

## HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES OF ACUTE KETOPROFEN INDUCED NEPHROPATHIC LESIONS IN RATS

AMIRA S. SADEK; MARWA F. ALI; SARY K. ABD ELGHAFAR and  
MOKHTAR TAHA

Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine,  
Assiut University, Egypt.

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### ABSTRACT

The current work was undertaken to evaluate the nephrotoxic effect of Ketoprofen on adult male rats. Eighteen rats were divided into two groups. Ketoprofen- received group (I) included 10 rats were administered Ketoprofen at a therapeutic dose of 13.5 mg/kg by I/M injection daily for 4 successive weeks. Five rats were randomly selected from group I and sacrificed at 2 and 4 weeks of the experiment. The control group (II) that received olive oil included 8 rats, where 4 rats were sacrificed after 2 weeks and the rest of rats were sacrificed after 4 weeks. Tissue specimens from kidneys of all groups were collected for histopathological examination as well as the serum was obtained for the determination of biochemical parameters. The histopathological examination of group I showed glomerular changes such as expanding of glomerular matrix, glomerular sclerosis and congestion of glomerular capillary in the cortex. Renal tubular degeneration and necrosis accompanied with infiltration of inflammatory cells in interstitial tissue in both cortex and medulla were also observed. The biochemical results revealed that animals in group I showed a significant increase in malondialdehyde, creatinine, and urea compared to the control group, while total antioxidant capacity was numerically decreased. In conclusion, the therapeutic dose of Ketoprofen caused damage in kidney tissue even if was taken for a short period as well as altered biochemical parameters.

**Keywords:** Ketoprofen, Nephrotoxicity, Histopathological examination, Biochemical parameters.

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### INTRODUCTION

Ketoprofen is known as 2-(3-benzoylphenyl)-propionic acid. It is derived from arylpropionic acid class of nonsteroidal anti-inflammatory drugs (NSAIDs) (Caldwell *et al.*, 1988). Ketoprofen is white or off-white in colour,

odourless, fine to a granular powder, highly lipophilic, soluble in strong alkali and also easily soluble in ethanol, chloroform, acetone, and ether, but it is insoluble in water at 20° C (Klasco, 2003).

Ketoprofen possesses good anti-inflammatory, antipyretic, and analgesic effects (Seymour *et al.*, 1996; Levoine *et al.*, 2004). Ketoprofen can be used in the treatment of rheumatic diseases such as rheumatoid arthritis and osteoarthritis (Medeiros *et al.*, 2020).

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*Corresponding author:* AMIRA S. SADEK

*E-mail address:* [amira.sayed4494@gmail.com](mailto:amira.sayed4494@gmail.com)

*Present address:* Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Egypt.

It is considered an essential part of current veterinary therapy, it can relieve pain and inflammation associated with musculoskeletal disorders in dogs, cats, horses and cattle, and it alleviates fever in acute mastitis in cattle (Shpigel *et al.*, 1994; Owens *et al.*, 1995; Arrijoja-Dechert, 2002; Grecu *et al.*, 2014). In addition, Ketoprofen helps in reducing joint swelling and works as a medication for arthritis (Zafar *et al.*, 2017).

Although Ketoprofen is regarded as a wide therapeutic drug, it may cause unwanted side effects (Villegas *et al.*, 2004). The side effects of Ketoprofen have resembled other NSAIDs (Fries *et al.*, 1993). Most of the patients administrated therapeutic doses of Ketoprofen for a short duration usually tolerate them well, but, with longer duration of treatment may cause the occurrence of high risk (Bennett *et al.*, 1996; Harirforoosh *et al.*, 2013). Ketoprofen can cause various forms of renal damage as acute kidney injury, renal papillary necrosis, acute interstitial nephritis, hyperkalemia, and sodium and fluid retention (Breyer and Harris, 2001). Furthermore, there were histopathological changes related to administration of Ketoprofen therapeutic dose such as atrophy and congestion in few glomeruli, degeneration of renal tubules and interstitial nephritis (Farag Allah, 2001).

The present study determined the nephrotoxic effect of administration of a therapeutic dose of Ketoprofen for 2 and 4 weeks. This was done via histopathological examination of kidney tissue sections, estimation of oxidative indices (total antioxidant capacity and lipid peroxidation) and kidney functions through detection of creatinine and urea in the blood.

## MATERIALS AND METHODS

### Materials:

#### Chemicals used:

Ketoprofen: Purchased from SIGMA Aldrich (St Louis, MO, USA).

Total antioxidant capacity (TAC) kit, Malondialdehyde (MDA) kit, Urea kit and Creatinine kit, were purchased from Biodiagnostic Company, Egypt.

### Experimental animals:

Eighteen adult male rats were obtained from the Laboratory Animal House, Faculty of Vet. Medicine, Assiut University. The rats were healthy, weighing about 180-200 gms. The animals were housed in cages under controlled temperature (25C°) and humidity. All animals received laboratory food and tap water ad libitum. They were housed in the laboratory for at least one week before the experiment for acclimatization. The time of the experiment was 4 weeks. The rats were randomly divided into 2 groups according to the following design:

#### Group 1: Ketoprofen administered rats:

Ten adult male rats were given Ketoprofen in a dose of 13.5 mg/kg (Farag Allah, 2001). Ketoprofen was dissolved in olive oil and given by I/M injection daily for 4 successive weeks. After 2 weeks, 5 rats were randomly selected and sacrificed by cervical dislocation, while the other 5 rats were sacrificed after 4 weeks.

#### Group 2: Control rats:

Eight rats were given only Ketoprofen vehicle (olive oil) in a similar dose and route of Ketoprofen administered group. Four rats were sacrificed after 2 weeks and the others were sacrificed after 4 weeks.

### Methods:

#### Histopathological examination:

After sacrificing the rats from different groups according to the assigned schedule, kidney tissue specimens were collected and fixed in 10% neutral buffered formalin solution for 24 hours and then routinely processed for conventional histopathological examination as follow:

Tissue specimens were washed in tap water and then kept in 70% ethyl alcohol overnight. Dehydration of the specimens was done by immersion in ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) for a half-hour each. Tissue specimens were cleared with xylene and embedded in paraffin wax and then blocked by fresh molten paraffin. Five-micron sections were cut and stained with hematoxylin and eosin stain (Bancroft and Stevens, 1982) for histopathological examination by light microscopy (Olympus CX31, Japan) with Digital Camera (Olympus Camedia C -5060, Japan).

#### **Histopathological scoring:**

All the microscopic lesions of the kidney for each group were presented in tables to demonstrate the type of lesion and its severity according to (Chen *et al.*, 2018) as follow:

Kidney lesions ranged from 0 to 4. Histopathological score is (0 = no lesions), (1= mild), (2= moderate), (3= severe) and (4= very severe lesions).

- **Glomerular lesions:** Histological injury of glomeruli was estimated as the percentage of glomeruli that showed glomerular congestion, glomerulosclerosis, glomerular collapse and glomerular basement membrane expansion. In each round of the experiment, 10 glomeruli were randomly selected in cortical fields and evaluated at bar =100  $\mu$ m in each kidney section, and an average score was calculated.
- **Tubular lesions:** Histological injury of renal tubules was evaluated as the percentage of tubules that showed tubular dilation, tubular atrophy, tubular epithelial cell necrosis and cast formation. At bar =100  $\mu$ m in each kidney section. Ten areas of renal tubules were randomly chosen per kidney for the assessment, and an

average score was calculated in each round of the experiment.

- **Tubulointerstitial lesions:** Tubulointerstitial injury was scored according to the degree of intertubular congestion and area of infiltration of inflammatory cells. A score of 0 was assigned when the section shows no damage, a score of 1 was assigned when less than 25% was present, a score of 2 was assigned when there was at least 50% but less than 75%, a score of 3 was assigned when there was at least 76% but less than 95%, and finally a score of 4 was assigned when there was at least 95%. At bar =100  $\mu$ m, the severity of tubulointerstitial injury was evaluated by examining 10 randomly selected fields in each kidney section stained with H&E in each round of the experiment.

#### **Biochemical estimations:**

Blood samples were taken from the medial canthus of the eye and collected in sterilized plain tubes (without anticoagulant) from all experimental animals before sacrificing. Blood samples were centrifuged then sera were separated by micropipette into epindorf tubes from all different groups and kept frozen at -20 °C till the time of estimation of the biochemical parameters.

Biochemical parameters were measured in the Central Laboratory of Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University by using of 6705 UV |Vis Spectrophotometer (JENWAY) as the following:

- 1- Total antioxidants capacity (TAC) was determined using a colourimetric assay kit according to (Koracevic *et al.*, 2001).
- 2- Malondialdehyde (MDA) was determined using a colourimetric assay kit according to (Ohkawa *et al.*, 1979).

3- Urea was determined using a colourimetric assay kit according to (Fawcett and Soctt, 1960).

4- Creatinin was determined using colourimetric assay kit according to (Larsen, 1972).

#### **Statistical analysis:**

The data were analyzed using the Statistical Package for Social Science program SPSS (version 16) software. For comparison between different experimental groups, one-way analysis of variance (one-way ANOVA) was used followed by the Duncan test as a Post Hoc test. The graphs were done by using the Prism program, version 5.01 (GraphPad Prism). The acceptance level for statistical significance was  $P < 0.05$ . All data were expressed as mean  $\pm$  S.E.

## **RESULTS**

#### **Histopathological findings:**

##### **Group I: Rats sacrificed 2 weeks post Ketoprofen administration:**

Microscopic examination of H&E stained tissue sections from the kidneys of the sacrificed rats administered Ketoprofen for 2 weeks revealed marked nephropathic lesions in both cortex and medulla. The cortical lesions could be classified into glomerular, tubular and interstitial lesions.

Consistent glomerular changes appeared in all 5 examined rats and affected the majority of the glomeruli. These changes were expressed by swelling of the glomerular tufts of capillaries with complete obliteration of Bowman's space. These swollen glomeruli were related to either expanded mesangial matrix with thickening of the glomerular basement membrane (Fig. 1a). The glomerular changes were associated with occasional congestion of the glomerular capillary tufts. Furthermore, periglomerular mononuclear cellular infiltration appeared in all 5 examined rats that exhibited focal

distribution (Fig. 1b). On the contrary, focal glomerular atrophy was seen in 2 rats. The atrophied glomeruli appeared shrunken with widened Bowman's space, decrease in the mesangial matrix and mesangial cells (Fig. 1c).

Microscopic examination of the cortical renal tubules showed variable forms of tubulonephrosis. Apoptosis of the renal tubular epithelium was a peculiar finding in 2 rats out of the 5 rats. The apoptotic cells were demonstrated as sporadic shrunken cells with dense nuclear fragments, eosinophilic cytoplasm, compact nuclear chromatin and surrounded by a clear halo. The diagnosed apoptotic changes were accompanied by haemoglobin nephrosis where an accumulation of eosinophilic pigment in the tubular epithelial cells was also found in 2 rats out of the 5 rats in a focal manner (Fig. 1d).

The angiopathic changes of the cortical interstitial tissue appeared in all 5 rats. These changes were manifested as congestion of the blood vessels, vacuolation of tunica media, desquamation of the vascular endothelium and perivascular infiltration of mononuclear inflammatory cells (Fig. 1e).

Regarding the vascular damage seen in the renal medulla, severe congestion was found in all examined cases. Besides, edema of the interstitial stroma was noticed as faint pink homogenous fluid infiltrated with mononuclear inflammatory cellular reaction in only 2 rats. Focal atrophy of the collecting tubules was evident in 2 rats out of the 5 examined cases (Fig. 1f).

##### **Rats sacrificed 4 weeks post Ketoprofen administration:**

Histopathological examination of these rats showed various nephropathic alterations that affected the glomeruli. These alterations were expressed by focal periglomerular mononuclear cellular

infiltration that appeared in all 5 examined rats (Fig. 2 a).

Focal global glomerulosclerosis was a distinctive glomerular finding revealed in 3 rats out of the 5 rats and it affected some glomeruli. The characteristic features of this lesion included replacement of mesangium with fibrosis, increase in the glomerular matrix; obliteration of the capillary lumen and hypocellularity, also, it was accompanied by intertubular haemorrhage that expressed in all examined rats (Fig. 2 b). In all 5 examined rats other glomerular lesions which involved most of the glomeruli were demonstrated. These lesions were manifested by dilatation and congestion of the glomerular tufts of capillaries, accompanied by complete obliteration of Bowman's space as a result of an expanded mesangial matrix with thickening of the glomerular basement membrane. These diagnostic glomerular lesions were associated with intertubular congestion in all 5 examined rats (Fig. 2c).

Another prominent finding, revealed in 3 rats out of 5 rats, was focal segmental glomerulosclerosis. Histologically, it was characterized by segments of sclerosis, obliteration of glomerular capillary lumen and an increase in glomerular matrix of some glomeruli that was accompanied with thickening in glomerular basement membrane without obliteration of urinary space (Fig. 2d). Periglomerular haemorrhage was found in 2 rats out of 5 examined rats affecting few glomeruli (Fig. 2e). Focal collapsing of glomerular segment causing a decrease in the glomerular matrix was also seen in 2 rats out of 5 rats, associated with intertubular haemorrhage (Fig. 2f).

A diffuse vacuolar degeneration was commonly observed in the cortical convoluted tubules and collecting ducts of all examined rats. It was characterized by cellular swelling and clear vacuoles present

in the cytoplasm of renal tubular epithelium. This feature was associated with mononuclear inflammatory cells infiltration in the interstitium and some tubular lumina contained sloughed cellular debris that caused occlusion of tubular lumen forming epithelial cast (Fig. 3a). Hyaline cast formation was seen in 3 rats out of 5 rats. This intratubular cast appeared as an eosinophilic proteinaceous homogenous cast associated with cellular flattening and irregularity of the lining epithelium (Fig. 3b).

The vascular changes were also revealed in all examined rats as perivascular mononuclear cellular infiltration, vacuolation of tunica media and desquamation of vascular endothelium. These vascular changes were accompanied by perivascular edema admixed with red blood cells (RBCs) and infiltrated with inflammatory cells that appeared in 2 rats out of 5 rats (Fig. 3c).

Histopathological examination of the renal medulla in all examined rats revealed morphological alterations in various segments of medullary tubules. These alterations were expressed by clear cytoplasmic vacuolar degeneration in renal tubular epithelium of the collecting ducts (Fig. 3d). Focal dystrophic calcification of medullary tubules accompanied the medullary tubular injury and was seen in 2 rats out of 5 rats as purple calcium deposits on the necrotic tubular epithelium. It was associated with intraluminal pale eosinophilic proteinaceous material (Fig. 3e). Moreover, focal intraluminal pale eosinophilic proteinaceous material associated with congestion in intertubular blood vessels were persistent lesions in the medullary tubules of all 5 examined rats (Fig. 3f).

#### **Group II: Control group:**

Histopathological examination of the renal tissue of the control rats showed normal

histological structure. The normal glomeruli had thin glomerular capillary loops and cellular constituent. The surrounding different types of renal tubules appeared normal without changes in the interstitial tissue (Fig. 4 a & b).

#### **Results of histopathological scoring:**

Histopathological scorings were carried out in the cortex and medulla using H&E stained tissue sections from the kidneys of rats administered Ketoprofen for 2 and 4 weeks as well as the control rats.

Histopathological scoring of rats sacrificed 2 and 4 weeks post Ketoprofen showed a significant increase in the glomerular, tubular, interstitial lesions compared with the control values.

The histopathological scoring of renal lesions in different groups was demonstrated in Table (1) and Graph (1).

#### **Biochemical results:**

##### **Kidney function parameters (creatinine and urea levels):**

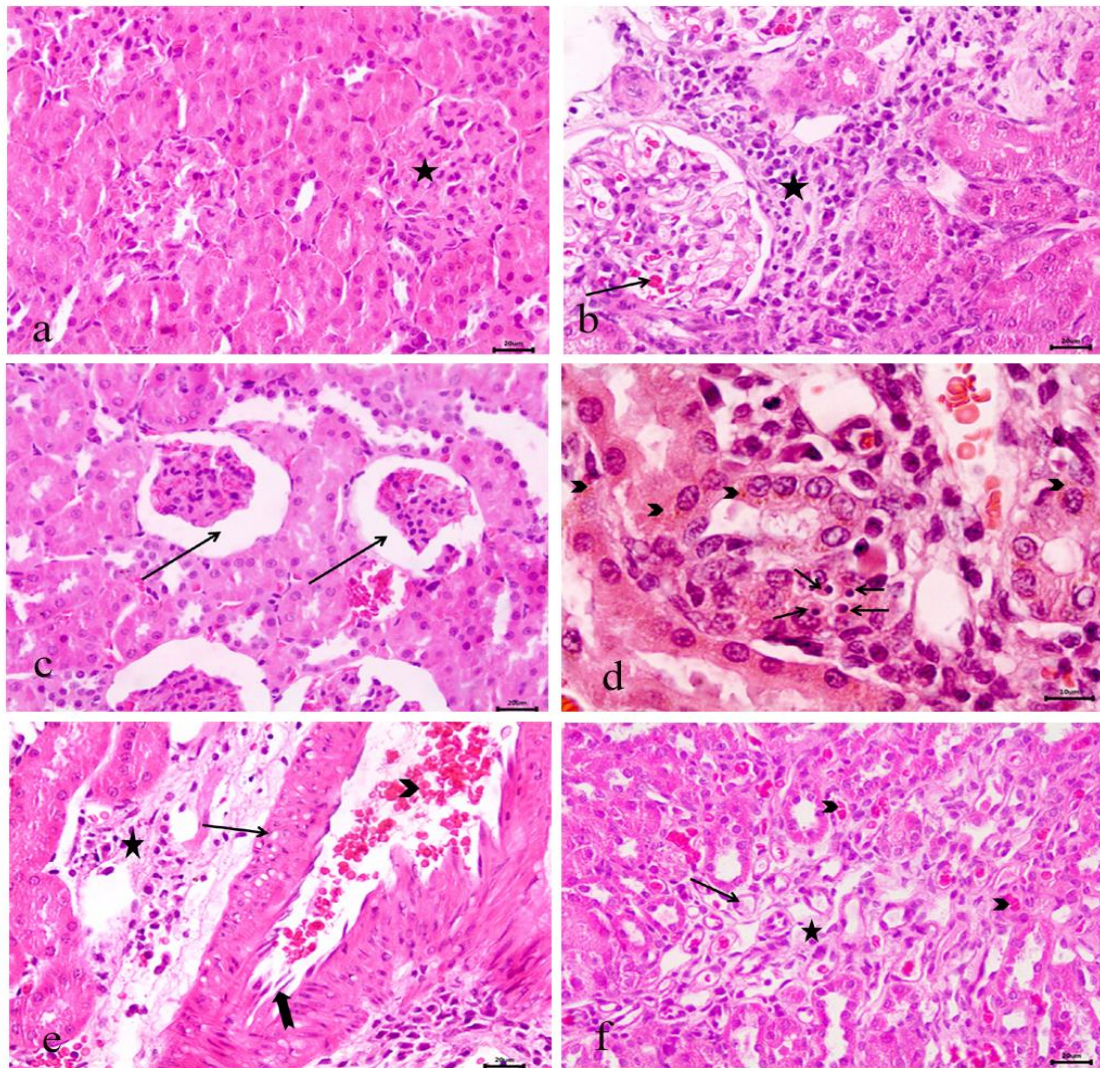
Serum biochemical analysis of Ketoprofen administered group for 2 weeks showed significantly changed values of urea and creatinine levels. Ketoprofen administered group showed a significant increase in the level of serum urea and creatinine when compared with the control group.

Evaluation of kidney function parameters of rats administered Ketoprofen for 4 weeks revealed a significant increase in serum urea concentration in comparison with the control group. Regarding the serum level of creatinine, there was a significant increase in Ketoprofen administered group as compared with the control one. Creatinine and urea levels in rats of different groups were demonstrated in Table (2) and Graph (2, 3, 4 and 5).

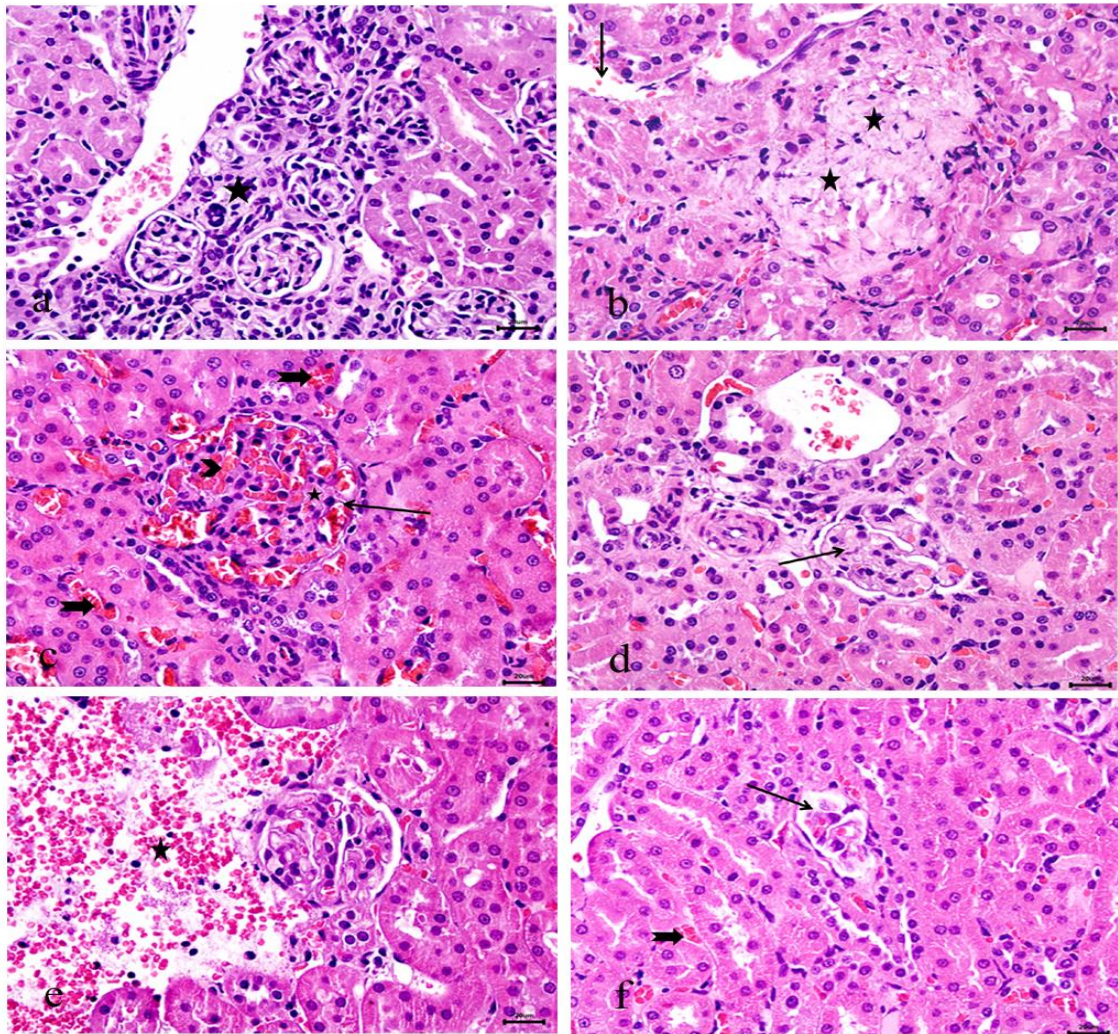
##### **Oxidative stress indices (MDA and TAC):**

Determination of serum levels of MDA and TAC exhibited that there was a significant elevation in the level of MDA in Ketoprofen administered group after 2 weeks compared with the control one, but the serum level of TAC in Ketoprofen administered group after 2 weeks was numerically decreased in comparison with control group. There was a significant increase in the serum level of MDA in Ketoprofen administered group after 4 weeks compared to the control one, however, the serum level of TAC was numerically decrease compared to the control group.

Selected biochemical parameters in rats of different groups were presented in Table (3) and Graph (6).

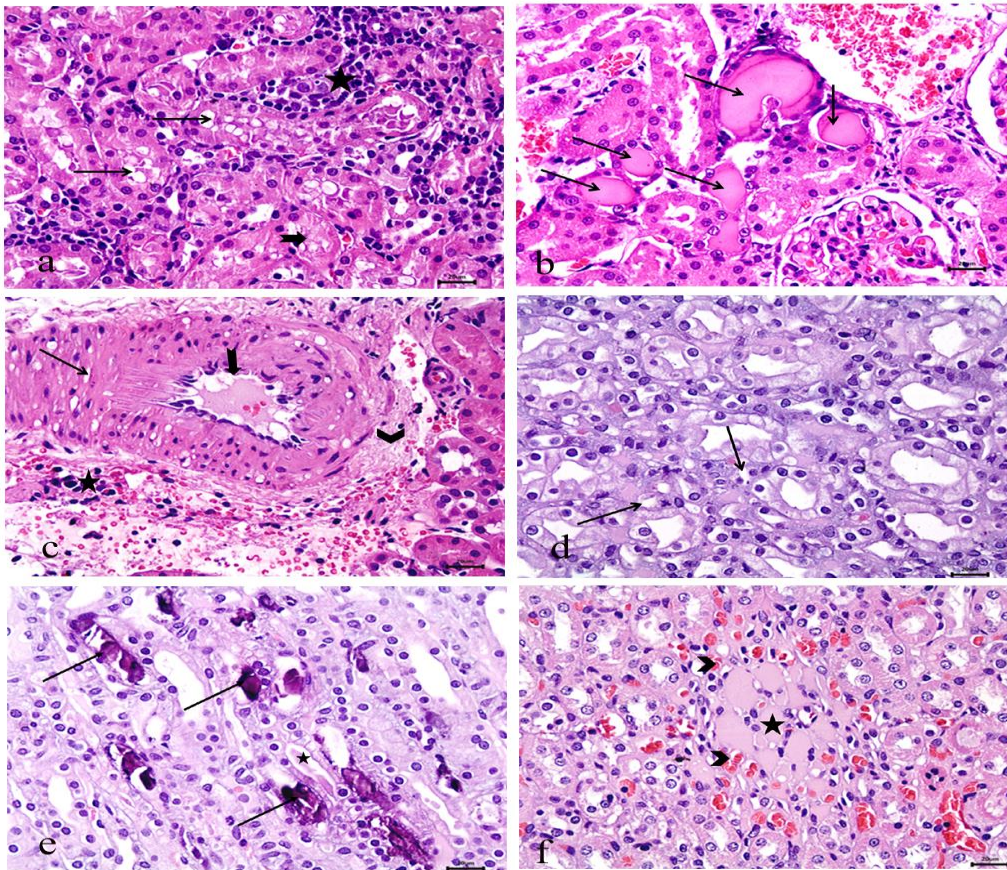


**Fig. 1:** Kidney, Ketoprofen administered group after 2 weeks showing (a) An expanded mesangial matrix with thickening of glomerular basement membrane and obliteration of Bowman's space (star). (b) Congestion of glomerular capillary (arrow) and periglomerular mononuclear cellular infiltration (star). (c) Shrunken glomeruli with widened of the Bowman's space (arrow). (d) Apoptotic cells (apoptosis) (arrow) and haemoglobin nephrosis (arrow head). (e) Congestion of blood vessels (arrow head), perivascular mononuclear cellular infiltration (star), vacuolation of tunica media (arrow), and desquamation of vascular endothelium (notched arrow). (f) Interstitial edema (star) with tubular atrophy (arrow) and congestion of intertubular blood vessels (arrow head) (H&E, bar= 20 um).

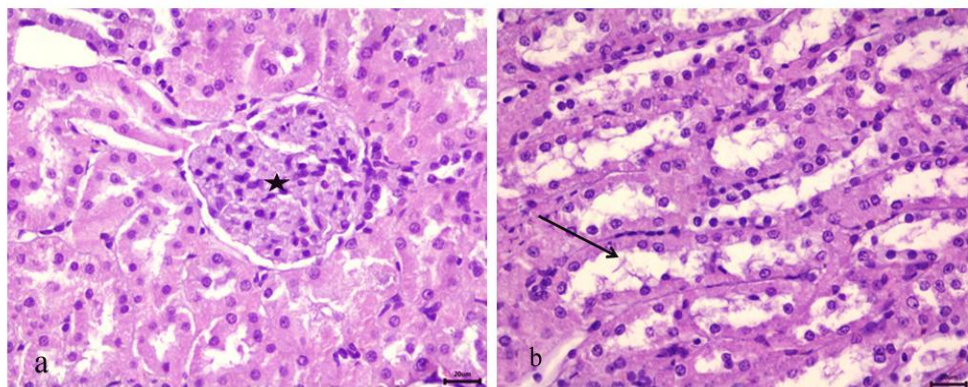


**Fig. 2:** Kidney, Ketoprofen administered group after 4 weeks showing (a) Periglomerular mononuclear cellular infiltration (star). (b) Focal global glomerulosclerosis (star) and intertubular haemorrhage (arrow). (c) Congestion of glomerular capillary tufts (arrow head), expanded mesangial matrix (star) with thickening of glomerular basement membrane, obliteration of Bowman's space (arrow) and congestion of intertubular blood vessels (notched arrow). (d) Focal segmental glomerulosclerosis accompanied with thickening in glomerular basement membrane (arrow). (e) Periglomerular haemorrhage (star). (f) Collapsing glomerular segment (arrow) and intertubular haemorrhage (notched arrow) (H&E, bar= 20 um).





**Fig. 3:** Kidney, Ketoprofen administered group after 4 weeks showing (a) Vacuolar degeneration of renal tubular epithelium (arrow), epithelial casts formation (notched arrow) and interstitial infiltration with mononuclear inflammatory cells (star). (b) Hyaline cast in renal tubular lumen (arrow). (c) Perivascular mononuclear cellular infiltration (star), vacuolation of tunica media (arrow), desquamation of vascular endothelium (notched arrow) and perivascular edema admixed with (RBCs) and infiltrated with inflammatory cells (arrow head). (d) Vacuolar degeneration in renal medullary tubules (arrow). (e) Dystrophic calcification in renal tubules (arrow) associated with intraluminal pale eosinophilic proteinaceous material (star). (f) Focal intraluminal pale eosinophilic proteinaceous material (star) and congestion in intertubular blood vessels (arrow head) (H&E, bar= 20 um).



**Fig.4:** Kidney cortex, control rats showing (a) normal glomeruli (star). (b) Kidney medulla, control rats showing normal medullary tubule (arrow) (H&E, bar= 20 um).

**Table 1:** Histopathological score (scale 0-4) of renal lesions observed by light microscopy in the kidney of rats Ketoprofen 2 weeks administered group, Ketoprofen 4 weeks administered group and control rats.

Renal lesions	Ketoprofen 2 weeks administered group	Ketoprofen 4 weeks administered group	Control rats
Glomerular lesions	3.60±0.24 <sup>a</sup>	4.00±0.00 <sup>a</sup>	0.60±0.24 <sup>b</sup>
Tubular lesions	3.20±0.37 <sup>a</sup>	4.00±0.00 <sup>a</sup>	0.60±0.24 <sup>b</sup>
Interstitial lesions	3.40±0.24 <sup>a</sup>	4.00±0.00 <sup>a</sup>	0.60±0.24 <sup>b</sup>

Means within the same row with different superscripts were significantly different at P< 0.05. Data were expressed as the mean ± S.E.

**Table 2:** Values of Kidney function parameters (creatinine and urea levels) in rats of different groups.

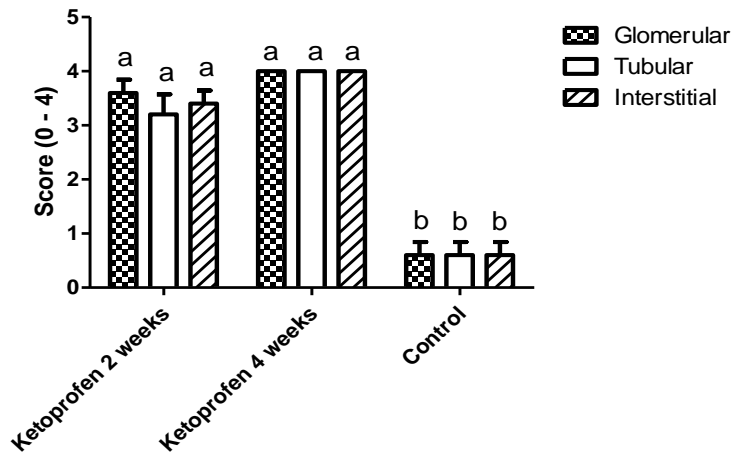
	Ketoprofen 2 weeks administered group	Ketoprofen 4 weeks administered group	Control rats
Urea (mg/dl)	64.43± 3.70 <sup>a</sup>	68.84±4.87 <sup>a</sup>	35.45±1.86 <sup>b</sup>
Creatinine (mg/dl)	2.15±0.30 <sup>a</sup>	1.58±0.06 <sup>a</sup>	1.11±0.05 <sup>b</sup>

Means within the same row with different superscripts were significantly different at P< 0.05. Data were expressed as the mean ± S.E.

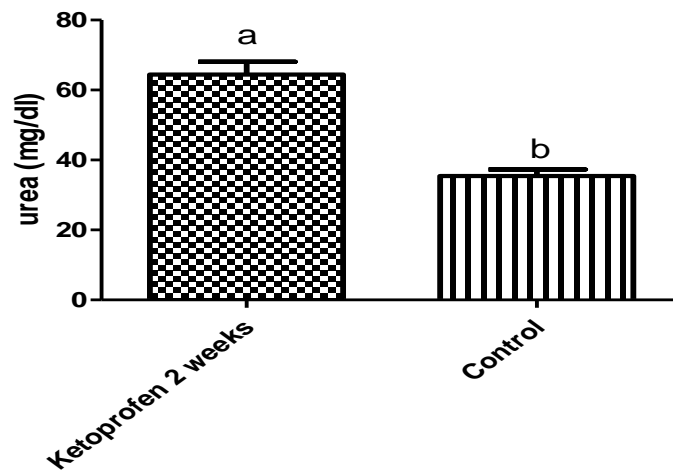
**Table 3:** Values of biochemical indices (MDA and TAC levels) in rats of different groups.

	Ketoprofen 2 weeks administered group	Ketoprofen 4 weeks administered group	Control rats
MDA (nmol/ml)	6.75± 0.61 <sup>a</sup>	16.89±1.85 <sup>a</sup>	2.19±0.43 <sup>b</sup>
TAC (mM/L)	1.13±0.25 <sup>a</sup>	1.29±0.05 <sup>a</sup>	1.48±0.21 <sup>a</sup>

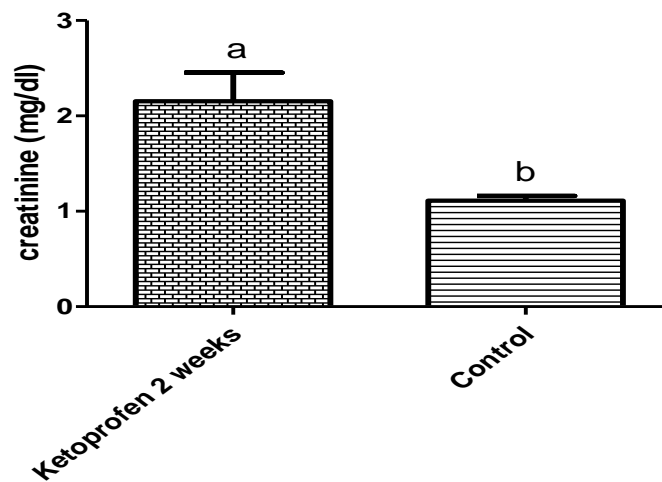
Means within the same row with different superscripts were significantly different at P< 0.05. Data were expressed as the mean ± S.E.



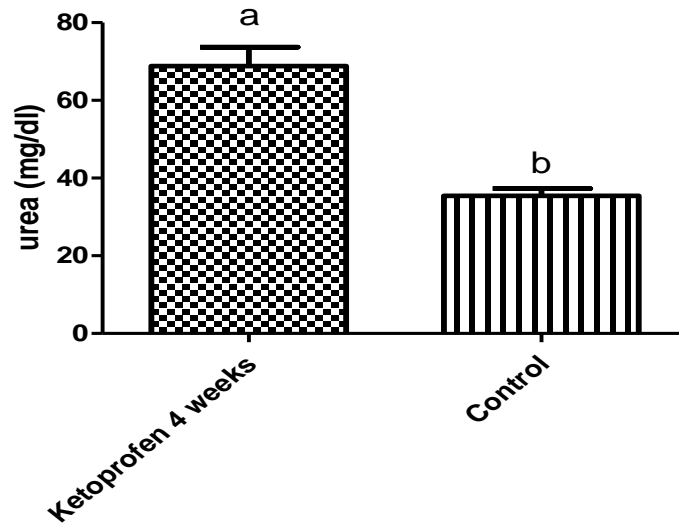
**Graph (1):** Histopathological score (scale 0-4) of renal lesions in Ketoprofen administered group and control group after 2 and 4 weeks. Means with different superscripts were significantly different at  $P < 0.05$ . Data were expressed as the mean  $\pm$  SE.



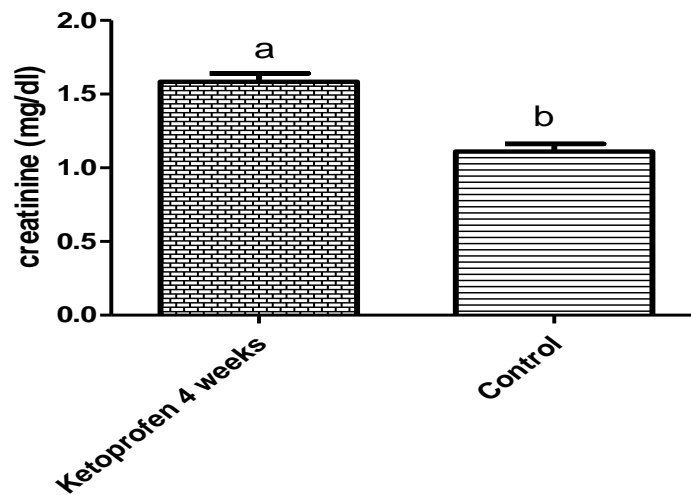
**Graph (2):** Values of urea (mg/dl) in Ketoprofen administered group and control group after 2 weeks. Means with different superscripts were significantly different at  $P < 0.05$ . Data were expressed as the mean  $\pm$  S.E.



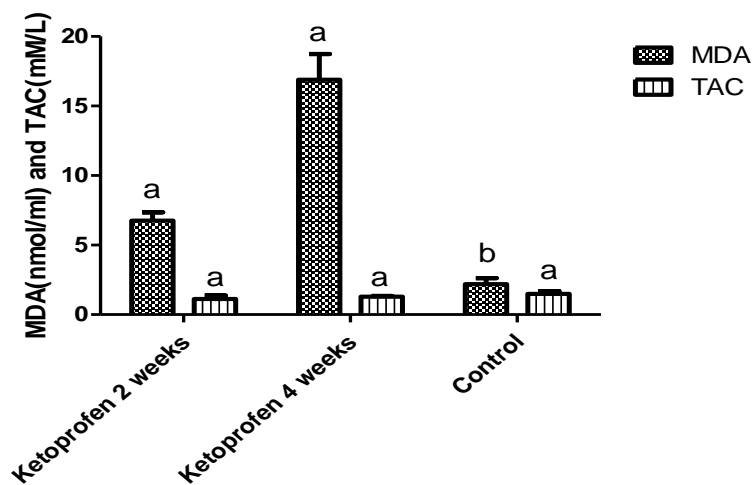
**Graph (3):** Values of creatinine (mg/dl) in Ketoprofen administered group and control group after 2 weeks. Means with different superscripts were significantly different at  $P < 0.05$ . Data were expressed as the mean  $\pm$  S.E.



**Graph (4):** Values of urea (mg/dl) in Ketoprofen administered group and control group after 4 weeks. Means with different superscripts were significantly different at  $P < 0.05$ . Data were expressed as the mean  $\pm$  S.E.



**Graph (5):** Values of creatinine (mg/dl) in Ketoprofen administered group and control group after 4 weeks. Means with different superscripts were significantly different at  $P < 0.05$ . Data were expressed as the mean  $\pm$  S.E.



**Graph (6):** Values of MDA (nmol/ml) and TAC (mM/L) in Ketoprofen administered group and control group after 2 and 4 weeks. Means with different superscripts were significantly different at  $P < 0.05$ . Data were expressed as the mean  $\pm$  S.E.

## DISCUSSION

In our study, we investigated the effect of a therapeutic dose of Ketoprofen on renal tissue in rats administered Ketoprofen in a dose of 13.5 mg/kg daily by intramuscular route for 4 weeks (Farag Allah, 2001). The rats were sacrificed 2 and 4 weeks post-dosing beside the control group. Tissue specimens from the kidneys were taken and subjected for histopathological examination as well as the serum was collected for biochemical analysis in the Ketoprofen administered group and control group.

Histopathological findings of tissue sections from the kidneys of the sacrificed rats administered Ketoprofen for 2 weeks revealed marked nephropathic lesions in both cortex and medulla. The cortical lesions could be classified into glomerular, tubular and interstitial lesions.

Concerning the glomerular lesions observed in our study, there were congestion of the glomerular capillary tufts, expanded mesangial matrix with thickening of the glomerular basement membrane, focal glomerular atrophy and periglomerular mononuclear cellular infiltration. Similar glomerular lesions were described by many authors (Tomic *et al.*, 2008; Awad *et al.*, 2014; El-Feky *et al.*, 2018).

In our work, there were variable forms of tubulonephrosis in the cortex as apoptosis of the renal tubular epithelium and haemoglobinc nephrosis. In addition to interstitial lesions expressed by congestion of the blood vessels, vacuolation of tunica media, desquamation of the vascular endothelium and perivascular infiltration of mononuclear inflammatory cells. Moreover, the medulla was also affected including interstitial edema, tubular

atrophy and congestion of intertubular blood vessels. Similar results were previously described and interpreted by Farag Allah (2001) who studied the effects of different doses of Ketoprofen in various periods on renal tissue.

Kent *et al.* (2007) reported on the similar effect of Ibuprofen in neonatal rats. Deniz (2019) proved that Ketoprofen can increase caspase-3 activity which is critical in inducing apoptosis of pancreatic cells. On the other hand, Safarchi *et al.* (2010) revealed only interstitial nephritis and glomerular hypercellularity in goats administered Flunixin meglumine, Ketoprofen, and Phenylbutazone. Mozaffari *et al.* (2010) stated that Ketoprofen caused only interstitial nephritis in miniature donkeys.

Regarding our histopathological observation after administration of Ketoprofen for 4 weeks, various morphopathological alterations of the glomeruli were manifested as focal segmental glomerulosclerosis, focal global glomerulosclerosis, congestion of the glomerular tufts of capillaries, expanded mesangial matrix with thickening of glomerular basement membrane and focal collapsing glomerular segment. The same glomerular changes were recorded by (Farag Allah, 2001). Furthermore, these findings could be supported by Mozaffari and Derakhshanfar (2011) who reported that administration of NSAIDs as Flunixin meglumine, Ketoprofen, and Phenylbutazone in fat-tailed sheep caused similar results such as glomerular sclerosis and glomerular hypercellularity.

Variable changes were also seen in the renal tubules of rats sacrificed 4 weeks post-dosing. These changes were expressed by vacuolar degeneration, interstitial infiltration with mononuclear

inflammatory cells, epithelial cast, hyaline cast formation and perivascular edema admixed with RBCs. Moreover, the medulla showed different alterations manifested as focal dystrophic calcification and intraluminal pale eosinophilic proteinaceous material in a focal manner. Raekallio *et al.* (2010) found similar results and proved that Ketoprofen can affect the renal tubules. Baisakh *et al.* (2014) recorded similar findings of administration of a therapeutic dose of Ibuprofen on renal tissue. Comparable findings were also seen by Talat *et al.* (2017) who studied the effect of Ibuprofen on renal tissue but their results were accompanied by no significant inflammatory reaction and mild glomerular congestion. Ketoprofen administration in this study showed multiple vascular changes. Owumi and Dim (2019) mentioned similar findings in their experimental studies on diclofenac sodium in kidney rats.

The kidneys are essential organs for the excretory function of the body hence; they receive about 25% of all cardiac output. They preserve homeostasis, metabolize and excrete a lot of exogenous substances, such as drugs (Rahman and Malcoun, 2014; Pathan *et al.*, 2018; Lucas *et al.*, 2019). Many research explained the action of Ketoprofen and its effect. As all NSAIDs, Ketoprofen acts by inhibiting the cyclooxygenase (COX) pathway of arachidonic acid (AA) metabolism (Kantor, 1986). Prostaglandins vasodilate the afferent arterioles of the glomeruli and maintain glomerular filtration rate (Patrono and Dunn, 1987; Oates *et al.*, 1988). Inhibition of COX pathway and the protective effect of prostaglandin by NSAIDs leads to activation of the lipoxygenase pathway, also increase the formation of leukotrienes which act as mediators of inflammation (Rainsford, 2007; Pountos *et al.*, 2011). Furthermore,

NSAIDs cause a decrease in ability of the kidneys to autoregulate blood flow (Gunson, 1983; Clive and Stoff, 1984).

Moreover, the acute tubular injury occurred by 2 mechanisms. The first one is the inhibitory effect on prostaglandin synthesis by NSAIDs, this leads to vasoconstriction of afferent renal arteriole and acute renal injury. The second one is acute interstitial nephritis characterized by localized inflammatory response and edema of the renal interstitium causing impairment in perfusion; this leads to renal cellular injury (Konder and Kudrimoti, 2003; Lucas *et al.*, 2019).

In the present study, the Ketoprofen administered group after 2 and 4 weeks showed significant increase in values of urea and creatinine levels when compared with the control group. These findings were in agreement with Raekallio *et al.* (2010) who recorded an increase in plasma concentration of urea and creatinine when evaluated the effect of Ketoprofen on the urinary enzyme. Similar results were obtained by El-Feky *et al.* (2018) who studied the effect of Ketoprofen on kidney functions. In addition, similar findings were reported by Talat *et al.* (2017) who stated the effect of Ibuprofen on kidney functions.

Urea and creatinine are metabolic waste products that are normally filtered by the glomeruli of the kidneys (Gaspari *et al.*, 1998). NSAIDs are known to alter renal function by decreasing the glomerular filtration rate due to inhibition of prostaglandin synthesis, which leads to retention of urea, creatinine and other nitrogen waste products that are normally removed by the kidneys (Bennett *et al.*, 1996; Bellomo *et al.*, 2012; Aprioku and Uche, 2013; Paueksakon and Fogo, 2017; Luciano and Perazella, 2018). Hence, serum concentrations of urea and

creatinine can indicate renal toxicity (Perrone *et al.*, 1992; Traynor *et al.*, 2006). By contrast, minimal changes in the levels of urea and creatinine were recorded by Borges *et al.* (2013). Muchhara *et al.* (2018) observed non-significant changes in serum urea and creatinine levels. Furthermore, Aprioku *et al.* (2014) mentioned that Ibuprofen administration to rats did not change serum levels of urea and creatinine in low and high doses at 7 days and in low dose at 14 days but these levels were increased in high dose at 14 and 28 days of the experiment.

In the current study, determination of serum levels of MDA and TAC proved that there was a significant elevation in the level of MDA in the Ketoprofen 2 and 4 weeks administered group than the control one. On the other hand, the serum level of TAC in the Ketoprofen 2 and 4 weeks administrated group was numerically decreased in comparison with the control group. Similar findings were described by many authors who concluded that Ketoprofen alters oxidative stress markers (Fefar *et al.*, 2016; El-Feky *et al.*, 2018; Deniz, 2019). Owumi and Dim (2019) studied the effect of diclofenac sodium on renal oxidative stress and recorded the same results.

There are various influences on oxidative stress and antioxidant-related parameters caused by NSAIDs (Orhan *et al.*, 1999). In renal ischemia, a decrease in intracellular levels of adenosine triphosphate (ATP) and a rapid increase in reactive oxygen species production was happened (Edelstein *et al.*, 1997; Dagher, 2000; Lee *et al.*, 2005). Malondialdehyde (MDA) is a useful marker of free radical-mediated damage and oxidative stress; as an end product of lipid peroxidation (Del Rio *et al.*, 2005). Lipid peroxidation is the most important source of free radicals to

cause injury. These free radicals directly damage cellular membranes and produce several secondary products which lead to extensive cellular damage (Romero *et al.*, 1998). Our observed results of antioxidant enzymes were supported by (Cheng *et al.*, 2013).

It could be concluded that administration of Ketoprofen for a short period in a therapeutic dose can cause various forms of nephropathic lesions in each of the glomeruli, tubules, and interstitium; furthermore, biochemical indices were also altered.

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## التغيرات النسيجية المرضية والبيوكيميائية للآفات الكلوية الحادة الناجمة عن الكيتوبروفين في الجرذان

اميره سيد صادق ، مروه فاروق على ، سارى خليل عبد الغفار ، مختار طه

Email: [amira.sayed4494@gmail.com](mailto:amira.sayed4494@gmail.com) Assiut University web-site: [www.aun.edu.eg](http://www.aun.edu.eg)

تم إجراء العمل الحالي لتقييم التأثير الكلوي للكيتوبروفين على ذكور الجرذان البالغة. تم تقسيم ثمانية عشر جرذاً إلى مجموعتين. تم إعطاء كيتوبروفين للمجموعة (I) التي تضمنت ١٠ جرذان بجرعة علاجية ١٣.٥ مجم / كجم عن طريق الحقن العضلي يومياً لمدة ٤ أسابيع متتالية. تم اختيار خمسة جرذان بشكل عشوائي من المجموعة الأولى حيث تم التضحية بها بعد اسبوعين و بعد ٤ أسابيع من التجربة. ضمت المجموعة الضابطة (II) التي تلقت زيت الزيتون ٨ جرذان ، حيث تم التضحية بـ ٤ جرذان بعد أسبوعين وتم التضحية ببقية الجرذان بعد ٤ أسابيع. تم جمع عينات أنسجة الكلى من جميع المجموعات للفحص التشريحي المرضي وكذلك تم الحصول على مصل الدم لتحديد المعايير البيوكيميائية. أظهر الفحص النسيجي المرضي للمجموعة الأولى تغيرات كيميائية مثل تمدد المصفوفة الكبيبية والتصلب الكبيبي واحتقان الشعيرات الدموية الكبيبية في القشرة. كما لوحظ تنكس ونخر كلوي أنبوبي مصحوب بارتشاح الخلايا الالتهابية في النسيج الخلالي في كل من القشرة والنخاع. أظهرت النتائج البيوكيميائية أن الحيوانات في المجموعة الأولى أظهرت زيادة معنوية في الملانوندايالدهيد والكرياتينين واليوريا مقارنة بالمجموعة الضابطة ، بينما انخفضت السعة الكلية لمضادات الأكسدة عددياً. في الختام ، تسببت الجرعة العلاجية من كيتوبروفين في تلف أنسجة الكلى حتى لو تم تناولها لفترة قصيرة بالإضافة إلى تغيير في المعايير البيوكيميائية.