Prophylactic Role of Curcumin against Tubular Atrophy caused by Aspirin in Kidneys of Adult Female Albino Rats

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ABSTRACT

Introduction: Aspirin the inhibitor of prostaglandin and thromboxane synthesis is used to relieve fever, inflammation and pain. Curcumin that exhibits anti-inflammatory, antioxidant and anti-atherosclerotic properties is obtained from the rhizome of curcuma longa plant. **Aims & Objectives:** To evaluate the nephroprotective effects of curcumin against tubular atrophy caused by aspirin in kidneys of adult female albino rats. **Place and duration of study:** The study was conducted in Anatomy Department of Federal Postgraduate Medical Institute, Lahore for 30 days. **Material & Methods:** 4 groups were made containing 15 rats in each group. Nephrotoxicity in the rats was induced with aspirin given by orogastric intubation (200 mg/kg bodyweight) in positive control group B and experimental groups C & D. Then rats of experimental groups C & D in addition to aspirin received 15 mg/kg & 30 mg/kg body weight of curcumin by orogastric intubation. **Results:** On histopathological examination, the proximal and distal convoluted tubules were observed normal in control group A (Fig-1) Atrophic changes in the proximal and distal convoluted tubules along with damage to the tubular epithelial cells were found in 11 rats (73.3%) in positive control group B, 4 rats (26.7%) in experimental group C and 3 rats (20.0%) in experimental group D (Fig-2, 3 & 4). The difference was found highly significant with p-value <0.001 (Table-1). **Conclusion:** The present study has shown that curcumin exerts profound effects in reducing the damage to proximal and distal convoluted tubules caused by aspirin in a dose dependent manner.

Key words: Aspirin, curcumin, tubular atrophy, proximal convoluted tubules, distal convoluted tubules, kidneys, adult female albino rats

INTRODUCTION

Aspirin has emerged as a cornerstone therapy available for treating pain, inflammation and cardiovascular diseases. Its history dates back to the use of willow bark by the Egyptian healers. Aspirin produces nephrotoxicity by inhibiting the synthesis of prostaglandins.¹ It inhibits the activity of the enzyme cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).² Aspirin causes atrophy of tubular epithelial cells by ischemia, atrophic changes in the proximal & distal convoluted tubules in the renal cortex and the inflammatory changes in the renal medulla.³

Medullary ischemia is the initial event in the development of nephrotoxicity. NSAIDs inhibit prostaglandin synthesis that decreases renal medullary blood flow.⁴ Studies have shown that sexually mature female rats are more predisposed to

aspirin induced nephrotoxicity than male and immature female rats.⁵ It has been found that females have lower concentration of serum aspirin esterase levels which degrade aspirin more slowly.⁶ Aspirin has various therapeutic uses in the prevention of cardiovascular diseases, the prevention & treatment of Alzheimer's disease and chemoprevention of colorectal cancer.⁷ It causes gastrointestinal disturbances which include nausea, vomiting, heartburn, indigestion, peptic ulcers and gastrointestinal bleed. Aspirin is contraindicated in patients with peptic ulcer disease, gastrointestinal bleeding, alcoholic intoxication and cerebral hemorrhage.⁸

Turmeric that possesses versatile properties belongs to Zingiberaceae family.⁹ Curcumin a chemical constituent of turmeric powder is a polyphenol obtained from the rhizome of the plant Curcuma Longa.¹⁰ It is used as a cooking spice and adds

flavor to the food.¹¹ Curcumin is frequently used in traditional Ayurvedic medicine. It has been extensively cultivated in Asian countries predominantly in India and China.¹² The commercially produced curcuminoid complex is composed of 71.5% curcumin I (diferuloylmethane), 19.4% curcumin II (demethoxycurcumin) and 9.1% curcumin III (bisdemethoxycurcumin).^{13,14} It also contains volatile oils, sugars, proteins and resins. The curcuminoid complex due to its resemblance with the saffron is also known as "indian saffron" and can be used as a substitute.¹⁵ Curcumin possesses anti-inflammatory, anti-atherosclerotic,¹⁶ antibacterial, antioxidant, antispasmodic, anti-protozoal, antihuman immunodeficiency virus and nematocidal activities.¹² The beneficial effects of curcumin were documented in one study for the treatment of swelling and sprains when applied locally over the affected area.¹⁷ Curcumin helps in wound healing and muscle regeneration, prevents gall stone formation and cataract, inhibits scarring, prevents liver and kidney toxicity, septic shock, cardiovascular disease, inflammatory bowel disease,¹⁸ and arthritis.¹⁹ Curcumin restores brain derived neurotrophic factors thus causing significant reversal of depression like behavior.¹⁸ It prevents DNA damage by inhibiting free radical generation and lipid peroxidation.²⁰ Curcumin reduces the profibrotic cytokines, VEGF, transforming growth factor-beta (TGF- β), connective tissue growth factor (CTGF), extracellular matrix proteins, fibronectin and collagen type IV. It also prevents structural damage by reducing glomerulosclerosis Index (GI), tubulointerstitial fibrosis and arteriolopathy.²¹ Curcumin down regulates the activity of cytochrome P450 that can lead to toxicity of some drugs.²² Various evidences indicate that curcumin acts as a bifunctional antioxidant with both direct and indirect antioxidant effects, such as scavenging reactive oxygen species and inducing the expression of cytoprotective proteins. Hence preventing oxidative stress and reducing anti-oxidant enzymes²³ which could be beneficial against drug induced nephrotoxicity. In previous studies the prophylactic role of curcumin against other nephrotoxic drugs has been observed.

The present study was designed to evaluate the prophylactic effects of curcumin against tubular atrophy caused by aspirin in kidneys of adult female albino rats.

MATERIAL AND METHODS

This experimental study was conducted in the Department of Anatomy, Federal Postgraduate Medical Institute, Lahore.

All rats were kept in cages in the animal house of the Department of Anatomy, PGMI, Lahore. The rats were allowed free access to food and water. A 12 hourly light: dark cycle was maintained at room temperature of 27°C. After acclimatization for 15 days, these rats were kept in separate cages divided into 4 groups A, B, C and D with proper labeling. 15 rats were distributed in each group. Body weight was measured before and at the end of experiment. Blood samples were taken from the rat's tail before the experiment and then on 15th and 30th day of experiment, for serum creatinine levels of all four groups. A lignocaine cream as a local anaesthetic was applied to the site 30 minutes prior to blood sampling.²⁴

Turmeric extract was prepared from PCSIR laboratory, Lahore. Quantification of extract was done through Gas Chromatography-Mass Spectrometry (GC-MS) from Chemistry Department of Forman Christian College, Lahore.

Turmeric extract was given to the rats through orogastric intubation.

Group A (Healthy Control Group): The rats received 10ml/kg body weight/day of distilled water.

Group B (Positive Control Group): 100mg/kg body weight/day of aspirin for 30 days was given.

Group C (Experimental Group I): 100mg/kg body weight/day of aspirin and 15mg/kg body weight/day of curcumin simultaneously for 30 days were given.

Group D (Experimental Group II): 100mg/kg body weight/day of aspirin and 30mg/kg body weight/day of curcumin simultaneously for 30 days were given.

After dissection, the kidneys were removed and studied histologically for the tubular damage.

Statistical analysis:

The data was entered and analyzed by using SPSS 22.0. The qualitative data for the tubular damage was stated by using frequency & percentage of each group. Chi-square test was used for the comparison among groups. P-value ≤ 0.05 was considered statistically significant.

RESULTS

The proximal and distal convoluted tubules were found normal in control group A (Fig-1) Atrophic changes in the proximal and distal convoluted tubules along with damage to the tubular cells were observed in 11 rats (73.3%) in positive control group B followed by 4 rats (26.7%) and 3 rats (20.0%) in experimental groups C & D respectively (Fig-2, 3 & 4). The difference was found highly significant with p-value <0.001. (Table-1)

Fig-1: Photomicrograph of kidney of adult albino rat of control group A showing: Glomerulus(G), Urinary space (US), Blood vessel (BV), Proximal convoluted tubule (PCT) and Distal convoluted tubule (DCT) (H&E,20x)

Fig-2: Photomicrograph of kidney of adult albino rat of positive control group B showing: Glomerulus (G), cytoplasmic vacuolization of Proximal convoluted tubule (blue arrow) and tubular atrophy of Distal convoluted tubule (green arrow). (H&E,20x)

Fig-3: Photomicrograph of kidney of adult albino rat of experimental group C showing less changes in: Blood Vessels (BV), Proximal convoluted tubule & Distal convoluted tubule. (H&E,20x)

Fig-4: Photomicrograph of kidney of adult albino rat of experimental group D showing: Glomerulus (G), least changes in the Proximal convoluted tubule & Distal convoluted tubule (H&E,20x)

	Tubular Damage					
	Present		Absent		Total	
	N	%	N	%	N	%
Group A	0	0.0	15	100.0	15	100.0
Group B	11	73.3	4	26.7	15	100.0
Group C	4	26.7	11	73.3	15	100.0
Group D	3	20.0	12	80.0	15	100.0

Chi-square = 23.50 p-value < 0.001

Table-1: Comparison of tubular damage among control groups given aspirin and experimental groups given aspirin & curcumin

The group wise comparison of tubular damage revealed a significant difference among control group A & experimental groups C and D from positive control group B with p-values <0.001, 0.028 and 0.010 respectively. The difference between control group A and experimental group C had a p-value 0.100. (Table-2 & Fig-5)

(I) Group	(J) Group	Chi-square	Df	P-value
Group A	Group B	14.35	1	<0.001**
	Group C	2.60	1	0.100
	Group D	1.48	1	0.224
Group B	Group C	4.80	1	0.028*
	Group D	6.56	1	0.010*
Group C	Group D	0.00	1	1.000

Table-2: Group wise comparison of tubular damage among control groups given aspirin and experimental groups given aspirin & curcumin (by using chi-square)

KEY

- A Control Group of rats
- B Positive control Group of rats
- C Experimental Group I of rats
- D Experimental Group II of rats
- N Number of rats in each group
- Df Degrees of freedom
- ** Highly significant difference ($P < 0.001$)
- * Significant difference ($P < 0.05$)
- + Insignificant difference ($P > 0.05$)

Fig-5: Graphic comparison of tubular damage among control groups given aspirin and experimental groups given aspirin & curcumin

DISCUSSION

Aspirin has been used as a famous, cheap and readily available drug. It shows versatile properties such as an anti-inflammatory, anti-platelet, antipyretic & analgesic actions.^{3,8} Curcumin is a spice, additive and a pigment commonly used in Asia.²⁵ In the present study the atrophic changes in the proximal and distal convoluted tubules along with damaged tubular epithelial cells in the cortex of the kidney were found in more number of rats in positive control group B followed by experimental groups C and D. The difference of damage of proximal and distal convoluted tubules among the four groups was found significant with p-value

<0.001 (Table-1). These observations were consistent with the results of the study performed by Molland et al. who observed tubular atrophy and an interstitial inflammatory cell infiltrate after aspirin administration. It may be secondary to tubular obstruction by fibrosis or may involve a direct toxic effect of aspirin on cortical tubules.²⁶ These results are also similar with the findings performed by Neha et al, Owen et al & Calder et al who proved that aspirin causes damage of the proximal and distal convoluted tubules in the cortex.²⁷ The underlying mechanism of tubular damage involves tubular vacuolization. It arises due to hypoxia, mitochondrial injury, free radical production and decreased production of ATP.²⁸ Acute tubular injury involves attenuation of the brush borders of the proximal tubules and detachment of the tubular cells from the underlying basement membranes.²⁹ TGF-beta has been shown to play a major role in the induction of tubular atrophy.³⁰

Less tubular changes were observed in the experimental groups C & D given curcumin in addition to aspirin. These findings are in relevance with the results of Tapia et al who states that curcumin has regenerative potential due to its antioxidant properties.³¹ Administration of curcumin restores the degenerated renal tubules and preserve normal renal structure.³²

CONCLUSION

The results of the present study indicated that the co-treatment of aspirin with the ethanolic extract of turmeric prevented aspirin induced damage of the proximal and distal convoluted tubules in adult female albino rats.

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