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CELECOXIB;

PATHOLOGICAL EFFECT ON BODY WEIGHT, ABSOLUTE AND RELATIVE WEIGHT OF KIDNEY WITH PROTECTION BY LYCOPENE IN ALBINO RATS; AN EXPERIMENTAL STUDY.

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ABSTRACT… Objective: To observe the absolute, relative weight of kidney and body weight of albino rats on celecoxib induced kidney with protection by lycopene. **Study Design:** Experimental study. **Place and Duration of study:** This study was conducted in BMSI (Anatomy department), JPMC, Karachi, from 4th May 2015 to 3rd June 2015. **Materials and Methods:** Forty healthy adult, male Albino rats, 90-120 days old, weighing 200-220gm was 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 decreased weight was observed in rats taking celecoxib. Slides which were stained with hematoxylin and eosinshowed general architecture of renal parenchyma, shape and arrangement of epithelial cells. Apoptosis, hemorrhage, necrosis and vacuolation seen in Celecoxib group, whereas renal architecture were ameliorated and reverted back in celecoxib along with lycopene receiving group. **Conclusion:** This study concludes that lycopene restored the body weight, absolute and relative kidney weight in celecoxib treated group.

Key words: Lycopene, Apoptosis, Celecoxib.

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INTRODUCTION

Different group of drugs are clinically used for pain management, inflammation and fever such as NSAIDs, glucocorticoids and disease-modifying ant rheumatic drugs (DMARDs). 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 which is responsible for pain and inflammation. Its production is regulated by enzymeCyclooxygenase. NSAIDs produce their action by inhibiting the production of prostaglandin (PGs) from arachidonic acid, under the control of 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 function of organs. It playsa key role in aggregation of platelets and homeostasis of kidneys.[4] COX-2 enzyme mostly activates in response of injury and inflammatory stimuli.[5,6] It metabolizes arachidonic acid to prostaglandinG_2.[7]

Selective COX-2inhibitors are more preferred for the therapeutic purpose over COX-1 inhibitors as they exhibit lesser side-effects. Celecoxib is a selective COX-2 inhibitor, most widely used analgesic in long-term therapy for pain and inflammation which inhibits the prostaglandin production by inhibiting COX-2 enzyme. It is achemo preventive and chemotherapeutic drug

and associated with angiogenesis and cell proliferation.[8] It is present in thick ascending limb, interstitial cells of papilla and macula densa of kidney.[9] It is associated with nephrotoxic effects like increase serum urea and creatinine.[10]

Celecoxib reduces glomerular filtration rate (GFR) and raise blood pressure.[11,12] It causes renal damage, tubular necrosis, dilatation of renal tubules and shrinking of glomeruli.[13] It inhibits gastrointestinal bleeding, ulcers and reduces gastrointestinal effects of NSAIDS.[14]

Dietary antioxidant bioflavonoid is one of the most common antioxidants which prevents oxidative damage caused by free radical. Lycopene is a bioflavonoid, having highest oxygen-quenching capacity. It elevates the serum level of catalase, glutathione and superoxide dismutase in renal tissue.[17] It gives red color to tomatoes and found in fruits and vegetables like watermelon, pink guavas and pink grape fruits etc.[18,19] It reduces the risk of cardiovascular diseases, glomerulosclerosis, congestion of vessels and diabetes.[20,21] It is a chemo preventive agent and effective in the prevention of cancer such as mammary tumor and prostatic cancer etc.[22] It prevents lipid peroxidation and decreases the risk of multiple sclerosis, age-related macular degeneration, and atherosclerosis etc.[23,24]

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. Lycopeneis a bioflavonoid, which is closely related to beta-carotene. Lycopenehas been shown to have 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 May2015-3rd June2015), in the Department of Anatomy, BMSI, JPMC, Karachi. Fortyhealthy adult Albino rats, 90-120 days old, weighing 200-220gm wereobtained 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 manubrumsterni 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.

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.

OBSERVATIONS ON BODY WEIGHT (G):

The mean value of initial body weight of control Group-A was 200.0±2.12gm and the final body weight was 202.0±0.83gm. No significant increase (P□0.05) was observed when initial body weight was compared with the final body weight of Group A (Table-I, Figure-1)

During the study, group B which was administered by celecoxib showed highly significant decrease in body weight as compare to control group A(P-0.001), while group C which was treated with lycopene showed highly significant increase in body weight as compare to group B(P-0.001) (Table-I, Figure-1).

OBSERVATIONS ON ABSOLUTE WEIGHT (G):

There was a significant increase (P□0.05) in the absolute weight of kidney observed in Group-B, when it was compared with Group-A. There was a significant decrease (P□0.05) in the absolute weight of kidney of Group-C, when it was compared with Group-B (Table-II, Figure-2).

OBSERVATIONS ON RELATIVEWEIGHT (G):

A highly significant increase (P□0.001) observed in the relative weight of kidney in Group-B animals, when it was compared with Group-A, whereas moderately significant decrease (P□0.005) in the relative weight of kidney in Group-C animals was observed, when it was compared with Group- B (Table-III, Figure-3).

Groups	Treatment given	Initial body weight	Final body weight	P-value	Statistical comparison	P-value
A (n=10)	ND	200.0±2.12	202.2±0.83	P>0.05*	B vs. A	P<0.001****
B (n=10)	Celecoxib	205.0±4.12	189.2±1.92	P<0.005***	C vs. A	P<0.005***
C (n=10)	Celecoxib + Lycopene	196.6±2.07	198.8±0.83	P>0.05*	C vs. B	P<0.001****

Table-I. Mean body weight (g) in different groups of albino rats

*Mean±SEM

Statistical analysis of the difference in the mean body weight within the same group and between different experimental groups of Albino Rats.
Non-significant*
Significant**
Moderately significant***
Highly significant****

*Mean±SEM
Statistical analysis of mean absolute weight of the kidney between different experimental groups of Albino Rats.
Non-significant*
Significant**
Moderately significant***
Highly significant****

*Mean±SEM
Statistical analysis of mean relative weight of kidney between different experimental groups of Albino Rats.
Non-significant*
Significant**
Moderately significant***
Highly significant****

Groups	Treatment given	Mean absolute kidney weight	Statistical comparison	P-value
A (n=10)	ND	0.82±0.10	B vs. A	P<0.05**
B (n=10)	Celecoxib	0.94±0.09	C vs. A	P<0.05**
C (n=10)	Celecoxib + Lycopene	0.91±0.07	C vs. B	P<0.05**

Table-II. Mean absolute weight of kidney (g) in different groups of albino rats

Groups	Treatment given	Mean relative weight of kidney	Statistical comparison	P-value
A (n=10)	ND	0.39±0.01	B vs. A	P<0.001****
B (n=10)	Celecoxib	0.51±0.06	C vs. A	P<0.001****
C (n=10)	Celecoxib + Lycopene	0.48±0.02	C vs. B	P<0.005***

Table-III. Mean relative weight of kidney (g) in different groups of albino rats

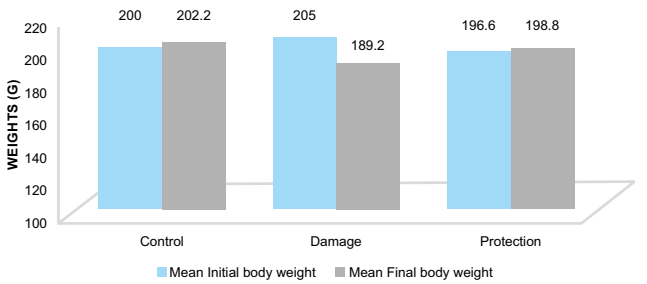

Figure-1. Mean initial & final body weight (G) in different groups of albino rats

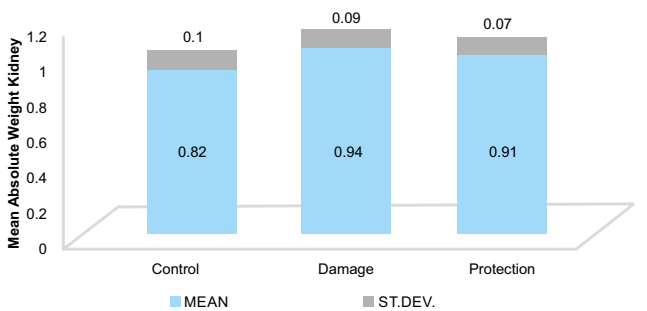

Figure-2. Mean absolute weight of kidney (G) in different groups of albino rats

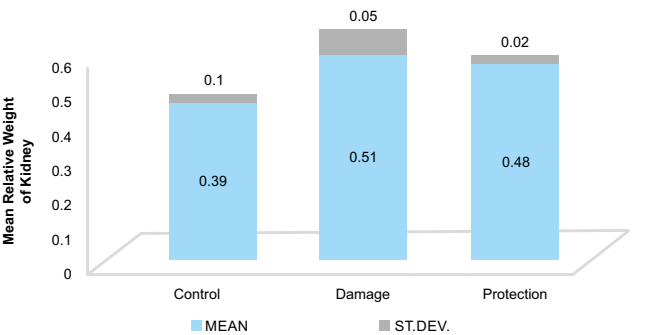

Figure-3. Mean relative weight of kidney (G) in different groups of albino rats

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).

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]

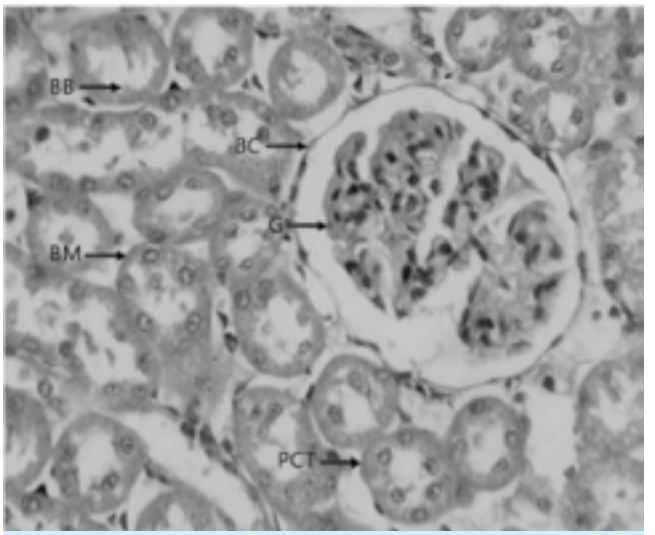

Figure-1a. Photomicrograph showing normal cytoarchitecture of kidney normal glomerulus, proximal and distal renal tubules in control group-A at 40x

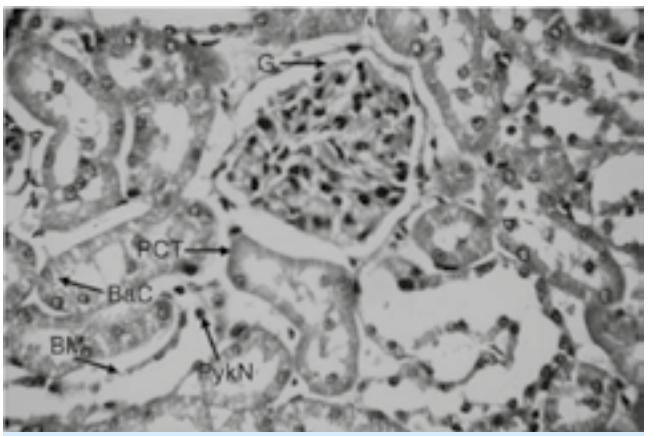

Figure-2a. Photomicrograph showing altered cytoarchitecture of kidney glomerulus has vacuolation, cells disrupted, apoptosis, brush border of proximal is absent and hemorrhagein group-B(celecoxib treated) at 40x

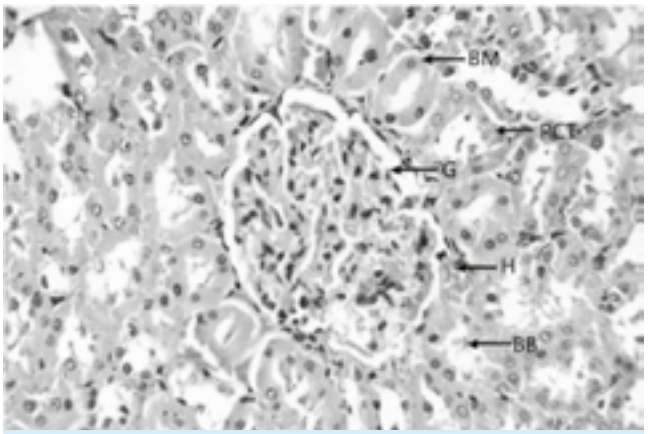

Figure-3a. Photomicrograph showing preserved cytoarchitecture of kidney glomeruli has less vacuolation, brush border of proximal is restored and not as much of hemorrhage in group-C (lycopene treated) at 40x

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]

The animals of celecoxib treated group B were lethargic and lost their body weight due to suppression of cell proliferation and angiogenesis.[6] It suppresses the anti-oxidant status by reducing glutathione content and enzymatic activity of superoxide dismutase. It raises TNF and free radicals, which caused apoptosis of cells and thus caused decreased body weight.[26]

The lycopene protected animals of group C were as active and similar to control and reduced less body weight than group B animals because it prevents oxidative damage to the tissue and reduces apoptosis of cells.[27,28] Lycopene produced

significant reduction in malondialdehyde (MDA) and elevation of glutathione (GSH) and catalase (CAT) activity, thus it improves health and digestive functions, thus increased the body weight. It provides protection against cellular damage caused by reactive oxygen species.[29,30]

A highly significant increase in absolute weight and relative weight of kidney in celecoxib treated animals due to decreased production of prostaglandin E2 in the kidney and inhibition of PGE2 lead to sodium retention, renal hypertrophy and thickening of glomerular basement membrane, thus increased the absolute and relative kidney weight.[13] A moderately significant decrease in the absolute and relative kidney weight was found in group-C animals because lycopene prevented the fibrosis and hydronephrosis of kidney thus decreased the absolute and relative weight of kidney to near normal.[31,32]

CONCLUSION

The above mentioned study concluded that celecoxib treated group showed significant decrease in body weight and increase in absolute and relative weight of kidney while celecoxib+lycopene treated group showed increase in body weight and decrease in absolute weight and relative weight of kidney. 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.

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Losers quit when they are tired.
Winners quit when they have won.

– Unknown –

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Sadia Sundus	Conceived manuscript, manuscript writing.	
2	Nazia Qamar	Final approval of article.	
3	Raheela Adil	Literature review.	
4	M. Faisal Fahim	Statistical analysis and review article.	