with regular brush border and centrally located spherical nucleus. (Fig-1a)

Microscopic examination of Group 2animals showed apoptosis and dilatation of renal tubules with pyknotic nuclei, infiltration ofproximal convoluted tubular cells. (Fig-2a). Microscopic examination of Group 3animals revealed restoration of renal architecture, slight dilatation of renal tubules with regular brush border, centrally located spherical nucleus. (Fig-3a)

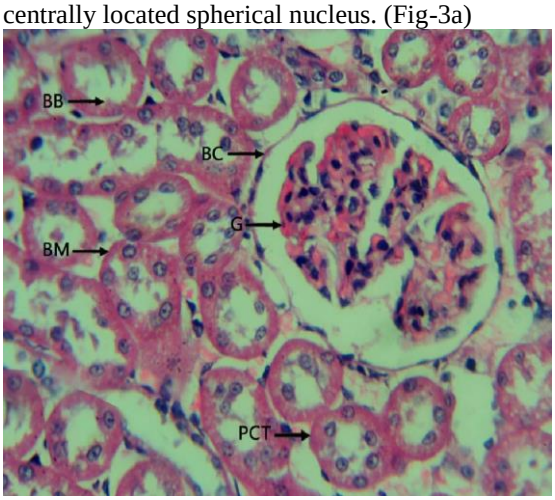


**Figure No.1a: Photomicrograph showing normal cytoarchitecture of kidney normal glomerulus, proximal and distal renal tubules in control group-1 at 40x**

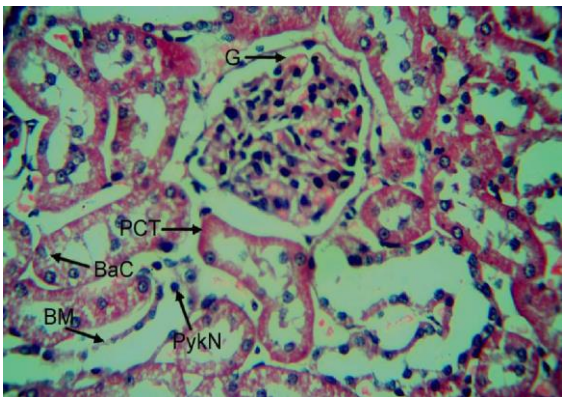


**Figure No.2a: H& E stained, 4 µm thick section of celecoxib treated rat kidney (Group-2) shows proximal convoluted tubules, congested and hemorrhagic slightly shrunken glomerulus and ballooning of cells (BaC) with pyknotic nuclei (PykN). (Photomicrograph x 400).**

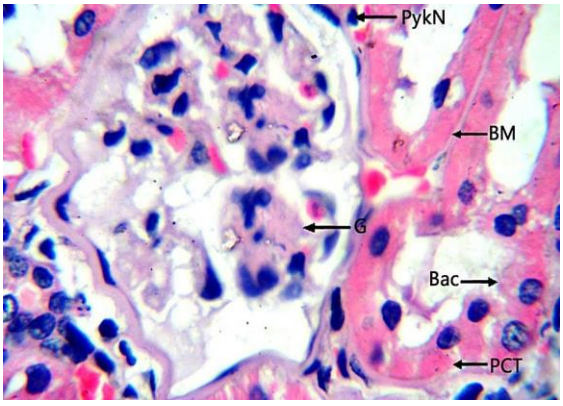


**Figure No.3a: H& E stained, 4 µm thick section of celecoxib treated rat kidney (Group-2) showing proximal convoluted tubule (PCT), slightly shrunken glomeruli (G)and ballooning of cells (BaC) with pyknotic nuclei (PykN). (Photomicrograph x 1000)**

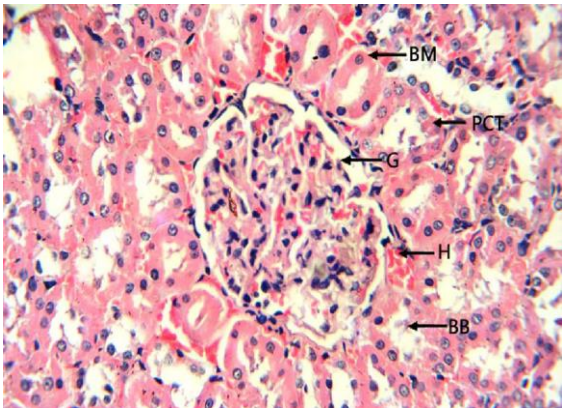


**Figure No.4a: Photomicrograph showing preserved cytoarchitecture of kidney glomeruli has less vacuolation, brush border of proximal is restored and not as much of hemorrhage in group-3 (lycopene treated) at 40x**

# DISCUSSION

Celecoxib is one of the subclasses of ANSAIDs, used for pain, dysmennorhoea, inflammation, redness, edema and fever.[1] Histological examination exhibits apoptosis, pyknosis, infiltration of mononuclear cells and tubular dilatation.[2]

Lycopene is a member of carotenoid family.[19] It possesses antioxidant activity and inhibits cellular damage because of reactive oxygen species (ROS). Red colored fruits such as tomato, pink grape fruit and vegetables contains lycopene.[20]It contributes in multiple biological activities such as aging prevention, anticancerous and anti-inflammatory agent.[21,22]

Group B animals were sluggish and reduced their body weight because of cytotoxicity and cell mediated immune injury which leads to apoptosis and pyknosis.[15]Pyknotic nuclei, degenerative and apoptotic changes were observed in proximal convoluted tubular cells as described by. [2]

Group C animals were healthy just like Group A and slight body weight is reduced as compared to Group B because it inhibits production of reactive oxygen species, thus reduces apoptosis and cellular damage.[24,25] Group C showed reversal of the renal parenchyma degenerated changes induced by celecoxib. Lycopene plays a substantial role in maintaining tissue and cellular integrity as described by.[27,28] who described that lycopene gives protection to cells against oxidative damage.

# CONCLUSION

This experimental study determined that Group B had significant decrease in body weight due to apoptosis with pyknotic nuclei, oxidative cellular damage and tubular dilatation however Group C exhibited increase in body weight, reversal of pyknosis and apoptosis. So this is our suggestion that don't use celecoxib frequently and if needed always used it with lycopene to decrease its adverse effects.

**Author's Contribution:**

| | |
|---|---|
| Concept & Design of Study: | Sadia Sundus |
| Drafting: | Sarwath Fatimee, Nadira Hameed |
| Data Analysis: | Jamil Ahmed Siddiqui, Imran Bakar, Shah Jabeen |
| Revisiting Critically: | Sadia Sundus |
| Final Approval of version: | Sadia Sundus |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

# REFERENCES

1. Jieqing Li, Qiongyu Hao, Wei Cao, Jaydutt V Vadgama, and Yong Wu2.Celecoxib in breast cancer prevention and therapy. Cancer Manag Res 2018;10:4653–4667.
2. Chou CI, Shih CJ, Chen YT, Ou SM, Yang CY, Kuo SC, et al. Adverse Effects of Oral Nonselective and cyclooxygenase-2-Selective NSAIDs on Hospitalization for Acute Kidney Injury. Medicine (Baltimore) 2016;95(9):e2645.
3. Harris TR, Kodani S, Rand AA, Yang J, Imai DM, Hwang SH. Hammock. Celecoxib Does Not Protect against Fibrosis and Inflammation in a Carbon Tetrachloride–Induced Model of Liver Injury. Molecular Pharmacol 2018;94:834–841.
4. Kianian F, Seifi B, Kadkhodaee M, Sajedizadeh A, Ahghari P. Protective effects of celecoxib on ischemia reperfusion–induced acute kidney injury: comparing between male and female rats. Iran J Basic Med Sci 2019;22(1):43–48.
5. Warth LC, Noiseux NO, Hogue MH, Klaassen AL, et al. Risk of acute kidney injury after primary and revision total hip arthroplasty and total knee arthroplasty using a multimodal approach to perioperative pain control. J Arthroplasty 201631;1:253-255.
6. Safaeian L, Hajhashemi V, Ajoodanian M. The effect of celecoxib on blood pressure and plasma oxidant/antioxidant status in co-administration with glucocorticoid in rat. Biomedicine & Pharmacotherapy 2018;108:1804-1808.
7. Hsu CC, Wang H, Hsu YH, Chuang SY, et al. Use of Nonsteroidal Anti-Inflammatory Drugs and Risk of Chronic Kidney Disease in Subjects with Hypertension 2015;66(3).
8. Ozer EK, Goktas MT, Kilinc I, Bariskaner H. Celecoxib administration reduced mortality, mesenteric hypoperfusion, aortic dysfunction and multiple organ injury in septic rats. Biomedicine Pharmacotherapy 2017;86:583-589.
9. Zhang Xinyu, Donnan PT, Bell S, Guthrie B. Non-steroidal anti-inflammatory drug induced acute kidney injury in the community dwelling general population and people with chronic kidney disease:

systematic review and meta-analysis. BMC Nephrol 2017;18:256.
10. Monirujjaman M, Aukema HM, Cyclooxygenase 2 inhibition slows disease progression and improves the altered renal lipid mediator profile in the Pkd2WS25/− mouse model of autosomal dominant polycystic kidney disease. J Nephrol 2019; 32: 401–409.
11. Ungprasert P, Cheungpasitporn W, Crowson CS. Individual non-steroidal anti-inflammatory drugs and risk of acute kidney injury: A systematic review and meta-analysis of observational studies. Eur J Internal Med 2015;26(4):285-291.
12. Noor d aziz, Ouda MH, Ubaid MM. Comparing the toxic effects of non-steroidal anti-inflammatory drugs (Celecoxib and Ibuprofen) on heart, liver and kidney in rats. Asian J Pharmaceutical Clin Res 2018;11(6):21 -24.
13. Gang D, Xiaguang C, Kanghua Y, Aiping W, Guangxuan Z. Combined effect of celecoxib and glucosamine sulfate on inflammatory factors and oxidative stress indicators in patients with knee osteoarthritis. Tropical J Pharmaceutical Res 2019; 18(2):397-402.
14. Sang XX, W Liu Y, Zhu L, Lu H, Ma J. Effect of Celecoxib on Surgical Site Inflammation after Total Knee Arthroplasty: A Randomized Controlled Study. Med Princ Pract 2018;27: 481–488.
15. Essawy SS, Bilasy SE, Mohammad HMF, Shaalan AAM. Effect of celecoxib and cisplatin combination on apoptosis and cell proliferation in a mouse model of chemically-induced colonic aberrant crypt foci. Egyptian J Basic and Applied Sci 2019;4(4):323-331.
16. Verhoeven F, Totoson P, Marie C, Tessier AP, Wendling D, Tournier-Nappey M, et al. Diclofenac but not celecoxib improves endothelial function in rheumatoid arthritis: A study in adjuvant-induced arthritis. Atherosclerosis 2017; 266:136-144.
17. Hosseinimehr SJ, Fathi M, Ghasemi A, Shiadeh SNR, Pourfallah TA. Celecoxib mitigates genotoxicity induced by ionizing radiation in human blood lymphocytes. Res Pharm Sci 2017; 12(1): 82–87.
18. Izzotti A, Maestra S, Micale RT, Pulliero A, Geretto M, Balansky R, et al. Modulation of genomic and epigenetic end-points by celecoxib. Oncotarget 2018;9(72):33656–33681.
19. Zhu R, Chen B, Bai Y, Miao T, Rui L, Zhang H. Lycopene in protection against obesity and diabetes: A mechanistic review. Pharmacological Res 2020;159:104966.
20. Mahmoodnia L, Mohammadi K, Masumi R. Ameliorative effect of lycopene effect on cisplatin-induced nephropathy in patient. J Nephropathol 2017;6(3):144–149.
21. Shalaby AM, El Shaer DF. Lycopene protects against renal cortical damage induced by nandrolone decanoate in adult male rats. Annals of Anatomy-Anatomischer Anzeiger 2019;224: 142-152.
22. Buyuklu M, Kandemir FM, Ozkaraca M. Benefical effects of lycopene against contrast medium-induced oxidative stress, inflammation, autophagy, and apoptosis in rat kidney. Human Experimental Toxicol 2015;34(35):1 40 – 144.
23. Xiao G, Zou J, Xiao X. Sialic acid-conjugated PLGA nanoparticles enhance the protective effect of lycopene in chemotherapeutic drug-induced kidney injury. IET Nanobiotechnol 2020;14(4): 341 – 345.
24. Taheri Z, Ghafari M, Amiri M. Lycopene and kidney; future potential application. J Nephropharmacol 2015;4(2):49–51.
25. Pandir D, Unal B, Bas H. Lycopene protects the diabetic rat kidney against oxidative stress-mediated oxidative damage induced by furan. Braz Arch Biol Technol 2016;59:135 – 140.
26. Yu K, Zhang J, Cao Z, Ji Q, Han Y, Song M, et al. Lycopene attenuates AFB 1-induced renal injury with the activation of the Nrf2 antioxidant signaling pathway in mice. Food Function 2018; 12:125 – 131.
27. Stojiljkovic N, Ilic S, Jakovljevic V. The encapsulation of lycopene in nanoliposomes enhances its protective potential in methotrexate-induced kidney injury model. Biological Efficacy Medicinal Plant Extracts in Preventing Oxidative Damage 2018;11.
28. Palabiyik SS, Erkekoglu P, Kızılgun M, Sahin G, Gumusel BK. Lycopene restores trace element levels in ochratoxin A-treated rats. Arhiv za higijenuradaitoksikologiju 2017;68(2):148 – 155.