Original Research Article

EFFECT OF LYCOPENE ON HISTOLOGICAL ALTERATIONS OF BASEMENT MEMBRANE & FIBROSIS IN RENAL INTERSTITIAL TISSUE OF CELEBREX INDUCED ALBINO RATS; AN EXPERIMENTAL STUDY.

ABSTRACT

Objective: To assess the histological alterations in basement membrane and fibrosis in renal interstitium of albino rats due to celebrex with enhancement by lycopene.

Study design: Experimental study.

Abode of study: Animal House of Jinnah Postgraduate institute, Karachi,

Materials and Methods:

COX-2 inhibitor and antioxidant medicines were used in this research work. These medications were orally administered in 40 male albino rats weighing 200-220gm for experimentation. Rats were housed in separate pens at 23ºC. Rats were arranged into 4 groups including control horde and three experimental hordes. The medications were dose up orally by gastric tube daily for one month. At completion of experiment, animals were dissected and tissues were well-preserved for staining.

Results: In second horde PAS stained kidney segments showed disrupted basement membrane of distended proximal convoluted tubules & ill-defined brush border and fibrosis in renal interstitium, but 3rd horde had intact basement membrane & well-define brush border at the luminal surface of proximal tubular epithelium and there was mild fibrosis in renal interstitium.

Conclusion: This study divulges that lycopene convalesce the disrupted basement membrane and fibrosis in second horde.

Key words: Antioxidant, PAS, Masson's Trichome, Basement Membrane(BM), Horde, Nuclear factor erythroid 2-related factor 2 (Nrf2),celebrex, nonsteriodal anti-inflamatory drugs(NSAIDs).

INTRODUCTION:

NSAIDs are the efficient inhibitors of COX enzyme and widely used medication against inflammation, fever, arthritic and painful conditions. It ceases bioconversion of arachidonic acid to prostaglandins by inhibiting COX enzyme production because Prostaglandins synthesis take place by mobilization of arachidonic acid from membrane phospholipids leading to production of PGH2 due to cyclooxygenase reaction. COX-2 regulates renal perfusion and helps in releasing renin even in liver cirrhosis, renal insufficiency & congestive heart failure so in patients of renal ischemia risk became increased after using celebrex because it reduces vasodilation due to prostaglandin secretion. [(1)] Their usage is interrelated with renal & gastrointestinal toxicity like renal insufficiency, gastric perforation & GI bleeding. [(2)] Celebrex is a selective COX-2 inhibitor and has vasculoprotective properties by exerting anti-inflammatory and anti-oxidant activities on macrophages & vascular endothelium deprived of influencing basal COX-1-dependent prostaglandin synthesis but it has increased risk of atherothrombosis. [(3,4)] It also decreases the basement membrane thickness of glomerulus, densities of foot processes, reduce mesangium and diameter of slit pore.[(5)] Celebrex causes infertility by affecting conception by inhibiting ovulation, gestation & constricted ductus arteriosus due to raised PGs as well as NO placental levels, cytoplasmic vacuolation and enlarge mitochondria. [(6)] It also produces interstitial fibrosis by collagen deposition, vascular congestion & emitted unchanged in urine and feces.[(7,8)] celebrex intrude the COX pathway thus decreases vasodilatory and anti-aggregatory prostacyclin whereas increases prothrombotic thromboxane A2 serum levels so it increases the cardiovascular risk. [(9)] It increases cellular dysfunction and acts as a chemopreventive agent by inhibiting COX-2 enzyme activity. [(10)]

Lycopene is a member of dietary carotenoid antioxidant group and it can reduce inflammatory cytokines serum levels thus enhanced redox balance of renal tissue & present in red pigment of tomato, papaya, pink guava watermelon, and red grapefruit.[11,12] It is also present in rich amount in blood plasma of human beings because of these dietary resources, primarily in LDL and VLDL due to their lipophilic affinity and in the adrenal gland, testes, liver and prostate gland and act as an anti-carcinogenic, antiautophagic, antiapoptotic and anti-ageing agent because it reduces PGs synthesis by amending cyclooxygenase enzyme activity therefore it provide fortification against prostatic, respiratory, GIT, uterine and liver cancers, nephropathy and cardio- vascular ailments.[13,14,15,16] It also boost up the antioxidant ability of renal tissue by triggering Nrf2 antioxidant signaling pathway which is a key transcription element for cellular oxidative stress reaction and it has strongest affinity to quench singlet oxygen too.[17,18] It plays a protective role against eye infection, coronary heart ailments, masculine infertility, osteoporosis, liver and kidney impairment.[19] Due to its extremely effective antioxidant capability for scavenging free radical by chemical reaction, so it plays a prophylactic role against oxidation of proteins, lipids, DNA and cellular damage. [20,21]

The above mentioned research studies determined that celebrex is related with various harmful effects. Consequently we assumed that renal interstitial fibrosis & thickened basement membrane may be associated with celebrex. The current experimental study was planned to inspect the beneficial efficiency of lycopene as compared with celebrex nephropathy.

MATERIAL AND METHODS

This is a 30 days experimental study, take place in animal house, JPMC, Karachi with the approval from ethical review board No.F-1-2/BMSI-E.COMT/039/JPMC. Forty adult rats in good physical condition, 90-120 days old age group, of about 200-220gm were achieved from laboratory of USA and nurtured in animal house in isolated coops. Evaluated for 1 week for their general health status and weighed prior the initiation of research and alienated into four clusters and administered celebrex and lycopene pigment 0.05g/kg orally. Dose calculation done according to their weight. Oral preparation of tab. Celebrex from Getz Pharma along with lycopene pigment capsule from General Nutrition Corporation, UK; was used in this research study. Calculated dose of Celebrex as well as lycopene 0.05g/kg [22,23] were administered to rats orally.

- G a: control.
- G b: Celebrex 0.05g/kg per oral. (Morbid group)
- G c: Celebrex with lycopene pigment 0.05g/kg per oral.
- G d: lycopene pigment 0.05g/kg per oral.

During the whole research period rats were keenly observed for any difference in their over-all conditions. Rats were again weighed in the end of experiment and sedate under ether before dissection. An upright cut was given by scalpel & kidneys were seen cautiously for any alteration in its gross structures and weighed on sartorious weighing scale. Kidney tissue was treated and sections were fixed in alcoholic formalin for periodic acid shift stain for 24 hours. Periodic Acid Schiff stain was used to observe basement membrane, brush border of proximal convoluted tubule, Bowman's capsule, cortical and medullary region (8X ocular and 40X

objective lens). [24] Masson's trichrome stain was used to observe collagen fibers of renal interstitial tissue (8X ocular and 40X objective lens). [(24)]

The statistical records was statistically evaluated by SPSS software version 20. Student paired T test was used to evaluated the statistical variances among experimental groups to test the significance in different groups. The variances were deliberated as statistically significant, if P-value was same or less than 0.05.

RESULTS

Microscopic observation of Renal Tissue on the basis of PAS (Periodic Acid Schiff Stain).

Ga:

PAS stained sections of group Ga revealed an intact basement membrane of glomeruli, Bowman's capsule and proximal tubular epithelium having well defined luminal brush border (Figure-1A).

Gb:

PAS stained kidney sections of group Gb animals showed dilated proximal convoluted tubules with ill-defined brush border and disruption of basement membrane (Figure-1B).

Gc:

PAS stained kidney sections of group Gc showed well-define brush border at the luminal surface of proximal tubular epithelium with intact basement membrane (Figure-1C).

Gd:

PAS stained slides of Gd group (lycopene pigment treated) revealed regular renal architecture. The effects of Gd (lycopene pigment treated) group were similar to Ga.

Microscopic observation of Renal Tissue on the basis of Masson's trichrome

Ga: Masson's trichrome tainted kidney segments of group Ga exhibited normal architectural distribution of collagen fibers with no signs of fibrosis (Figure-2A).

Gb: Masson's trichrome tainted kidney segments of group- Gb animals exhibited moderate fibrosis around glomerulus and in the renal interstitial tissue (Figure-2B).

Gc: Masson's trichrome tainted kidney segments of group-Gc exhibited mild fibrosis around glomeruli and few collagen fibers in the renal interstitial tissue (Figure-2C).

Gd: Masson's trichrome tainted slides of Gd group (lycopene pigment treated) exhibited normal renal pattern. The effects of Gd (lycopene pigment treated) group were comparable to Ga.

Figure No.1A: PAS stained, 4 µm thick section of control rat kidney showing Proximal convoluted tubule(PCT) with well-defined brush border(BB), glomeruli(G) with bowman's capsule(BC), intact basement membrane(BM) (Photomicrograph x 400)

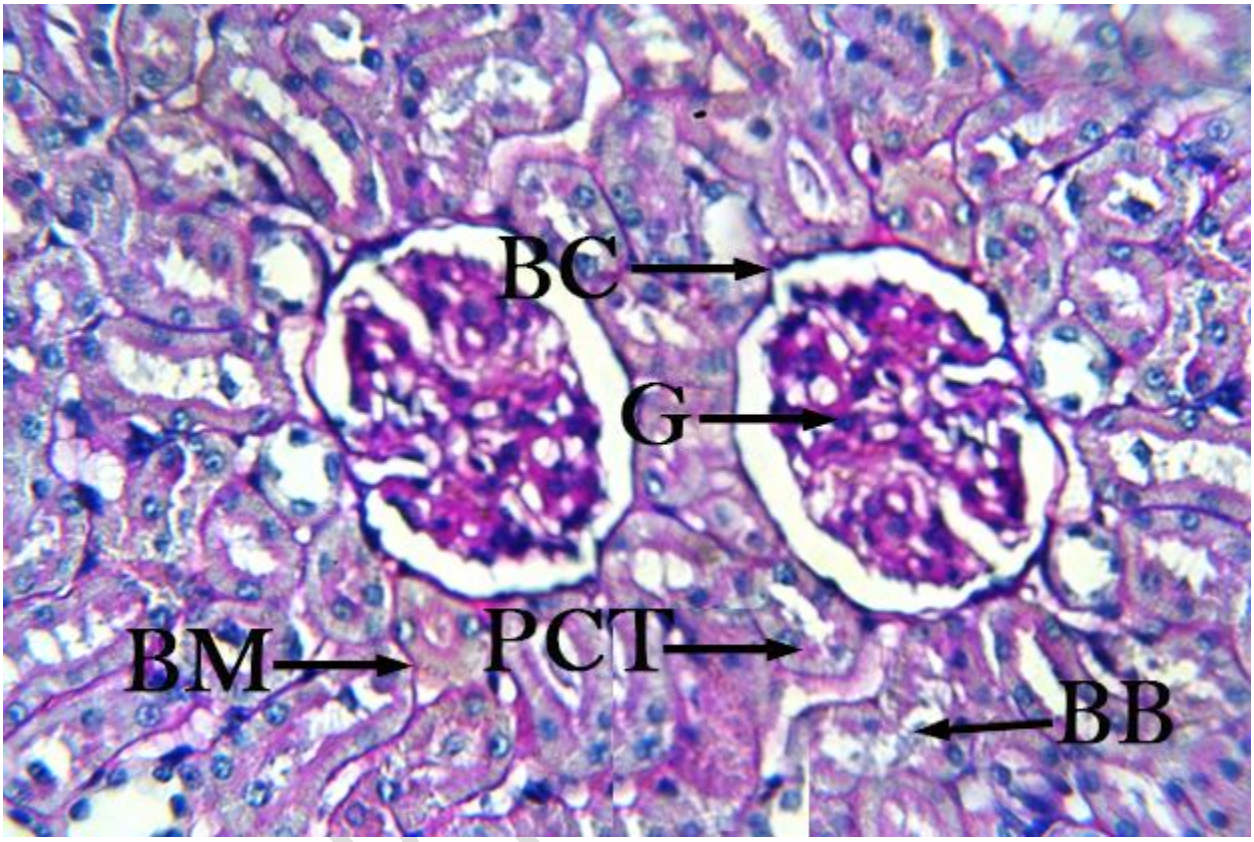


Figure-1B: PAS stained, 4 µm thick section of rat kidney showing dilated Proximal convoluted tubule (PCT) with Epithelial Cast (E) , Ballooning (BaC) and necrosis (Nec) of cells with pyknotic nuclei (Pykn), disruption of basement membrane (BM) and shrinkage of glomeruli (G) in Celebrex treated group. (Photomicrograph x 400).

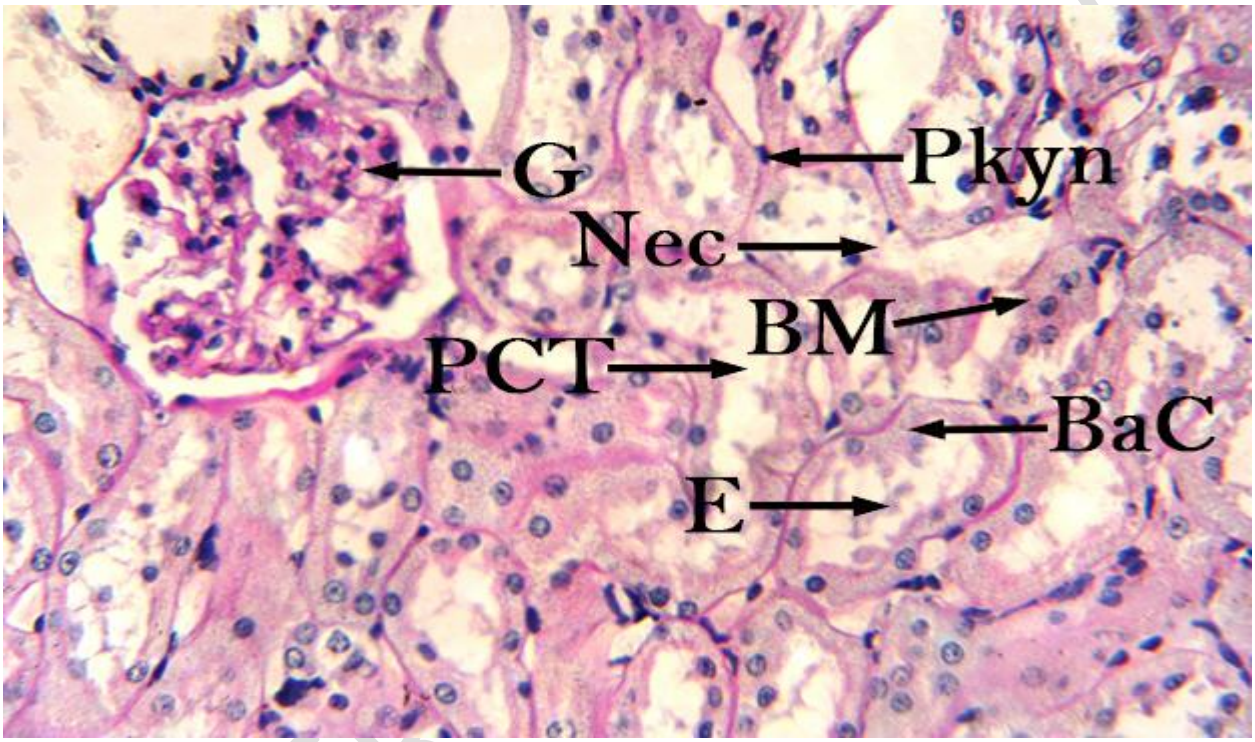


Figure-1C: PAS stained, 4 µm thick section of rat kidney showing architecture of renal parenchyma, Proximal convoluted tubule(PCT) with brush border(BB), glomeruli(G), mild necrosis (Nec) with pyknotic nuclei (Pykn), intact basement membrane(BM) in Celebrex + Lycopene pigment treated group. (Photomicrograph x 400)

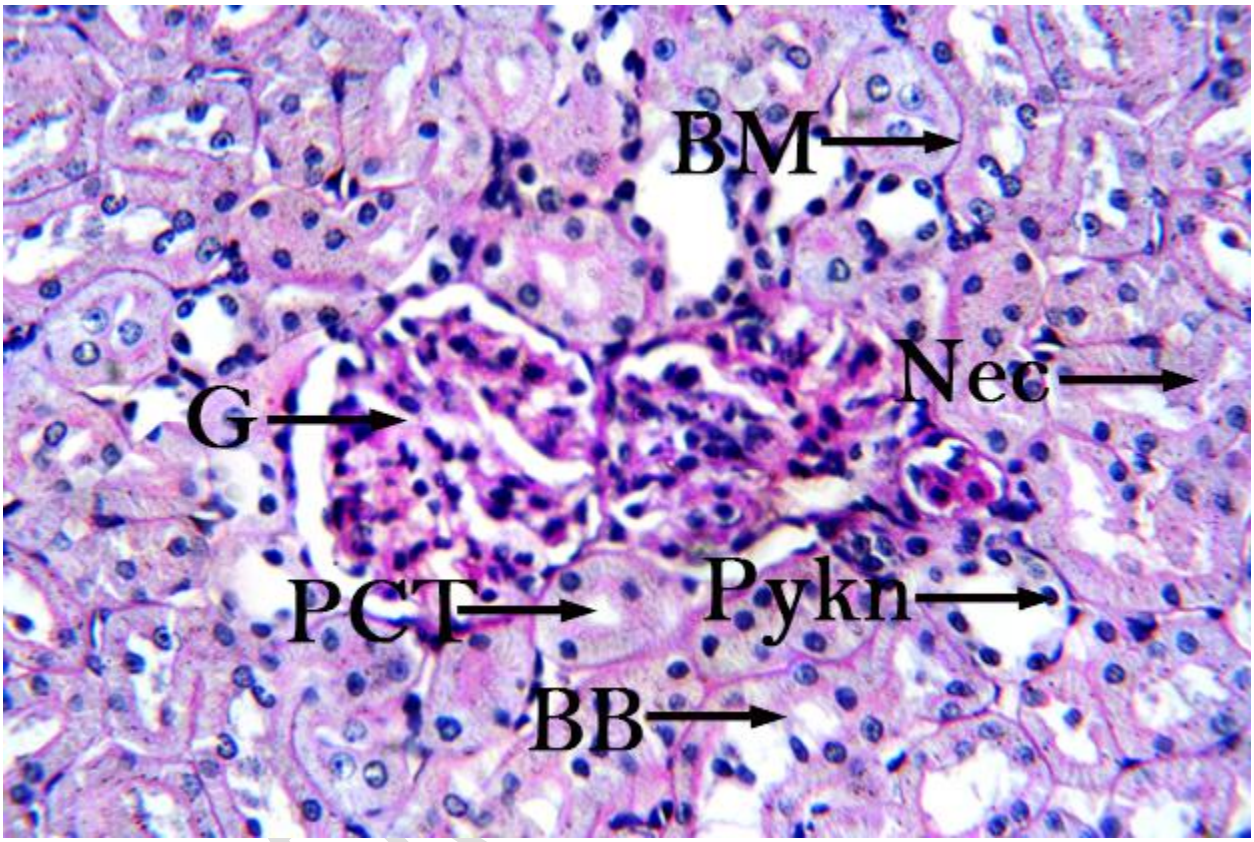


Figure-2A: Masson's Trichrome stained, 4 µm thick section of control rat kidney showing normal architecture of renal parenchyma Proximal convoluted tubule(PCT) with well defined brush border(BB), glomeruli(G) with bowman's capsule(BC), intact basement membrane(BM) (Photomicrograph x 400)

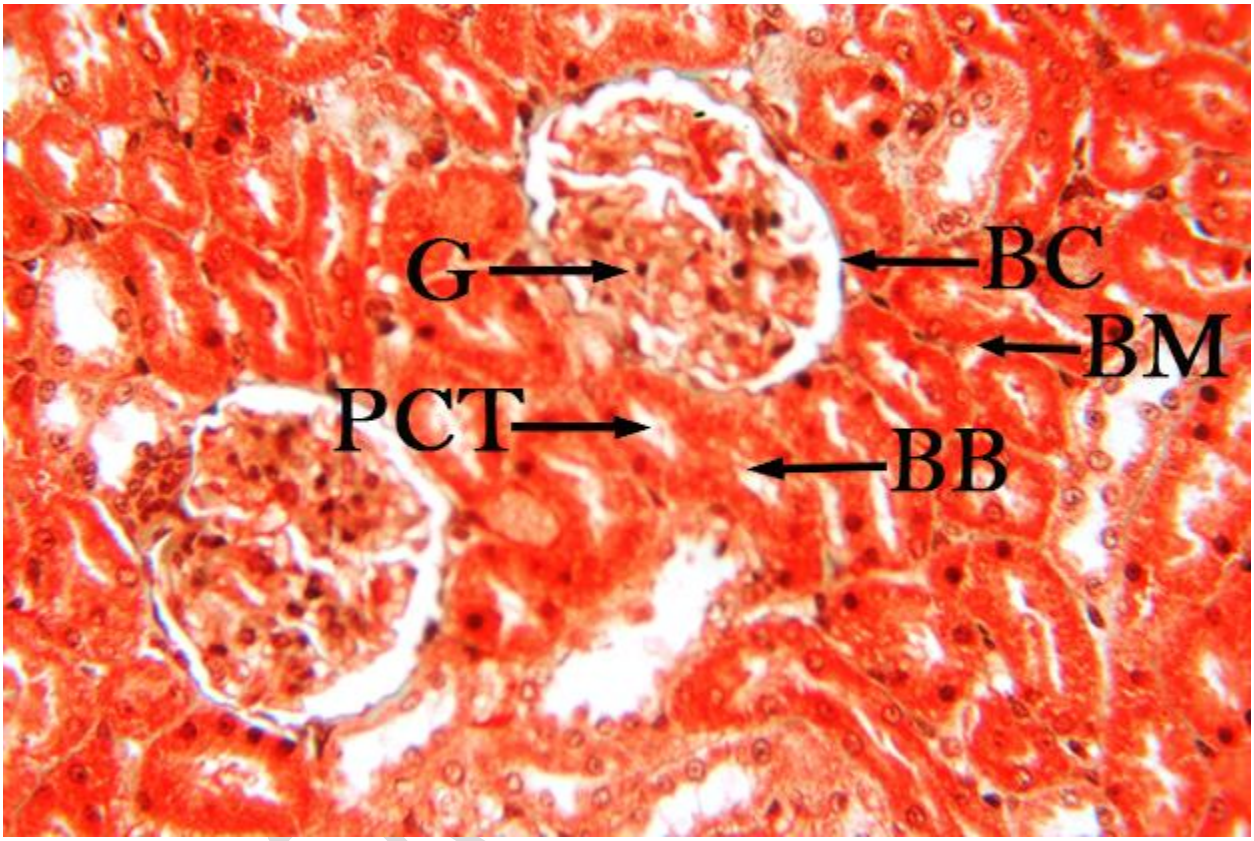


Figure-2B: Masson's Trichrome stained, 4 µm thick section of rat kidney showing dilated Proximal convoluted tubule (PCT), necrosis (Nec) of cells with pyknotic nuclei (Pykn), interstitial fibrosis (F) around glomeruli (G) in Celebrex treated group. (Photomicrograph x 400).

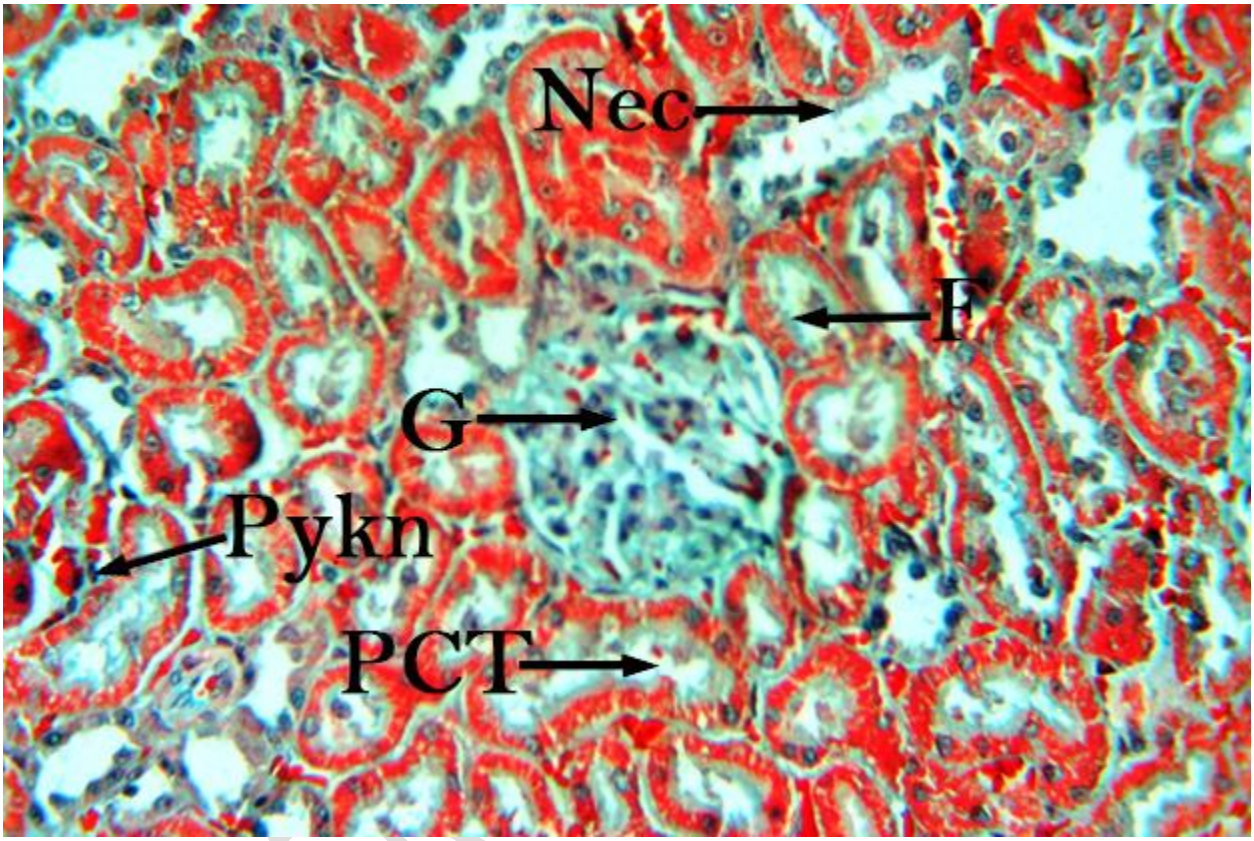
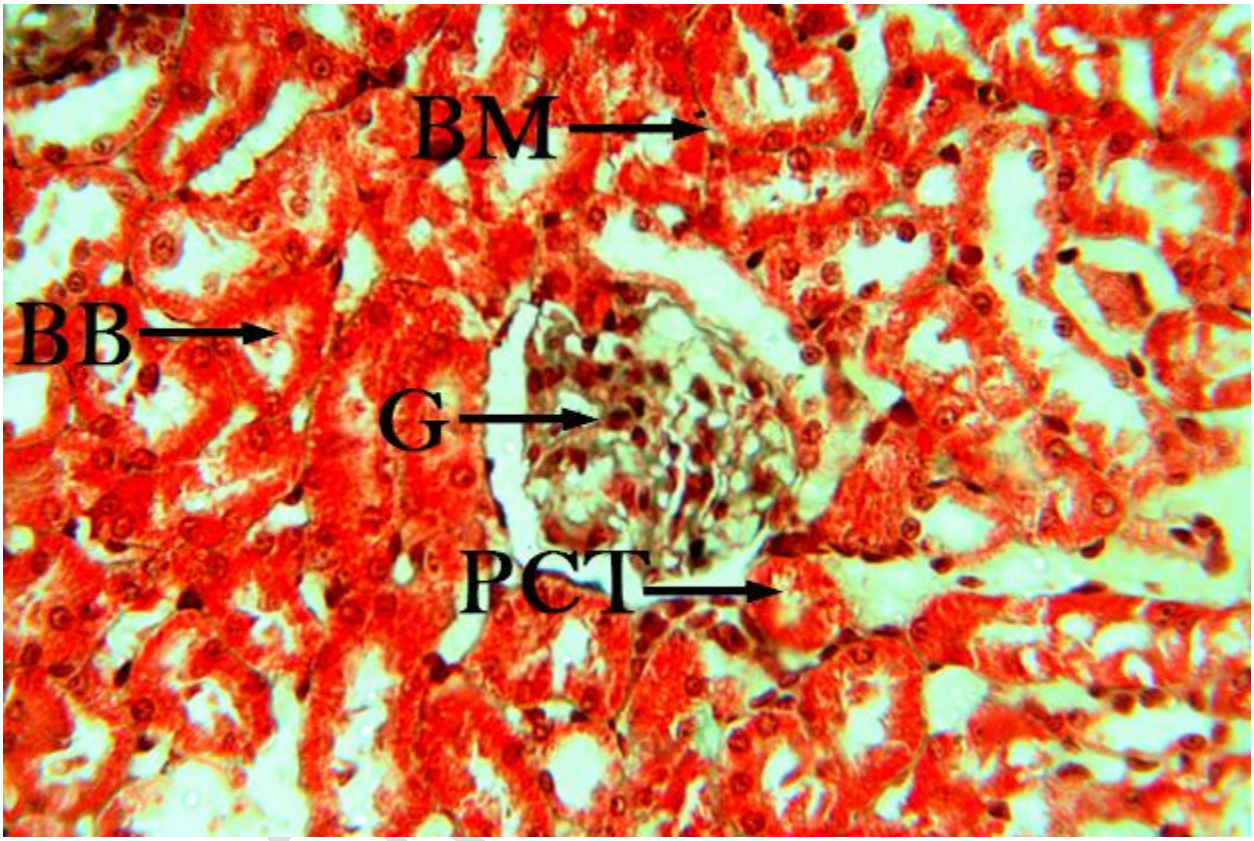


Figure-2C: Masson's Trichrome stained, 4 µm thick section of rat kidney showing architecture of renal parenchyma, Proximal convoluted tubule(PCT) with brush border(BB), mild fibrosis around glomeruli(G), intact basement membrane(BM) in Celebrex + Lycopene pigment treated group. (Photomicrograph x 400)



DISCUSSION:

NSAIDs are the main drugs of choice for inflammation, pain, temperature and other diseases for about hundred years. In 1971 vane documented enzyme cyclooxygenase inhibitors as anti-inflammatory agent which block the prostaglandins biosynthesis essential for renal blood flow, diuresis and renal functions like perfusion, water handling, and renin release in the kidney. Therefore risk of renal ischemia will increase if we use celebrex. [1,2] Due to inhibition of prostaglandins celebrex can decreases the thickness of glomerular basement membrane, mesangial area, structure of podocytes, peritubular inflammatory cellular infiltration,

degeneration of lining epithelial cells, endoplasmic reticulum shattering, also mitochondrial atrophy, chromatin clomping, malformation of nuclear membrane and slit pore diameters.[5,6]

Lycopene belong to carotenoids fat-soluble pigments found in tomatoes &its products, red grape fruit, watermelon and red vegetables. It is a very effective antioxidant against free radical scavenging agents by chemically reacting with it, thus inhibits cellular damage and prophylactic against oxidation of DNA, proteins & lipids.[11,13,14] Lycopene act as an anti- carcinogenic agent especially prostate cancer, respiratory & gastrointestinal cancers. It is also protective against cardiovascular diseases & sperm toxicity after chemotherapy and decreases DNA fragmentation index.[17,19]

PAS stained kidney sections of group Gb animals were showed dilated proximal convoluted tubules with ill-defined brush border and disruption of basement membrane. Analogous results were also illustrated by.[1,4,6] Masson's trichrome stained kidney sections of group- Gb animals were showed moderate fibrosis around glomerulus and in the renal interstitial tissue. Same consequences were also discussed by. [7,8]

PAS stained kidney sections of group Gc showed well-define brush border at the luminal surface of proximal tubular epithelium with intact basement membrane. Analogous outcomes were also illustrated by. [12,14] Masson's trichrome tainted kidney sections of group-Gc exhibited mild fibrosis around glomeruli and few collagen fibers in the renal interstitial tissue. Same results were also deliberated by. [17,18,19]

CONCLUSION:

Research work determined that Gb rats had disrupted basement membrane, moderate fibrosis around glomerulus and in the renal interstitial tissue however Gc rats showed intact basement

membrane with mild fibrosis around glomeruli and few collagen fibers in the renal interstitial tissue. Therefore our assumption from this research is that celebrex is harmful for renal tissue and lycopene is beneficial for human body due to its anti-oxidative effects.

LIMITATION OF STUDY

Funds are not adequate otherwise we will do it in further depth.

ETHICAL APPROVAL

Animal Ethic committee approval had been taken from our department.

COMPETING INTERESTS

Authors have declared that no competing interests exist**.**

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