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

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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-benzothiazene-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 NSAIDs, 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 Germ any) 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).

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.

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 1. Tissue dehydration protocol.

<table>
<thead>
<tr>
<th>Ethanol (%)</th>
<th>75%</th>
<th>85%</th>
<th>95%</th>
<th>95%</th>
<th>100%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hours)</td>
<td>01 hour</td>
<td>01 hour</td>
<td>01 hour</td>
<td>01 hour</td>
<td>01 hour</td>
<td>01 hour</td>
</tr>
</tbody>
</table>

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).
Table 2. Haematoxylin and Eosin staining of tissue section.

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

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.

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Fig. 1.
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).

Fig. 2.
Kidney sections of rabbits (Group C) revealed marked shrinkage of glomeruli with widened bowman’s spaces.

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

Histopathological observations 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).](image)

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.](image1) ![Fig. 10.](image2)

Photomicrograph of Kidney section of group C, showing Shrunk 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.](image3)

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.

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. 12.

Fig. 13.

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).

Fig. 14.

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.
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 present study, 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 agreement 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 shrunked 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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**References**


