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# Effects of Ibuprofen on Kidneys of Albino Rats.

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**ABSTRACT**

Ibuprofen is one of the most commonly prescribed Non-steroidal anti-inflammatory drug in osteo-arthritis, rheumatoid arthritis, fever, mild to moderate pain and primary dysmenorrhoea. Due to its easy accessibility, abuse of the drug is very common, leading to its various side effects. The present study was designed to see the effects of Ibuprofen on kidneys of albino rats over scheduled period of time at therapeutic doses. Total of 72 albino rats were divided equally into control and experimental groups and treated with distilled water and 50 mg of ibuprofen for six weeks respectively. The effects were seen every week by sacrificing six from the experimental group and six from the control group. Morphological assessment of Hematoxylin and Eosin stained sections of the kidney tissues were done along with micrometry. The different morphological changes including shrunken glomeruli, increased capsular spaces, congested blood vessels along with dilated and thickened renal tubules in later weeks of treatment. Interstitium revealed moderate fibrosis and edema with chronic inflammatory cell infiltrate. Ibuprofen causes tubulo-interstitial nephritis on prolonged use.

**Keywords:** Non-steroidal anti-inflammatory drugs, Micrometry, Tubulo-interstitial nephritis, Ibuprofen.

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## INTRODUCTION

Pain is an unpleasant sensation occurring in varying degrees of severity as a consequence of injury, disease or emotional disorder. It has both neurophysiological and psychological components. Pathophysiological mechanisms involve neural pathways and a variety of pain producing substances and modulating mechanisms. The products implicated in pain genesis are acetylcholine, serotonin, histamine, bradykinin, prostaglandins, substance P, somatostatin, cholecystokinin, vasoactive intestinal polypeptide, cytokine like IL-1, platelet activating factor, noradrenaline and endogenous opioid peptides.

Ibuprofen is one of the most commonly prescribed NSAID in osteo-arthritis and rheumatoid arthritis [1]. They are one of the largely prescribed drugs in fever, mild to moderate pain and primary dysmenorrhea and their toxic effects are also frequent. The side effects are widespread, includes GI dyspesia, peptic ulceration, nephrotoxicity, dermatological lesion, central nervous system effects like change of mood, hallucination. However, spectrum of nephrotoxicity is the most frequently encountered side-effect associated with different types of analgesic abuse [2]. Spuhler and Zollinger had first described analgesic induced chronic interstitial nephritis [3]. Keeping the above facts in mind, the present study was designed to see the effect of ibuprofen on kidney of albino rats over a scheduled period of time at therapeutic doses. The histomorphologic changes of renal parenchyma and micrometry findings of different segments of renal tubules were correlated with the finding of previous workers.

## MATERIALS AND METHODS

The present study was undertaken in the Department of Anatomy with 72 albino rats as subjects after ethical clearance. All rats were approximately of same weight (150-200gm), kept in similar environmental and dietary conditions. The rats were divided into Experimental Group and Control Group comprising of 36 rats each. The control group received 2 ml of distilled water in two divided doses. The experimental group was given 50 mg (120mg/kg) which is the therapeutic dose of Ibuprofen, dissolved in 2 ml of distilled water per day, orally in two divided doses.

Six rats from each group (control and experimental) were sacrificed at weekly intervals for six weeks. The animals were given chloroform anaesthesia and abdomen was opened. Kidneys were taken out, formalin fixed and processed. The histology slides were prepared with H&E stain. Slides were seen under the light microscope and the measurements were taken using the ocular micrometer.

The ocular micrometer was standardized and calibrated with a stage micrometer slide. The ocular lens containing the micrometer disc was placed on the microscope. The object to be measured was focused and the reading was taken in ocular units. The number of ocular units was multiplied by the calibration factor for that specific microscope, objective and ocular micrometer.

The calibration of ocular micrometer was done by comparing ocular micrometer scale with a calibrated stage micrometer. The stage micrometer is a microscope slide that has a carefully calibrated scale each division of which measures 0.01mm units. The calibrated stage micrometer was placed on the stage and its zero line was adjusted with that of ocular micrometer. Then another point was noted where two lines of both micrometer scale coincides. The number of divisions on stage micrometer and ocular divisions on ocular micrometer was noted.

$$\text{Calibration factor (µm/ocular unit)} = \frac{\text{Stage micrometer divisions (mm)} \times 1000 \text{ um}}{\text{Ocular micrometer divisions (mm)}}$$

In malpighian corpuscle the diameter of the Bowman's capsule, thickness of the capsular space and diameter of the glomerulus were measured. For tubules, diameter, wall thickness and lumen size were measured. The statistical analyses were done using one way ANOVA with SPSS (Stastical Packages for Social Studies) software.

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## RESULTS

The present study was designed to analyse the effect of Ibuprofen, a commonly used NSAID for the treatment of variety of common chronic and acute inflammatory conditions on the kidney of the albino rats, comparing the treated group with the control. The histological morphometry of kidney tissue were studied over six weeks period [Table 1-5] and relevant statistical analyses were done to substantiate the significance of the study.

In H &E sections, malpighian corpuscle showed changes in $5^{th}$ and $6^{th}$ week like shrunken glomrulie, increase in capsular space (Fig. 1) as compared to the control group (P value <0.0001). Proximal and distal convoluted tubules were dilated with vacuolated lining epithelial cells (Fig. 2). Loop of Henle and collecting tubule were dilated with decrease lumen size. The changes were mild in $1^{st}$ two weeks and gradually increased and associated with hyalinization and necrosis. Tubular morphology was not clear in many tubules in $6^{th}$ group.

Interstitium of renal cortex was infiltrated with sparse inflammatory cells in early weeks. In $5^{th}$-$6^{th}$ week, the interstitium showed significant oedema and areas of fibrosis with dilated and congested blood vessels (Fig. 3).

The P values for different segment of renal tubules are < 0.0001, considered significant. Variation among column means is significantly greater than expected by chance. Confidence Intervals are 95%.

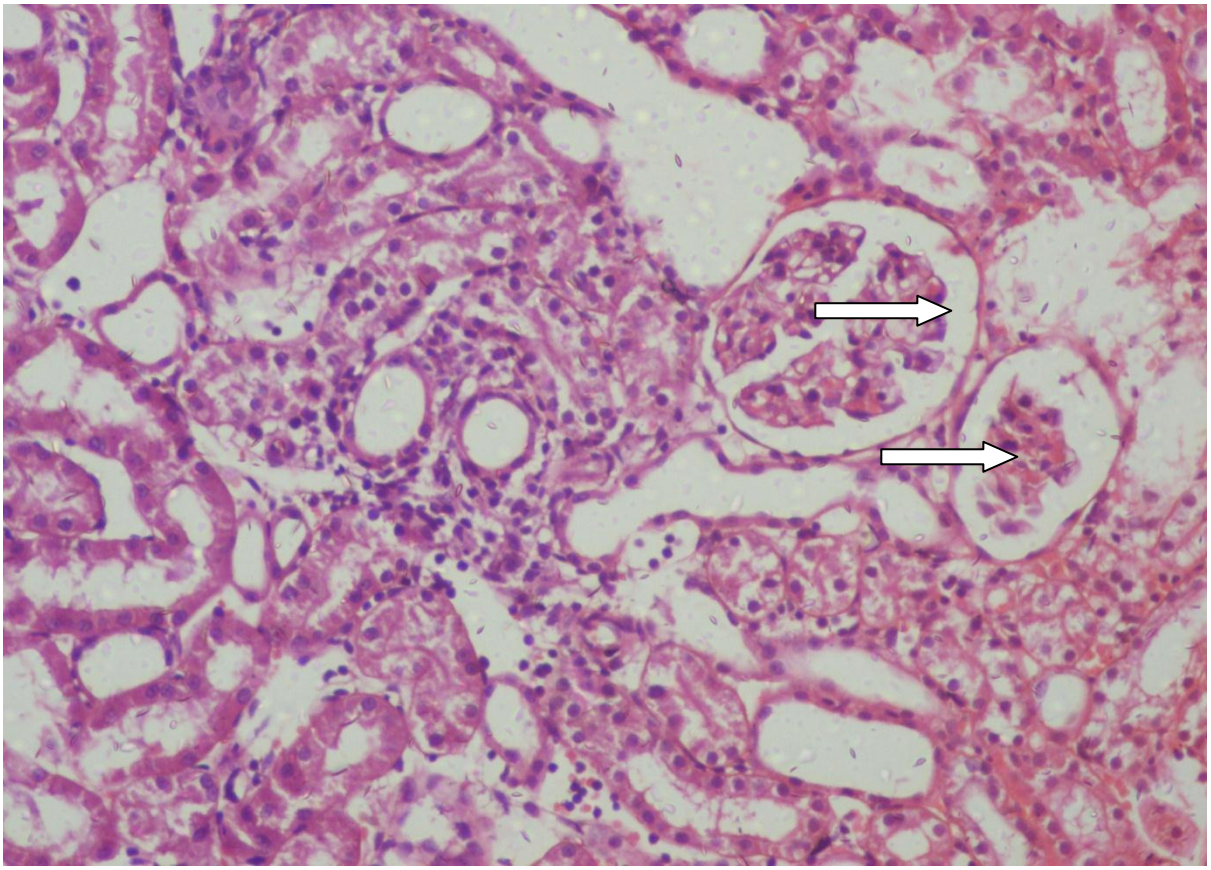

**Figure 1: Bowman's capsule showing grossly increased capsular space & shrunken glomeruli**

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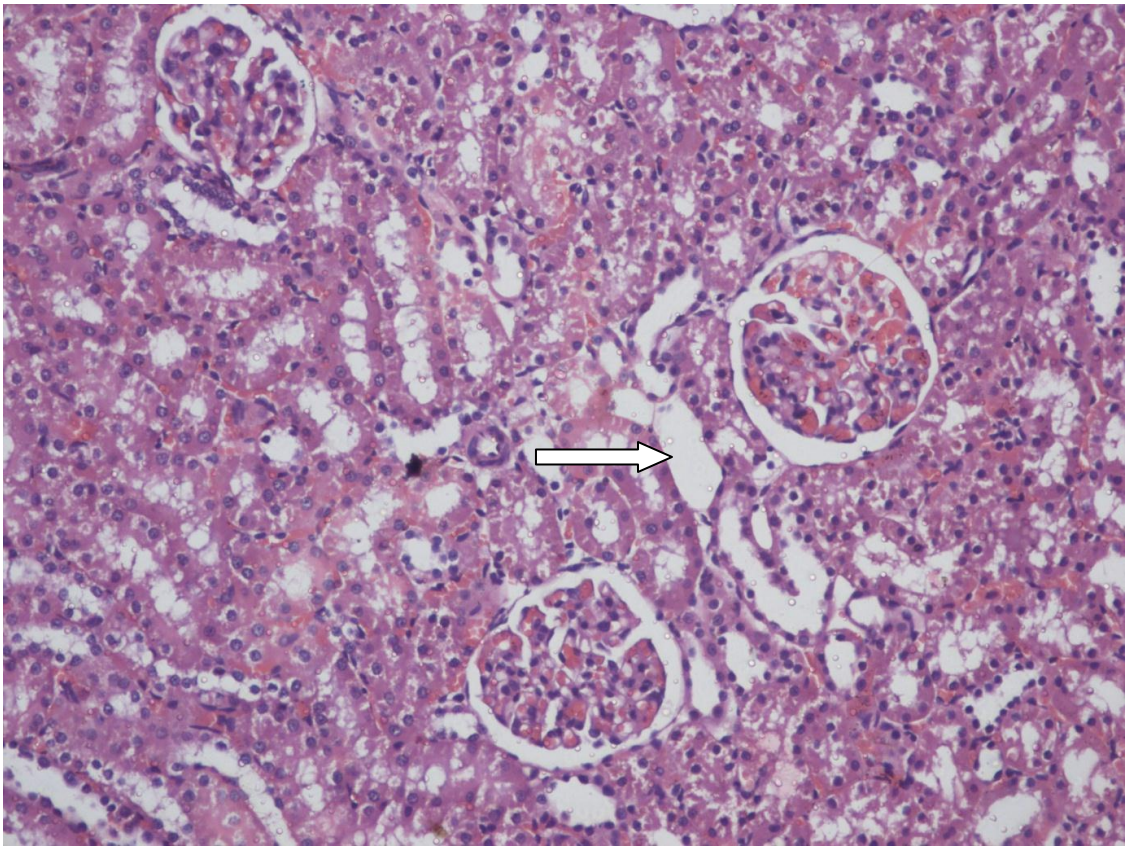

**Figure 2: Oedematous Interstitium & hydropic degeneration of epithelial cells of the tubules**

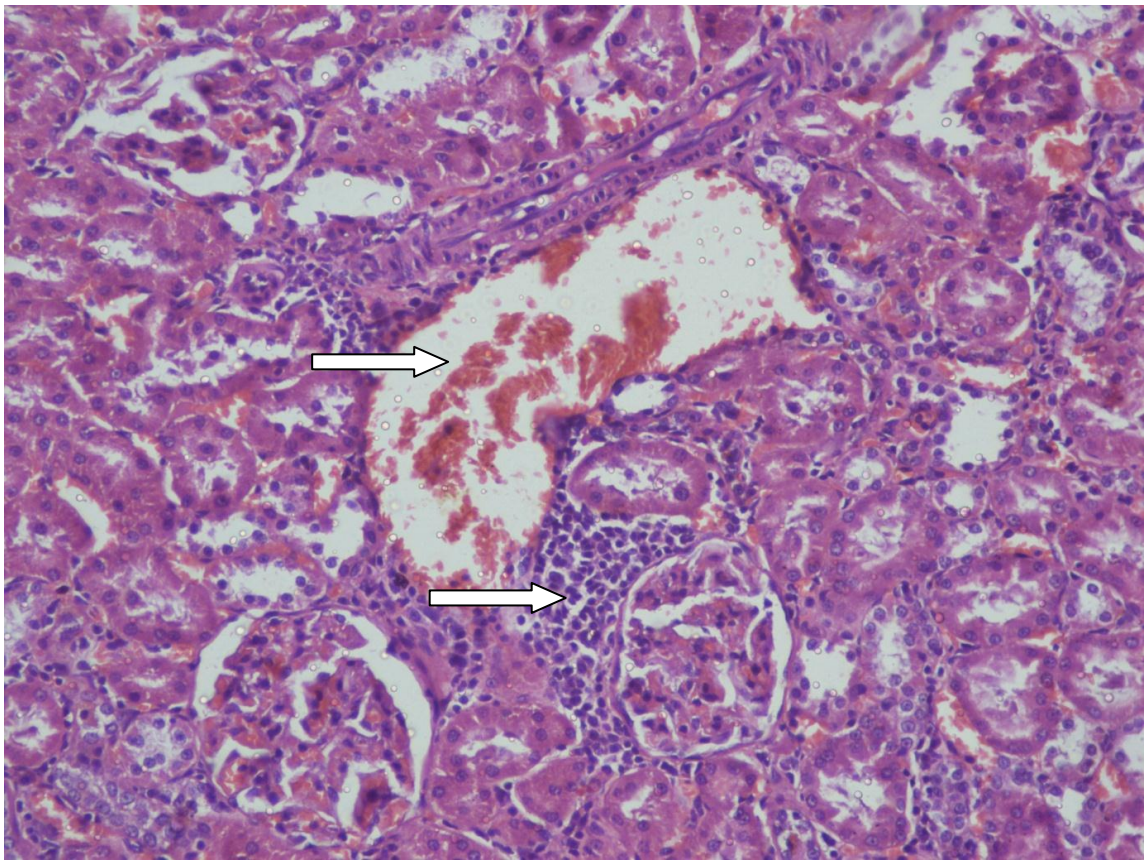

**Figure 3: Dilated and congested blood vessels with deep inflammatory infiltration**

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**Table 1: Changes in Malpighian Corupscle**

| Duration (in weeks) | Diameter Bowman's Capsule (in µm) | | Capsular space (in µm) | | Glomerulus (in µm) | |
|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated |
| I | 88.2 | 87.8 | 11.4 | 10.6 | 65.8 | 66.1 |
| II | 86.4 | 86.8 | 8.5 | 8.5 | 62.5 | 63.4 |
| III | 84.4 | 84.3 | 8.6 | 8.6 | 58.4 | 58.5 |
| IV | 82.5 | 83.6 | 8.8 | 8.7 | 58.8 | 58.6 |
| V | 83.5 | 83.2 | 8.8 | 17 | 59.3 | 48.5 |
| VI | 84.3 | 83.3 | 9.3 | 20 | 61.2 | 44.5 |

**Table 2: Proximal Convoluted Tubule**

| Duration (in weeks) | Diameter (in µm) | | Thickness (in µm) | | Lumen (in µm) | |
|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated |
| I | 28 | 31.5 | 7.5 | 11.5 | 12.9 | 9.9 |
| II | 24.5 | 30.1 | 6.1 | 10.1 | 11.7 | 7.6 |
| III | 24 | 32.5 | 6.4 | 13.5 | 11.4 | 6.5 |
| IV | 24.8 | 39.6 | 7.1 | 15.8 | 13.3 | 5.6 |
| V | 24.5 | 40.7 | 6.9 | 16.8 | 12.9 | 5.8 |
| VI | 25 | 46.7 | 8.4 | 20.5 | 11.9 | 4.8 |

**Table 3: Distal Convoluted Tubules**

| Duration (in weeks) | Diameter (in µm) | | Thickness (in µm) | | Lumen (in µm) | |
|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated |
| I | 24.9 | 30.7 | 6.3 | 11.2 | 10.8 | 7.4 |
| II | 27.4 | 30.78 | 8 | 11.8 | 11.7 | 7 |
| III | 28.1 | 35.3 | 8.4 | 14.2 | 13.6 | 6.5 |
| IV | 24 | 42 | 7.5 | 18.4 | 12.3 | 4.3 |
| V | 23.5 | 44.8 | 7.4 | 23 | 10.8 | 3.9 |
| VI | 22.3 | 46.3 | 7.9 | 24.9 | 10 | 3.2 |

**Table 4: Collecting Tubule**

| Duration (in weeks) | Diameter (in µm) | | Thickness (in µm) | | Lumen (in µm) | |
|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated |
| I | 20.1 | 31.1 | 5.9 | 14.4 | 9.4 | 6 |
| II | 23 | 31.3 | 6.2 | 16.2 | 8.4 | 5.6 |
| III | 20.9 | 35.1 | 7.2 | 14.5 | 9.1 | 5.1 |
| IV | 20.5 | 44.6 | 7 | 20.5 | 10 | 4.2 |
| V | 20.6 | 45.3 | 6.7 | 21.5 | 9.5 | 3.3 |
| VI | 20.4 | X | 6.3 | X | 6.5 | X |

**Table 5: Blood Vessels**

| Duration (in weeks) | Thickness (in µm) | |
|---|---|---|
| | Control | Treated |
| I | 46.4 | 46.3 |
| II | 44.8 | 66.5 |
| III | 44.7 | 71.5 |
| IV | 46 | 75 |
| V | 50.9 | 70 |
| VI | 48.3 | 80 |

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## DISCUSSION

Nonsteroidal anti-inflammatory drugs (NSAIDS) are most commonly prescribed medication for pain management [4]. Most NSAIDS act as non-selective inhibitor of the enzyme cyclo-oxygenase inhibiting both COX - 1 and COX - 2 isoenzymes. This enzyme catalyzes the formation of prostaglandins from the precursor of arachidonic acid.

Prostaglandins maintain renal blood flow (RBF) and glomerular filtration rate (GFR) especially in fluid depleted states. Locally synthesized prostaglandins $PGI_2$ (Prostacyclin), $PGE_2$ and $PGD_2$ cause vasodilation, diminish vascular resistance and enhance renal perfusion with redistribution of blood flow from the renal cortex to nephrons in the Juxtamedullary region, $PGE_2$ and to a linear extent $PGF_{2\alpha}$ cause diuresis and natriuresis by inhibiting the transport of sodium and chloride in thick ascending limb of loop of Henle and the collecting ducts. PGE-1 tends to antagonise the action of antidiuretic hormone (Vasopressin). Lastly prostacyclin in concert with $PGE_2$ serves to maintain the glomerular filtration rate.

In volume depleted states, renin-angiotensin - aldosterone axis is stimulated with increased renin, angiotensin and aldosterone production resulting in renal vasoconstriction and increased sodium & chloride reabsorption. There is increased sympathetic outflow which increases vascular tone. In this setting prostaglandins provide compensatory vasodilation of renal vasular bed and ensure adequate renal blood supply precluding acute renal functional deterioration. $PGE_2$, $PGD_2$ and to lesser degree prostacyclin causes vasodilation by depressing norepinephrine release. $PGE_2$ antagonizes the vasoconstrictive action of angiotensin II on afferent arterioles. NSAIDs by inhibiting prostaglandin synthesis promotes unopposed vasoconstrictive action of leukotrienes, angiotensin II, vasopresin, endothelin and Catecholamines. This produces different nephrotoxic effects of NSAIDS [5-7].

In the present study, effects of Ibuprofen were observed on histomorphology of renal parenchyma of albino rats. Malpighian corpuscles were affected with longer duration of treatment($5^{th}$-$6^{th}$ week), with moderate increase in the capsular space and decrease in glomerular size, unlike work by *Yasmeen et al. and Ejaz et al.* , which describes tubulo-intestitial lesion without glomerular involvement [8,9].

There was significant increase ($p<0.001$) in diameter of PCT, DCT and CT in treated groups which may be attributed to hydropic degeneration, necrosis and hyalinization. The changes were mild in the first and second group, become moderate in the third and severe in the fourth, fifth and sixth groups. In the sixth group, tubular morphology of most of the tubules were not clear. The findings are supported by works of *Ucheya & Igweh , Ejaz et al. and Yasmeen et al* [8-10].

There is gradual increase in interstitial fibrosis and edema seen with increase in duration of treatment, comparable to findings of *Abaton et al and Henrich* [5,6]. Analgesic nephropathy is also associated with vascular changes with gradual increase in duration of treatment which includes increase in thickness of vessel walls and congestion of blood vessels.

## CONCLUSION

Analgesic nephropathy is a tubulo-interstitial disease characterized by-Mild to moderate increase in the capsular space and decrease in glomerular size, necrosis and hydropic degeneration of renal tubules; Interstitial changes include gradual increase in inflammatory infiltrate and congestion of blood vessels. Therefore, Ibuprofen at therapeutic dose for longer duration of time can cause damage to the renal parenchyma. Hence, it should be judiciously used in patients with chronic renal insufficiency.

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