



Evaluation of COX-2 Inhibitor Injurious Effects on Proximal Convoluted Tubular Diameter of Kidney with Favorable Impact of Lycopene in Albino Rats on the Basis of Microscopic Features

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To analyze the harmful effect of COX-2 inhibitor on proximal convoluted tubular diameter of kidney, amendment by lycopene.

Research Design: Experimental Research.

Abode of Research: Animal House, JPMC, Karachi.

Materials and Methods: 90-120 days old, forty healthy adult male Albino rats of 200-220gm weight were taken for this study and distributed into 4 cliques, set 1 was chosen as control, in Set 2 Celecoxib was given 0.05g/1000g by gavage, in set 3 Celecoxib was given 0.05g/1000g by gavage with lycopene 0.05g/1000g by gavage and set 4 lycopene was given 0.05g/1000g by gavage for 30 days. At accomplishment of study, animals were sacrifice and tissues were preserved for staining.

Results: Inset 2 proximal convoluted tubular diameter became distended due to apoptosis of lining epithelial cells. They turn into low cuboidal from simple cuboidal cells along with ill-defined brush border at the luminal surface due to scarce microvilli and had disrupted basement membrane, however renal structure were amended in set3 which were given celecoxib with lycopene.
Conclusion: This study reveals that lycopene amended the apoptotic changes of set2.

Keywords: Celebrex; nephrotoxicity; tubular necrosis; chemo preventive.

1. INTRODUCTION

NSAIDs are highly effective pain relieving medications, which have anti-inflammatory, antipyretic, and analgesic effects and prevents COX-1 & COX-2 cyclooxygenase enzymes synthesis. They cease the arachidonic acid metabolism by preventing cyclooxygenase (COX) enzyme production that is necessary for prostaglandin (PG) formation [1,2]. Properties of prostaglandins are to regulate renal functions and retain vascular and Broncho dilation, therefore in damage of tissue COX-2 & PGs production activates pain and inflammation [3]. Celecoxib is a COX-2 inhibitor and has decrease adverse effects on GIT but it can raise dysfunction of kidney by interstitial collagen deposition [3,4]. Reduced PGE2 plays an influential role in the pathogenesis of kidney by decreasing fluid metabolism and hemodynamics as well as tissue damage[5,6]. Celecoxib significantly enhanced cell dysfunction as anticancer effect [7-10]. Celebrex has comparable analgesic effect as NSAIDs, but they have decrease adverse effects such as platelet dysfunction and gastrointestinal bleeding [11,12]. It is also effective in postoperative pain [13,14]. It causes tubular necrosis with thicken glomerular basement membranes[15].

Lycopene a representative of carotenoid family, is the most effective antioxidant, antiapoptotic, radical scavenging, and chelating agent, mostly found in fruits and vegetables. It decreases the hazards of mycotoxins, bacterial toxins, and chemical toxins [16]. It protects cellular damage, DNAs, lipids, and proteins caused by free radicals through *in vivo* reaction and neutralization from reactive oxygen species [17,18]. Lycopene has a nephroprotective effect in tubular necrosis [19]. It exhibited a vital chemoprotective prospective against nephrotoxicity[20]. It suppresses oxidative stress and improve damage at cellular level [21].

Tomatoes contain lycopene and flavonoids which can neutralize free radical so that it improves kidney damage. It reduces free radicals 10 times

as compared to vitamin E and 20 times as compared to vitamin C [22]. Because of conjugated double bonds, it fortifies the antioxidant ability, inhibits tumor marker production and prevents lipid peroxidation at cell membrane level [23]. It is proved that lycopene is 100 times more effective than vitamin E in quenching singlet oxygen because of its asymmetric carbon skeleton and unsaturated bonds [24]. It reduces inflammatory cytokines serum level and improved redox steadiness of the Renal tissue [25].

In the meantime, we did not observe experimental study about histo-pathological alterations because of Celecoxib along with antioxidant influence of lycopene, thus this chance is taken to instigate this research work and associate the outcomes with previous studies.

2. MATERIALS AND METHODS

This is a one month research work and forty Albino rats of 3 to 4 Months old, weighing 200-220gm were attained from USA laboratory and reared in JPMC animal house and were numbered, weighed and kept in separate cages. Retained for 1 week to evaluate their general status, earlier the commencement of experiment till the end and separated into four sets and orally administer Celecoxib and lycopene 50 mg/kg. The dose was calculated for each animal according to their weight. In this study Tab. Celecoxib made by Getz Pharma was used in the form of oral preparation by dissolving in normal saline and lycopene soft gel capsule made by General Nutrition Corporation, UK; was given in the form of oral preparation by dissolving in corn oil. Calculated amount of Celecoxib 0.05g/1000g body weight [26] and lycopene 0.05g/1000g body weight [27] according to experimental dose were given to the experimental animals.

- G 1: control.
- G 2: Celecoxib 0.05g/1000g by gavage. (Diseased group)

- G 3: Celecoxib with lycopene 0.05g/1000g by gavage
- G 4: lycopene 0.05g/1000g by gavage

All animal were weighed prior the commencement of experiment and retained in animal house. Throughout the experiment animals were deeply observed for any variation in their general conditions and activities. They were weighed again at the termination of experiment and sacrificed by sedation under ether. A vertical cut was given by penknife. Kidneys were visualized prudently for any change in its gross features and weighed by Sartorius balance. Renal tissue was processed and sections were taken and slides were prepared with H&E stain. SPSS version 20 were used.

3. RESULTS

3.1 G 1

G1 animals were stayed in their best of health, their dietary habits and reaction to Stimuli were satisfactory till the end of experiment.

3.2 G 2

G2 animals observed unhealthy and lethargic. Their dietary habit reduced and reaction to stimulus was listless.

3.3 G 3

G3 animals seemed relatively well, energetic as compare to G2. Their food intake were usual.

3.4 Histological Observation of Renal Tissue

3.4.1 G1

The H&E histological slide of G1 appeared completely normal architecture of renal parenchyma without any indication of degeneration. The luminal surface of renal tubules exhibited low columnar cells with eosinophilic granular cytoplasm and basophilic

spherical nuclei, well-defined brush border, intact basement membrane, glomeruli and Bowman's capsule.(Fig.1a).The mean value of tubular diameter of renal tubules of G1 was 20.33±3.1µm (Table-1, Fig.1a).

3.4.2 G2

The H&E stained histological slide of G1 exhibited different degenerative changes in the renal tissue. Renal tubules were distended because lining epithelial cells became low cuboidal with ill-defined brush border at the apical surface and had interrupted basement membrane. (Fig.1b).

The mean value of tubular diameter of G2 was 65.83±2.6µm. An extremely major increase (P<0.001) was perceived in the mean value of tubular diameter of G2as compare to G1 (Table-1 & Fig.1b).

3.4.3 G3

The H&E stained histological slide of G3 appeared slightly dilated tubules and the lining epithelium with microvilli at the apical surface and intact basement membrane. Occasional pyknosis were detected. (Fig. 1c). The mean value of tubular diameter of G3 was 21.66±4.2µm. There was an extremely major decrease (P<0.001)) was perceived in the mean value of tubular diameter of G3 animals when compared with G2 and minor raise (P>0.05) was detected in the mean value of tubular diameter of G3 as compared to G1 (Table-1 & Fig.1c)

3.4.4 G4

The H&E stained histological slide of G4 presented entirely typical renal architecture. Lycopene doesn't alter the morphology and histology of renal tissue and the outcomes of G4 were analogous G1.

Arithmetical assessment of the mean proximal convoluted tubular diameter of kidney between Albino Rat groups.

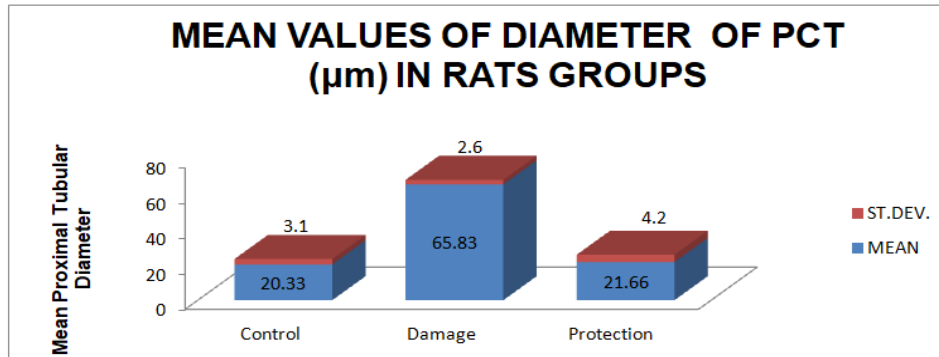
Table 1. Mean values of diameter of Pct (µm) in rats Groups

Groups	Treatment given	Mean value of tubular diameter of proximal tubules(µm)
G1 (n=10)	ND	20.33±3.1
G2(n=10)	Celecoxib	65.83±2.6
G3 (n=10)	Celecoxib + Lycopene	21.66±4.2

Mean±SEM

Arithmetical assessment	P-value
G2 vs. G1	P<0.001
G3 vs. G1	P>0.05
G3 vs. G2	P<0.001

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



Graph 1. Mean values of diameter of pct (µm) in rats groups

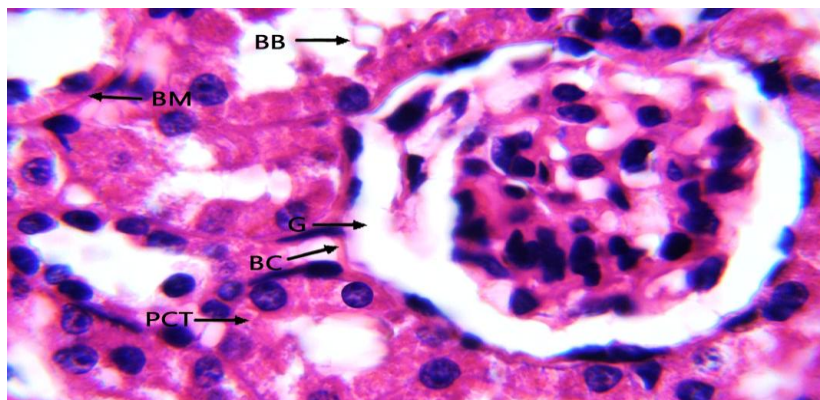


Fig. 1a. Photomicrograph showing normal cytoarchitecture of kidney in G1 at 100x.(BB=basal border, BM= basement membrane, G=glomeruli, PCT=proximal convoluted tubule, BC=ballooning of cell)

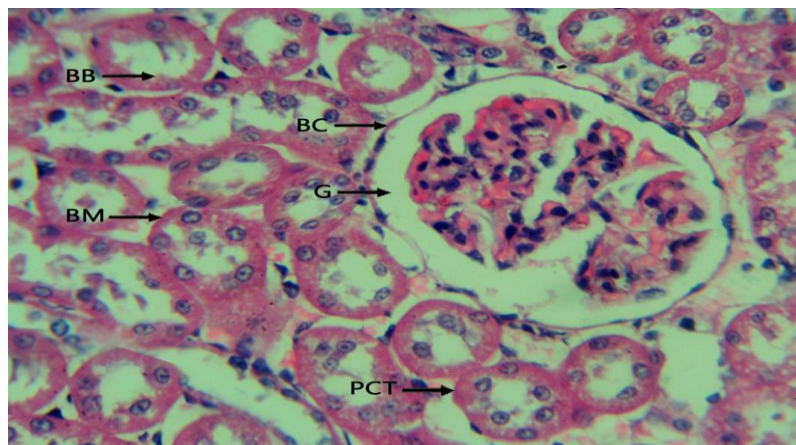


Fig. 1b. H& E stained, 4 µm thick section in G2 shows proximal convoluted tubules, congested and hemorrhagic slightly shrunken glomerulus and ballooning of cells (BC) with pyknotic nuclei (PykN). (Photomicrographx 400)

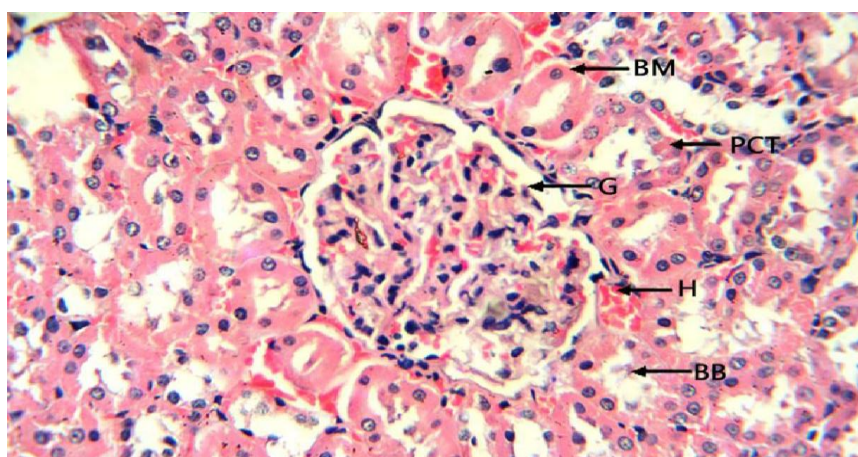


Fig. 1c. Photomicrograph showing preserved cytoarchitecture of kidney glomeruli has less vacuolation, brush border of proximal is restored and not as much of hemorrhage in G3 (lycopene treated) at 40x

4. DISCUSSION

NSAIDs are extremely effective drugs, prescribed globally, which have anti-inflammatory, antipyretic, and analgesic effects. They cease the arachidonic acid metabolism by preventing cyclooxygenase (COX) enzyme production that is necessary for prostaglandin (PG) formation. COX-2 inhibitors like celecoxib can alter microscopic architecture of renal tissue therefore raises dysfunction but minimizes GI problems due to frequent usage[1, 2].

Tomatoes contain lycopene and flavonoids which can neutralize free radical so that it improves kidney damage. It reduces free radicals 10 times as compared to vitamin E and 20 times as compared to vitamin C[22].

G2 animals presented with distended renal tubules because lining epithelial cells became low cuboidal with ill-defined brush border at the apical surface and had interrupted basement membrane. Similar outcomes were also explained by[4,7].

G3 animals presented with slightly distended renal tubules as compared to G2. The lining epithelium had microvilli at the apical surface and intact basement membrane. Occasional pyknosis were noticed because lycopene inhibits degenerative changes in renal tissue. Similar outcomes were also explained by[17,18].

5. CONCLUSION

Experiment concluded that G2 animals had distended renal tubules because lining epithelial

cells became apoptotic but G3 animals presented with occasional pyknosis and slightly distended renal tubules as compared to G2. Hence our conclusion from this experimental study is that cox-2 inhibitor effect renal architecture i.e tissue damage, so please avoid the frequent usage of celecoxib habitually and if obligatory don't use it without lycopene, in order to minimize its detrimental effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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