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Histopathological effect of Meloxicam (Preferential COX-2 inhibitor NSAID) on liver and kidney of Rabbit

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Key words: Hepatotoxicity, Histopathology, Meloxicam, Nephrotoxicity, Rabbit Model

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Abstract

The present study was aimed to evaluate the histopathological effect of meloxicam, a preferential COX-2 inhibitor NSAID on functional status of liver and kidney of rabbit. Meloxicam was administered to rabbits divided in two different treatment groups. Group B and C were given therapeutic (1.5mg/kg b.w.) and double dose (3.0mg/kg b.w.) of meloxicam respectively for seven consecutive days. Control group (A) was left untreated. Histopathological studies of liver and kidneys of rabbits in Group B, treated therapeutic dose showed mild alterations in liver (slight dilation of sinusoids and central vein, with mild kupffer cell proliferation) and kidney (slight dilation in distal convoluted tubules and slight disruption of proximal convoluted tubules) on day 5 post treatment which completely reversed to normal on day 10 post treatment. In contrast, marked alterations in liver (severe necrosis and vacuolation of hepatocytes, disruption of bile duct and severe central vein dilation) and kidney (severe shrinkage of glomerulus with widened bowman's spaces, vasoconstriction of arterioles, congested and disrupted nuclei of distal convoluted tubules, obliterated lumens of proximal convoluted tubule and mild inflammatory cellular infiltration) were observed at day 5 post treatment in Group C, which were persistent till day 10. It was concluded that the effect of meloxicam is dose and time dependent, which was reversible with therapeutic dose, whereas persistent with double dose.

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Introduction

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are a class of drugs having anti-inflammatory, antipyretic and analgesic properties (Cooper *et al.*, 2009). They are mostly used in animals for the relief of pain, fever and inflammation (Mahmood *et al.*, 2010). NSAIDs exert their effects via inhibition of the enzyme cyclooxygenase (COX), and this ultimately inhibits the conversion of arachidonic acid (a dietary fatty acid) to prostaglandins during inflammation (Modi *et al.*, 2012). Meloxicam is a preferential COX-2 inhibitor, an oxicam derivative belonging to the enolic acid group of NSAIDs mostly used in cattle, buffalo, goat and dog in a number of inflammatory conditions. It is chemically referred as 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazi ne-3-carboxamide-1,1dioxide (Mahmood *et al.*, 2010).

It is frequently used in domestic animals for the treatment of laminitis, mastitis, myositis, pleuritis, pneumonia, premature labor, sprain, synovitis, severe and prolonged inflammation accompanying with musculoskeletal ailments, and for managing post-operative pain. It has 12 times more selectivity in inhibiting COX-2 activity over COX-1 (Kay-Mugford *et al.*, 2000; Wani *et al.*, 2014). It has been shown to be a best substitute for diclofenac after ban in 2005-06 due to catastrophic decline in vulture population upto 95% in the subcontinent since 1990 (Prakash *et al.*, 2003). Meloxicam is described to be safer as it produces considerably lower occurrence of gastrointestinal adverse effects in contrast to diclofenac and naproxen (Hawkey *et al.*, 1998; Wojtulewski *et al.*, 1996). It causes lower occurrence of nephrotoxicity, therefore, has been largely replaced the diclofenac (Mahmood *et al.*, 2010). However there are several reports indicated that meloxicam also caused hepatotoxicity, nephrotoxicity and G.I.T ulcerations (Mahaprabhu *et al.,* 2011).

Meloxicam though a preferential COX-2 inhibitor and safer NSAID as compared to other NSADs, still shown to produce histologically extensive nephrotoxic and hepatotoxic effects in a number of studies, such as those carried out by (Al- Rekabi *et al.*, 2009; Mahaprabhu *et al.*, 2011; Burukoglu *et al.*, 2014). Meloxicam is widely used clinically in veterinary practice (Budsberg *et al.*, 2002). Keeping in view the above reports meloxicam might cause toxic effects on liver and kidney in large animals for longer duration, therefore the current project was intended to assess this frequently used drug in two different doses in rabbit model.

Materials and methods

Eighteen clinically healthy rabbits of domestic breed with approximately 3 months age, mixed sex and weighing approximately 3 kg, were purchased from local market of Hyderabad, Sindh Pakistan. The rabbits were kept at Animal House, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam and were allowed for 15 days to acclimatize the new environment. The rabbits were offered standard rabbit feed (Imperial rabbit feed Germany) and fresh water ad labitum.

For histological examination laparotomy was performed to remove kidney and liver. For this purpose 3 rabbits from each group were slaughtered through halal method on day 5 and the remaining at the end of the study i.e on day 10th of the last dose administered. The histological procedure were performed at postgraduate Laboratory of Department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam Pakistan. The histopathological examination was performed by the following procedure.

Fixation

The kidney and liver samples were preserved for twenty-four hours in 10 % buffered formalin for fixation.

Washing

The fixed tissues were transferred to cassettes for washing twice for five minutes in Phosphate Buffered Solution (PBS), the tissue cassettes were labelled and were put into the container of linear automatic tissue processor (HT Company).

Dehydration

For dehydration, the cassettes were run through ascending grades of ethanol 75 – 100% (Merck Germany). The dehydration process completed in 6 hours.

Clearing

For clearing the tissues, absolute xylene (Merck Germany) was used. For this purpose, the cassettes containing tissues were transferred to pure xylene twice in separate containers for 30 minutes each.

Infiltration

The tissues were kept in melted paraffin wax (65° C) twice using 100 % histological paraffin wax (Merck Germany) for 1 hour each for infiltration.

Embedding

Tissue embedding was done by using a tissue embedding center (model: HT company). The tissues were embedded in paraffin blocks using plastic molds. Melted wax were poured into the molds after proper positioning of the tissue before cooling. Cooling plate (HT Company) was used for cooling the molds containing tissue quickly to solidify the melted wax (Table 1).

Sectioning

The tissue paraffin block containing tissue held in a manual microtome (Kedee Company). 5 µm sections/ribbons were cut and after that the sections were stretched in a warm water bath (Company: Gallenkamp England) at 42° C.

Mounting

2 – 3 sections of paraffin ribbons were transferred to each microscope slide at its lower 1/3rd.

The slides were kept in hot air oven (Company: Gallenkamp England) at 420° C overnight for drying and fixation of sections to slides.

Staining

The slides with tissue sections were stained by an automatic stainer.

The containers of the machine were filled with required volume and concentrations of various reagents (Table 2).

Mounting

On completion of staining process, a small drop of the mounting medium; DPX (Distrene, plasticizer, xylene) was added to the slide and cover slip was kept on the sample. The slides were air dried for 1 hour at room temperature and were observed under lower and higher magnification of the microscope.

Microscopic examination

The prepared slides were observed under low (10X) and high (40X) magnification of the microscope for histopathological analysis.

Results

Histological observations of liver and kidney tissues (Control)

Tissues of liver and kidney sections of the control group were observed in normal condition.

Table 1. Tissue dehydration protocol.

Ethanol (%)	75%	85%	95%	95%	100%	100%
Time (hours)	01 hour	01 hour	01 hour	01 hour	01 hour	01 hour

Hepatocytes, central and portal veins, sinusoids and bile duct were observed to be normal in the control group (Figure 1 & 2). Glomerulus, bowman's space, proximal and distal convoluted tubules of kidney of the control group were also seen normal (Figure 3 & 4).

Histopathological observations of liver and kidney tissues (Day 5 post treatment)

Sections of liver tissue of rabbits (Group B) treated with therapeutic dose of meloxicam (1.5mg/kg b.w) for seven days, showed normal cellular architecture of hepatocytes. However there was mild dilation of central vein and sinusoids, Kupffer cell proliferation of a mild degree was also noticed (Figure-5). On the other hand, group C rabbits treated with double dose, sacrificed on day 5 post treatment revealed severe necrosis of hepatocytes adjacent to central vein and disruption of the cellular integrity of bile duct whereas in some animals, the cytoplasm of the hepatocytes appeared to be vacuolated.

Table 2. Haematoxylin and Eosin staining of tissue section.

S. No.	Process	Reagent	Reagent % / Processing time					
1	Dewaxing	Xylene	10 minutes	10 minutes				
2	Rehydration	Ethanol	100 %	100 %	95 %	95 %	85 %	75 %
			03 minutes	03 minutes	03 minutes	03 minutes	03 minutes	03 minutes
3	Washing	Running tap water	10 seconds					
4	Staining with Hematoxylin	Hematoxylin (Merck)	10 minutes	10 minutes				
5	Washing	Running tap water	05 seconds					
6	Differentiation	0.5 % Acid (Hcl) Alcohol	01 second					
7	Washing	Running tap water	05 seconds					
8	Bluing	Ammonia water (0.2%)	01 minute					
9	Washing	Running water	05 seconds					
10	Dehydration	Ethanol	75 %	85 %				
			03 minutes	03 minutes				
11	Staining with Eosin Y	Eosin Y (0.25%)	90 seconds					
12	Dehydration & Differentiation	Ethanol	95 %	95 %	100 %	100 %		
			03 minutes	03 minutes	03 minutes	03 minutes		
13	Clearing	Xylene	10 minutes	10 minutes				

The liver of rabbits (Group C) also showed severe dilation of the central vein (Figure-6 & 7). There was marked inflammation of peri-portal area and severe swelling of hepatocytes (Figure 7).

Kidney sections of the rabbits being administered therapeutic dose of meloxicam (1.5mg/kg b.w) for seven days showed mild dilation of distal convoluted tubules and slight disruption of the proximal convoluted tubules.

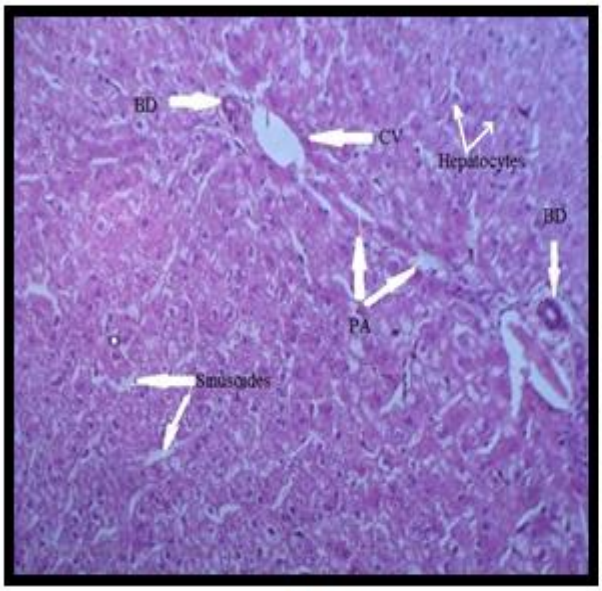

Fig. 1.

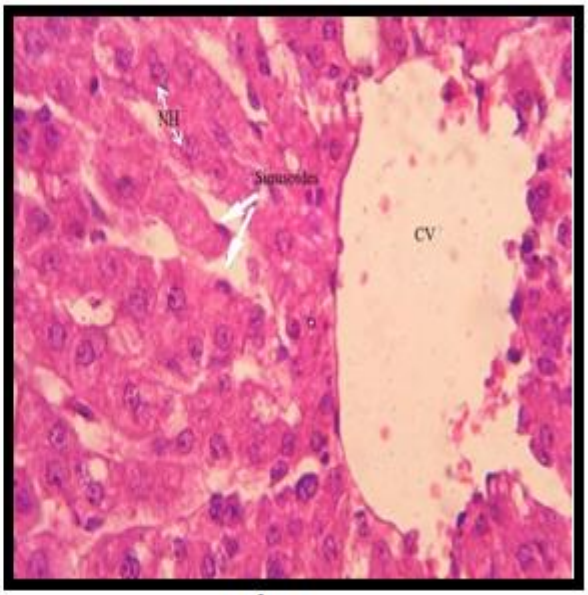

Fig. 2.

Photomicrographs of Liver section of Control group, showing Normal central vein (CV), Hepatocytes, Sinusoids, Portal area (PA) and Bile duct (BD). (10X, 40X H&E).

The glomerulus was seen to be unchanged with a normal texture of bowman's space (Figure 8).

Kidney tissues of rabbits (Group C) revealed marked shrinkage of glomeruli with widened bowman's spaces.

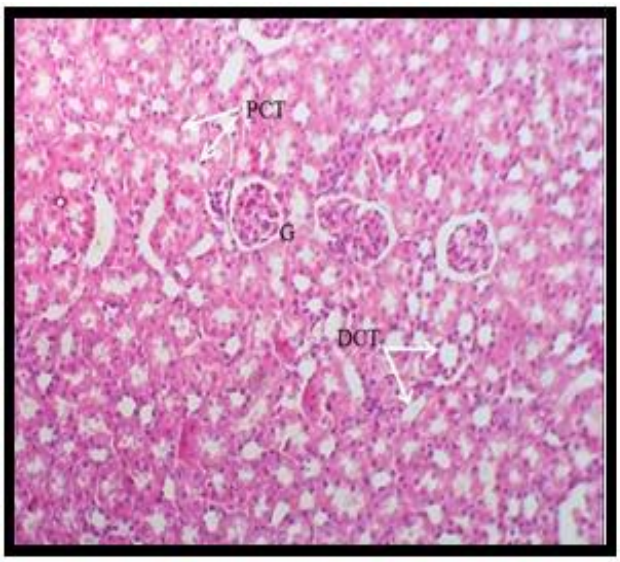

Fig. 3.

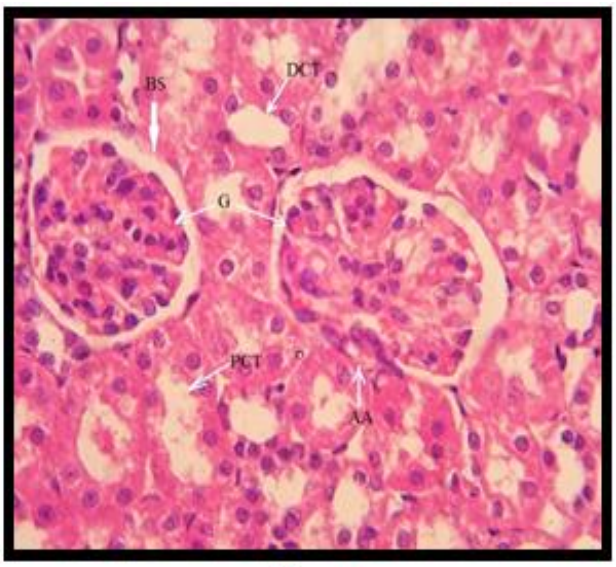

Fig. 4.

Photomicrographs of Kidney section of Control group, showing Normal Glomerulus (G), Proximal Convoluted Tubule (PCT), Bowman's Space (BS), Afferent Arteriole (AA) and Distal Convoluted Tubule (DCT). (10X, 40X H&E).

The proximal convoluted tubules were appeared with obliterated lumens while distal convoluted tubules appeared to be congested with disturbed nuclei.

Mild inflammatory cellular infiltration and hyperemia in intertubular spaces were also seen (Figure 9 & 10).

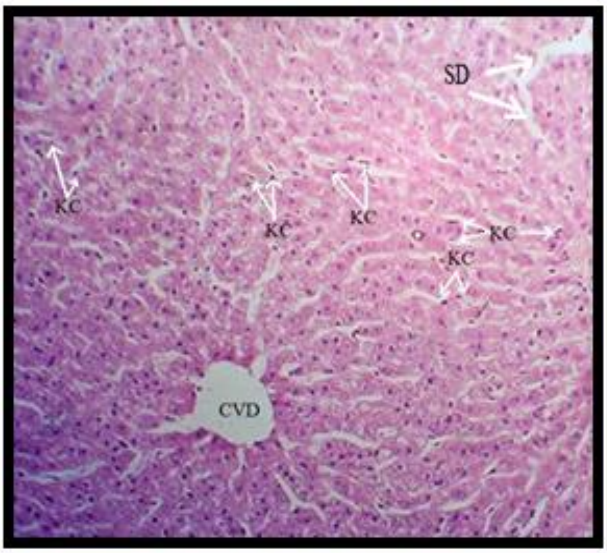

Fig. 5. Group-B

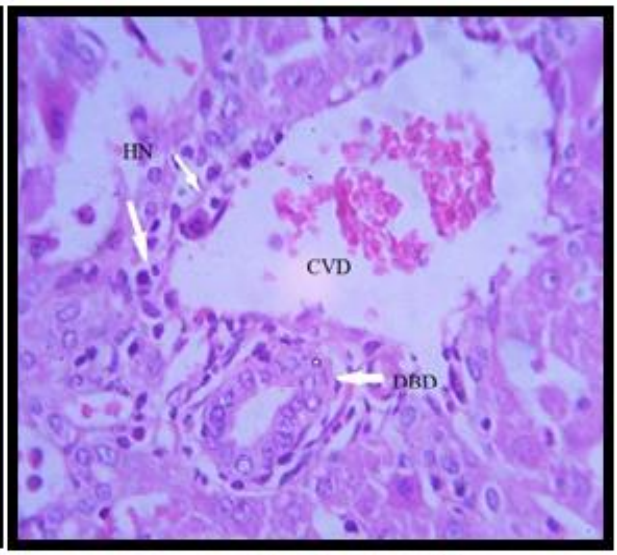

Fig. 6. Group-C

Photomicrographs of Liver section of group B, showing Central Vein Dilation (CVD), Siunsoidal dilation (SD), Disrupted Bile duct (DBD), Hepatic necrosis (HN) and Kupffer cell proliferation (KC). (Day 5), (10X, H&E).

Histopathological observations of liver and kidney tissues (Day 10 post treatment)

Histopathological observation of liver sections of group B treated with therapeutic dose (1.5mg/kg b.w) showed normal hepatocytes. The central vein was observed with normal architecture (Figure 11).

The hepatocytes surrounding the central vein were observed to possess darkly staining condensed nuclei, and significant necrosis of the hepatocytes around the peri-portal and central vein area in Group C (Figure-12 & 13).

Central vein dilation with necrosis in hepatocyte around central vein and bile duct and dilation of the sinusoids were found persistent in group C (Figure-14). Kidney sections of group B on day 10 were seen to be in a normal structure.

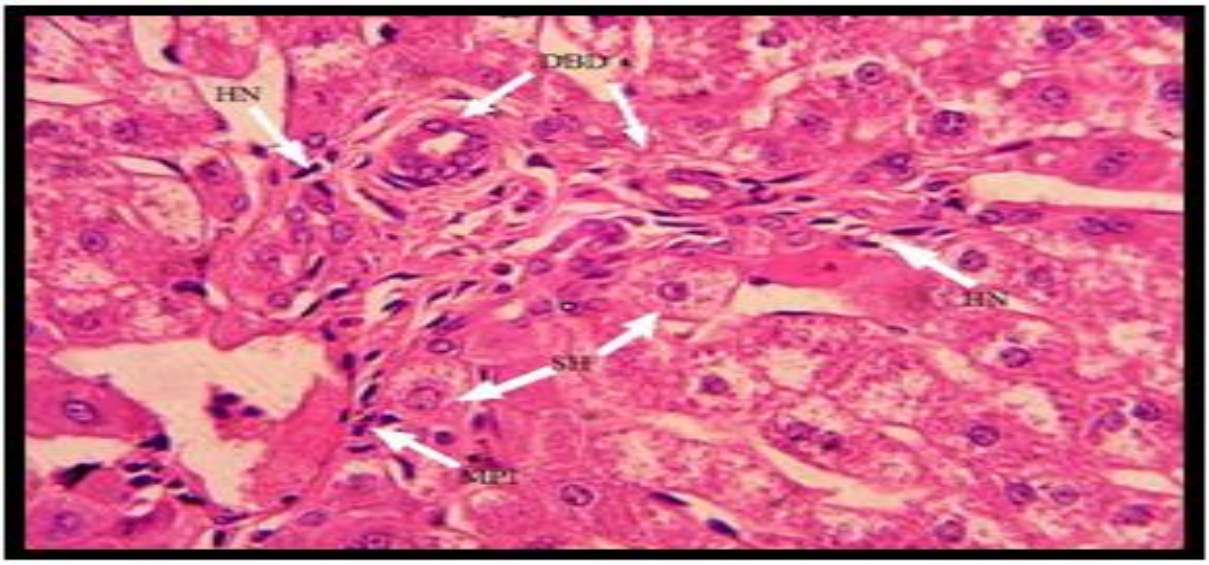

Fig. 7. Photomicrograph of Liver section of group C, showing Hepatic Necrosis (HN), Swollen Hepatocytes (SH), Disrupted Bile Duct (DBD) and Massive Peri-portal inflammation (MPI). (Day 5), (40X, H&E).

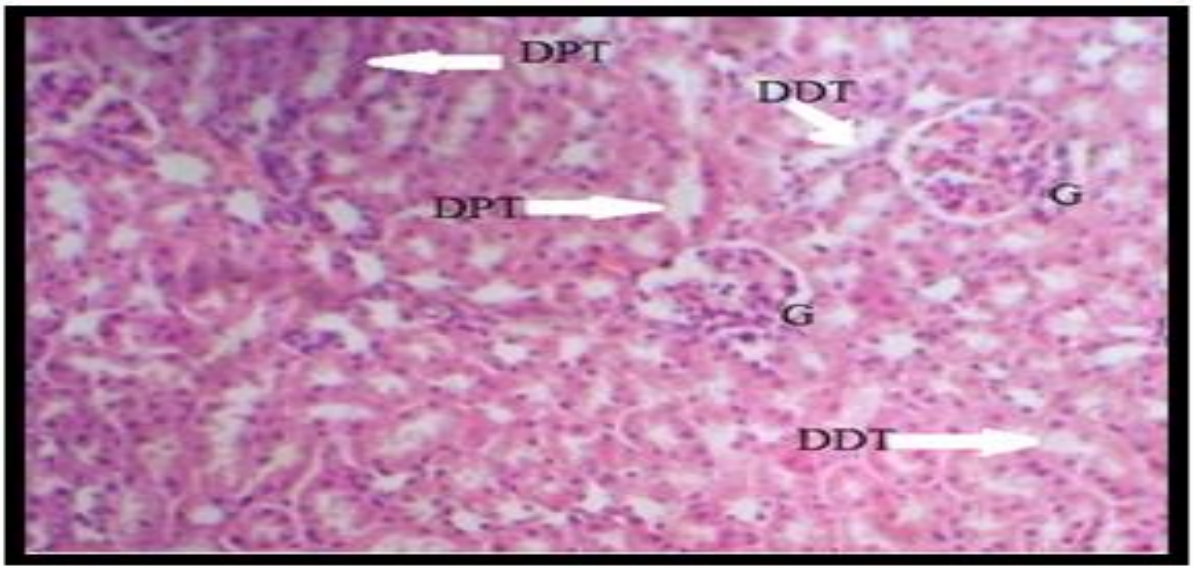

Fig. 8. Photomicrograph of Kidney section of group B, showing Normal Glomerulus (G), Disrupted proximal tubules (DPT), and Distal Tubule Dilation (DDT). (Day 5), (10X, H&E).

The glomerulus, proximal and distal convoluted tubules, and afferent arterioles resumed to their normal histological state as compared to day 5 (Figure 15).

In contrast the kidney sections of rabbits of group C were seen to have persistent and continuous alterations as seen on day 5 i.e. severe shrinkage of glomeruli with wide bowman's spaces and vasoconstriction of arterioles (Figure 16).

Discussion

Group B (Therapeutic Dose)

Histopathologically in Group B (Therapeutic dose), there was mild type of alterations such as mild dilation of central vein and slight dilation of sinusoids of liver cells and slight dilation of renal tubules in kidneys on day 5, whereas the glomerulus and the bowman's space were in normal condition. On the other hand,double dose of meloxicam on day 5 showed severe central vein dilation, hepatic necrosis, and swollen hepatocytes, disrupted bile duct and massive peri-portal inflammation, whereas the kidney sections showed severe shrinkage of glomerulus with widened bowman's space.

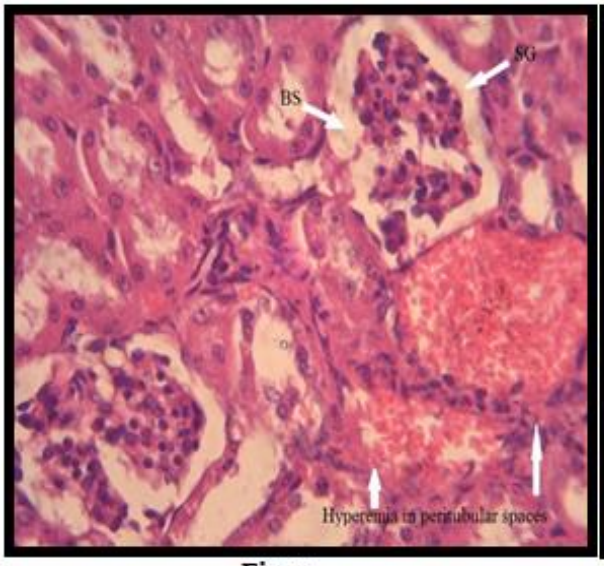

Fig. 9.

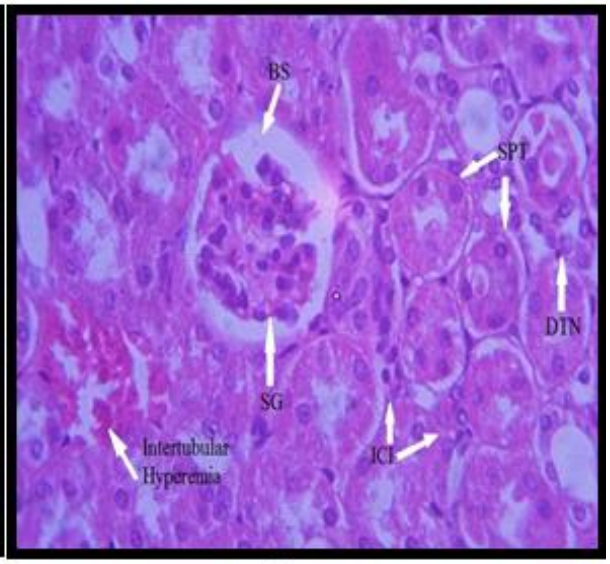

Fig. 10.

Photomicrograph of Kidney section of group C, showing Shrinked Glomerulus (SG), Widened Bowman's space (BS), Inflammatory cellular Infiltration (ICI), Disrupted Tubular Nuclei (DTN), Swollen Proximal Tubules (SPT) and Hyperemia in Peri Tubular spaces. (Day 5), (40X, H&E).

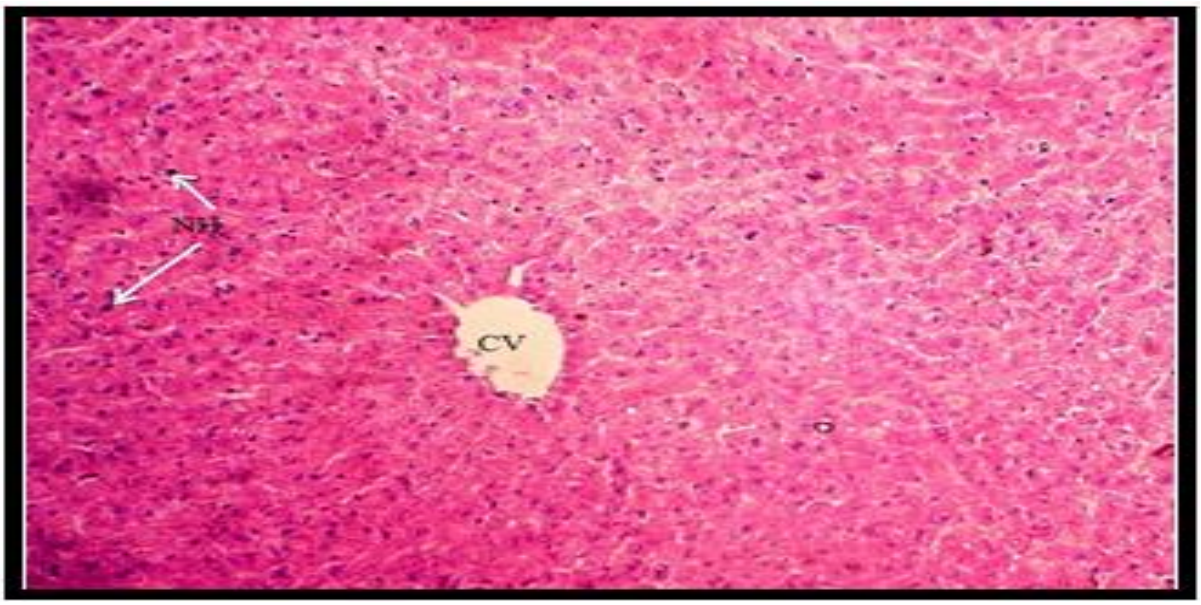

Fig. 11. Photomicrograph of Liver section of group B, showing Normal hepatocyte (NH) and Central vein (CV). (Day 10), (10X, H&E).

The changes observed with the administration of therapeutic dose on day 5 in liver and kidney tissue almost resume to the normal structure on day 10,

However, those treated with double dose did not return to normal and were in consistency to those observed on day 5th. This recommencement of liver and kidney structure of Group B to normal might be due to the fact that as the drug has been eliminated from the blood, the tissues were recovered as the alterations were mild and did not accumulate highly in tissues. Since, Fredholm *et al*., 2013 reported that meloxicam at 1 mg/kg accumulated up to 5 days in plasma of rabbit and the levels of drug dropped off after cessation of therapy.

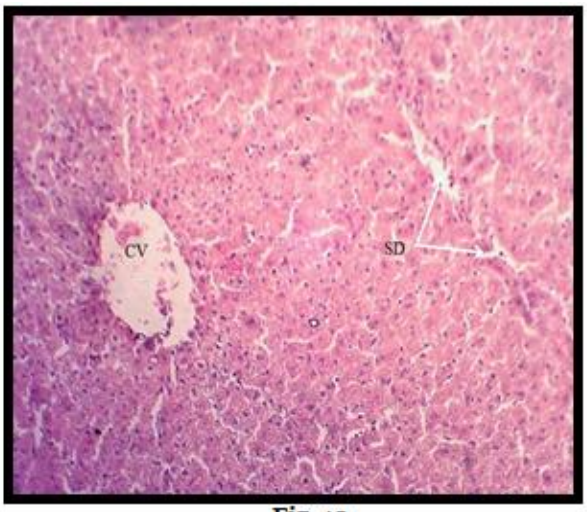

Fig. 12.

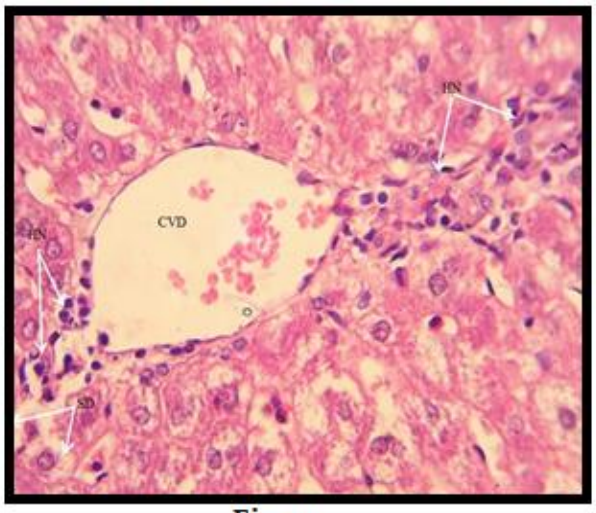

Fig. 13.

Photomicrograph of Liver section of group C, showing Sinusoidal Dilation (SD) Hepatic Necrosis (HN) and Dilation of Central vein (CV). (Day 10), (10X, 40X H&E).

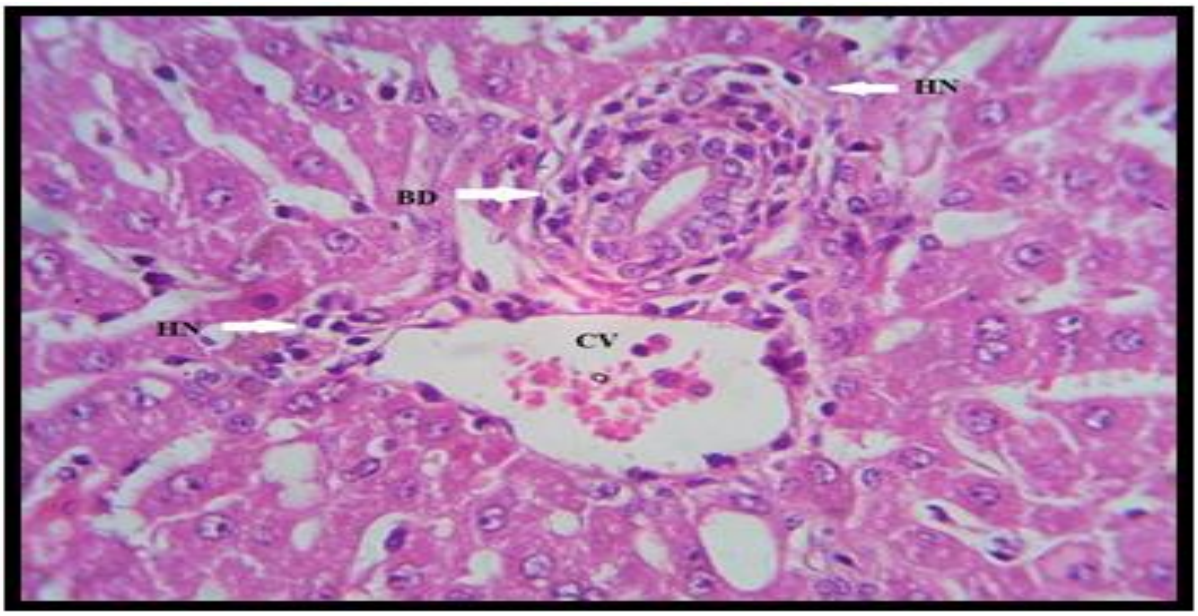

Fig. 14. Photomicrograph of Liver section of Group C, showing Central vein dilation (CV), Hepatic Necrosis around bile duct and central vein (HN). (Day 10), (40X, H&E).

Group C (Double Dose)

On the other hand meloxicam with double dose revealed marked alterations in liver and kidney tissues, this was supported by Al-Rekabi *et al*., 2009 who reported severe necrosis, haemorrhages of hepatocytes with three-fold dose of meloxicam, and with therapeutic dose too in rat model, and stated that meloxicam can accumulate in liver and kidney at higher level.

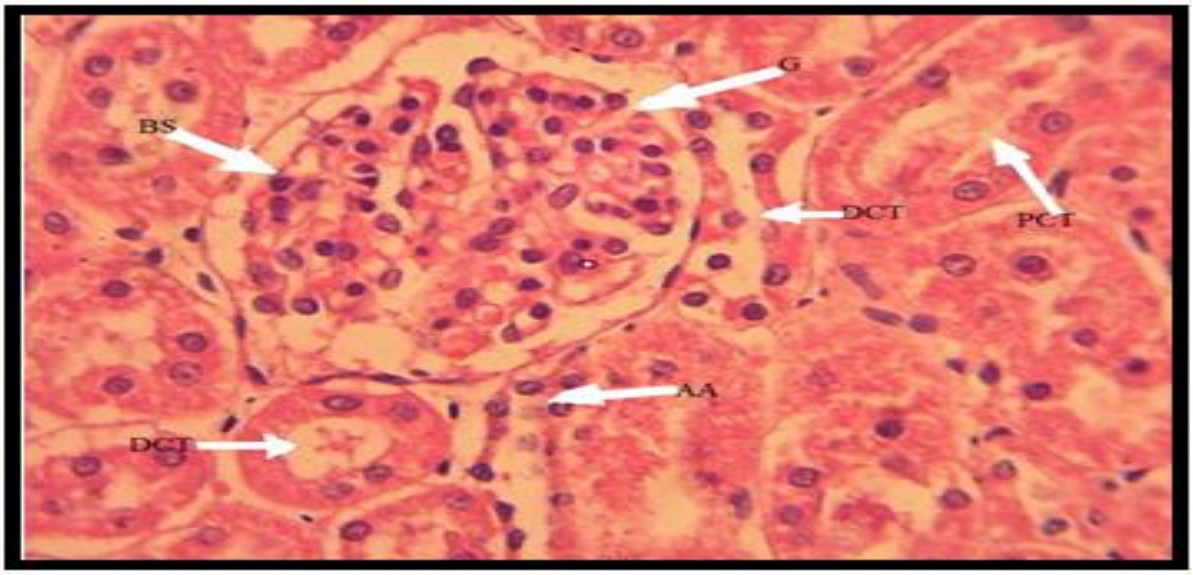

Fig. 15. Photomicrographs of Kidney section of group B, showing Normal Glomerulus (G), Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT), Bowman's Space (BS) and Afferent Arteriole (AA). (Day 10), (40X, H&E).

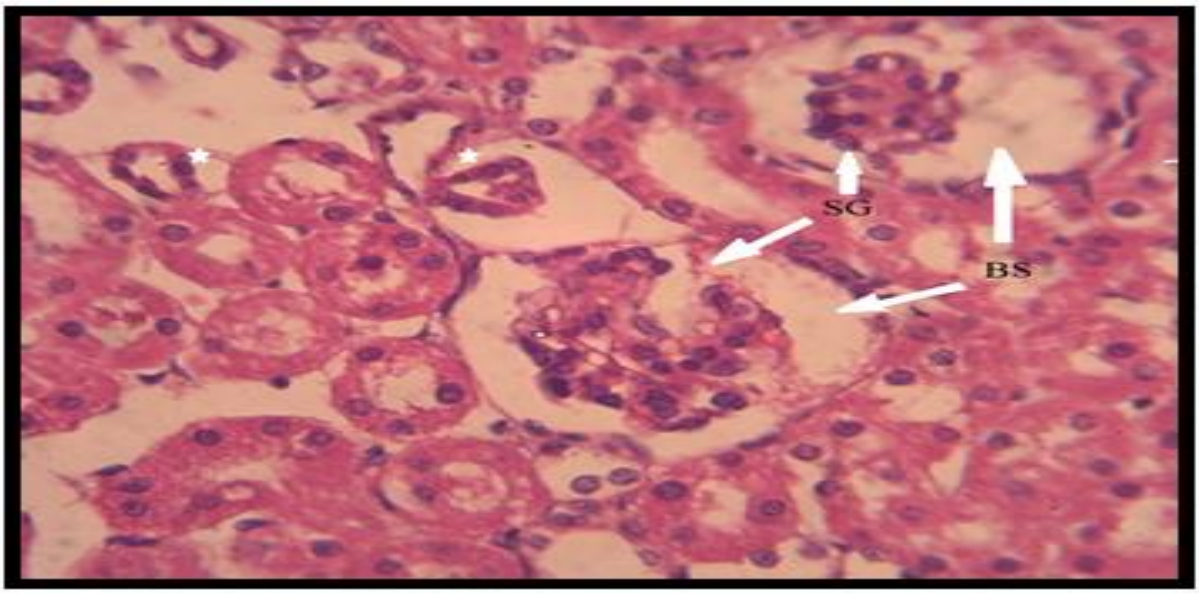

Fig. 16. Photomicrograph of Kidney Section of group C, showing Shrinked Glomerulus (SG), widened Bowman's Space (BS) and Vasoconstriction of arteriole (Star). (Day 10), (40X, H&E).

The therapeutic findings were in contrast to those observed by Al-Rekabi *et al.*, 2009 this might be due to treatment for a long time.

In the presentstudy, the drug was administered for a shorter period i.e. seven days. The histopathological findings of liver, in Group C, were also supported by Ebaid *et al.*, 2007 who found vacuolated hepatocytes and dilation of blood sinusoids with one-week administration of piroxicam to mice.

This marked alterations in liver cells may be attributed to increased lipid peroxidation in liver. Yukiko *et al.*, 1977 suggested that vacuolation of hepatocytes may be due to retention of water inside hepatocytes, causing edema, which may have occurred due to the reduction of energy necessary for the regulation of ion concentration inside the cells. The alterations found with double dose of meloxicam were in agreeance with

those found by Burukoglu *et al.,* 2014 who reported shrinkage of Bowman's capsule, dilation of distal tubules and vasoconstrictions of arterioles with meloxicam administration. Similar findings were also reported by Ebaid *et al.*, 2007 who reported shrinked glomerulus with widened bowman's spaces.

Glomerular shrinkage might be due to the higher concentration of meloxicam in the blood which affected capillary constriction resulted in a decreased glomerular filtration rate.

Conclusion

Histologically, therapeutic dose of meloxicam caused only slight deformity in liver and kidney sections on day 5 which returned to almost normal on day 10, whereas marked alterations were observed in liver and kidney tissue sections on day 5 which were consistent till day 10 with double dose of meloxicam. It was concluded that the effect of meloxicam was time and dose dependent.

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