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Nano-curcumin Attenuates Nephropathic Lesions Induced by Chronic Ketoprofen Administration in Rats: Role of Cyclooxygenase-1

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Abstract

Ketoprofen can relieve pain and inflammation associated with many diseases but, it may cause unwanted side effects especially in kidneys. Curcumin nanoparticles (CurNPs) have a protective effect related to their anti-inflammatory and antioxidant properties. The present study examined the protective role of cyclooxygenase-1 (COX-1) in kidney induced by CurNPs against Ketoprofen-induced nephropathic injury. Twenty adults male Wistar rats were randomly assigned into four groups (n=5); Ketoprofen administered group, Ketoprofen and CurNPs- treated group, CurNPs treated group and control group. Animals were sacrificed 6 weeks post-administration. Blood serum samples were used for evaluation of urea, creatinine, lipid peroxidation (MDA) and total antioxidant capacity (TAC) levels. Kidney specimens were collected for histopathology and COX-1 expression was studied as well. The histopathological results of kidney in Ketoprofen administrated group showed focal segmental and global glomerulosclerosis, periglomerular fibrosis, intratubular casts and lytic necrosis of renal tubular epithelium. The pathological lesions were decreased to be mild changes in kidney of Ketoprofen and CurNPs- treated group. Immunhistochemical examination of COX-1 showed negative expression in Ketoprofen group which was attenuated in Ketoprofen and CurNPs- treated group. The biochemical examination revealed that animals in Ketoprofen administrated group showed significant increase in urea, creatinine, and MDA while TAC levels were numerically decreased. These results were attenuated in Ketoprofen and CurNPs- treated group. Co-administration of CurNPs with Ketoprofen caused reduction in kidney parameters and MDA with numerical improvement in TAC. In the present study, CurNPs have obvious protective effects on nephropathic lesions induced by Ketoprofen.

KEYWORDS
CurNPs, COX-1, Immunohistochemistry, Ketoprofen, Nephropathic lesions, NSAIDs

INTRODUCTION

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) belonging to aryl propionic acid derivatives (Rençber *et al*., 2009). It has antipyretic, anti-inflammatory, and analgesic effects (Seymour *et al*., 1996; Levoin *et al*., 2004). Ketoprofen can alleviate pain and inflammation related to musculoskeletal affections in dogs, cats, horses and cattle, furthermore it relieves fever in cattle with acute mastitis, so it is regarded as an essential drug of current veterinary therapy (Shpigel *et al*., 1994; Owens *et al*., 1995; Grecu *et al*., 2013).

Ketoprofen causes undesirable side effects, although it is considered a wide therapeutic drug (Villegas *et al*., 2004). Administration of Ketoprofen therapeutic doses for a short duration can be tolerated by most patients, but, the occurrence of high risk may result from a longer duration of treatment (Bennett *et al*., 1996; Harirforoosh *et al*., 2013). NSAIDs are often prescribed only for the short-term in veterinary practice to avoid the increase in incidence and severity of side effects with prolonged use but NSAIDs for long periods is necessary for diseases such as osteoarthritis (Baltoyiannis *et al*., 2001). Administration of high doses and longer half-lives of NSAIDs increases the risk of chronic kidney diseases to occur (Musu *et al*., 2011; Chiu *et al*., 2015). Moreover, longer periods of administration of Ketoprofen therapeutic dose causes histopathological changes such as glomerular shrunken and congestion, accumulation of cellular debris in the renal tubular lumen and intertubular hemorrhage (Farag Allah, 2001).

Curcumin is a polyphenolic naturally occurring compound extracted from the rhizomes of Curcuma longa (Maheshwari *et al*., 2006; Altenburg *et al*., 2011). Some factors limit the therapeutic use of curcumin such as low aqueous solubility and bioavailability, less absorption, rapid metabolism, low penetration and targeting efficacy (Flora *et al*., 2013a). Using nanoparticles for increasing solubility and bioavailability of lipophilic compounds such as curcumin was documented by Freitas (2005).

In numerous studies, curcumin nanoparticles exhibited superior therapeutic benefits over free curcumin (Murali *et al*., 2013). Curcumin nanoparticles produced effective results against cancers, brain tumors, also liver, heart and kidney affections (Mohanty and Sahoo, 2010; Lim *et al*., 2011; Shimatsu *et al*., 2012). Curcumin nanoparticle has superior ability to remove free radi-

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cals with enhanced anti-lipid peroxidation compared to free curcumin (Basnet *et al*., 2012). Curcumin nanoparticles are useful in the prevention, treatment and diagnosis of numerous diseases as a result of their very small size, and large surface area (Flora *et al*., 2013 a).

Cyclooxygenase-1 (COX-1) is located in the glomeruli, collecting ducts, and afferent and efferent arterioles, guaranteeing that the kidney's physiological functions, such as hemodynamic control and glomerular filtration rate, are maintained (Goetz Moro *et al*., 2017).

Like all NSAIDs, Ketoprofen works by blocking the cyclooxygenase (COX) route of arachidonic acid metabolism (Kantor, 1986). The COX enzyme has 2 forms, COX-1and COX-2 (Shibata *et al*., 2005). COX-1 is found in most cells. It is a constitutive enzyme that possesses many physiologic functions, including platelet aggregation and protection of gastric mucosa (Crofford, 1997). COX-1 can maintain normal kidney function, it has a role in hemodynamic modulation and glomerular filtration rate preservation (Kummer and Coelho, 2002; Moore *et al*., 2015). Ketoprofen blocks both types of COX enzymes, which are essential in prostaglandins formation (Villegas *et al*., 2004; Oh *et al*., 2006). NSAIDs can elevate the levels of leukotriene in the lipoxygenase pathway and decrease the vasodilator effect of prostaglandin following the suppression of the COX pathway (Shastri *et al*., 2001). Moreover, the capacity of the kidneys to maintain the blood flow was declined by NSAIDs (Cliveand Stoff, 1984). High exposure to NSAIDs can lead to the occurrence of chronic kidney diseases (Gooch *et al*., 2007). The results of chronic kidney diseases were interstitial fibrosis and sclerosis of the glomeruli (Kamata *et al*., 2015).

The current study aimed to investigate the possible ameliorative impact of curcumin nanoparticles on the nephrotoxic effect of chronic administration of Ketoprofen. In this work, an attempt was made to highlight the role of COX-1 in kidney tissue and whether curcumin nanoparticles can mitigate nephropathological lesions through their modulation.

MATERIALS AND METHODS

Chitosan nanoparticles

Synthesis of encapsulated CurNPs

Nano-curcumin were synthesized and characterized as reported previously (Yadav *et al*., 2016). Briefly, Tween 80 (0.5 ml) was added to 20 ml of a 0.15% solution of chitosan (Nano-gate company- Egypt) in dilute acetic acid (35 mM) with constant stirring for 1 h. Then 250 µl of curcumin (which obtained from sigma chemical company, USA.) solution (25 mg/ml in chloroform) was added in aliquots of ~ 20 µl with stirring. The solution was stirred for a further 1 h, after which 0.5 ml of 20% sodium sulfate solution was added dropwise with stirring and stirring was continued for another 30 minutes. To crosslink the nanoparticles, 0.1 ml of glutaraldehyde was added to the solution and stirring continued for another 30 minutes. Finally, 1 ml of 10% sodium metabisulfite was added to the solution and stirred for another 30 minutes.

Encapsulation efficiency and loading capacity

The centrifugation method was performed to determine the encapsulation efficiency (EE). The particles were precipitated, and then the absorbance of the supernatant was measured to determine the unloaded drug. The encapsulation efficiency was estimated by using a UV-Vis spectrophotometer (Cary series UV-Vis-NIR, Australia).

Size & Shape of CurNPs

Transmission electron microscope (TEM) was performed on JEOL JEM-2100 high-resolution TEM at an accelerating voltage of 200 kV, respectively.

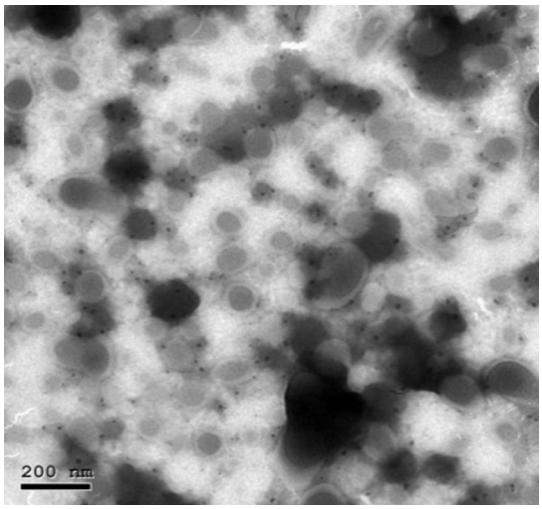

Fig. 1. Show the TEM image of the prepared curcumin loaded chitosan nanoparticles

Determination of encapsulation efficiency and loading capacity

The curcumin was detected at 420 nm. The EE parameter was calculated as follow: EE%=(Wt-Wf)/Wt *100

Where Wt and Wf describe the total drug added and the drug extracted into the supernatant, respectively (Abd Ellah *et al*., 2019).

Initial concentration = 0.32 mg per ml.

Free concentration = 0 .0515 mg/ml.

Encapsulation eff. = (Intial Con.-Free Conc.)/(Intial Con) * 100 = 84.375 %

Loading capacity (LC %) can be calculated by the amount of total encapsulated drug divided by the total nanoparticle weight. LC = 17.58 %

Properties of CurNPs

The color of CuNPs is yellow slurry which formed in the suspension. The average size after its formation is 60 ±20 nm which appear spherical in shape.

Experimental animals

Twenty male rats were obtained from the laboratory animal house, Faculty of Vet. Medicine, Assiut University, Egypt. The rats were healthy, weighing about 180-200 g. All rats were housed in separate cages under controlled laboratory conditions of temperature, humidity, and light. The rats were allowed free access to standard food and tap water. For acclimatization to the laboratory conditions, the rats were kept in the laboratory for one week at least before the beginning of the experiment. The rats were randomly divided into four groups based on the following design:

Ketoprofen administered group (Group 1): 5 adult male rats were given Ketoprofen (Sigma Aldrich, St Louis, MO, USA) 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 6 successive weeks.

Ketoprofen and CurNPs- treated group (Group 2): 5 adult

male rats were given Ketoprofen in the same dose and route as in the first group (Farag Allah, 2001). The rats were prevented from drinking water for 12 h before dosing the nanoparticle. Meanwhile, each rat of this group was treated by oral administration of CurNPs in a daily dose of 15 mg/kg for 6 successive weeks (Yadav *et al*., 2016).

Rats administered CurNPs only (Group 3): 5 adult male rats were deprived of drinking water for 12 h before dosing the nanoparticle. Then the rats were given CurNPs in the same dose and route as in the second group.

Control rats (Group 4): 5 adult male rats were kept at normal conditions of laboratory temperature and humidity then, were administered only Ketoprofen vehicle (olive oil) in a similar dose and route as Ketoprofen administered group.

At the end of the study (after 6 weeks), blood serum samples for biochemical analysis and kidney specimens were collected for histopathological and immunohistochemical examinations.

All the samples were collected under the permission in accordance with the local license. All experiments were performed in experimental units of Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University approved by The National Ethical Committee of The Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to The OIE standards for use of animals in research in accordance with ARRIVE guidelines. All efforts were made to minimize the number of animals used and their suffering.

Biochemical analysis

Serum samples were collected from 3 experimental animals from each group for biochemical analysis. The 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 following: Urea was determined using a colorimetric assay kit according to Fawcett and Scott (1960). Creatinine was determined using a colorimetric assay kit according to Knud Larsen (1972). Malondialdehyde (MDA) was determined using a colorimetric assay kit according to Ohkawa *et al*. (1979). Total antioxidants capacity (TAC): was determined using a colourimetric assay kit according to Koracevic *et al*. (2001). All kits measured by spectrophotometric method; using kit applied by Bio diagnostics, Egypt.

Histopathological examination

After sacrificing, Fresh specimens from the kidney of rats of all experimental groups were collected and fixed in 10% neutral buffered formalin. Tissue specimens were processed routinely, sectioned at 4 µm thickness, and stained with hematoxylin and eosin (H&E) for histopathological examination by light microscopy (Olympus, CX31; Tokyo Japan) and photographed using a digital camera (Toupview, LCMos10000KPA, china) (Bancroft and Stevens, 2019). Specific stains were carried out on kidney slides whenever needed including PAS stain was applied to the kidney tissue section (Chen *et al*., 2018). Sirius red stain was applied to kidney tissue sections as a specific stain for collagen fibers (Segnani *et al*., 2015). The stained tissue sections were examined under a light microscope and photographed.

Histopathological scoring

The histopathological lesions of renal tissue were evaluated microscopically in all groups at 10X power and showed in tables to detect the type and severity of lesions depending on (Chen *et al*., 2018) as follow:

The renal damage ranged from 0 to 4. Histopathological score 0 indicated no lesions, 1 as mild, 2 as moderate, 3 as severe and 4 as very severe lesions.

Glomerular lesions

The glomerular injury was evaluated as the percentage of glomeruli that revealed sclerosis collapsed glomerular segment, atrophy of the glomerular tufts, periglomerular fibrosis and expansion of the basement membrane of the glomerulus. The scoring was done using H&E stained kidney sections examined by light microscope, 10 glomeruli in the cortical fields were selected randomly.

Tubular lesions

The renal tubular damage was estimated as the percentage of tubules that exhibited necrosis, casts formation and accumulation of proteinaceous material in the tubular lumen. Under the light microscope, randomly 10 regions of renal tubules in H&E stained kidney sections were selected.

Tubulointerstitial lesions

The tubulointerstitial damage was assessed based on the area of infiltration of inflammatory cells and tubulointerstitial fibrosis extension. Ten randomly selected fields in H&E stained kidney section were observed to evaluate the severity of the tubulointerstitial injury. when the kidney section appears no lesions a grade of 0 was assigned, however, when less than 25% was found a grade of 1was indicated when there was at least 50% but less than 75% was found a grade of 2 was indicated, a grade of 3 was indicated when the lesions were present at least 76% but less than 95%, and when there was at least 95% of lesions a grade of 4 was assigned.

Immunohistochemistry examination

Paraffin sections from the kidney were used for immunohistochemical detection of COX-1 at the end of the study. The tissue sections (3µm thick) were deparaffinized and washed by distal water. Heat-induced antigen retrieval was applied in a water bath using citrate buffer (pH 6) for 20 minutes. The endogenous peroxidase activities were removed with 3% hydrogen peroxide (H_2O_2). Sections were then incubated in primary antibody overnight at 4°C in a humidified chamber for COX-1 (obtained from US Biological life sciences) diluted in phosphate-buffered saline (PBS). Econo Tek biotinylated Anti polyvalent was applied and incubated for 30 minutes. Then the sections were rinsed four times for 5 min each with Phosphate-buffered saline, and the sections were incubated in Econo Tek HRP Conjugate for 30 minutes at room temperature. A mixture of DAB chromogen was visualized in the sections, and the DAB substrate was then incubated for 10 minutes. Sections were washed with distilled water then counterstained with hematoxylin and dehydrated and mounted. Positive immunoreactions looked at the brown coloration. Negative controls were performed by neglecting the primary antibody, which resulted in negative immunoreactivity.

Statistical analysis

The data were analyzed using the Statistical Package for So-

cial Science program SPSS (version 16) software. For comparison between different experimental groups, a 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 ± Standard error (S.E).

RESULTS

Biochemical results

Kidney function parameters (urea and creatinine levels) after 6 weeks

Estimation of urea concentration in the serum of rats in all groups revealed a significant increase in the urea level in the rats that received Ketoprofen compared to rats treated with Ketoprofen and CurNPs and control rats. Furthermore, the rats treated with Ketoprofen and CurNPs exhibited a significant reduction in the urea level when compared with the rats that received Ketoprofen. On the contrary, CurNPs only treated rats appeared to significant decrease compared to the Ketoprofen and CurNPs treated rats.

Detection of creatinine concentration in the serum of rats in all groups demonstrated that there was a significant increase in the creatinine level in the rats received Ketoprofen compared to Ketoprofen and CurNPs treated rats and control rats. However, Ketoprofen and CurNPs treated rats revealed a numerically increase compared to CurNPs only treated rats.

In different groups, the urea and creatinine values after 6 weeks were demonstrated in Fig. 2 and 3.

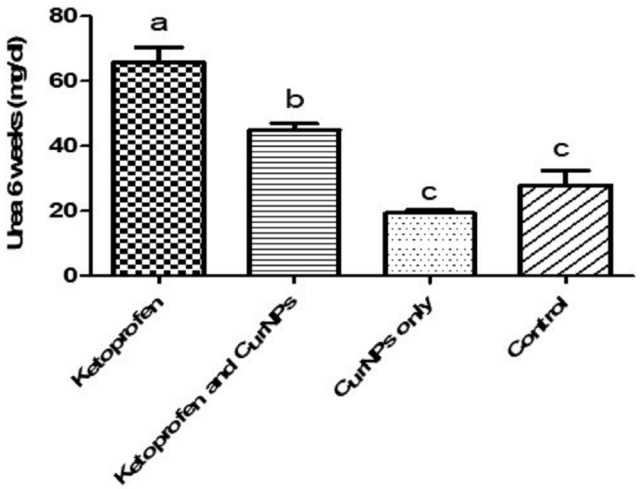

Fig. 2. Values of urea (mg/dl) in different experimental groups after 6 weeks. Means with different superscripts letters were significantly different at P< 0.05. Data were expressed as the mean ± S.E and (n of each group= 3).

Oxidative stress indices (Malondialdehyde and Total antioxidant capacity) after 6 weeks

The rats that administrated Ketoprofen only showed a significant increase in serum malondialdehyde level when compared to Ketoprofen and CurNPs treated rats and control rats. Rats treated with Ketoprofen and CurNPs showed a significant decrease in the serum level of malondialdehyde compared to Ketoprofen received rats and numerically increase compared to CurNPs only treated rats. The rats treated with CurNPs only exhibited a numerically decrease when compared with Ketoprofen and CurNPs treated rats.

Evaluation of total antioxidant capacity in the serum of rats that received Ketoprofen revealed a numerically decline compared to Ketoprofen and CurNPs treated rats and control rats. CurNPs only treated rats exhibited a numerically increase in comparison with Ketoprofen and CurNPs treated rats.

In different groups, the malondialdehyde and total antioxidant capacity levels at the end of the study were demonstrated in Fig.4.

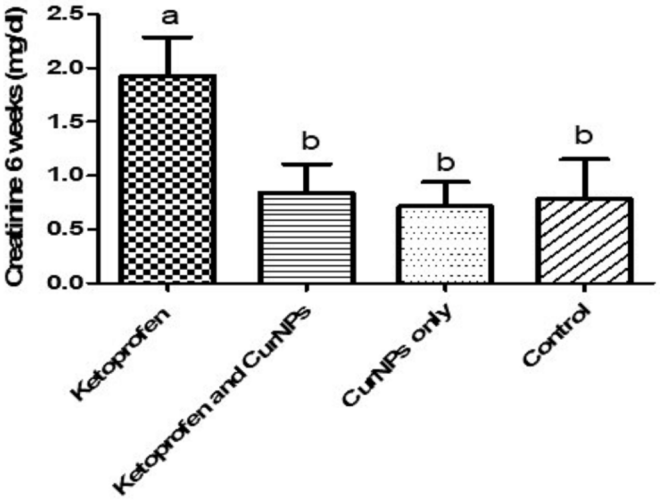

Fig. 3. Values of creatinine (mg/dl) in different experimental groups after 6 weeks. Means with different superscripts letters were significantly different at P< 0.05. Data were expressed as the mean ± S.E and (n of each group = 3).

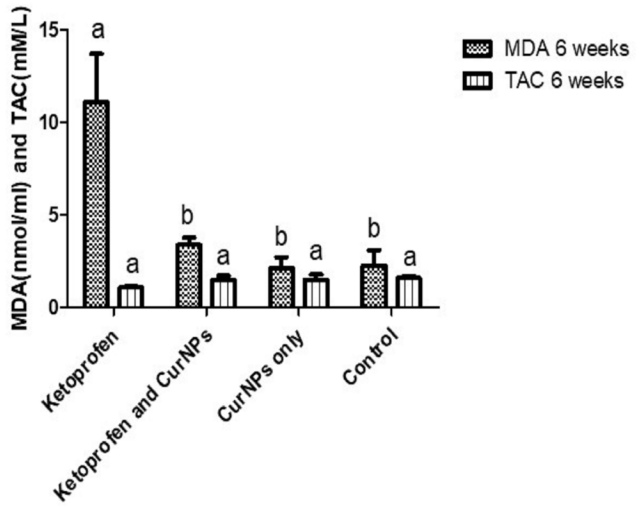

Fig. 4. Values of MDA (nmol/ml) and TAC (mM/L) in different experimental groups after 6 weeks. Means with different superscripts letters were significantly different at P< 0.05. Data were expressed as the mean ± S.E and (n of each group= 3).

Histopathological findings

Ketoprofen administered group (The first group)

Histopathological examination of renal tissue sections exhibited distinctive alterations in renal cortex and medulla of rats after 6 weeks dosing of Ketoprofen. The cortical lesions could be classified into glomerular, tubular, and interstitial lesions.

Histopathological examination of kidney sections of this group revealed severe glomerular alterations. The prominent finding in this group 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 a glomerular matrix of some glomeruli. It was also accompanied by accumulation of eosinophilic material (Fig. 5A), that quite clear by PAS stain (Fig. 5B). Focal global glomerulosclerosis was the most characteristic glomerular feature in this group also affected few glomeruli. It was found in 3 rats out of 5 rats, and expressed by diffuse replacement of mesangium with fibrosis, increase in the glomerular matrix; obliteration of the capillary lumen and hypocellularity (Fig. 5C). Focal global glomerulosclerosis was confirmed by sirius red stain (Fig. 5D). The peculiar glomerular distortion was Bowman's capsule metaplasia affecting 3 rats out of 5 rats. Microscopically the squamous epithelium of Bowman's capsule parietal cells changed into cuboidal epithelium in some glomeruli (Fig. 5E). Furthermore, in 3 rats out of 5 rats, collapsed glomerular segment causing a decrease in the glomerular matrix was also seen in some glomeruli (Fig. 5F). Periglomerular fibrosis was another advanced glomerular lesion that appeared in 3 rats out of 5 rats in few glomeruli, associated with atrophy of the glomerular tufts (Fig. 5G). Periglomerular fibrosis accompanied by thickening of the Bowman's corpuscle basement membrane was seen in 2 rats, confirmed by sirius red stain (Fig. 5H).

The histomorphological alterations of the cortical tubules were very clear in this group. Some tubular lumens contained sloughed cellular debris and occlusion of tubular lumens forming epithelial casts; it was also seen in all examined rats (Fig. 5I). Intratubular red blood cells (RBCs) casts were a distinctive feature of some cortical tubules, predominated in all examined rats. RBCs casts tend to appear microscopically as compacted erythrocytes inside the tubular lumen, intratubular haemorrhage, accompanied with flattening of the epithelium of tubules, tubular damage, and lytic necrosis of the epithelium of cortical tubules (Fig. 5J). The eminent lesion of the cortical tubules in this group was lytic necrosis of the epithelium of tubules observed in 3 rats out of 5 rats. This lesion manifested in some renal tubules as tubular lumen filled with damaged tubular epithelium admixed with erythrocytes and few leukocytes (Fig. 5K). Microscopic examination of kidney sections stained by H&E revealed that all examined rats exhibited obvious cortical interstitial changes. These changes were expressed by interstitial cellular reaction of mononuclear inflammatory cells as a frequent lesion of all examined rats (Fig. 5L). Furthermore, apparent blood vessels alterations were demonstrated in all examined rats. These alterations were represented by a mixed thrombus that appeared as a large thrombus located inside the lumen of blood vessels (Fig. 5M). The thrombus consisted of RBCs, leukocytes and fibrin (Fig. 5N). Focal area of intraluminal pale eosinophilic proteinaceous material was seen in all rats in different parts of the medullary tubules (Fig. 5O) and confirmed by PAS stain (Fig. 5P).

Ketoprofen and CurNPs treated group (The second group)

Microscopic examination of H&E stained sections from the renal tissue in this group revealed mild glomerular alterations

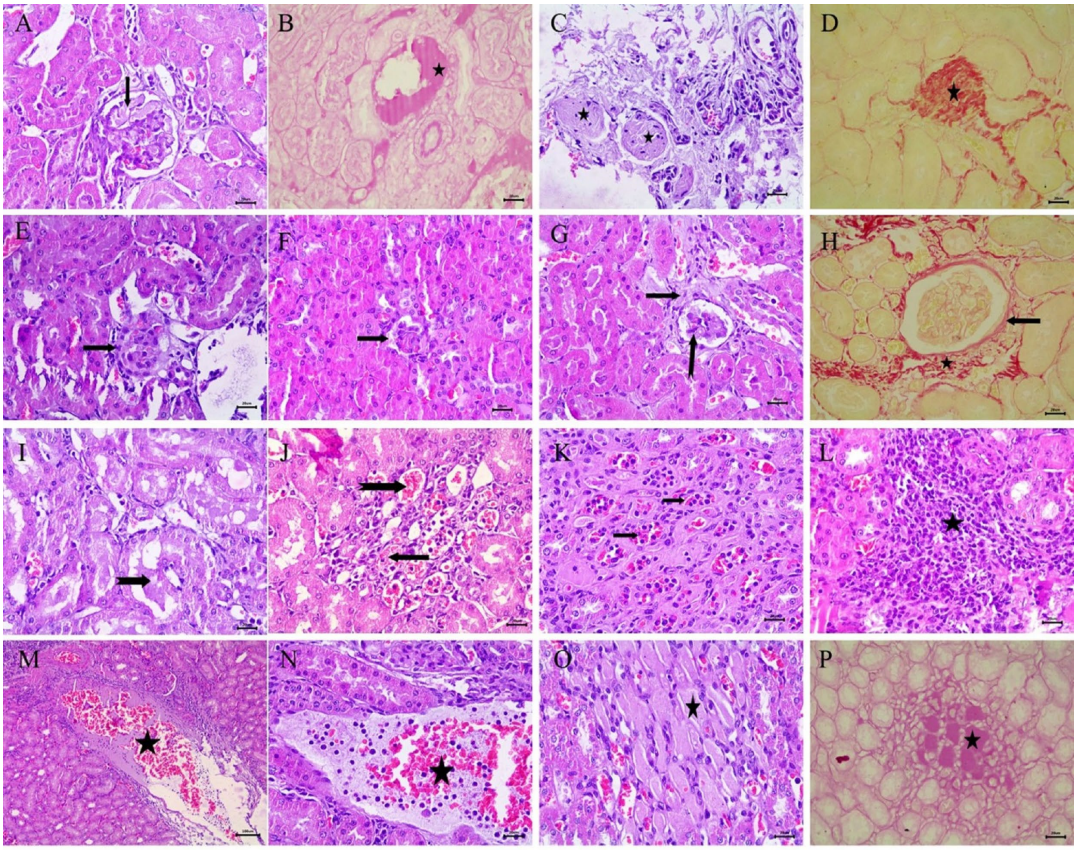

Fig. 5. Kidney, Ketoprofen administered group after 6 weeks showing (A) Focal segmental glomerular sclerosis with an accumulation of eosinophilic material (arrow) (H&E, bar = 20 um). (B) Focal segmental glomerulosclerosis with an accumulation of eosinophilic material (star) (PAS, bar = 20 um). (C) Focal global glomerulosclerosis with replacement of mesangium with fibrosis (star) (H&E, bar = 20 um). (D) Focal global glomerulosclerosis (star) (Sirius red, bar = 20 um). (E) Bowman's capsule metaplasia, the squamous epithelium of parietal cells converted into cuboidal. (arrow) (H&E, bar = 20 um). (F) Collapsed glomerular segment causing a decrease in the glomerular matrix (arrow) (H&E, bar = 20 um). (G) Periglomeruolar fibrosis (arrow) is accompanied by atrophy of glomerular tufts (notched arrow) (H&E, bar = 20 um). (H) Periglomeruolar fibrosis (star) and thickening in Bowman's capsule basement membrane (arrow) (Sirius red, bar = 20 um). (I) Some tubular lumens contain sloughed cellular debris forming epithelial casts (notched arrow) (H&E, bar = 20 um). (J) Intratubular haemorrhage of renal tubules forming RBCs cast (notched arrow) also, lytic necrosis of the tubular epithelium (arrow) (H&E, bar = 20 um). (K) Lytic necrosis of the tubular epithelium and the lumen filled with damaged tubular epithelium admixed with erythrocytes and few leucocytes (arrow) (H&E, bar = 20 um). (L) Interstitial cellular reaction of mononuclear inflammatory cells (star) (H&E, bar = 20 um). (M) Mixed thrombus inside the lumen of blood vessels (star) (H&E, bar= 100 um). (N) Mixed thrombus consisted of fibrin, RBCs and leucocyte (star) (H&E, bar = 20 um). (O) Focal area of intraluminal proteinceous material inside the tubular lumen (star) (H&E, bar = 20 um). (P) Focal area of intraluminal proteinceous material inside the tubular lumen (star) (PAS stain, bar= 20 um).

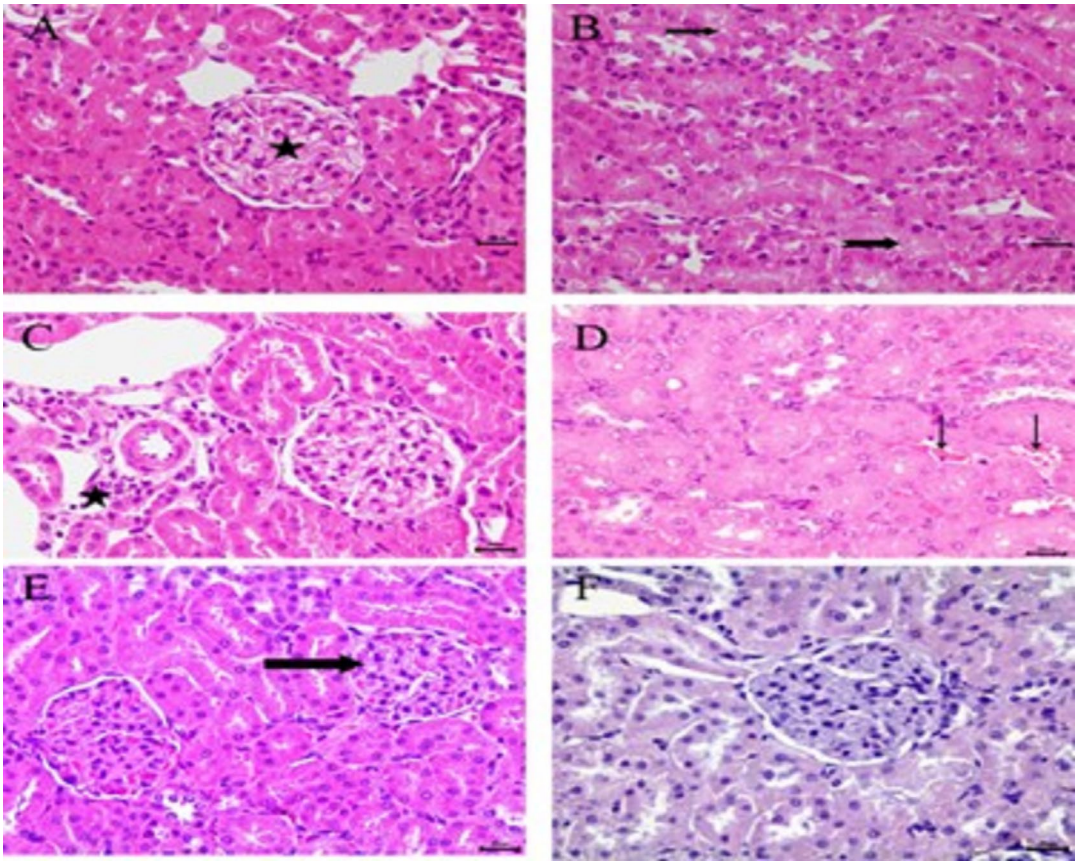

Fig. 6. Kidney, Ketoprofen and CurNPs treated group after 6 weeks showing (A) thickening in the basement membrane of the glomerulus and expanded mesangial matrix that leads to incomplete occlusion of Bowman's space (star). (B) Mild granular degeneration (arrow) with a slight amount of tissue debris in the lumen of tubules (notched arrow). (C) Mild interstitial inflammatory cells infiltration (star). (D) Intertubular haemorrhage (arrow). CurNPs only treated group after 6 weeks showing (E) Mild congestion in glomerular capillary tufts (arrow). Control rats showing (F) normal glomeruli (H&E, bar= 20 um).

observed in 4 rats out of all 5 examined rats. These alterations were manifested by few glomeruli showed incomplete obliteration of Bowman's space due to expanded mesangial matrix and thickening of the basement membrane of the glomerulus (Fig. 6A).

Histopathological examination of the renal cortex exhibited very mild cortical changes in this group. Such changes appeared in 4 rats out of 5 rats and were characterized by mild granular degeneration of renal tubular epithelium, associated with a slight amount of tissue debris in some renal tubular lumen (Fig. 6B). Slight cortical interstitial alterations were demonstrated in kidney sections in this group. The mild angiopathic injury was seen in 4 rats out of 5 rats, expressed by mild interstitial infiltration of mononuclear inflammatory cells (Fig. 6C). Furthermore, intertubular haemorrhage was revealed in 3 rats out of 5 rats (Fig. 6D).

Rats administered CurNPs only (The third group)

Microscopic examination of tissue sections from the kidneys of the sacrificed rats exhibited normal architecture of both cortex and medulla except a few rats showed mild changes. Mild congestion in glomerular capillary tufts was seen in 3 rats out of 5 rats (Fig. 6E).

Control untreated rats (The fourth group)

Microscopic examination of H&E stained tissue sections from the kidneys of the sacrificed rats revealed histological features of normal cortex and medulla. In this group, rats showed normal Malpighian renal corpuscles, proximal and distal convolut¬ed tubules. The renal cor¬puscles showed a central tuft of glomer¬ular capillary loops lined with flat endothelial cells. Moreover, these capillary loops were surrounded by mesangial cells and mesangial matrix with normal morphology. In addition, Bowman's space appeared normal and cleared of any cell debris. The cortical tubules preserved the normal histological structure and lining epithelium (Fig. 6F).

Histopathological scorings were carried out in the cortex and medulla in kidney sections from all rats in different groups that were sacrificed after 6 weeks post-dosing. The glomerular, tubular and interstitial lesions were significantly elevated in the Ketoprofen administered rats when compared with Ketoprofen and CurNPs treated rats and control rats. Furthermore, the Ketoprofen and CurNPs treated rats showed a significant decrease in comparison with the Ketoprofen administered rats. By contrast, the CurNPs treated rats and control rats were revealed significantly decreased in compared to Ketoprofen and CurNPs treated rats.

The histopathological scoring of renal lesions in different groups after 6 weeks was demonstrated in Table 1.

Table 1. Histopathological score (scale 0 - 4) of kidney lesions detected by light microscope in the renal tissue of rats in all experimental groups after 6 weeks.

Renal lesions	Ketoprofen administered group	Ketoprofen and CurNPs-treated group	Rats administered CurNPs only	Control rats
Glomerular lesions	4.00±0.00^{a}	2.00±0.00^{b}	0.80±0.37^{c}	0.60±0.24^{c}
Tubular lesions	4.00±0.00^{a}	2.40±0.24^{b}	0.60±0.24^{c}	0.60±0.24^{c}
Interstitial lesions	4.00±0.00^{a}	1.60±0.40^{b}	0.60±0.24^{c}	0.60±0.24^{c}

Means within the same row with different superscripts letters were significantly different at P< 0.05. Data were expressed as the mean ± S.E and (n of each group = 5).

Immunhistochemical findings

Immunhistochemical examination of immunoreactivity in rats received Ketoprofen revealed that glomeruli, endothelial cells lin-

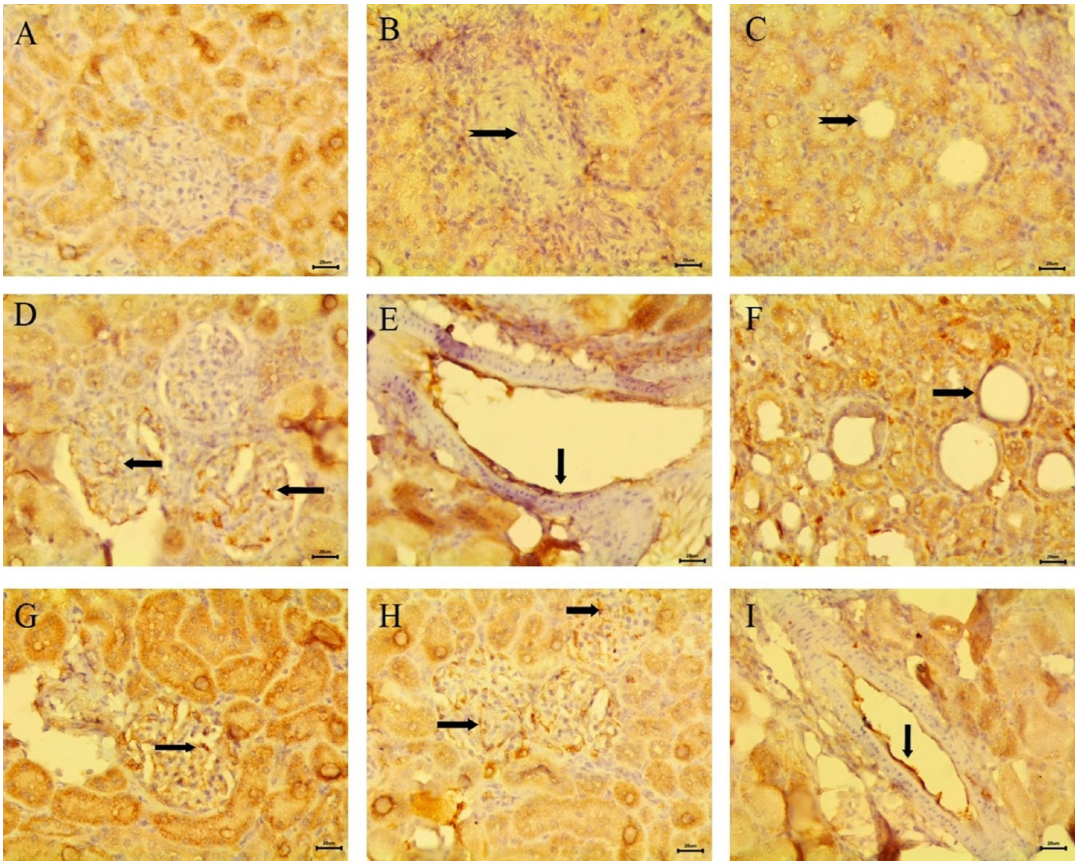

Fig. 7. Immunhistochemical examination of immunoreactivity of COX-1. A B&C) Ketoprofen administered group showing negative expression of COX-1 reaction in the glomeruli, lining endothelial cells or lining epithelium of collecting ducts (notched arrow). D, E &F) Ketoprofen and CurNPs- treated group showing moderate positive expression of COX-1 in the lining endothelial cells, lining epithelium of collecting ducts and glomerulus (arrow). G) CurNPs administrated group showing strong positive expressions of COX-1. H &I) Control rats showing moderate positive expressions of COX-1.bar=20.

ing blood vessels and renal epithelium were negatively expressed for COX-1 (Fig. 7 A, B &C). While Ketoprofen and CurNPs treated group revealed moderate positive staining of COX-1 reaction (Fig. 7 D, E &F). Also, a strong positive expression of COX-1 was exhibited in CurNPs treated group (Fig. 7G). In the control group, the glomerulus and renal epithelium showed moderate expressions of COX-1 with intense brown color (Fig. 7 H &I).

DISCUSSION

In the current work, the curative effect of CurNPs against Ketoprofen induced chronic nephrotoxicity was estimated in rats. The rats in all groups were sacrificed after 6 weeks of the experiment.

The biochemical investigation in this study exhibited that, there was a significant increase in the urea and creatinine levels in the rats that received Ketoprofen compared to control rats. Field *et al*. (1999) stated similar results and reported higher values of urea and creatinine in serum with the chronic use of NSAIDs. Aprioku *et al*. (2014) proved that rats received high doses of Ibuprofen at 14 and 28 days showed an elevation in urea and creatinine levels in serum. Estimation of urea and creatinine levels is considered an indicator of kidney function and filtration rate. The decline in the glomerular filtration rate associated with chronic kidney diseases lead to a decrease in the excretion of urea and creatinine levels in urine but increase their concentrations in blood (Higgins, 2016). However, Borges *et al*. (2013) mentioned that there was a slight alteration in renal parameters in Ketoprofen received dogs. Muchhara *et al*. (2018) studied the Ketoprofen effect on rats for 28 days and observed non-significant changes in the levels of serum urea and creatinine.

In the current study, there were significant changes in serum malondialdehyde and total antioxidant capacity levels in rats received Ketoprofen appeared significant increase in the level of malondialdehyde in the serum compared to control rats, however, the serum level of total antioxidant capacity was numerically declined. Many authors stated similar findings and found that malondialdehyde and total antioxidant capacity can be altered by Ketoprofen (Fefar *et al*., 2016; El-Feky *et al*., 2018; Deniz, 2019). Furthermore, Owumi and Dim (2019) reported the same results during the evaluation of the oxidative stress indices in the kidney induced by diclofenac sodium.

In this work, CurNPs ameliorated the biochemical parameters that altered by Ketoprofen and this by detection of a significant decrease in serum urea and creatinine concentration in rats treated with Ketoprofen and CurNPs when compared with the rats that administrated Ketoprofen only. Similar results obtained by many authors proved that CurNPs improved renal dysfunction through a reduction in the concentrations of urea and creatinine in serum (Sankar *et al*., 2013; Chattopadhyay *et al*., 2018; Ansar *et al*., 2019; Sudirman *et al*., 2019).

The obtained results were supported by Chen *et al*. (2017) who recorded that CurNPs decreased serum concentrations of urea and creatinine in rats intoxicated with glycerol. Mailafiya *et al*. (2020) observed that when rats treated with CurNPs exhibited a reduction in the levels of urea and creatinine in serum. Furthermore, El-Gizawy *et al*. (2020) found that CurNPs declined serum urea and creatinine values induced by cisplatin. Similarly, Anwar *et al*. (2020) mentioned that curcumin loaded chitosan nanoparticles reduced urea and creatinine values.

According to, many studies which proved that CurNPs had anti-inflammatory and antioxidant properties, this restored normal kidney function and improved high concentration of urea and creatinine (Hanai *et al*., 2009; Sankar *et al*., 2013).

In the current study, rats treated with Ketoprofen and CurNPs showed a significant reduction in the serum level of malondialdehyde compared to Ketoprofen received rats; however, the total antioxidant capacity level was numerically elevated.

Flora *et al*. (2013b) reported that CurNPs ameliorated the renal oxidative stress through decreased reactive oxygen species

(ROS) levels and increased glutathione (GSH) levels. In addition, Yadav *et al*. (2016) stated that CurNPs increased anti-oxidant activity and declined lipid peroxidation levels in rats intoxicated with fluoride and arsenic. Helli *et al*. (2021) mentioned that malondialdehyde level was decreased and the total antioxidant capacity level was increased in patients administrated capsules of CurNPs.

The ability of CurNPs to reduce ROS and increase the activities of the antioxidant enzymes was recorded by Ibrahim *et al*. (2019) and Anwar *et al*. (2020). Moreover, the obtained results were supported by Ansar *et al*. (2019) who demonstrated that CurNPs elevated antioxidant status in renal tissue and reduced oxidative damage. Mailafiya *et al*. (2020) reported that CurNPs increased superoxide dismutase activity and reduced MDA level in rats intoxicated with lead.

The capability of CurNPs to decrease oxidative stress was associated with the methoxy and phenolic groups present on the phenyl ring in the curcumin structure which explains its ability to scavenge the free radical. This phenolic group inhibits the SH group oxidation and protects the protein oxidation. Also, curcumin can react directly with reactive species and elevated the activities of some antioxidant enzymes (Swarnakar *et al*., 2005; Ak *et al*., 2008; Gera *et al*., 2017). Curcumin elevated the endogenous scavenger of free radical as GSH; also it inhibits lipid peroxidation (Dickinson *et al*., 2003).

Concerning the histopathological findings for chronic Ketoprofen administration, severe glomerular alterations were expressed by focal segmental glomerulosclerosis and focal global glomerulosclerosis. Ouda *et al*. (2018) mentioned similar glomerular lesions during study the toxic effect of NSAIDs on kidney of rats. Liu *et al*. (2017) reported that NSAIDs caused focal segmental glomerulosclerosis which progresses into global sclerosis.

In both cortical and medullary tubules advanced tubular changes were observed in this study such as intratubular RBCs casts. Resemble findings were mentioned by Sabry *et al*. (2014) who reported the diclofinac sodium effect on mice fetuses maternally treated. Wood *et al*. (2013) stated similar tubular lesions of nephrotoxic NSAIDs in rats. Moreover, similar kidney lesions were mentioned by Sadek *et al*. (2021) who reported the Ketoprofen nephrotoxic effect on rats received Ketoprofen daily for one month. Fogazzi *et al*. (2012) explained intratubular RBCs casts formation, also mentioned that NSAIDs causes interstitial inflammation that leads to disruption of interstitial vessels, with the consequent extravasation of RBCs in the interstitum and hence an invasion of the lumen of tubules through the gaps of the basement membrane of tubules.

Regarding the interstitial alterations of the current study were characterized by the interstitial cellular reaction of mononuclear inflammatory cells. Alabi and Akomolafe (2020) mentioned that diclofenac affected on the kidney of rats and cause similar lesions.

In the present study rats were receiving Ketoprofen for 6 weeks revealed angiopathic changes such as mixed thrombus inside the lumen of blood vessels. Cure *et al*. (2020) stated the risk of NSAIDs in the formation of thrombus. NSAIDs increased the possibility of thrombus formation that is relevant to the imbalance between thromboxane and prostacyclin as a result of COX-2 blocking (Wise, 2014). Furthermore, COX-2 inhibition lead to PGI2 inhibition which has an antithrombotic effect (Goetz Moro *et al*., 2017).

The histopathological findings that have been shown in the Ketoprofen and CurNPs treated group, we found that CurNPs can reduced the severity of histopathological lesions induced by Ketoprofen in the glomeruli, tubules and interstitium. In Ketoprofen and CurNPs treated group; we revealed that few glomeruli showed incomplete occlusion of Bowman's space due to expanded mesangial matrix and thickening of the basement membrane of the glomerulus. Furthermore, the renal cortex exhibited very mild cortical alterations as mild granular degeneration of the epithelium of renal tubules, slight cortical interstitial lesions and mild angiopathic changes. From these findings, CurNPs can alleviate the lesions caused by Ketoprofen and this confirmed by many researchers. The ameliorative effect of CurNPs was observed by Sandhiutami *et al*.,(2019) who described that CurNPs decreased necrosis of the epithelial cells and reduced the damage of renal histology. The obtained results were supported by Mailafiya *et al*. (2020) who recorded that CurNPs reduced pathological changes such as atrophy and necrosis of the glomerulus. Anwar *et al*. (2020) stated that CurNPs at low doses decreased the histopathological lesions. The protective effect appeared in the glomerular tufts that were less contracted and in mild necrosis but at high dose, most of the lesions were disappeared.

Moreover, Ansar *et al*. (2019) reported that CurNPs had a protective effect against congestion in glomeruli and degeneration of tubules. Similarly, El-Gizawy *et al*. (2020) stated that CurNPs decreased renal lesions such as tubular necrosis and proteinaceous casts deposition in the lumen of renal tubules.

Improvement of the histological alterations with CurNP is related to its antioxidant and its ability to protect tissue (Sankar *et al*., 2013). Also, CurNPs have an anti-inflammatory effect, because they can decrease the lipoxygenase enzymes that stimulate inflammation (Hanai *et al*., 2009). CurNPs could be very efficient because they easily penetrate cell membranes because of their small diameter and large surface area (Flora *et al*., 2013 a). Furthermore CurNPs reduce the levels of inflammatory cytokines like tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) (Boarescu *et al*., 2019).

By contrast, Mosa *et al*. (2019) mentioned that CurNPs given with hydroxyapatite nanoparticles induced nephrotoxicity, exhibited a mild degree of amelioration such as wide lumen renal tubules, but some tubules have still degenerated in rats treated with CurNPs for 45 days with a dose of 15mg/kg.

This study investigated the expression of COX-1 in renal tissue of all experimental groups using immunhistochemical analysis. The immunoreactivity in rats received Ketoprofen revealed that glomeruli, endothelial cells lining blood vessels and renal epithelium of collecting ducts were negatively expressed for COX-1. In the control group, the glomerulus and renal epithelium appeared moderate positive expressions of COX-1 with intense brown color. In the present study we reported that Ketoprofen inhibited COX-1 in renal tissue, these results agreed with many authors. Meskell and Ettarh (2011) revealed similar results when studied the NSAIDs effect on the renal COX-1 expression using immunohistochemistry. The authors reported the expression of COX-1 protein was in the glomerulus, parietal cells of the Bowman's capsule, endothelial cells of renal blood vessels, and collecting ducts of control mice. In contrast, indomethacin treated mice, with minimal expression in the glomerular capsule, endothelium of renal blood vessels and collecting ducts. Furthermore, nimesulide treated mice; appeared reduction in COX-1 expression in renal tissue with very minimal staining in the proximal convoluted tubule, however, in the collecting duct and endothelium of blood vessels focal cytoplasmic immunostaining was seen.

Similar immunolocalization of COX-1 was mentioned by Pelligand *et al*. (2015) who found that in the afferent arteriole and glomeruli of normal kidneys cat, COX-1 staining was slight, however, strongly immunopositive staining was revealed in the collecting duct. Khan *et al*. (2016) stated that in the endothelial cells of renal blood vessels and epithelial cells of collecting ducts, COX-1 immunoreactive protein was found of all species in their study.

In the current study, the expression of COX-1 in renal tissue did not decrease or inhibit by CurNPs treatment. Immunhistochemical examination of immunoreactivity in the Ketoprofen and CurNPs treated group showed moderate positive staining of COX-1 reaction. Also, a strong positive expression of COX-1 was exhibited in CurNPs treated group. Similar immunohistochemical results were described by Goel *et al*. (2001) who proved that in the cancer cells of the human colon, curcumin inhibits COX-2 but not COX-1 expression so; curcumin treatments are not reduced COX-1 protein expression. (Ghosh *et al*., 2012) stated that the basal expression of COX-1 in the macrophages of nephrec-

tomized rats did not alter by curcumin administration. Chun *et al.* (2003) reported that curcumin inhibited COX-2 expression in mouse skin. Similarly, Morsy *et al.* (2013) reported that curcumin decreased COX-2 expression in renal tissue through immunohistochemical staining of kidney rats moreover; curcumin had an anti-inflammatory effect and ameliorated nephrotoxicity.

CONCLUSION

Ketoprofen administration for long time lead to more advanced lesions and chronic kidney diseases. Furthermore, Ketoprofen alters kidney functions and oxidative stress indices as well. CurNPs administration ameliorate the nephropathic lesions induced by Ketoprofen administration by stimulation of COX-1 expression which has a protective effect .Moreover, CurNPs improved renal kidney functions and oxidative stress indices.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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