

ORIGINAL ARTICLE

EVALUATION OF HEPATOTOXIC AND NEPHROTOXIC EFFECTS AFTER 2 WEEKS TREATMENT OF PIROXICAM SULFONATED DERIVATIVES IN ALBINO RATS

Ghazala Shaheen, Syed Hamid Habib*, Syeda Huma Khizar, Safiullah**, Muhammad Omar Malik*, Naeem Khan**, Shafique Ahmad Tariq*

Khyber Medical University Institute of Medical Sciences, Kohat, *Institute of Basic Medical Sciences, Khyber Medical University Peshawar, **Drug Control Health Department Khyber Pakhtunkhwa, Peshawar, Pakistan

Background: New pharmacotherapy with good efficacy and less side effects is required for management of pain. Use of Piroxicam is associated with side effects. Sulfonated piroxicam derivatives (SPD) have been introduced to minimized adverse effects associated with its use. In this study we aim to find hepatotoxic and nephrotoxic effects of SPD in Albino rats after 14 days of treatment. **Methods:** This was an experimental study 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 having two compounds (compound I and II) in a dose of 10 mg/Kg and 20 mg/Kg respectively were administered for 14 days. Blood was obtained for liver function tests (LFTs) and renal function tests (RFTs). Furthermore, histology of liver and kidney were performed after sacrificing rats. **Results:** Data was analysed with SPSS using one way ANOVA with post hoc analysis. Among the LFTs alkaline phosphatase values were significantly high for compound II when used in the 20mg/kg concentration than control (348IU/L vs 210.25IU/L, $p=0.004$). The Alanine aminotransferase and aspartate aminotransferase concentrations were not significantly different between the groups ($p=0.14$, $p=0.21$ respectively). There was significant difference in blood urea among the groups ($p=0.03$) and in post hoc comparison the main difference was observed in the compound II with 20 mg/Kg concentrations ($p=0.08$). The mean final score observed for liver injury (mean range 2–3.5) and kidney injury (2.5–3) are suggestive of less pronounced effect on both liver and kidney. **Conclusion:** The Piroxicam sulfonated derivatives show minimal and reversible toxic effects on liver and kidney after 2 weeks treatment.

Keywords: Piroxicam, Hepatotoxicity, Nephrotoxicity

Pak J Physiol 2019;15(3):27–33

INTRODUCTION

Globally, about 20% of the population suffers some type of pain.¹ Pain can be either acute or chronic depending on the duration.² Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and narcotic analgesics are most commonly used drugs for management of both acute and chronic pain worldwide.³ Benefits associated with use of NSAIDs over opiates are it has less chances of drug abuse with good efficacy.⁴ These drugs have good potency and are in use in clinical practice since long. NSAIDs are generally prescribed to relieve pain due to chronic conditions such as osteoarthritis and other rheumatologic and arthritic conditions.⁵ However, prolonged use of NSAIDs are associated with many complications such as gastrointestinal, respiratory and cardiovascular problems. Hypersensitivity reactions can occur with NSAIDs use⁶, along with hepatotoxicity⁷ and nephrotoxicity.⁸ Due to multiple side effects of this class of pain relieving drugs new pharmacotherapy are in practice these days.

Piroxicam is a type of NSAID belonging to the group of oxicam that has shown to have high permeability and low solubility. Piroxicam is nowadays commonly used for the treatment of pain in conditions

such as dysmenorrhea, musculoskeletal pain, rheumatoid arthritis and osteoarthritis due to longer duration of action.⁹ Nevertheless, oxicam group is preferred over other NSAIDs but these drugs are not spared from related side effects. Among the main reasons for withdrawal of drugs from market are due to their hepatic and renal toxicity.¹⁰ Therefore, minimizing the adverse effects of NSAIDs can further improve the safety and increase their use. The adverse effects of these drugs are studied in animal models by analysing the serum biomarkers and histomorphological evaluations.

In order to minimize the risks associated with use of NSAIDs, Biology Oriented Drug Synthesis (BIODS) have introduced/synthesized Sulfonated Piroxicam Derivatives (SPD) as a new pharmacotherapy. The anti-nociceptive activity of SPDs have been assessed in animal models and results suggest significant decline in pain sensation in the peripheral tissues.¹¹ Previously, we have studied the short term effects of SPD drugs in different concentrations and were found to be safe in therapeutic doses.¹² The present study was conducted with the aim to assess the

hepatotoxic and nephrotoxic effects of SPD in albino rats after 2 weeks of treatment.

MATERIAL AND METHODS

This was an experimental study carried out at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar, Pakistan after obtaining approval of the Institutional Review Board of KMU. All the procedures were carried out in accordance with the ethical guidelines approved by the ethical committee.

Two compounds of SPD were used in current study. The chemical structures of both compounds are as follows:

Compound I (white colour): '2-methyl-1, 1-dioxo-3-[(pyridin -2 yl amino) carbonyl]-1,2-dihydro-11⁶, 2-benzothiazin-4-yl 2,4-dichlorobenzenesulfonate'.

Compound II (yellow colour): '2-methyl-1, 1-dioxo-3-[(pyridin-2-yl amino) carbonyl]-1, 2, 3, 8a-tetrahydro-11⁶, 2-benzothiazin-4-yl 4-chlorobenzenesulfonate'.

These compounds were synthesized and provided in powdered form by Department of Pharmacy, University of Peshawar. Injectable solutions of drugs were prepared by mixing compounds in distilled water to form known concentration and administered according to body weight.

A total of 24 healthy albino rats weighing between 150 to 250 grams were purchased from animal house facility at Peshawar Medical College, Peshawar. Animals were acclimatized for one week in the animal house facility at KMU. Afterwards, they were randomly divided into 5 groups; including 4 experimental and 1 control group. Group I and II received compound I in concentration of 10 mg/Kg and 20 mg/Kg body weight respectively. Group III and IV received compound II in concentration of 10 mg/Kg and 20 mg/Kg body weight respectively. Group V was control group that received normal saline. Drugs were administered through the intraperitoneal route in accordance with the body weight and all animals were in optimal conditions with a 12 hours dark and light cycle with food and water provided ad libitum.

After 14 days of treatment, all rats were anaesthetized with injection sodium thiopental (60 mg/Kg intraperitoneal). Blood samples were collected via aseptic procedure through cardiac puncture and animals were sacrificed through cervical dislocation. Blood was centrifuged at 3,000 rpm for 20 minutes to obtain serum and stored at -80 °C. Serum was analysed for Blood Urea (Urea), Serum Creatinine (Creat), serum Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and total serum bilirubin (TSB) using standard kits (Merck) via Micro Lab 300. For histological evaluation, liver and kidneys were dissected. These tissues were fixed in 10% neutral

formalin solution and processed in automated tissue processor into paraffin embedded tissue blocks. Four micrometre thin sections were cut and stained by eosin and haematoxylin stain for histopathological evaluation using the criteria done by Gokakin *et al.*¹³ Statistical analysis was performed using SPSS-22. All data were checked for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and histograms, and were normally distributed. Data were presented as Mean±SD. Control and different groups (compound and doses) were compared by ANOVA, with Tukey post hoc testing for individual comparison.

RESULTS

Weight of all the animals was recorded at start and end of 14 days treatment. Weight gain was observed only in the control group during the 2 weeks period while it almost remained similar in the experimental groups (Table-1).

The biochemical parameters of Liver and Renal functions were analysed for all the groups at the end of 14 days of treatment. In comparison of LFTs, statistically significant difference was observed in ALP between the groups ($p=0.004$). In comparison of RFTs, only blood Urea levels were significantly different between the groups ($p=0.03$) (Table-2). No significant difference was observed in serum Creatinine levels and TSB between groups (Table-2).

The liver and kidney morphology was assessed using the Gokakin *et al* scoring system ranging from 0 to 14.¹³ The mean score of histopathological changes after 14 days of intervention are shown in Table-3. In liver histology, hyperaemia, haemorrhage, mononuclear cell infiltration, vacuolar degeneration along with cloudy swelling of hepatocytes and necrosis were checked in all groups. The changes were more marked in higher dosage concentrations in both compound I and II in comparison to control group. Significant difference was mainly observed as necrosis of the liver tissue. Multiple comparisons showed significant difference in controls versus group II ($p=0.02$) and group IV ($p=0.02$). In kidneys histology; hyperaemia, haemorrhage, mononuclear cell infiltration, necrosis of tubular epithelium, tubular epithelial degeneration, and glomerular mesangial cell hyperplasia were assessed. There were mainly increased hyperaemia observed in treatment with both compounds at higher concentration, i.e., controls vs group II ($p=0.02$) and controls vs group IV ($p=0.02$).

Table-1: Weights in each group of animals at day 0 and day 14 of experiment

Weight	Control	Gp I	Gp II	Gp III	Gp IV
Day 0	230±22.73	196.5±72.83	230±12.02	179.5±44.54	230±35.35
Day14	243±15.75	193.5±30	227±11.3	178±43.84	226±33.23

All values are in grams (Mean±SD)

Table-2: Biochemical parameters between control and different experimental groups after 14 days of treatment

Parameters	Control	Group I	Group II	Group III	Group IV	<i>p</i>
ALT (IU/L)	29.5±3.42	31.5±3.54	31.5±2.12	33±1.41	37±1.42	0.143
AST (IU/L)	210±15.12	231±14.14	237.5±13.43	231.5±16.26	235±9.89	0.213
ALP (IU/L)	210.25±14.89	220±18.38	223±8.49	287±28.28	348±55.15	0.004
TSB (mg/dl)	0.45±0.13	0.45±0.07	0.45±0.07	0.5±0.141	0.55±0.07	0.830
Urea (mg/dl)	20.75±3.5	26.5±2.12	29±2.83	20.5±2.12	29±2.83	0.032
Creat(mg/dl)	0.55±0.06	0.35±0.07	0.45±0.07	0.4±0.14	0.5±0.14	0.208

All data is presented as Mean±SD. One way ANOVA was used for comparison between groups.

Table-3: Mean histopathological scoring of liver after 14 days of treatment

Liver score	Control	Group I	Group II	Group III	Group IV	<i>p</i>
Hyperaemia (1)	0	0	0	1	0	0.311
Cloudy swelling of hepatocyte (1)	0	0	0	0.5	0	0.311
Vacuolar degeneration (2)	0	0	0	0	1	0.311
Mononuclear cell infiltrations (2)	0	2	0	0	0	-
Necrosis in 1 -3 hepatocytes (2)	0	0	2	1	2	0.008
Necrosis in >3 hepatocytes (3)	0	0	0	0	0	-
Haemorrhage (3)	0	0	1.5	0	0	0.311
Total (14) Mean±SD	0±0	2±1.414	3.5±2.121	2.5±0.707	3.5±1.414	

Table-4: Mean histopathological scoring of kidney after 14 days of treatment

Kidney score	Control	Group I	Group II	Group III	Group IV	<i>p</i>
Hyperaemia (1)	0	0.5	1	1	0	0.08
Glomerular space expansion (1)	0	0.5	1	0.5	1	0.071
Glomerular mesangial cell hyperplasia (2)	0	0.5	0	1	0	
Tubular epithelial degeneration (2)	0	0	0	0	0	0.381
Mononuclear cell infiltrations (2)	0	1	1	0	2	0.103
Tubular epithelial necrosis (3)	0	0	0	0	0	-
Haemorrhage (3)	0	0	0	0	0	-
Total(14) Mean±SD	0±0	2.5±2.12	3±1.14	2.5±2.12	3±0	

DISCUSSION

The concept of BIODS is relatively new that is used for the exploration of medicines from advance medicine.¹⁴ To identify new bioactive small molecules as described in literature.¹⁵ The NSAID piroxicam belongs to the oxamicam class according to the chemical classification of drugs that is widely used these days.¹⁶⁻¹⁸ Main advantages are its prolonged life and lesser adverse effect on the stomach causing peptic ulcer.¹⁹ The inflammatory and pain causing COX-2 receptors are selectively blocked via the enolic component of the piroxicam. SPD are derivatives of the piroxicam analgesics synthesized by the BIOS recently. These compounds were also found to have potent analgesic activity and were found to be safe.^{20,21} The compound had been tested in animal models for its acute toxicity.¹¹

In the following study, two sulphonated piroxicam derivatives were tested in different concentration to test their effects on kidney and liver after 14 days of daily injections in albino rats. Raised serum levels of ALP were observed when drug was used in higher concentration, i.e., 20 mg/Kg body weight. However, serum levels of other biochemical parameters (ALT, AST and TSB) were comparable between experimental and control groups. This finding suggests that both drug might be safe when used in lower doses and need to be used with caution in higher

doses. Similar observations are reported in our previous study suggesting slightly more damaging effects after 7 days of these compound use.¹² The findings were further confirmed by histological evaluation of these drugs.

Similar reports are found in literature for different analogues of NSAIDs.²² The findings of biochemical parameters were also checked through histology of liver. The histopathological score of liver and kidney damage was significantly raised ($p<0.05$) in animals treated in higher concentrations (20 mg/Kg body weight dose) for 14 days as compared to control rats. Similar findings were observed previously after 7 days treatment with SPD.¹²

Liver and kidneys are the major organs responsible for drug metabolism and therefore mainly affected by the toxic effects.²³ Different liver enzymes are present in cytoplasm of liver cells and damage causes increased levels in blood.²⁴⁻²⁷ Polyunsaturated fatty acids of endoplasmic reticulum in the liver cells are damaged by reactive free radicals produced by drug and toxin metabolism.²⁸ These in turn leads to rise in the hepatic enzymes in serum; in accordance with the cellular damage. To counteract the toxic effects of drugs ROS (reactive oxygen species) are excessively generated and the antioxidant reserves deplete drastically. The production of excessive amount of ROS can also lead to nucleic acid damage.

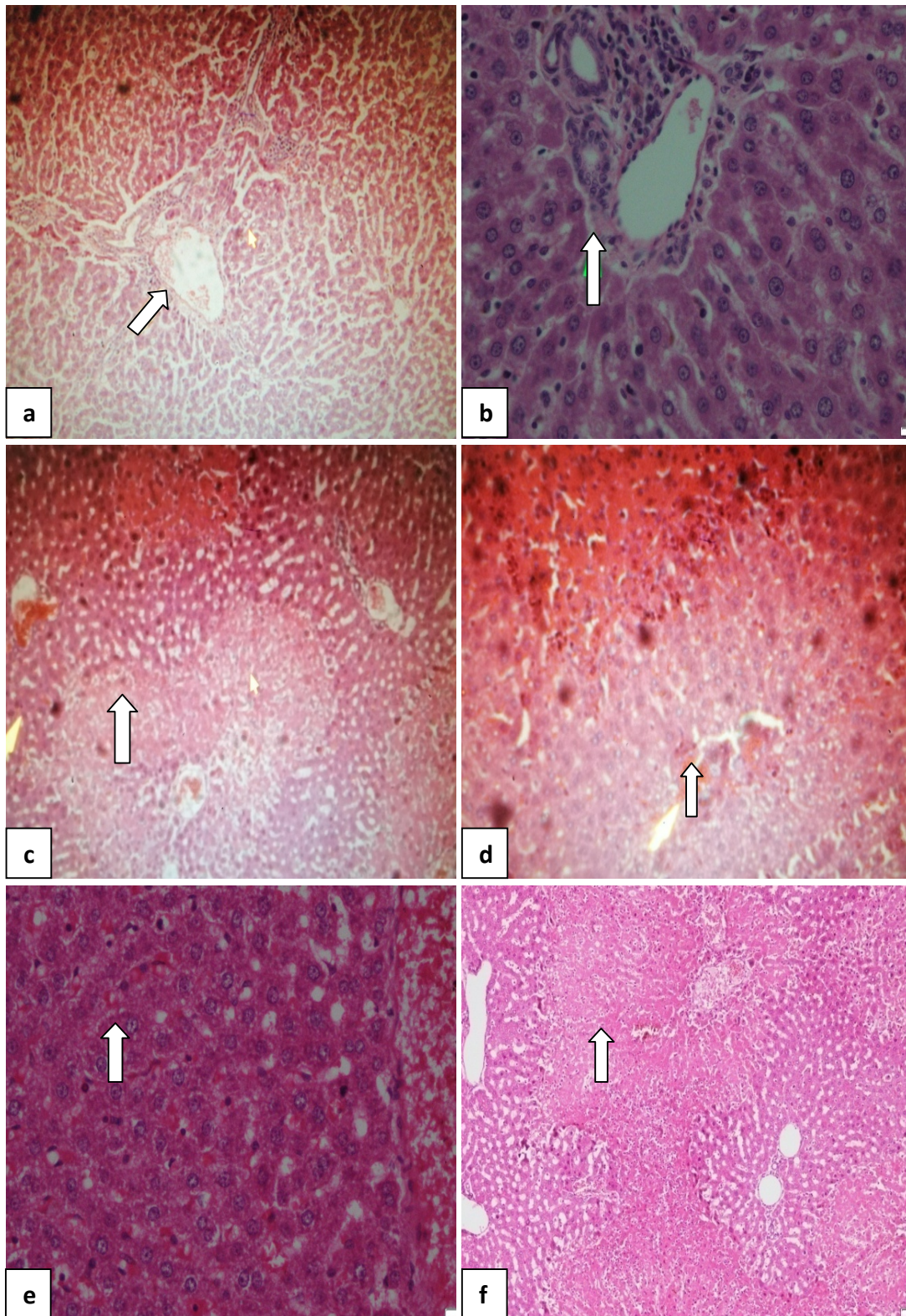


Figure-1: Photomicrographs of Liver Parenchyma.

a. & b. Liver parenchyma of normal control rat (arrow showing portal triad) c. Liver parenchyma of group II after 14 days of SPD treatment (arrow shows necrosis) d. Liver parenchyma of group II after 14 days of SPD treatment (arrow shows haemorrhage) e. Liver parenchyma of group III after 14 days of SPD treatment (arrow shows haemorrhage) f. Liver parenchyma of group IV after 14 days of SPD treatment (arrow shows necrosis).

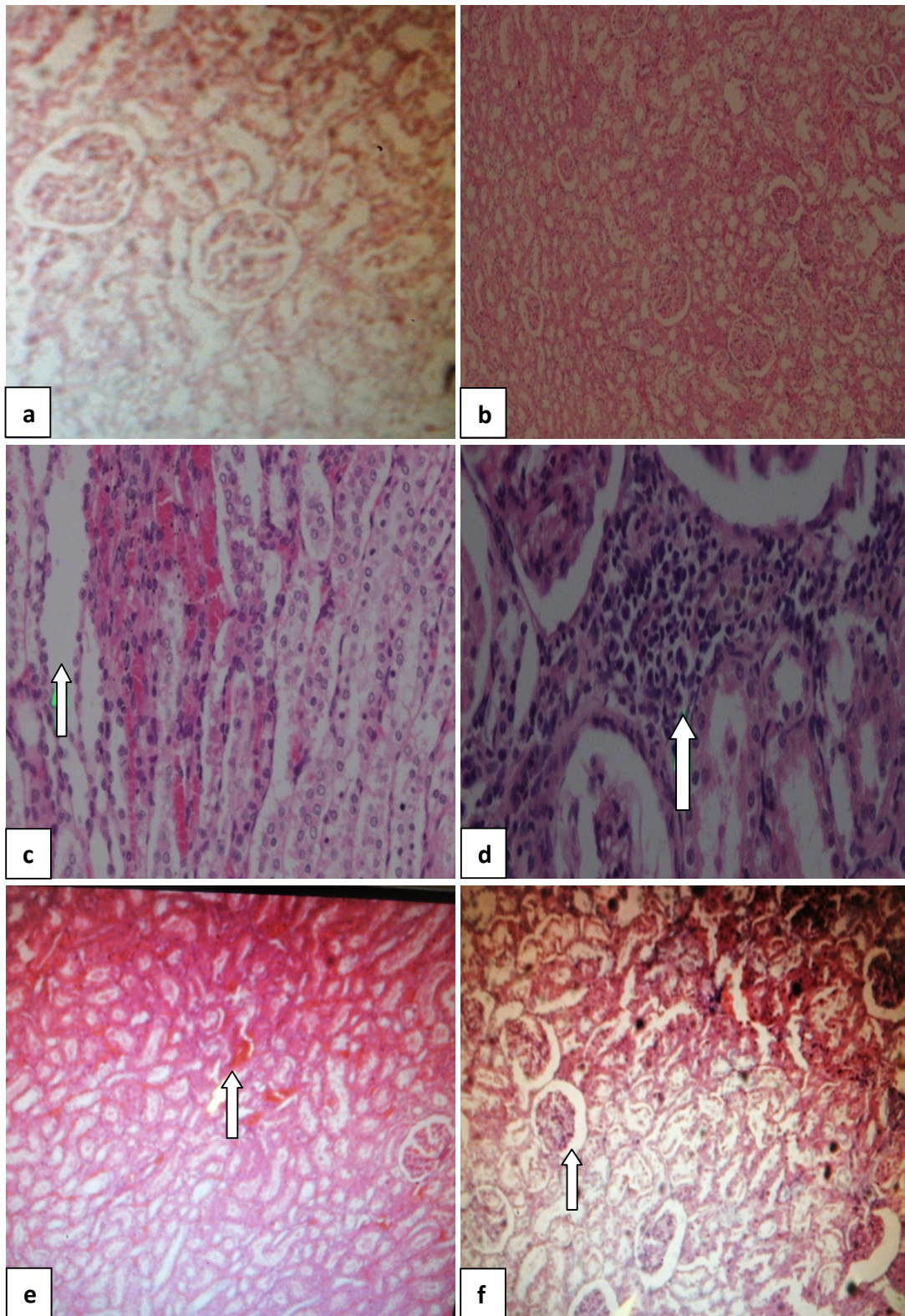


Figure-2: Photomicrographs of Liver Parenchyma.

a. & b. Histology of kidney parenchyma in normal control rats c. Kidney Histology of group I after 14 days of SPD treatment (arrow shows hyperemia) d. Kidney histological findings in group II after 14 days of SPD treatment (arrow shows mononuclear cell infiltrations) e. Kidney histological findings in group III after 14 days of SPD treatment (arrow shows haemorrhage) f. Liver parenchyma of group IV after 14 days of SPD treatment (arrow shows mesangial space expansion)

This can further lead to inactivation of sulphhydryl antioxidants, lipid peroxidation of cell membrane and inhibition of DNA repair.²⁹ It is well established that hepatotoxicity is one of the common reason for withdrawal of drugs from markets despite preclinical and clinical trials.¹⁰

One of the first response to cell injury is the cytoplasmic vacuolation of the liver and kidney cells in all type of injuries. It is the unique ability of hepatic cells to regenerate even if the damage occurs at the mitochondrial levels. Furthermore, it is observed that liver cells in rats can proliferate and there is auto reversal of damage within two weeks after cessation of toxins exposure.³⁰ The results of our study after 14 days use of SPD when compared to 7 days exposure¹² suggest the ability of liver for regeneration after injury, which is a peculiar property of liver cells.³⁰ Similarly, drugs can have toxic effects on kidneys and results in the increased levels of blood urea, serum creatinine and uric acid due to renal cell damage.³¹ Kidneys are also responsible for homeostasis of the body and helps in metabolism of many toxins and drugs. Many toxins and drugs such as xenobiotics and their metabolites are removed from the body via kidneys.³² It is observed that kidney tubules have great capability to regenerate damage cells in a short period of time in animal models.³³ The results of our study suggest that the drug is comparatively more safer when used in lower concentrations.

STUDY LIMITATIONS

We could not include positive control which would have made comparisons more reliable whether the damage seen in this study was comparable to other standard NSAIDs. Only two dose concentrations were used. However, higher dose concentrations and longer duration studies would provide a broader picture of the SPD. Furthermore, the sample size was small for the biochemical comparisons.

CONCLUSION

Sulphonated piroxicam derivatives can lead to focal degenerative changes along with biochemical derangements in both liver and kidneys when used in higher concentrations. However, these changes seem to be reversible and further larger pre-clinical studies are recommended for clinical use.

REFERENCES

- Fornasari D. Pain mechanisms in patients with chronic pain. *Clin Drug Investig* 2012;32(suppl 1):45–52.
- Lumley MA, Cohen JL, Borszcz GS, Cano A, Radcliffe AM, Porter LS, *et al.* Pain and emotion: a biopsychosocial review of recent research. *J Clinical Psychol* 2011;67(9):942–68.
- Borsook D, Hargreaves R, Bountra C, Porreca F. Lost but making progress —Where will new analgesic drugs come from? *Sci Transl Med* 2014;6(249):249sr3.
- Brune K, Patrignani P. New insights into the use of currently available non-4 steroidal anti-inflammatory drugs. *J Pain Res* 2015;8:105–18.
- Stauffer ME, Taylor SD, Watson DJ, Peloso PM, Morrison A. Definition of nonresponse to analgesic treatment of arthritic pain: an analytical literature review of the smallest detectable difference, the minimal detectable change, and the minimal clinically important difference on the pain visual analog scale. *Int J Inflam* 2011;2011:231926.
- Kowalski ML, Makowska JS, Blanca M, Bavbek S, Bochenek G, Bousquet J, *et al.* Hypersensitivity to nonsteroidal anti-inflammatory drugs (NSAIDs) –classification, diagnosis and management: review of the EAACI/ENDA and GA2LEN/HANNA. *Allergy* 2011;66(7):818–29.
- Pandit A, Sachdeva T, Bafna P. Drug -induced hepatotoxicity: A review. *J Appl Pharm Sci* 2012;2(5):233–43.
- Möller B, Pruijm M, Adler S, Scherer A, Villiger PM, Finckh A. Chronic NSAID use and long-term decline of renal function in a prospective rheumatoid arthritis cohort study. *Ann Theum Dis* 2015;74(4):718–23.
- Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S. Effect of biofield treatment on spectral properties of paracetamol and piroxicam. *Chem Sci J* 2015;6:098. doi:10.4172/2150-3494.100098.
- Jiang Y, Gu L, Zhang R, Zhang Y, Zhang L, Ju P, *et al.* Evaluation of the indicative roles of seven potential biomarkers on hepatonephrotoxicity induced by Genkwa Flos. *J Ethnopharmacol* 2014;158(Pt A):317–24.
- Ullah S, Saeed M, Halimi SMA, Fakhri MI, Khan KM, Khan I, *et al.* Piroxicam sulfonates biology-oriented drug synthesis (BIODS), characterization and anti-nociceptive screening. *Med Chem Res* 2016;25(7):1468–75.
- Ali A, Shaheen G, Tariq SA, Ullah S, Sultana N, Khan N, *et al.* Evaluation of hepatotoxic and nephrotoxic effects of piroxicam sulfonated derivatives. *Pak J Physiol* 2017;13(4):18–22.
- Gökakın AK, Atabey M, Deveci K, Sancakdar E, Tuzcu M, Duger C, *et al.* The effects of sildenafil in liver and kidney injury in a rat model of severe scald burn: a biochemical and histopathological study. *Ulus Travma Acil Cerrahi Derg* 2014;20(5):319–27.
- Wilk W, Zimmermann TJ, Kaiser M, Waldmann H. Principles, implementation, and application of biology-oriented synthesis (BIOS). *Biol Chem* 2010;391(5):491–7.
- Van Hattum H, Waldmann H. Biology-oriented synthesis: harnessing the power of evolution. *J Am Chem Soc* 2014;136(34):11853–9.
- Lombardino JG, Wiseman EH. Sudoxicam and related N-heterocyclic carboxamides of 4-hydroxy-2H-1,2-benzothiazine 1,1-dioxide. Potent nonsteroidal antiinflammatory agents. *J Med Chem* 1972;15(8):848–9.
- Manohara C, Sanganal S, Prem K, Swamy K, Phani A. Improved dissolution rate of Piroxicam by fusion solid dispersion technique. *Sci Technol Arts Res J* 2014;3(1):44–7.
- Richy F, Scarpignato C, Lanas A, Reginster JY. Efficacy and safety of piroxicam revisited. A global meta-analysis of randomised clinical trials. *Pharmacol Res* 2009;60(4):254–63.
- Kacem Y, Kraiem J, Kerkeni E, Bouraoui A, Hassine BB. Synthesis and pharmacological profile of 6-methyl-3-isopropyl-2H-1,2-benzothiazin-4(3H)-one 1,1-dioxide derivatives: non-steroidal anti-inflammatory agents with reduced ulcerogenic effects in the rat. *Eur J Pharm Sci* 2002;16(4-5):221–8.
- Chérié Lignière G, Montagnani G, Alberici M, Acerbi D. Plasma and synovial fluid concentrations of piroxicam during prolonged treatment with piroxicam pivalic ester. *Arzneimittelforschung* 1987;37(5):560–3.
- Rabasseda X, Hopkins S. Ampiroxicam: a prodrug of piroxicam devoid of gastrointestinal toxicity. *Drugs Today* 1994;30(7):557–64.
- 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):82–9.

23. Zimmerman HJ, Seeff LB. Enzymes in hepatic disease In: Goodly EL (Ed) Diagnostic enzymology Philadelphia: Lea and Febiger; 1970.p. 1–38.
24. Sureshkumar S, Mishra S. Hepatoprotective activity of extracts from *Pergularia daemia* Forsk. against carbon tetrachloride-induced toxicity in rats. *Pharmacogn Mag* 2007;3(11):187–91.
25. Packer JE, Mahood JS, Mora-Arellano VO, Slater TF, Willson RL, Wolfenden BS. Free radicals and singlet oxygen scavengers: Reaction of a peroxy-radical with β -carotene, diphenyl furan and 1,4-diazobicyclo(2,2,2)-octane. *Biochem Biophys Res Commun* 1981;98(4):901–6.
26. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective activity of cinnamon ethanolic extract against CCl₄-induced liver injury in rats. *EXCLI J* 2012;11:495–507.
27. Kuriakose GC, Kurup MG. Hepatoprotective effect of *Spirulina lonar* on paracetamol induced liver damage in rats. *Asian J Exp Biol Sci* 2010;1(3):614–23.
28. Farooq Y, Khan MA, Farooq MA. Liver function tests, red cell indices and oxidative stress in healthy male sprague dawley rats. *Pak J Physiol* 2015;11(3):10–3.
29. Mossalam HH, Yousuf YA. Hepatotoxic potential of leflunomide drug in adult male albino rats. *Al-Azhar Assiut Med J* 2013;11:284–309.
30. Mershiba SD, Dassprakash MV, Saraswathy SD. Protective effect of naringenin on hepatic and renal dysfunction and oxidative stress in arsenic intoxicated rats. *Mol Biol Rep* 2013;40(5):3681–91.
31. Goering PL, Waalkes MP, Klaassen CD. Toxicology of Cadmium. In: Goyer RA, Cherian MG, (Eds). *Toxicology of Metals*. Berlin, Heidelberg: Springer Berlin Heidelberg;1995. p.189–214.
32. Apte U, Gkretsi V, Bowen WC, Mars WM, Luo JH, Donthamsetty S, *et al.* Enhanced liver regeneration following changes induced by hepatocyte-specific genetic ablation of integrinlinked kinase. *Hepatology* 2009;50(3):844–51.
33. Berger K, Moeller MJ, (Eds). Mechanisms of epithelial repair and regeneration after acute kidney injury. *Semin Nephrol* 2014;34(4):394–403.

Address for Correspondence:

Dr. Shafiq Ahmad Tariq, Institute of Basic Medical Sciences, Khyber Medical University Peshawar, Pakistan.

Email: shafiq.ibms@kmu.edu.pk

Received: 18 Apr 2019

Reviewed: 12 Sep 2019

Accepted: 16 Sep 2019