ORIGINAL ARTICLE
EVALUATION OF HEPATOTOXIC AND NEPHROTOXIC EFFECTS OF PIROXICAM SULFONATED DERIVATIVES

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Background: Pain management requires new pharmacotherapy with good efficacy and less side effects. Piroxicam is used routinely in clinical practice but it is associated with side effects. To minimize the chances of adverse effects, sulfonated piroxicam derivatives (SPD) have been introduced. We sought to find hepatotoxic and nephrotoxic effects of SPD in Albino rats.

Methods: An experimental study on SPD was carried out at the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar. Healthy 24 albino rats were divided into 5 groups. One control group and four experimental groups (compound I and II, each with a dose of 10mg/kg and 20mg/kg) received treatment for 7 days. Liver function tests (LFTs), renal function tests (RFTs) and histology of liver and kidney specimens was performed after culling rats. The difference between median values of samples was assessed using Kruskal-Wallis test with post-hoc (for LFTs and RFTs). SPSS-21 was used for all statistical analysis and p≤0.05 was considered statistically significant.

Results: The alanine aminotransferase (ALT) values were significantly high in the 20 mg/Kg group than control for both compounds (p=0.03, p=0.001 respectively). The aspartate aminotransferase (AST) values were significantly high in the 10 mg/Kg and 20 mg/Kg group than control for compounds II (p=0.01, p=0.0001 respectively). The alkaline phosphatase (ALP) values were significantly high in the 20 mg/Kg group than control for compounds II (p=0.002). The blood urea values were significantly high in the 20 mg/Kg group than control for compounds II (p=0.008). The mean final score of liver injury in all experimental groups (mean range 5–7) was less suggesting that the damage in liver was less pronounced. Renal injury was more pronounced in the 20 mg/Kg dose for both compound I and compound II (mean score 7) compared to 10 mg/Kg dose (mean score 4).

Conclusion: Piroxicam sulfonated derivatives can cause focal changes in liver and kidney which might be reversible. The changes are less pronounced for compound I with a low dose.

Keywords: Piroxicam, Hepatotoxicity, Nephrotoxicity

INTRODUCTION

It is estimated that worldwide, approximately 20% of adults experience a type of pain, which is broadly classified as acute or chronic. Chronic pain is one of the main reasons that people seek medical care. It leads to personal suffering and it also has adverse effect on individual productivity at work place. Pain management requires new pharmacotherapy with good efficacy and less side effects. At present, pharmacotherapy for pain management mainly includes non-steroidal anti-inflammatory drugs (NSAIDs) and narcotic analgesics. Patients expect at least 50% reduction in the intensity of pain without risk of experiencing adverse effects. Non-steroidal anti-inflammatory drugs are preferred by practitioners because they are in clinical use since long with good efficacy and low drug abuse potential.

Piroxicam is a type of NSAID with low solubility and high permeability. It is widely used as an analgesic and anti-inflammatory agent in clinical practice. Piroxicam is preferred for the treatment of pain associated with rheumatoid arthritis and osteoarthritis than other drugs due to its long half-life (40 hrs), which makes once a day dosage possible. It is also used in musculoskeletal disease, dysmenorrhea, postoperative pain and rheumatic disease. However, like other NSAIDS, the use of piroxicam is associated with side effects including gastrointestinal, cerebrovascular, hypersensitivity, respiratory, bone related, hepatotoxicity and nephrotoxicity.

Thus, the benefit of NSAIDs could further be improved through controlling the adverse effects. This will clearly have a large market potential. Withdrawal of drugs from market can occur because of two main causes i.e. renal and hepatic adverse events. Serum biochemistry measurements and histopathology tests can be used to evaluate hepatic and renal damage in pre-clinical animal models.

To minimize the chances of adverse effects, sulfonated piroxicam derivatives have been introduced. The anti-nociceptive activity of sulfonated piroxicam derivatives has been evaluated in rat model and it has been found to have significant peripheral pain relieving effect. Thus, we conducted the current study to find...
RESULTS
Analyses of biochemical parameters (LTFs and RFTs) after the use of experimental compound I and II is shown in Table-1. In LFTs, a statistically significant difference between the median values of groups was observed for alanine aminotransferase (ALT) (normal range, 17.5–30.2 IU/L) (p=0.001). In pairwise comparison the difference between the control vs group III (p=0.03) and control vs group V (p=0.001) was statistically significant. In addition, a statistically significant difference between the median values of groups was observed for aspartate aminotransferase (AST) values (normal range 45.7–80.8 IU/L) (p=0.0001). In pairwise comparison the difference between the control vs group IV (p=0.01) and control vs group V (p=0.0001) was statistically significant. Furthermore, a statistically significant difference between the median values of groups was observed for alkaline phosphatase (ALP) values (normal range 56.8–128 IU/L) (p=0.001). In pairwise comparison the difference between the control vs group V (p=0.002) was statistically significant. For total serum bilirubin (TSB) (normal range 0.2–0.55 mg/dl), no statistically significant difference was observed (p=0.16).

In RFTs, a statistically significant difference between the median values of groups was observed for blood urea values (normal range 15–21 mg/dl) (p=0.002). In pairwise comparison the difference between the control vs group V (p=0.008) was statistically significant. Finally, for serum creatinine (normal range 0.2–0.8 mg/dl), no statistically significant difference was observed (p=0.25) (Table-1).

Table-1: Comparison of median values of study variables across the groups after 7 days of treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>LFTs (median)</th>
<th>RFTs (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (IU/L)</td>
<td>AST (IU/L)</td>
</tr>
<tr>
<td>Control</td>
<td>32.5</td>
<td>198</td>
</tr>
<tr>
<td>Group I</td>
<td>39</td>
<td>254</td>
</tr>
<tr>
<td>Group II</td>
<td>44</td>
<td>277</td>
</tr>
<tr>
<td>Group III</td>
<td>38</td>
<td>246</td>
</tr>
<tr>
<td>Group IV</td>
<td>48</td>
<td>482</td>
</tr>
<tr>
<td>p value</td>
<td>0.001*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*p<0.05

The histopathologic scoring for assessing liver and kidney injury are shown in Table 2 and Table 3. The control group was without any liver and kidney injury. However, focal areas of injury were noted in both liver and kidney specimens with both the compounds (Table-2 and 3).

Liver injury was assessed based on hyperemia, cloudy swelling of hepatocyte, vacuolar degeneration, mononuclear cell infiltrations, necrosis in 1–3 hepatocytes, necrosis in more than 3 hepatocyte and hemorrhage. The mean final score of liver injury in all
experimental groups (range 5–7) was less suggesting that the damage in liver was less pronounced (Table-2, Figure-1).

A similar pattern of overall less pronounced injury was observed in kidney specimens with total mean score of renal injury in a range of 4–7. However, renal injury was more pronounced in the 20 mg/Kg dose for both compound I and compound II (total mean renal injury score 7 for both) compared to 10 mg/Kg dose (total mean renal injury score 4 for both) (Table-3, Figure-2).

### Table-2: Mean histopathological scoring of hepatic injury after 7 days of treatment in experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hyperemia</th>
<th>Cloudy swelling of hepatocyte</th>
<th>Vacular degeneration</th>
<th>Mononuclear cell infiltrations</th>
<th>Necrosis in 1–3 hepatocytes</th>
<th>Necrosis in &gt;3 hepatocytes</th>
<th>Haemorrhage</th>
<th>Total Mean Score (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1.5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Group III</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Group IV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Group V</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

**Figure-1: Histopathological changes in liver after 7 days of treatment. a) control group (white arrow) in control group. White arrows showing focal haemorrhage (b) and necrosis (c & d)**

### Table-3: Mean histopathological scoring of renal injury after 7 days of treatment in experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hyperemia</th>
<th>Glomerular space expansion</th>
<th>Glomerular mesangial cell hyperplasia</th>
<th>Tubular epithelial degeneration</th>
<th>Mononuclear cell infiltrations</th>
<th>Tubular epithelial necrosis</th>
<th>Haemorrhage</th>
<th>Total Mean Score (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>0</td>
<td>0.5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1.5</td>
<td>4</td>
</tr>
<tr>
<td>Group III</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Group V</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the current study, two sulfonated piroxicam derivative compounds (I and II) were tested for their effect on liver and kidney histology and biochemical changes in LFTs and RFTs. Overall, both liver and renal damage were observed in histology, which was reflected in changes in biochemistry of rats, i.e., elevated LFTs and RFTs. Although, neither the hepatic, nor the renal injury was lethal to rats, compound II was found more toxic and compound I with 10 mg/Kg was least toxic.

Small molecules in relation to biological systems are studied by chemical biologists. The concept of BIOS (biology oriented synthesis) is the beginning of the exploration of advanced medicine. It is the art of creation and collection of compounds. Piroxicam is included in oxicam class of drugs on the basis of chemical classification. Piroxicam is routinely used as a pain-killer in medicine. Piroxicam sulfonated derivatives have been synthesized by BIODS (biology oriented drug synthesis). These derivatives were found safe and effective analgesics.

We designed the current study to assess the hepatic and renal toxicity profile of newly synthesized sulfonated derivatives of piroxicam.

The major organ responsible for secretion of clotting factors, enzymes involved in drug metabolism and excretion of waste products from the body is liver. Liver damage occur by drugs, cholesterol overload, alcohol intake, bile duct obstruction and viruses. ALT, AST, ALP and TSB are released into blood after hepatocytes are damaged. Reactive oxygen species (ROS) are normally generated by liver cells because of their ability to actively involve in various metabolic processes.
functions. After administration of drugs, ROS can be excessively generated and antioxidant reserves are depleted. ROS can cause nucleic acid damage, sulfhydryl antioxidants inactivation, initiation of lipid peroxidation of cell membrane and inhibition of DNA repair in liver cells. Hepatotoxicity can be assessed quantitatively by measuring serum levels of AST, ALT, ALP and TSB, as these are markers of hepatic damage. Normal values of these enzymes are different in rats \(^{16}\) than in humans but their rise above normal range show hepatic damage. \(^{23}\) Drug induced hepatic damage is one of the most common cause of withdrawal of drug from market in spite of careful preclinical and clinical studies. In the current study, all the hepatic markers were elevated after the use of piroxicam derivatives. The rise in the levels of hepatic markers displayed dose dependent response for both compound I and compound II. In addition, AST values were significantly higher for compound II (both doses) than control pointing towards a more hepatotoxic effect. These findings thus show that compound I with 10 mg/Kg dose might be a better option with less chances of toxicity.

Furthermore, cytoplasmic vacuolation is one of the first responses of cell to all types of injuries. \(^{17}\) Thus, vacuolar degeneration and other parameters were assessed histologically in tissue specimens. Focal hepatic damage was observed after the use of piroxicam derivatives for compound I and II. However, even after partial hepatectomy, liver cell of rats are regenerated after two weeks. \(^{24}\) Thus, we presume that the hepatic damage in the current study could potentially be recovered after discontinuation of piroxicam derivatives.

Drug administration can result in increased levels of blood urea and serum creatinine. \(^{25}\) In the current study, blood urea levels were significantly increased for compound II with 20 mg/Kg dose pointing towards a potential renal damage. This result was reflected histologically and renal damage was observed with hyperemia, glomerular space expansion, mesangial cell proliferation and focal haemorrhage. However, again renal damage was focal and less pronounced for lower dose. Kidney tubules have great capacity to regenerate damage cell within a week. \(^{26}\) Thus, we presume that renal damage in the current study especially for lower doses of both compounds could be recovered after discontinuation of piroxicam derivatives. The study is limited by a relatively small sample size and the lack of inclusion of a positive control group with higher doses of piroxicam derivatives

**CONCLUSION**

Piroxicam sulfonated derivatives can cause focal changes in liver and kidney which might be reversible. The changes are less pronounced for compound I with 10 mg/Kg dose. In addition, these piroxicam sulfonated derivatives could potentially be used as analgesic with prior exclusion of hepatic and renal impairment in further pre-clinical studies and phase 1 clinical trials.

**REFERENCES**

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