Histopathological changes induce by piroxicam administration in kidneys of adult male albino mice *Mus musculus*

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Abstract
Piroxicam is one of non-steroidal anti-inflammatory drugs (NSAIDs) which is widely used in treating rheumatic disorders. The current study aimed to detect the deleterious effect of piroxicam pertaining to the chronic administration in male albino mice *Mus musculus.* A total number of (64) adult male albino mice were utilized in this study, they were randomly distributed into four main groups, the first three groups were orally treated with concentrations (50,100,150) mg/kg of piroxicam respectively, the forth group considered as control group were orally treated with distilled water for eight weeks, kidney samples were collected every two weeks for the histological study.
This study recorded the presence of histopathological changes in the kidney of the treated mice with the concentration (50) mg/kg represented by hemorrhage, vascular congestion, calcium casts formation inside the lumens of renal tubules, while the groups treated with (100)mg/kg of the drug showed a fibrin deposition inside renal tubular lumens, infiltration of inflammatory cells, cytoplasmic vacuolation in the epithelial cells of the renal tubules and sloughing epithelium of renal tubules. The histological examination of mice kidneys treated with (150) mg/kg showed the appearance of hyalinization, shrinkage and complete loss of glomeruli, pyknosis of nuclei as well as the presence of oedema, in addition there were a remarkable decrease in the glycogen and protein contents of cells. The statistical results of the present study revealed a significant differences ($P<0.05$) in the mean diameters of renal glomeruli for all concentrations of the experiment for the period of eight weeks also there were significant decrease in the mean diameters of proximal convoluted tubules in the period of four weeks of the treated animals with concentrations (100 and 150) mg/kg as compared with control group. While the diameter of distal convoluted tubulesshowed a significant decrease with the concentrations (100 and 150)mg/kg for the periods six and eight weeks as compared with control group. It is clear from this study that piroxicam has drastic toxic effects on kidney tissue as represented by the observed histopathological changes.

Key words: Histopathological changes, Piroxicam, Kidney, Albino mice

التغيرات المرضية النسجية الناجمة عن التجريع بعقار البيروكسيكام في كلى ذكور الفئران البيض البالغة *Mus musculus*

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الخلاصة

يعد عقار البيروكسيكام احدالعقاقيرغيرالستيرويديةالمضادةللالتهابوالمستخدمةعلىنطاقواسعفيعلاجالاضطراباتالروماتيزمية. تهدف الدراسة الحالية الكشف عن التأثيرالضارلعقار البيروكسيكام نتيجة التجريع المزمن في ذكورالفئرانالبيض *Mus musculus* .

استعمل في الدراسة الحالية (64) ذكرا بالغامن الفئران البيض، وزعت الحيوانات عشوائيا إلى أربع مجموعات رئيسية، تم تجريع المجاميع الثلاثة الأولى فمويابتركيز (50، 100، 150) ملغم / كغم من عقار البيروكسيكام على التوالي، واعتبرت المجموعة الرابعة كمجموعة سيطرة حيث جرعت بالماء المقطر لمدة ثمانية أسابيع، تم جمع العينات كل أسبوعين لاغراض الدراسة النسجية.

سجلت الدراسة وجود تغيرات نسجية في كلى الفئران المجرعة بتركيز (50) ملغم / كغم تمثلتبحدوث النزف، احتقان الأوعية الدموية، و تكون قوالب الكالسيوم داخل تجاويف النبيبات الكلوية، في حين أظهرت المجاميع المجرعة بتركيز (100) ملغم/ كغم من العقار ترسب مادة الليفين داخل تجاويف النبيبات الكلوية، ارتشاح الخلايا الالتهابية، تفجي الخلايا الظهارية في النبيبات الكلوية وانفصال ظهارة النبيبات الكلوية، واظهر الفحص النسجي لكلى الفئران المجرعة بتركيز (150) ملغم / كغمحدوثالتنكس الزجاجي، انكماش وفقدان تام للكبيبات، تغلظ النوى وكذلك حدوثا لوذمة، بالإضافة الى حصولانخفاض ملحوظ في محتويات الخلايا من الكلايكوجين والبروتين.

أوضحت النتائج المورفولوجية لهذه الدراسة حدوث فروق معنوية ($P < 0.05$)في معدل أقطارالكبيبات الكلوية لجميع تراكيز التجربةلمدة ثمانية أسابيع كما لوحظ حدوث انخفاض ملحوظ في معدلأقطار النبيبات الملتوية الدانيةفي المدة اربعة أسابيع للمجاميع المجرعة بتركيز(100، 150)ملغم/كغممقارنةمعمجموعةالسيطرة،في حين أظهرت النتائج المتعلقة بالنبيبات الملتوية القاصية حدوث انخفاض ملحوظفيالتراكيز(100،150)ملغم/ كغم لمدة ستة وثمانية أسابيع مقارنة مع مجموعة السيطرة.

يتضح من خلال الدراسة الحالية ان لعقار البيروكسيكام تأثيرات سمية كبيرة في نسج الكلية والمتمثلة بالتغيرات المرضية النسجية الانفة الذكر.

الكلمات المفتاحية: التغيرات المرضية النسجية،بيروكسيكام،كلية ، فئران بيض

Introduction

Piroxicam is one of the most popular non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of inflammatory conditions and rheumatic disorders, it is useful in the management of ankylosing spondylitis, acute musculoskeletal disorders and dysmenorrhea [1]. It is belong to oxicam group, a group of non –steroidal anti-inflammatory, analgesic and antipyretic drugs, it is readily absorbed following oral administration and its long plasma half-life allows once daily dosing which is possible advantage in improving compliance [2].

The unwanted effect of NSAIDs is involved in the inhibition of cyclooxygenase enzyme (COX) pathway, inhibition prostaglandins which prevents ovulation in rat [3]. The recent studies showed that piroxicam has many side effects on intestinal, digestive system, represented by ulcers, gastritis [4].

NSAIDs also caused pregnant mice abortion and congenital anomalies in mice fetuses [5], furthermore there were side effects of piroxicam on the testes of male mice [6]. Ebaid *et. al.* [7] also found that injecting piroxicam caused a remarkable histopathological symptoms in the liver and kidney of male albino mice.

This study was done to evaluate the histological alterations in kidneys of adult male albino mice that induced by piroxicam.

Materials and Methods

Animals

A total number of (64) healthy adult male albino mice *Mus musculus* were utilized in this study they were collected from Iraqi national center for drug control and research, Baghdad, Iraq. weighing between (25-30) gm and age approximately (8-10) weeks old, They were randomly distributed into four main groups of five mice for each, they were housed in cages and were kept in the laboratory under constant conditions for at least one week before use, they were fed a standard commercial diet.

Experimental design

The drug used in this study was piroxicam in capsule form of (20) mg for each, concentrations (50, 100, 150) mg/kg were prepared daily and they were given orally using stomach cannula for the periods two, four, six and eight weeks three of the four groups were orally treated with the concentrations (50, 100, 150) mg/kg respectively, the forth group considered as control group were orally treated with 0.1 ml of distilled water for eight weeks.

Histological & Histochemical preparations

Animals from control and treated groups were sacrificed, dissected and small pieces of the kidney were quickly removed, then fixed in formalin fixative for 22 hours followed by changing the fluids with 70% alcohol after washing the specimens for many times, after washing, specimens were dehydrated, embedded and then sectioned to 5 µ thickness, for the histological examinations, sections were stained with haematoxylin and eosin [8].

In the histochemical study, sections were stained with periodic acid schiff's method to demonstrate carbohydrates [8] and with bromophenol blue to demonstrate total proteins [9].

Morphometric measurements

The morphometric measurements were done under light microscope using ocular micrometer stage after calibrating the ocular lenses with micrometer stage for each magnification power[10].

Statistical analysis

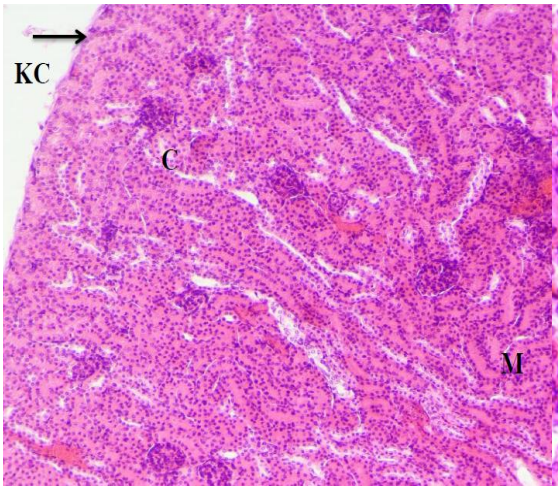
The statistical analysis were done by using the statistical analysis system (SAS) program to demonstrate the effect of drug concentration and period in different standards, which was performed by comparing the significant differences of the control groups and the treated groups for each period with the Least significant differences (LSD) at the level ($P<0.05$) [11].

Results

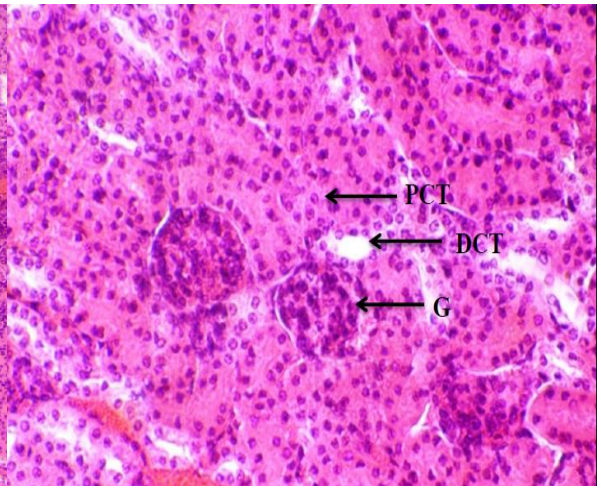
Histological examination of kidney tissue

Control group

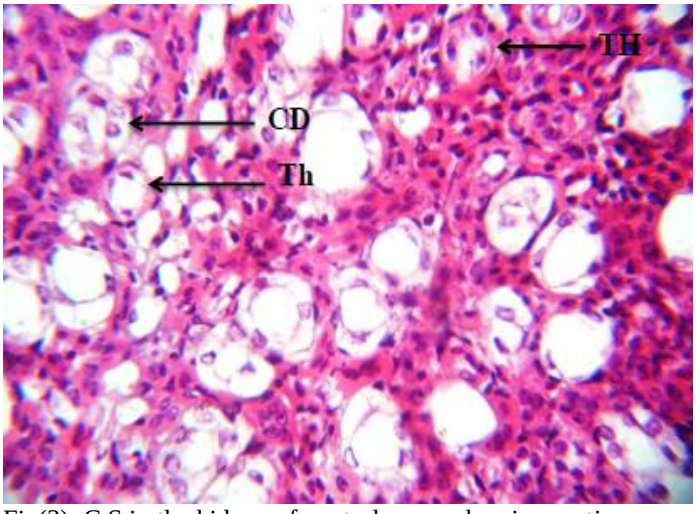
Histological sections of this group appeared with normal histological structure represented by a kidney surrounded by a capsule and it is differentiated into an outer region called cortex which contains renal corpuscles and sections of proximal and distal convoluted tubules, and an inner region called medulla which showed sections of thick and thin segments of Henle's loop as well as sections of the collecting ducts figures (1,2,3).



Fig(1): C.S in the kidney of control group showing Kidney capsule (KC), cortex (C) and medulla (M) (H&E, 100X).



Fig(2):C.S in the kidney of control group showing Distal convoluted tubule (DCT), Glomerulus (G) and Proximal convoluted tubule (PCT)(H&E, 400X) .

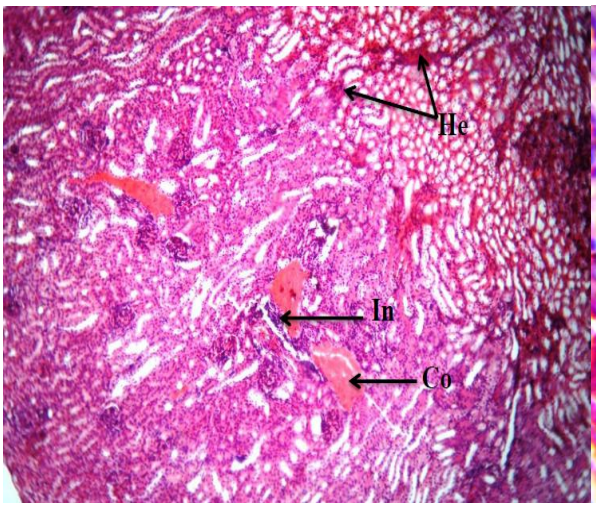


Fig(3): C.S in the kidney of control group showing sections

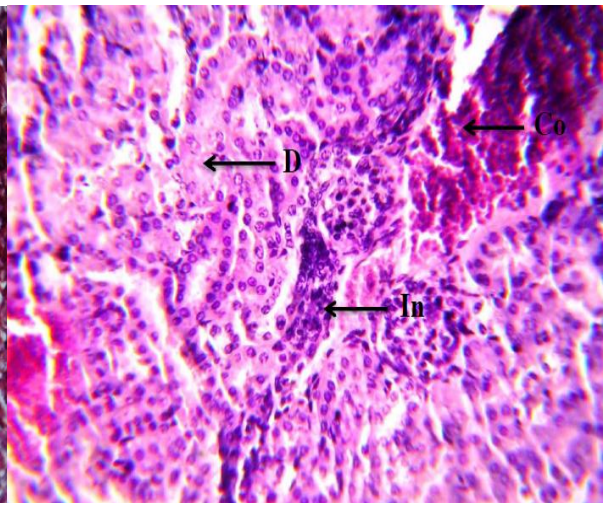
of collecting ducts (CD), Thick segments (TH) and Thin segments (Th) of Henle's Loop(H&E, 400X).

Groups treated with (50) mg/kg of piroxicam

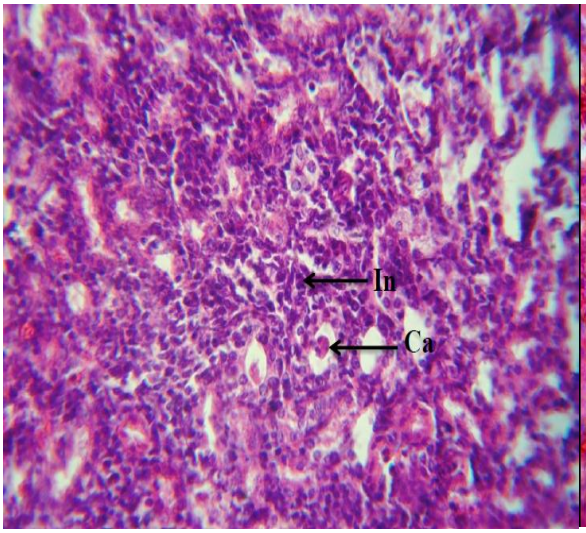
The examination of kidney sections of treated mice with (50) mg/kg of piroxicam for different periods revealed many pathological changes compared with control group. These changes are represented by vascular congestion and hemorrhage after 2 weeks, infiltration of leukocytes after 4 weeks of administration as well as the beginning of calcium casts formation after 6 and 8 weeks of administration figures (4,5,6,7).



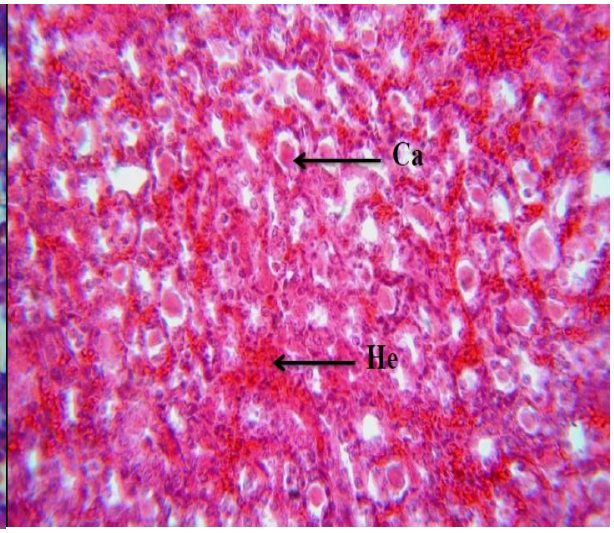
Fig(4):C.S in the kidney of group treated with (50)mg/kg for 2 weeks showing the congestion of blood Hemorrhage (He) and Infiltration of leukocytes (In)(H&E, 100X)



Fig(5): C.S in the kidney of group treated with (50) mg/kg for 4 weeks showing the congestion of blood vessels (Co), Degeneration of tubular epithelium (D) and Infiltration of leukocytes (In)(H&E, 400X)



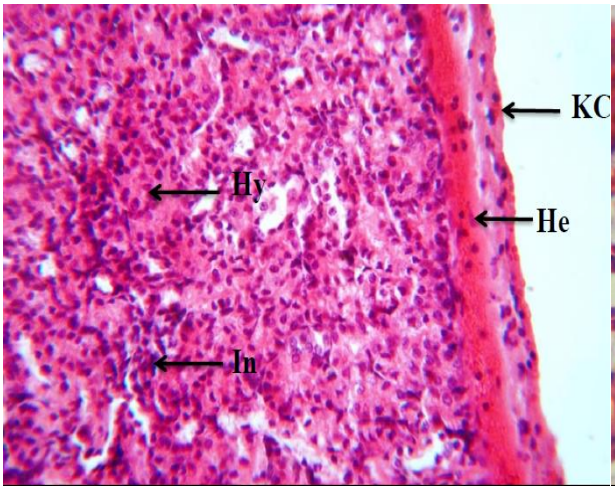
Fig(6): C.S in kidney of group treated with (50)mg/kg for 6 weeks showing the beginning of calcium casts formation (Ca) and the Infiltrationof leukocytes(In)(H&E, 400X)



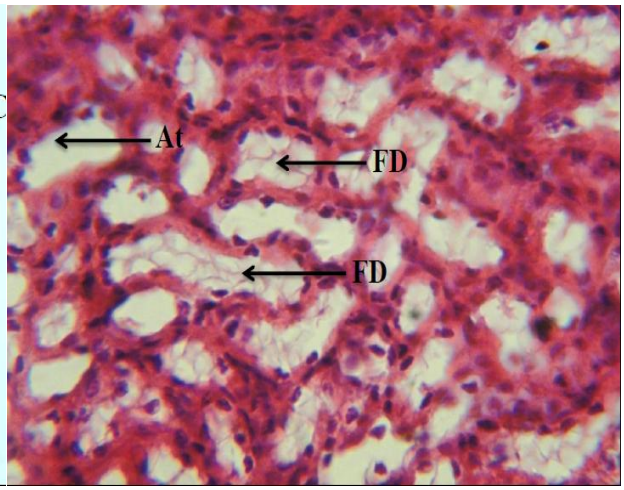
Fig(7): C.S in kidney of group treated with (50)mg/kg for 8 weeks showing the formation of calcium casts (Ca) and the hemorrhage (He)(H&E, 400X)

Groups treated with (100) mg/kg of piroxicam

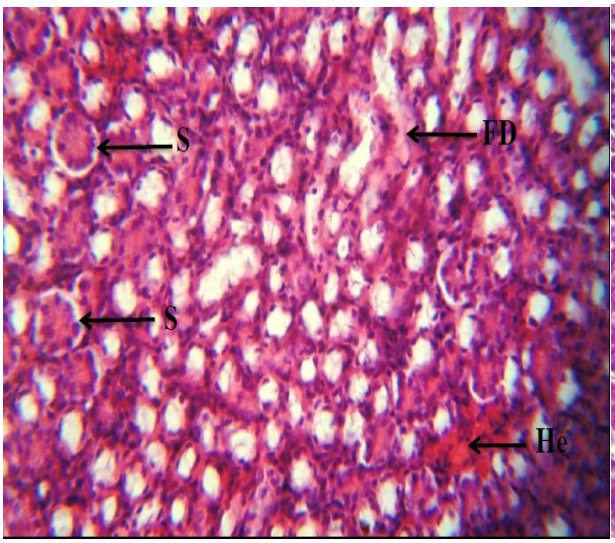
The results of microscopic examination showed many histopathological changes in the kidney of treated mice with (100)mg/kg of piroxicam for different periods of exposure these changes included the increased thickness of kidney capsule, subcapsular hemorrhage, hypertrophy of epithelial cells and the Infiltration of leukocytes after 2 weeks of administration figure (8), the sloughing epithelium in renal tubules , fibrin deposition inside tubular lumens, atrophy of tubular epithelium and hemorrhage were shown after 4 and 6 weeks figures (9,10), while after 8 weeks of administration there was a shrinkage and loss of some glomeruli in the kidney sections figure(11).

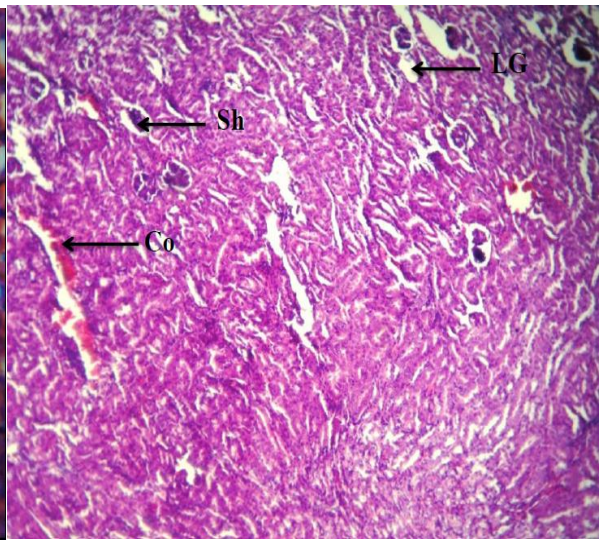


Fig(8): C.S in the kidney of group treated with (100) mg/kg for 2weeks showing the subcapsular hemorrhage(He), hypertrophy(Hy) of epitheliumand the Infiltration of leukocytes (In), kidney capsule (KC).(H&E, 400X)



Fig(9): C.S in the kidney of group treated with (100) mg/kg for 4 weeks showing the Atrophyof tubular epithelium (At) and fibrindeposition (FD)(H&E, 400X)



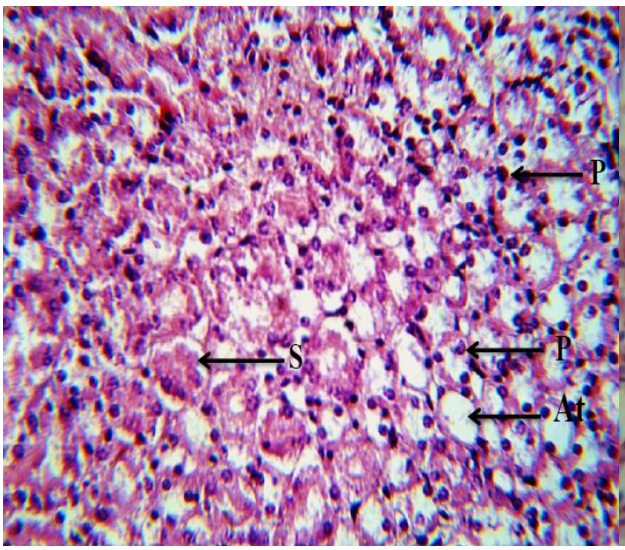


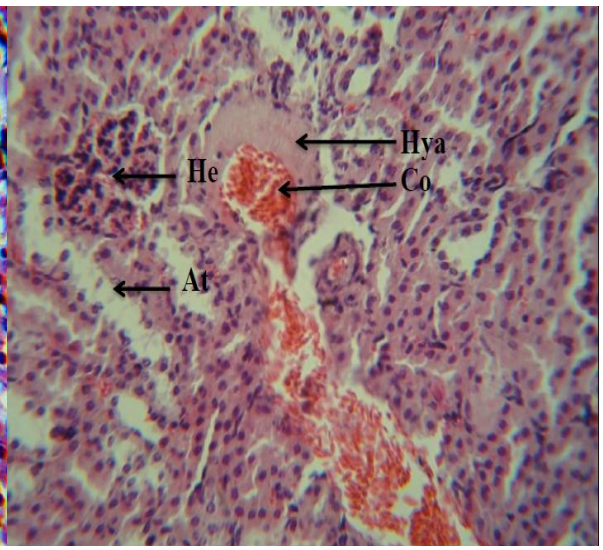
Fig(10): C.S in the kidney of group treated with (100)mg/kg for 6weeks showing the fibrin deposition (FD),sloughingepithelium (S)and hemorrhage(He)and completely loss of glomeruli (LG)(H&E, 100X).

Fig(11):C.S in the kidney of group treated with (100)mg/kg for 8weeks showing the congestion (Co), shrinkage(Sh) (S)and hemorrhage(He)and completely loss of glomeruli (LG)(H&E,400X).

Groups treated with (150) mg/kg of piroxicam

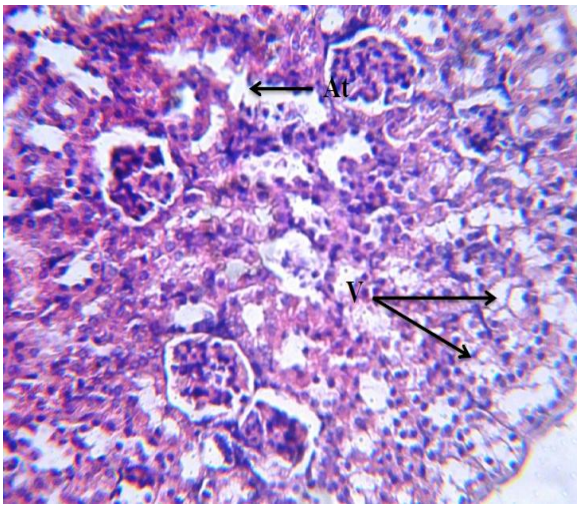
The examination of mice kidneys treated with (150) mg/kg of piroxicam showed severe changes that represented by pyknosis of nuclei, atrophy and sloughing of epithelium after 2weeks of administration figure(12).The appearance of hyalinization around the congested blood vessels was observed after 4 weeks figure(13), whereas after 6 and 8 weeks of administration the changes were vacuolation of tubular cytoplasm and atrophy of tubular epithelium as well as the presence of odema that occupied a large area of the kidney tissue figures (14,15).



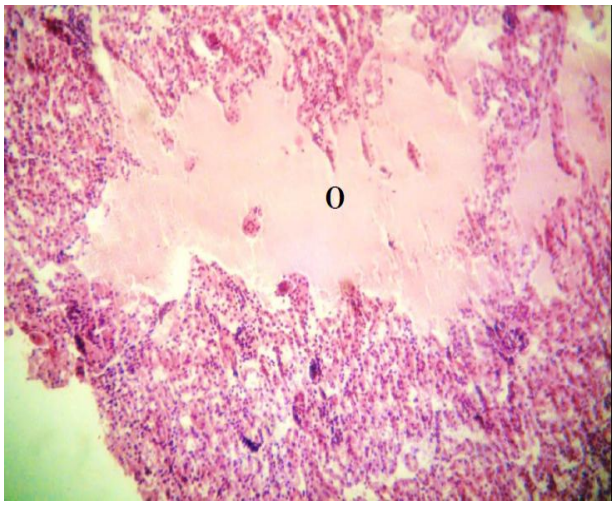


Fig(12):C.S in the kidney of group treated with (150)mg /kg for 2weeks showing theatrophyof tubularEpithelium(At),pyknosis (P)and sloughing (S)(H&E,400X).

Fig(13):C.S in the kidney of group treated with (150)mg/kg for 4weeks showing the atrophy (At), congestion(Co), epitheliumhyalinization (Hya)and hemorrhage(He)(H&E,400X)



Fig(14): C.S in the kidney of group treated with (150)mg/kg for 6weeks showing the atrophyof epithelial cells (At) and vacuolationof tubular cytoplasm (V) (H&E,400X).

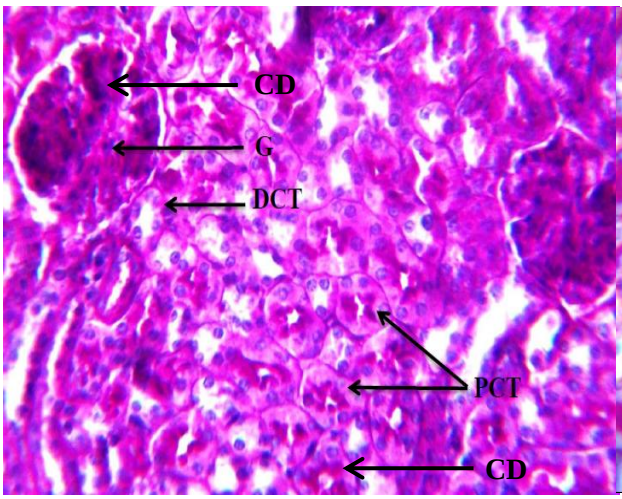


Fig(15): C.S in the kidney of group treated with (150)mg/kg for 8weeks showing the odema (O)(H&E,400X).

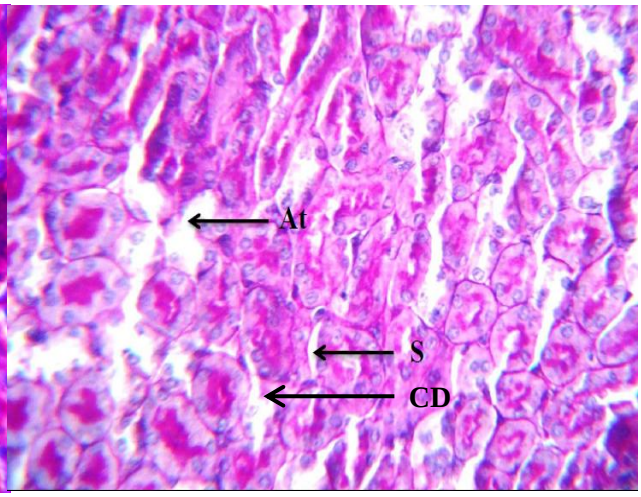
Histochemical changes in the kidney

Control kidney sections stained with periodic acid shiff's (PAS) showed a positive reaction to the stain as an indication for the existence of carbohydrates in the brush border of proximal convoluted tubules and the basement membrane of renal tubules figure (16).

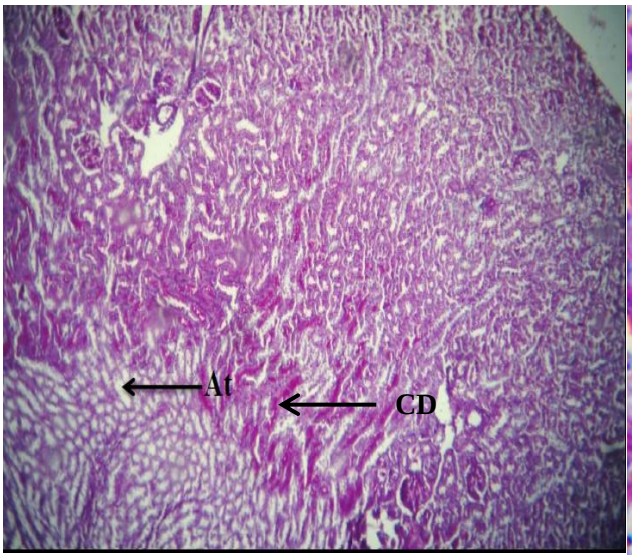
Kidney tissue of treated mice with (50,100,150)mg/kg of piroxicam after 8 weeks of administration showed a depletion of carbohydrate content. The reduction increases with the increment of concentration and administration period figures (17,18,19).



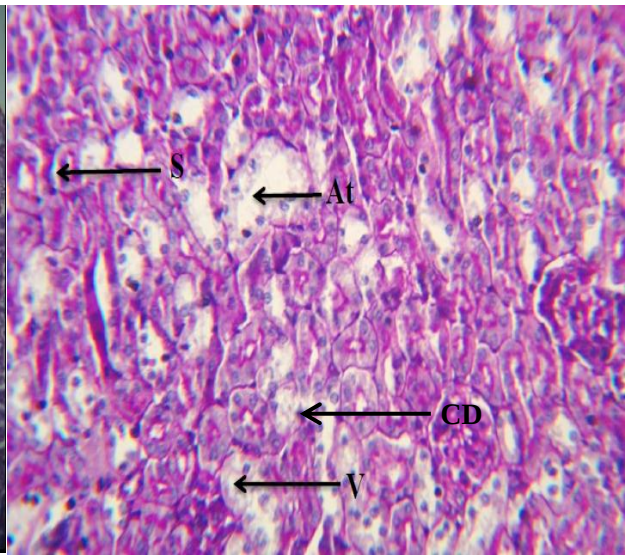
Fig(16):C.S in the kidney of control group showing Carbohydrate deposition(CD),distal convoluted tubule (DCT), glomerulus (G) and proximal convoluted tubule(PCT)(PAS, 400X).



Fig(17):C.S in the kidney of group treated with(50) mg/kg for 8weeks showing atrophy (At) and carbohydrate deposition(CD) sloughing epithelium (S) (PAS,400X).

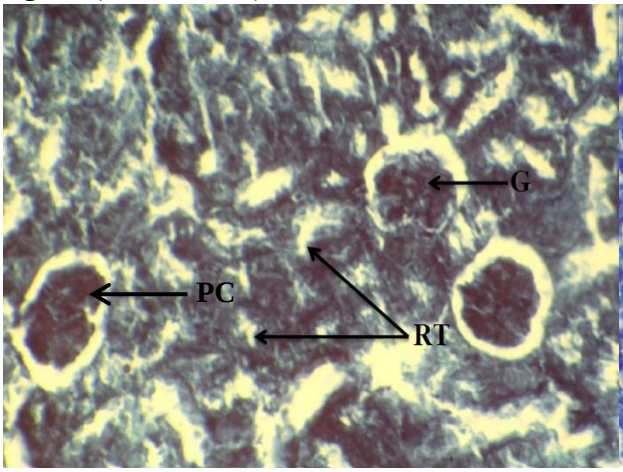


Fig(18):C.S in the kidney of group treated with (100) mg/kg for 8weeks showing atrophy (At), carbohydrate deposition(CD) (PAS,100X).

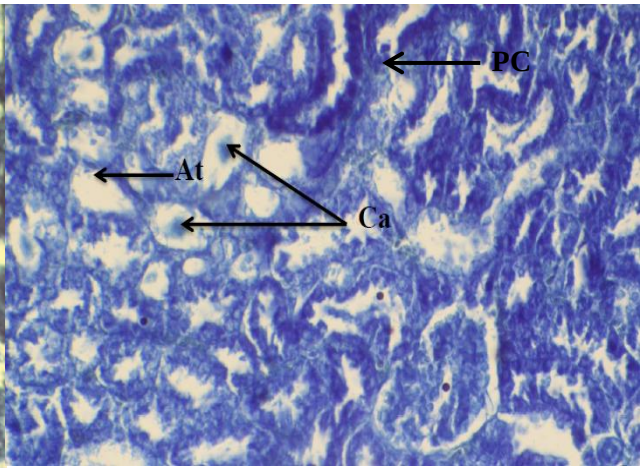


Fig(19):C.S in the kidney of group treated with (150) mg/kg for 8weeks showing atrophy (At),carbohydrate deposition (CD), sloughing epithelium (S) and Vacuolation (V) (PAS,400X).

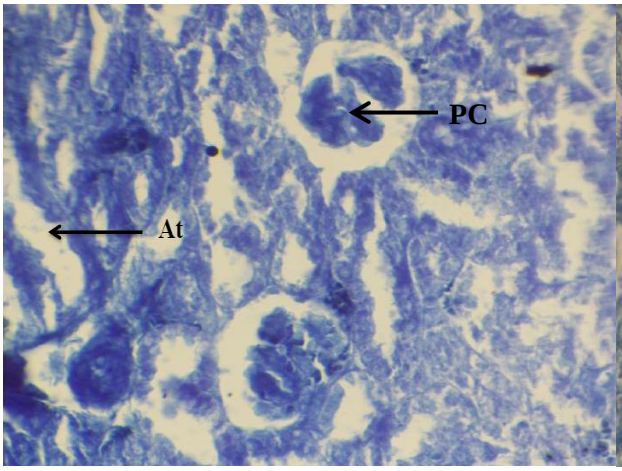
The control sections stained with Bromophenol blue (Bpb) showed normal protein content which is equally distributed in glomeruli and renal tubules, while there were a reduction in protein content in the kidney tissue of treated mice with (50,100,150) after 8weeks of administration figures(20,21,22,23)



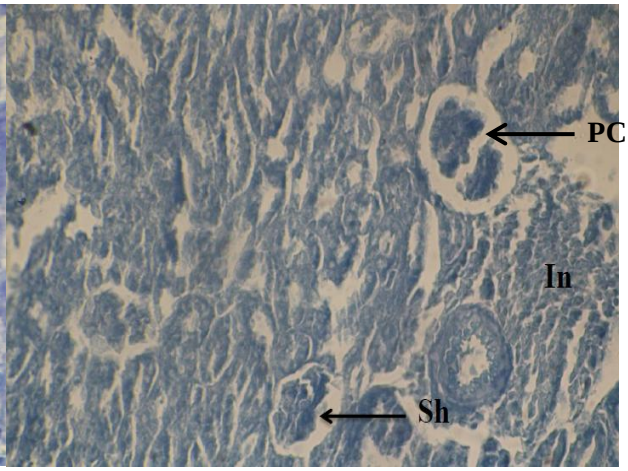
Fig(20):C.S in the kidney of control group showing the protein content (PC) inglomerulus (G),renal tubules (RT)(Bpb, 400X)



Fig(21):C.S in the kidney of group treated with (50) mg/kg for 8weeks showing atrophy (At) , calcium casts(Ca) and protein content (PC) (Bpb,400X).



Fig(22):C.S in the kidney of group treated with (100) mg/kg for 8weeks showingAtrophy (At) and protein content (PC) (Bpb, 400X)



Fig(23):C.S in the kidney of group treated with (150) mg/kg for 8weeks showing infiltrationof leukocytes (In) , shrinkage of glomerulus (Sh) and protein content (PC) (Bpb, 400X)

Morphometric results

The morphometric results of the present study revealed significant differences ($P<0.05$) in the mean diameters of renal glomeruli for all concentrations of piroxicam for the period of eight weeks compared with control group, while the other periods did not show any significant differences (Table 1).Also there were non-significant decrease in the mean diameters of proximal convoluted tubules in the treated groups with concentration of (50,100,150) mg/kg in all periods except the period four weeks of the treated animals with concentrations (100,150) mg/kg that showed a significant decrease ($P<0.05$) in comparison with control group (Table 2). The results also clarified that there were significant differences ($P<0.05$) in the mean diameters of distal convoluted tubules of treated groups with concentration (100,150) mg/kg for six and eight weeks of administration, while the group treated with concentration (50)mg/kg of piroxicam did not show any significant differences over the whole administration period (Table 3).

Table (1): The effect of Piroxicam on the mean diameter of renal glomeruli (µm) of male mice compared with the control group

Drug concentration mg/kg	Mean diameter of renal glomeruli (µm) ± S.E				L.S.D
	Periods (weeks)				
	2	4	6	8	
Control (0)	50.5±1.9	49.8±1.5	48.5±1.6	50.5±1.9	NS 5.85
50	38.0±1.7	36.5±1.8	31.3±1.6	36.0±1.6	*7.88
100	39.0±3.1	41.0±1.7	33.0±3.0	32.0±1.3	*9.24
150	39.0±2.3	38.0±2.0	27.0±2.6	26.0±1.6	*7.94
L.S.D	NS 6.79	NS 5.93	NS 6.77	*4.70	----

NS: Non-significant, *(P<0.05)

Table (2): The effect of Piroxicam on the mean diameter of Proximal convoluted tubule (µm) of male mice compared with the control group

Drug Concentration mg/kg	Mean diameter of proximal convoluted tubule (µm) ± S.E				L.S.D
	Periods (weeks)				
	2	4	6	8	
Control (0)	28.2±1.1	28.0±1.0	28.0±1.1	28.5±1.2	NS 1.85
50	26.3±0.8	27.0±0.7	24.1±0.9	22.6±0.6	*4.87
100	26.5±1.1	23.5±0.6	23.5±0.7	22.0±0.6	*4.15
150	25.0±0.5	22.2±0.6	22.7±0.6	21.7±0.6	*3.59
L.S.D	NS 2.45	* 2.93	NS 2.35	NS 1.91	----

NS: Non-significant, *(P<0.05)

Table (3): The effect of Piroxicam on the mean diameter of Distal convoluted tubule (µm) of male mice compared with the control group

Drug concentration mg/kg	Mean diameter of distal convoluted tubule (µm) ± S.E				L.S.D
	Periods (weeks)				
	2	4	6	8	
Control (0)	25.6±0.6	24.5±0.9	25.2±0.5	25.8±0.8	NS 2.25
50	25.7±0.9	27.0±0.7	26.5±0.9	25.8±0.6	*4.06
100	25.5±0.8	26.2±0.6	23.5±0.7	21.2±0.4	*3.57
150	25.0±0.5	25.0±0.6	22.0±0.5	20.7±0.4	*3.74
L.S.D	NS 2.89	NS 2.29	*2.36	*1.69	----

NS: Non-significant, *($P<0.05$)

Discussion

In this study, we tried to identify the effect of piroxicam in the kidneys of adult male albino mice. It was clear that its effect was time dependent, there were increased effects with prolonged time of dose administration this result is in agreement with[7].

The histological examination revealed that there were histopathological changes in the kidney of treated mice with the concentrations (50, 100, 150)mg/kg of piroxicam and were more severe by using high concentrations (100, 150) mg/kg , the histopathological changes of groups treated with (50) mg/kg showed severe hemorrhage, congestion, formation of calcium casts, these findings in agreement with Baisakh *et. al.* foundation [12] , furthermore these changes were accompanied by invasion of inflammatory cells to the interlobular tissues in a trial to minimize the injury [13] .

There was also a remarkable histopathological changes in the most groups treated with piroxicam that represented by a vascular congestion that may be related to the use of drug that cause an acute inflammation this and led to change the blood flow inside blood vessels, this change may cause a relaxation and an extension in these blood vessels, thus the blood will accumulate into the vessels [14].

The hemorrhage observed in the present study may be caused by the continuous inflammation which is resulted from the effect of drug especially with high concentrations, and led to the shred of the endothelial cells that line the blood capillaries. These findings in agreement with [15].

Results also showed formation of calcium casts in the tubular lumen, this may attributed to the elevation of hydrogen peroxidase H_2O_2 that cause the nephrotoxicity which is responsible for changing the permeability of mitochondrial cellular membrane; Hence the amount of calcium taken by the mitochondria will increase and with the existence of oxygen, the mitochondria will break down and the calcium will be released in the kidney tissue [16].

The present study also showed the presence of histopathological symptoms in the groups treated with (100) mg/kg of piroxicam that represented by fibrin deposition inside the tubular lumens, these findings also observed by [17] who stated that fibrin deposition is attributed to the inhibition of cyclooxygenase-1 by NSAIDs which is responsible for prostaglandins production in different positions of kidney tissue.

The sloughing of tubular epithelium would happen in a trial to increase the distance, so that the drug will not reach the blood stream as much as possible to exclude its harmful effect [18].
The shrinkage and loss of some glomeruli that observed in this group, may be caused by the mesangial cell processes that retracted due to the contraction of their filaments [12].
Results also showed the appearance of hyalinization in the kidney of groups treated with 150 mg/kg of piroxicam after 8weeks of administration is probably caused by the accumulation of abnormal protein substances resulted from dissolution of amino acids especially the immunoglobulins and carbohydrates resulted from cell death [19].
Regarding the histochemical changes observed in this study under piroxicam administration, results showed a reduction in the polysaccharides and total proteins in the kidney tissue, The decrease in carbohydrate content could be attributed to the increased stress on organs, which lead to high energy consumption and allowed an equalized pressure to be exerted upon them [20,7] or may be due to the depletion of mucopolysaccharide in tissue which is attributed to the turbulence of Golgi apparatus [21].
The decrease in protein content could be attributed to the depletion of ribosomal granules of the rough endoplasmic reticulum [21] or it may be caused by the disruption of lysosomal membrane under the effect of various toxicants leading to the liberation of their hydrolytic enzymes in the cytoplasm resulting in marked lysis and dissolution of the target materials [7].

The morphometric study regarding the measurements of glomerular diameters revealed a significant decrease in the mean diameters of glomeruli in groups treated with (50,100,150)mg/kg of piroxicam for the period 8 weeks, this findings are in agreement with the results of [17].Also measuring diameters of proximal convoluted tubules showed non-significant differences except the period of 4 weeks of treated groups with (100,150) mg/kg showed a significant decrease; while the results of distal convoluted tubules showed a significant decrease in the periods (6,8) weeks for the groups treated with (100,150)mg/kg. these results are consistent with many researchers included [22&23].

The main reason behind changing in the diameters of glomeruli, proximal and distal convoluted tubules is using NSAIDs that may reduce the collagen content in the tissue which probably has a big role in supporting the wall of renal tubules and ducts, therefore, the reduction of this material resulted in changing the tissue features [23].
Safari *et. al.*[24] suggested that using NSAIDs may cause free radicles releasing that is related with causing damages to renal tubules and glomeruli.

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