British Journal of Medicine & Medical Research

21(4): 1-8, 2017; Article no.BJMMR.32315
ISSN: 2231-0614, NLM ID: 101570965

Effect of Meloxicam on Hematological and Kidney Histopathological Changes in Male Mice

Najat M. H. Mohammed[1*], Ezzat El-Drieny[1], Ibrahim S. Eldurssi[2] and Masouda El-Agory[1]

[1]Department of Histology, Faculty of Medicine, Omar El-Mukhtar University, El-Bayda, Libya.
[2]Department of Zoology, Faculty of Science, Omar El-Mukhtar University, El-Bayda, Libya.

Authors' contributions

This work was carried out in collaboration between all authors. Author NMB designed the study, wrote the protocol and managed the analyses of the study. Author EED did examine and comment on prepared sections, wrote the first draft of the manuscript. Author IED managed the literature searches and sacrificed the animals. Author MEA performed tissue sampling and processing. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2017/32315
Editor(s):
(1) Toru Watanabe, Department of Pediatrics, Niigata City General Hospital, Japan.
Reviewers:
(1) Olga Berillo, McGill University, Canada.
(2) Abdullahi M. Nuhu, Cst. Kaduna Polytechnic, Kaduna, Nigeria.
(3) Nyoman Kertia, Gadjah Mada University, Indonesia.
Complete Peer review History: http://www.sciencedomain.org/review-history/18985

Original Research Article

Received 20th February 2017
Accepted 13th April 2017
Published 9th May 2017

ABSTRACT

Introduction: Non-steroidal anti-inflammatory drugs are class of medications that frequently utilized as analgesic and anti-inflammatory therapy. Meloxicam is type of NSAIDs that classified as selective COX 2 inhibitor, which is considered more protective than traditional nonselective COX1 inhibitor. However, meloxicam was approved by other researches in recent years to cause renal damage.
Aim of the Study: Therefore, the present study aimed to investigate effect of meloxicam on kidney of mice related with some blood parameter studies.
Methods: Fifteen male mice were divided into two groups; one group of five mice served as control and received only distilled water. While the second group was treated with meloxicam 0.4 mg/kg which administrated daily by oral gastric gavage for 10 days. After 24 hours of the last dose animals were sacrificed, their kidneys were then removed and processed for histological

**Corresponding author: E-mail: najat.mohammed@omu.edu.ly;*

examination by light microscope. Blood samples were collected by right atria and analyzed for complete blood count. The data of blood studies was presented as mean ±SEM.

Results: Sections obtained from meloxicam treated animals showed focal areas of glomerular sclerosis, shrunken glomeruli appeared with reduction of blood capillaries, presence of acidophilic materials in the capsular space and proliferation of parietal layers of bowman's capsule forming crescent shape, which further cause obliteration of urinary space. Tubules showed degenerative changes in the form of cytoplasmic vacuolization and nuclear pyknosis, PAS staining revealed depletion of carbohydrate materials of cell coats giving negativity of the stain. There was interstitial mononuclear cell infiltration and hemorrhage. While evaluation of complete blood picture showed non-significant differences (P>0.05) in Hb, HCT, RBCs count, WBCs count, MCV, MCH and MCHC of both treated animals and control group.

Conclusion: It should be concluded that meloxicam is like other traditional non-selective COX 1 inhibitor in terms of their toxic effect on renal tissue.

Keywords: Non-steroidal drugs; meloxicam; kidney and COX2 inhibitor.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have been currently used as analgesics, antipyretic and anti-inflammatory medications [1]. They show therapeutic effect in many inflammatory diseases such as osteoarthritis, Alzheimer's and cancer [1,2]. However, prescription of these drugs is associated with many side effects on human body as they can exert structural damage and tendency of pathological lesion to the kidney [1,3].

Previously the most common NSAIDs used are non-selective types, which show their therapeutic effect by decreasing prostaglandin synthesis through inhibition of cyclooxygenase enzymes (COX). Two isoforms as COX-1 and COX-2 enzyme have been recognized [2,4,5].

COX-1 enzyme is present in all body tissues and is responsible for generating prostaglandins that are normally important for maintaining organ function, intestinal integrity and blood hemostasis. While COX-2 enzyme is responsible for generating prostaglandin (PE2) induced by pathological conditions, it is expressed on kidney and vessels endothelium [2,4].

Non selective-NSAIDs act by inhibiting both COX-1 and COX-2 however, the desired effect that is needed is to inhibit only COX-2 over than COX1 [2]. Now a new version of NSAIDs called selective COX-2 inhibitor which act only on COX-2. Meloxicam is designed to leave protective effect of COX-1 products and inhibit only COX 2 which is might be useful than ns-NSAIDs.

Although some animal studies have showed that meloxicam can produce toxico-pathological damage to the renal tissue [2,3,6], as well as a group of clinical observation associated with blood disorders like anemia and cytopenia due to oral administration of non-steroidal drug aspirin [7]. Suggesting an inhibitor of the drug to bone marrow haemopoiesis. Al-Rekabi et al., have concluded that meloxicam caused significant decrease in hemoglobin level and neutropenia and that was time dependent doses [8].

The aim of this study was to investigate kidney histological changes of meloxicam as well as blood parameters study in adult male mice.

2. MATERIALS AND METHODS

The study was conducted in Histology Department, Faculty of Medicine, Omar Al-Mukhtar University. Fifteen albino adult male mice were used and their average weight 23 g. These animals were obtained from experimental animal house of Veterinary Faculty. They were maintained under standard condition in the laboratory for at least one week before use and standard food and tap water were provided ad libitum throughout experiment. They were divided into two groups; the first served as control group (5 animals) housed in a cage. While, the other group is treatment one (10 animals) treated with meloxicam.

Experimental animals received 0.4 mg/Kg of meloxicam which equalizes to human therapeutic dose (15 mg/Kg) [3]. Dosing solution of meloxicam (Mobic, Boehringer, Ingelheim, Germany) was prepared by dissolving one tablet of 15 mg in 75 ml of distilled water and each animal was given 0.05 ml of freshly prepared solution once daily in the morning after food supplement by gastric gavage for 10 days. While,

animals of control group was dosed with distilled water. At the end of experiment animals were anesthetized by chloroform and sacrificed and kidney organs removed from each animal as well as blood obtained from right atrium of heart and collected in tube containing anticoagulants (dipotassium EDTA). Then organs fixed by 10% buffered formalin and processed by routine histopathological technique.

The prepared slides were stained by Hematoxyline and Eosin (H&E) and Periodic acid Schiff's (PAS) stain. Blood samples were analyzed by Sysmex analyzer for complete blood count (CBC) including erythrocyte count, hemoglobin (Hb), hematocrit (HCT), leukocyte count (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Results of blood presented as (Mean ± SEM) and *P* value<.05 considered significant.

3. RESULTS

3.1 Histological Examination

Light microscopic examination of sections obtained from the control group stained with H&E showed normal histological structure of kidney in the form of normal glomeruli with urinary spaces and glomerular capillary tufts, epithelial lining of convoluted tubules and renal medulla (Fig. 1A). Moreover, PAS staining showed normal positive reaction of glomerular and tubular basement membranes as well as brush borders of proximal convoluted tubules (Fig. 4A). While, kidney sections treated with meloxicam showed histopathological changes in glomeruli including a slight dilatation of urinary spaces (Fig. 1B), mild congestion of blood capillaries (Fig. 1B), obliterated capsular spaces (Fig. 1C), adhesions between parietal and visceral layers of bowman capsule (Fig. 1D) and proliferation of mesangial cells (Fig. 2E). Proliferation of parietal layer of bowman capsule forming crescent shape surrounding some glomeruli (Fig. 2E) was also observed. Shrinkage of glomeruli, reduction and focal segmentation of blood capillary tufts (Figs. 2; F and G) (Fig. 2H) were seen.

Degenerative changes of epithelium of convoluted tubules were seen. The cells of proximal tubules showed cytoplasmic vacuolization and rarefaction (Figs. 3A and C). Nuclei with clumped chromatin were seen protruding towards the lumens (Fig. 3A). Hyaline casts were shown inside tubular lumens (Fig. 3B). Cortical (Fig. 3D) and medullary hemorrhage (Fig. 1D) and interstitial mononuclear cell infiltration were observed (Fig. 2E).

PAS stained sections revealed severe damage to the tubular brush borders as it appeared thin, interrupted (Fig. 4B) or completely lost (Fig. 4B and C). A presence of homogenous acidophilic materials in the capsular space (Fig. 4B).

3.2 Blood Examination

Blood samples were analyzed for complete blood picture (Hb, HCT, RBC count, WBC count, MCV, MCH and MCHC). Hematological parameters of control and meloxicam group showed that there was moderate decrease in terms of hemoglobin, hematocrit, erythrocytes, leukocyte count and mean cell volume (Table 1). However, it was not statistically significant difference. A slight increase was seen in terms of mean cell hemoglobin and mean cell hemoglobin concentration in treated animals but as compared with control was non-significant ($p>0.05$).

4. DISCUSSION

NSAIDs are the most popular analgesics that frequently described in osteoarthritis related diseases. Traditional non selective COX 1 inhibitors have been implicated with serious side effects. Shifting to a new version of NSAIDs (selective COX 2inhibitor) drug is believed to be more protective and saver in terms of gastrointestinal and renal tolerability than using traditional COX1 inhibitor [1,2]. However, it has been noted recently that this drug may induce renal damage since COX2 enzyme is normally expressed in kidney structures; glomerulus, vasculature and tubules [2,9].

In the present study, meloxicam as one of selective COX 2 inhibitor was administrated to experimental mice to investigate how could affect human kidney structure as well as blood parameters at therapeutic dose.

The present study showed many renal histological changes carrying a lot of meaning and requiring a precise discussion. Results showed many glomerular changes including congestion of blood capillaries, obliteration of capsular spaces, crescent formation and acidophilic (PAS +ve) materials in urinary spaces. Appearance of congestion was

considered the onset of glomerulonephritis [6]. The presence of acidophilic homogenous material by H&E in capsular space is a good marker of leakage of protein most probably fibrin indicating epithelial and endothelial injury causing increase of permeability to protein which in turn acts as a soluble mediator stimulating parietal layer of bowman capsule to proliferate causing crescent shape [10]. This abnormal proliferation will lead to obliteration of urinary spaces.

The present study has shown proliferation of mesangial cells and matrix. It is considered a pathological sign of glomerulosclerosis, coinciding with some investigators who found that COX2 inhibitors induce stress marker expression on mesagnial cells [1].

Some sections showed glomerular shrinkage with reduction of capillary tuft as well as segmentation of capillary loop which collectively refer to focal segmental glomerulosclerosis [10,11]. This was in agreement with other researchers which said that glomerular atrophy may be due to capillary constriction to minimize drug toxic effect [12].

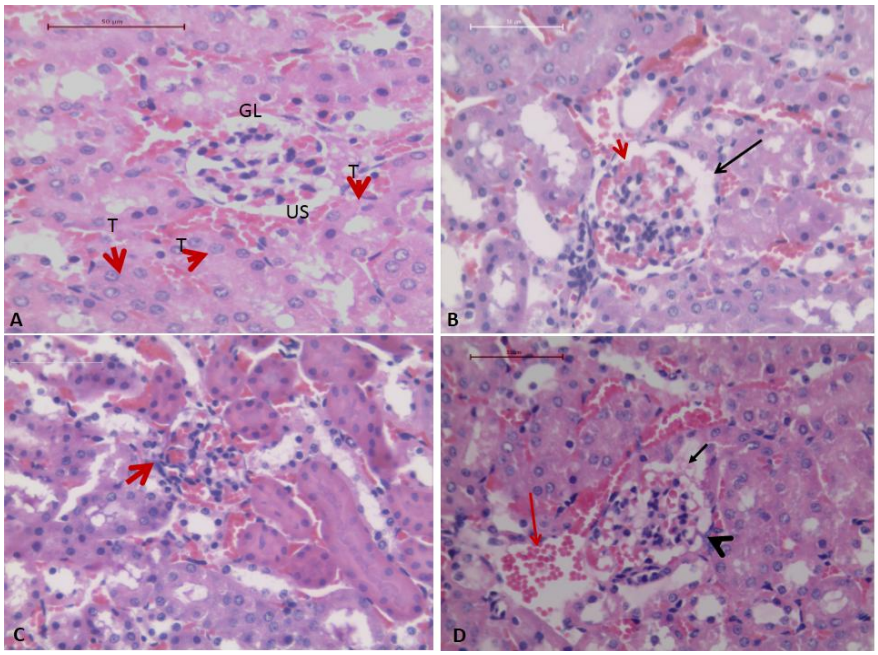

Fig. 1. Light micrographs of kidney sections. A. Control kidney representing normal structure of glomerulus (GL), normal urinary space (US) and convoluted tubules (T). In meloxicam treated groups (B,C, &D). B. Kidney glomerulus appeared with widened capsular space (thin arrow) and capillary tuft congestion (Thick red arrow).C. Glomerulus appeared with obliterated capsular space (red arrow). D. Adhesion between parietal, acidophilic materials in the capsular space (black arrow) and visceral layers of bowman capsule of glomerulus (black arrow head) and interstitial medullary hemorrhage (red arrow). Mic. Mag x200(A) x400 (B,C&D). H&E

Table 1. Represent effect of meloxicam on blood parameters of mice in comparison to the control group

	Control		Treated group
RBC ($X10^{12}$/L)	7.11±0.22	Mean ± S.E.M	6.52±0.37
Hb (g/dl)	11.67±0.096	Mean ± S.E.M	10.92±0.63
HCT (%)	35.27±0.28	Mean ± S.E.M	32.88±1.90
MCV (fL)	55.01±2.46	Mean ± S.E.M	50.21±0.43
MCH (Pg)	18.95±0.79	Mean ± S.E.M	19.78±0.19
MCHC (g/dL)	34.99±2.44	Mean ± S.E.M	39.5±0.15
WBC($x10^9$/l)	5.47±0.48	Mean ± S.E.M	5.45±0.99

**Mean ± S.E.M = Mean values ± Standard error of means*

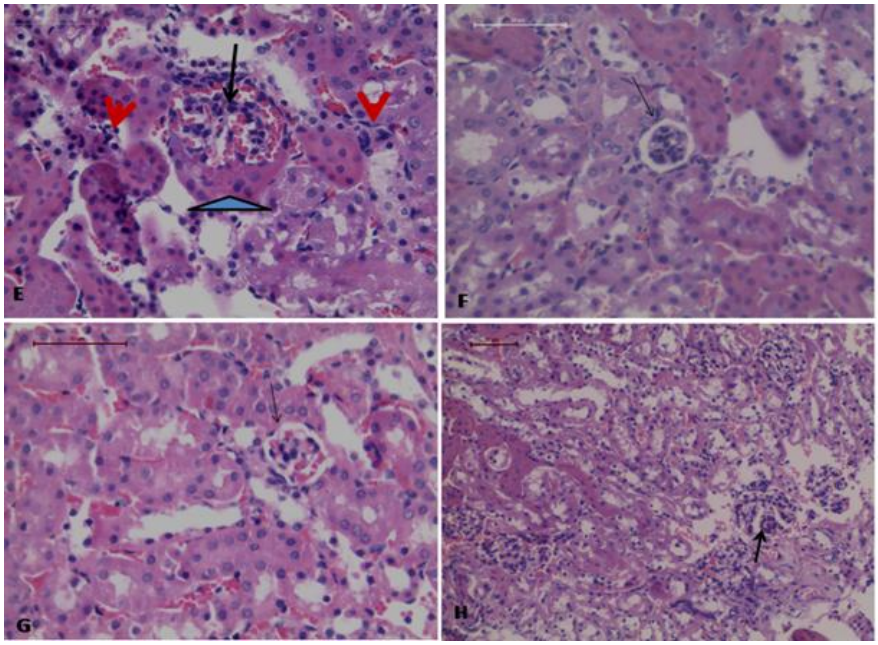

Fig. 2. Kidney of meloxicam treated mice. E. Glomerulus showing crescent formation (blue arrow head), mononuclear cell infiltration in interstitium (red arrow head) and proliferation of mesangial cells (black arrow). F&G. Treated kidney appeared shrinked glomerulus as well as reduced blood capillaries. H. Focal segmentation of capillary tufts (arrow). Mic. Mag ×400 (E,F&G) and x 200 (H). H&E

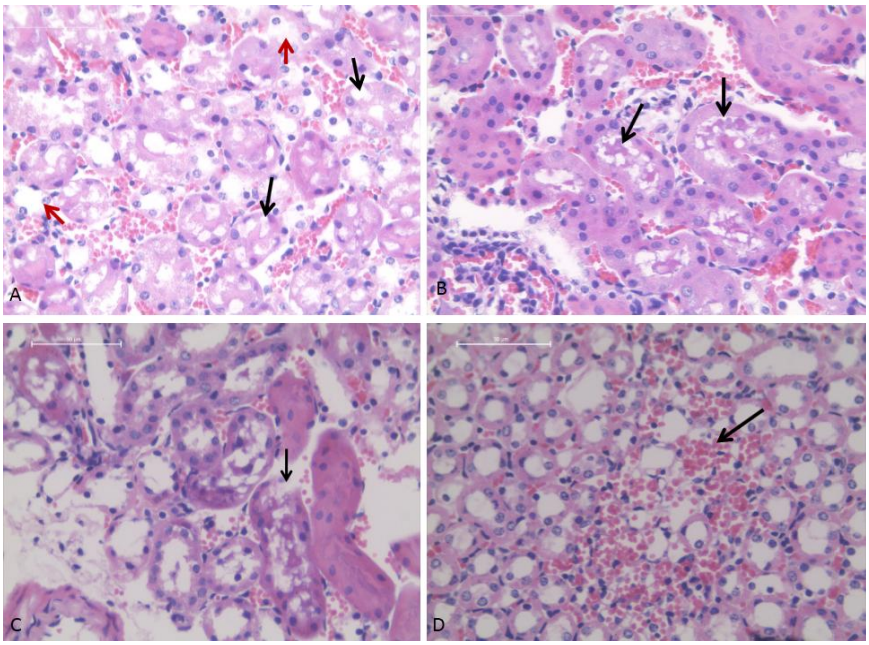

Fig. 3. Light micrographs of kidney treated with meloxicam. A. Degenerated tubules appeared with vacuolated cytoplasm (black arrow) & nuclei protrusion towards lumen (red arrow head). B. Presence of hyaline casts inside tubules (arrow). C. Rarefaction of tubular epithelial cytoplasm (arrow). D. interstitial medullary hemorrhage appeared between tubules. Mic. Mag x400 (AC&B), x200 (D) H&E

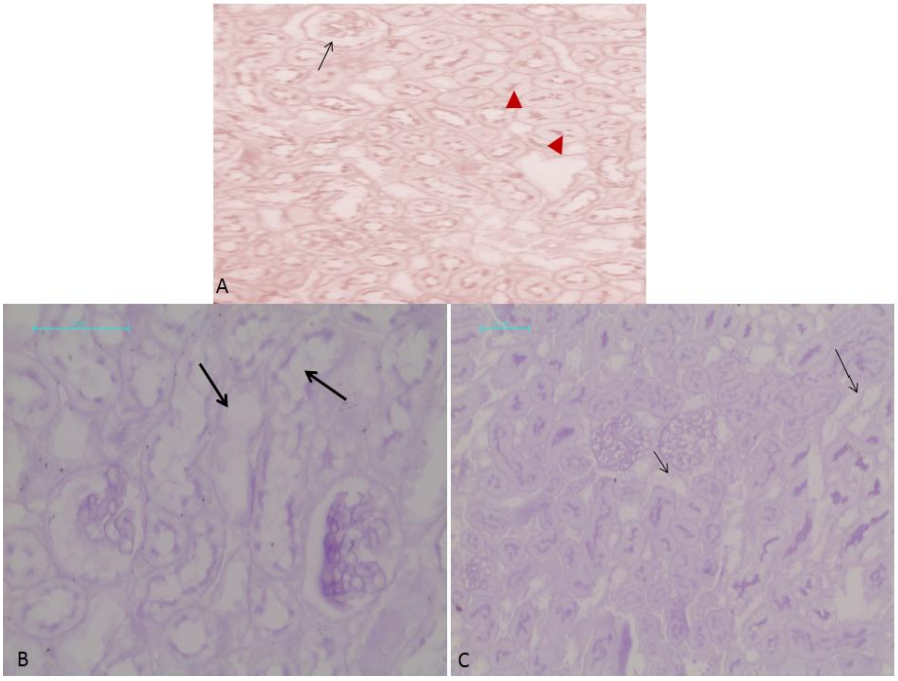

Fig. 4. Light micrographs of kidney. A. Control kidney shows normal PAS positive reaction for glomerulus basement membrane (black arrow) & brush borders of tubules (arrow head). B,C represent meloxicam treated kidney. B shows discontinuity & interruption of brush borders of most of convoluted tubules (arrow) C. Tubules appeared completely lost basement membrane & brush borders (black arrow) Mic. Mag, x400 (B), x200 (A&C) PAS

Hemorrhage appeared in the cortex and medulla which is caused by decreasing of prostaglandin vasodilator as a result of COX2 inhibition mechanism of meloxicam and increasing vasoconstriction resulting in rupture of blood vessels [13]. Some studies have demonstrated that hemorrhage was mediated by renin-angiotensinogen system [14].

Regarding to renal tubules, they appeared with degenerative epithelial changes as vacuolization and rarefaction of cytoplasm, nuclear pyknosis and even complete damage of tubular brush borders. In the same time, hyaline casts were evident in renal tubules.

Some studies suggested that tubular damage may be due to ischemia as a result of inhibition of prostaglandin production in renal arterioles [1]. In addition, Ebiad et al., found that piroxicam induces brush border damage which were attributed to increased stress on organ causing high consumption of carbohydrates [12] and consequently depletion of carbohydrate contents of the cell coat and hence weak or negative PAS staining of the brush border.

Previous ultrastructural findings of other researchers manifested podocytes injury in rabbits after administration of voltaren. These changes were in the form of fusion of foot processes with focal obliteration of filtration slits resulting in massive proteinuria and hence hyaline casts were found in the renal tubules [2,11,15]. Jorge Vega et al., demonstrated that meloxicam causes tubulo-interstitial nephritis associated with proteinuria manifested histologically in the form of hyaline casts [16].

Presence of mononuclear cell infiltration in interstitium indicates defense mechanism against inflammation. Hemorrhage and necrotic debris of damaged tubules could initiate inflammatory reaction as well as inflammatory cascade. These results were coinciding with the results of other researches [2,3,12].

There was a slight decrease in terms of Hb, RBC count and MCV as a result of effect of meloxicam on hematological parameters, however it was not significant (P>0.05). This because the present study used only the permitted therapeutic dose which is in agreement with previous findings

which showed that meloxicam effect is dose dependent and when it is administrated with high dose significant decrease in blood indices was seen [8].

5. CONCLUSION

It could be concluded from this study that meloxicam is like other traditional non-steroidal drugs and might cause considerable degree of nephrotoxicity at therapeutic dose level as represented by the observed histopathological changes and reporting of these events is to increase awareness and to better understanding of the risk factors of unlimited use of meloxicam. However, further research is needed to examine that the toxic effects of meloxicam are reversible or not.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Bioethics Committee at Biotechnology Research Center (BEC-BTRC), Protocol No. BEC-BTRC-02-2017.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the invaluable help and assistance to Histology Department of Medical Faculty, Omer Al-Mukhtar University as well as staff members of Veterinary collage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Andalib S, Naeinin AM, Gajani A, Asl NA, Abdollahi A. A comparative study pertaining to deleterious effects of diclofenac sodium and meloxicam on kidney tissue in rats. Excli Journal. 2011; 10:149-154.
2. Mahmoud FY, Abou-Alghait AT, Rateb A, Esam S. Comparative study on the effect of long term administration of non-steroidal anti-inflammatory drugs meloxicam and ketoprofen on the structure of the kidney and gastric mucosa in adult albino rats. Egypt J Histol. 2011;33:722-734.
3. Ahmed MY, Abdul-Fattah JH, Aziz FM. The protective role of vitamin C against the hepatotoxic and nephrotoxic effect of meloxicam in male mice. Journal of Pharmacy and Biological Sciences. 2015; 10(5):69-73.
4. Horl W. Non-steroidal anti-inflammatory drugs and the kidney. Pharmaceuticals. 2010;3:2291-2321.
5. Ricciotti E, Garret A. Gerald F. Prostaglandin and inflammation. Arterioscler Thromb Vasc Biol. 2011; 31(5):986–1000.
6. Burukoglu D, Bayu C, Taplamacioglu F, Sahin E, Bektur E. Effects of non-steroidal anti-inflammatory meloxicam on stomach, kidney and liver of rats. Toxicology and Industrial Health. 2014;32(6):1-7.
7. Elyazji N, Aziz I, Ashour A. Some hematological and biochemical changes associated with aspirin administration in albino rats. Al-Aqsa University Journal. 2015;9(1):63-76.
8. Al-Rekabi F, Abbas D, Hadi N. Effects of subchronic exposure to meloxicam on some hematological, biochemical and liver histopathological parameters in rats. Iraqi Journal of Veterinary Sciences. 2009; 23:249-254.
9. Modi G, Mody S, Patl H, Dudhatra G, Kumar A, Avale M. Toxicopathological overview of analgesic and anti-inflammatory drugs in animals. Journal of Applied Pharmaceutical Science. 2012; 2(1):149-157.
10. Kumar V, Abbas A, Aster J. Pathological basis of disease. 9th ed. Pheladelphia: Elsevier Saunders; 2014.
11. Jefferson J, Nelson P, Najafian B, Shankland S. Podocytes disorders; Core curriculum. Am J Kidney Dis. 2011;58(4): 666-677.
12. Ebaid H, Dkhil MA, Danfour MA, Tohamy A, Gabry MS. Piroxicam induced hepatic and renal histopathological changes in mice. Libyan J Med. 2007;2(2):82-89. DOI: 10.4176/070130
13. Weir M. Renal effects of nonselective NSAIDs and coxibs. Cleve Clin J Medicine. 2002;69(1):53-58.
14. Jadav R, Patel B, Joshi D, Patel G, Raval S. Toxipathological studies of

experimentally induced meloxicam toxicity in Wister rats (*Rattus norvegius*). Wayamba Journal of Animal Sciences. 2014;870-875.

15. Taib N, Jarrar B, Mubarak M. Ultra structural alterations in renal tissues of rabbits induced by diclofanic sodium (Voltaren). Saudi Med Journal. 2004; 25(10):1360-1365.

16. Vega J, Goecke H, Mendez G, Guarda F. Nephrotic syndrome and acute tubular necrosis due to meloxicam use. Ren Failure J. 2012;34(10):1344-1347. DOI: 10.3109/086022

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18985