International Journal of Science, Environment and Technology, Vol. 7, No 3, 2018, 925 – 932

ISSN 2278-3687 (O)
2277-663X (P)

EVALUATION OF TOXIC POTENTIAL OF KETOPROFEN ON HEMATO-BIOCHEMICAL PARAMETERS FOLLOWING SUBACUTE INTRAMUSCULAR ADMINISTRATION IN WISTAR RATS

Muchhara, J.A., Sankhala, L.N., Champawat, M., Bhavsar, S.K., Thakar, A.M. Dadhaniya, P.K., Vachhani, K.V. and Patel C.D.
Department of Pharmacology and Toxicology
College of Veterinary and Animal Science
Anand Agriculture University, Anand-388001, Gujarat, India
E-mail: allensankhala@gmail.com (**Corresponding Author*)

Abstract: Present study was planned to investigate the toxic potential of ketoprofen on haematological and biochemical parameters following long term intramuscular administration in wistar rat. A total number of 48 wistar rats divided in four groups were exposed to different doses of ketoprofen by intramuscular route for 28 days. In the present study highly significant reduction in RBCs, Hb, PCV and MCHC in high dose group in both male and female animals was observed. While highly significant increase in MCV in male rats and significant increase in MCV in female rats was seen. In present study there is no significant difference in WBC count, monocytes and eosinophils in male and female animals of all the groups. There is significant increase in neutrophils and decrease in lymphocytes in male and female rats of high dose group. While significant decrease in basophils in male animals of high dose group was observed, whereas female did not show any significant differences. There is significant decrease in platelet count in both male and female in high dose of ketoprofen in the present study. In present study no significant changes were observed in AST, ALT and ALP level. There is significant decrease in the total plasma protein concentration, albumin and globulin in male of medium dose group (6 mg/kg) and male and female of high dose group (9 mg/kg) compare to control in the present study. In present study there were no significant changes in glucose and cholesterol level. Significant rise in triglycerides level was found only in female rats of high dose group indicating impairment of lipid metabolism in animals. No significant changes in urea as well as serum creatinine level were observed in the present study.

Keywords: Haematological, Lymphocytes, Plasma protein, Ketoprofen.

Introduction

Ketoprofen, 2-(benzoyl-3-phenyl) propionic acid, is a non-steroidal anti-inflammatory drug belonging to the arylpropionic acid group and was synthesized in 1967. It was introduced into human medicine in France and Great Britain in 1973. In Veterinary therapeutics, Ketoprofen is given by oral and parenteral (Intravenous, Intramuscular or subcutaneous) routes for the treatment of inflammatory and painful conditions of the bones, joints and muscular-skeletal systems in cattle, horses, dogs and cats (European Medicines Agency, 1995). Ketoprofen's

*Received May 2, 2018 * Published June 2, 2018 * www.ijset.net*

antiinflammatory effect is believed to be due to inhibition of both cylooxygenase-1 (COX-1) and cylooxygenase-2 (COX-2) which leads to the inhibition of prostaglandin synthesis (Johnston and Fox, 1997). However, it is recommended that ketoprofen treatment should be limited to a maximum of five consecutive days to reduce the risk of gastrointestinal effects. Ketoprofen can cause gastrointestinal irritation that may lead to ulceration. Most of the toxicity studies are conducted in laboratory animals following oral administration. As the drug is used parentraly in domestic animals, toxicity data following long term intramuscular administration in laboratory animals were lacking. Looking to the paucity of literature on sub acute toxicity of Ketoprofen following intramuscular administration in animals, the present study has been planned to study the toxic effect of daily intramuscular administration of Ketoprofen formulation for 28 days in Wistar rats.

MATERIALS AND METHODS

Experimental Animals

The present study was conducted on 7-8 weeks old adult healthy male and female Wistar Rats. The live body weight range was within ± 20 % of the mean body weight for each sex at the time of randomization. The experiment was conducted at the Animal House of Cadila Pharmacutetical Ltd., Dholaka (Gujarat). The experimental protocol was approved by Institutional Animal Ethical Committee. The rats were housed in standard polypropylene cages and provided with standard pelleted feed and water *ad libitum*. Ketoprofen Injection (Neoprofen 10%, Vetnex- Ranbaxy, India) was obtained from open market. Each ml contains 100 mg of ketoprofen

Experimental Protocol

All the selected animals were kept under acclimatization for minimum 5 days before grouping and dosing. Fourty eight (24 Male + 24 Female) rats were randomly divided into four groups. Each group consists of 6 male and 6 female animals. Group I served as control and received only vehicle (Water for injection) till 28 days of dosing period. Group II, III and IV received Ketoprofen at dose of 3 mg/kg, 6 mg/kg and 9 mg/kg respectively intramuscularly till day 28 of dosing period.

After completion of treatment period of 28 day, blood was collected from all male animals on day 29 and all female animals on day 30 after overnight fasting under carbon dioxide anesthesia. The blood samples collected by cardiac puncture and transferred in two different containers. Vaccutainers coated with EDTA (Ethylene Diamine Tetra Acetic acid) were used for haematological investigation. The hematology parameters were analyzed using automatic

haematology analyzer Advia 120. Plain micro centrifuge tubes of 1.5 ml capacity were used to collect blood for serum separation. Clotted blood containing micro centrifuge tubes were centrifuged and serum was separated. Separated serum was used for biochemical analysis using Hitachi 902 automatic biochemical analyzer.

STATISTICAL ANALYSIS

The statistical procedure used for analysis of data was unpaired two tail Student's `t` test where the $p < 0.05$ has been considered as statistical significant and $p < 0.01$ as highly significant.

Result and Discussion

Result of daily intramuscular administration of ketoprofen for 28 days on various haematological parameters is as shown in table-1.In the present study highly significant reduction in RBCs, Hb, PCV and MCHC in high dose group in both male and female animals was observed. While highly significant increase in MCV in male rats and significant increase in MCV in female rats was seen. The reduction of RBCs, Hb and PCV suggested the existence of anaemia. The calculation of MCV helps in the morphological classification of anaemia. The increase MCV in the present study indicated the increase in the size of erythrocytes and, hence, anaemia was macrocytic anaemia. The decreased Hb suggests the hypochomic anaemia (Jain, 1986). Overall, the anaemia encountered in the present study is macrocytic hypochromic anaemia. Similar observation of reduction in Hb and PCV were noticed after the administration of certain NSAIDs in rats (Velankar *et al.,* 1999) and loxoprofen in dogs (Peter *et al*., 2003). On the contrary, Velankar *et al.,* (1999) reported that NSAIDs like Diclofenac sodium, Ibuprofen and Aspirin did not produce any significant changes in total erythrocyte count, plasma protein concentration, MCV, MCH, and MCHC level in rats. Decrease in hematocrit or hemoglobin values may be possibly because of gastrointestinal bleeding or microbleeding and/or hemodilution caused by fluid retention (Klasco, 2003). The macrocytic anaemias are most frequent following haemorrhages. The outpouring of less mature erythrocytes (reticulocytes) in response to the anaemia accounts for the increase in MCV. To substantiate these findings, the studies on gastro duodenal mucosa in the present investigation revealed multiple hemorrhages and ulceration with ketoprofen which have simultaneously decreased RBCs, Hb, PCV and increased MCV. The long term administration of anti-inflammatory agents, as evident from the present study cause continuous loss of blood in a series of small hemorrhages resulting in chronic hemorrhagic anaemia.

In present study there is no significant difference in WBC count, monocytes and eosinophils in male and female animals of all the groups. There is significant increase in neutrophils and decrease in lymphocytes in male and female rats of high dose group. While significant decrease in basophils in male animals of high dose group was observed, whereas female did not show any significant differences.

Similarly, no significant changes were observed in total leukocyte count and differential lymphocyte count in dogs treated with Aspirin (100 mg/kg), Diclofenac sodium (15 mg/kg), Ibuporfen (10 mg/kg) , Nimesulide (5 mg/kg)., and Serratiopeptidase (2 mg/kg) daily orally for 16 days (Sharma, 2002).

There is significant decrease in platelet count in both male and female in group IV at high dose of ketoprofen in the present study. Platelet formation is regulated by prostaglandins. Inhibition of prostaglandins leads to reduction in platelet formation. So, there may be decrease in platelet count in case of NSAIDs therapy (Cashman, 1996). Similarly, Niemi *et al.,* (1997) compared the effect of intravenous ketoprofen, ketorolac and diclofenac on platelet function in human volunteers. They found that diclofenac inhibited adrenaline induced platelet aggregation less (median maximal aggregation 22.5%) than ketoprofen (18.3%) and ketorolac (15.7%). They concluded that Ketoprofen, ketorolac and diclofenac caused a reversible platelet dysfunction.

Result of daily intramuscular administration of ketoprofen for 28 days on various biochemical parameters is as shown in table-2.In present study no significant changes were observed in AST, ALT and ALP level. It indicated that ketoprofen has no any adverse effect on liver function. Similarly Ramesh *et al*., (2001) studied the nimesulide toxicity following oral administration (2 mg/kg daily for four days) in eight dogs. They found that levels of AST, ALT and ALP in the treated group did not differ significantly from the control group.

There is significant decrease in the total plasma protein concentration, albumin and globulin in male of group III (6 mg/kg) and male and female of group IV (9 mg/kg) compare to control in the present study. Decrease in globulin is highly significant in male of group IV while significant in female of group IV. Similar observation of reduction in total plasma protein was reported in domestic and laboratory animals following exposure to various NSAIDs. MacAllister *et al.,* (1993) studied the relative toxicity of phenylbutazone, flunixin meglumine and ketoprofen in healthy adult horses. Phenylbutazone and ketoprofen treated horses had a significant decrease in serum total protein and albumin concentrations. Peter *et al.,* (2003) evaluated the toxicity profile of loxoprofen sodium in Dogs. In both males and

females, there was a statistically significant reduction in serum protein and albumin compared with the control group.

Plasma proteins are formed by lymphocytic tissuses and the liver. A low plasma protein observed in blood loss, hepatopathy and malnutrition. Loss of protein during inflammation or ulceration of the gastrointestinal tract could lead to impaired absorption as well as loss of plasma protein due to injured mucosal cells. Damage to the kidney is also responsible for the loss of plasma protein and, causes their low concentration (Jain, 1986).

In present study there were no significant changes in glucose and cholesterol level. Significant rise in triglycerides level was found only in female rats of group IV indicating impairment of lipid metabolism in animals. It is difficult to explain the reasons for this kind of change in these parameters except for disturbance in digestion and absorption.

Jelic *et al.,* (1985) investigated the *in vivo* effects of acetylsalicylic acid (2.7 to 4 g), diclofenac (75-150 mg), indomethacin (75-200 mg), ibuprofen (600-1600 mg), and ketoprofen (150-200 mg) on the concentrations of various blood constituents on human patients suffering from rheumatic disorders. Total protein, glucose, calcium, and inorganic phosphate were not significantly affected by any of these drugs. Ketoprofen had no definite influence on any constituent. Acetylsalicylic acid induced an increase in cholesterol, triglyceride, and iron; albumin, uric acid, and creatinine decreased with ibuprofen therapy. Urea nitrogen increased in patients treated with diclofenac or indomethacin.

No significant changes in urea as well as serum creatinine level were observed in the present study. Urea is excreted by glomerular filtration and excretion depends on the hydration status of the animals. In well hydrated animals urea is almost excreted, majority is reabsorbed in dehydrated animals. If an animal is, dehydrated urea level may rise to 4x - 5x than normal level. Ramesh *et al*., (2001) studied the Nimesulide (2 mg/kg b.wt. twice daily for four days) toxicity in dogs and found that there was progressive increase in creatinine levels in Nimesulide treated dogs.

Based on the findings observed in this study, the NO OBSERVED ADVERSE EFFECT LEVEL (NOAEL) of Ketoprofen, when administered to Wistar rats by intramuscular route, over a period of 28 days was found to be– 6 mg/kg/day. Ketoprofen was not found hepatotoxic or nephrotoxic as there were no changes in serum biochemical parameters related to liver or kidney function.

Table 1: Effect of daily intramuscular administration of ketoprofen for 28 days on various haematological parameters in Wistar rats

Group No.	Dose (mg/kg)	Sex	RBC (X 10^6/µL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Hb (g/dL)
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
I	Control	M	8.41 ± 0.14	43.07 ± 1.11	51.20 ± 0.74	18.37 ± 0.34	36.41 ± 0.76	15.42 ± 0.32
II	3	M	8.29 ± 0.28	44.43 ± 1.29	53.63 ± 0.93	18.63 ± 0.29	35.25 ± 0.36	15.45 ± 0.45
III	6	M	8.00 ± 0.26	40.10 ± 1.20	52.33 ± 0.82	17.73 ± 0.19	34.90 ± 0.39	14.85 ±0.30
IV	9	M	6.06 ± 0.38**	35.23 ± 1.76**	58.42 ± 1.29**	18.28 ± 0.19	31.35± 0.38**	11.05 ± 0.63**
I	Control	F	7.95 ± 0.16	42.30 ± 0.87	53.23 ± 0.94	19.55 ± 0.26	36.73 ± 0.22	15.55 ± 0.26
II	3	F	7.70 ± 0.16	41.63 ± 0.74	54.08 ± 0.64	19.08 ± 0.24	35.97 ± 0.36	15.20 ± 0.37
III	6	F	7.56 ± 0.31	40.85 ± 1.41	55.27 ± 0.65	19.02 ± 0.17	35.93 ± 0.31	14.87 ± 0.21
IV	9	F	5.37 ± 0.53**	32.02 ± 2.32**	60.48 ± 2.45*	19.18 ± 0.42	31.82 ± 0.71**	10.23 ± 0.92**

Group No.	Dose (mg/kg)	Sex	PLT (X 10^3/µL)	WBCs (X 10^3/µL)	Differential WBCs (%)				
					Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
			Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
I	Control	M	1110.67 ± 63.48	17.09 ± 1.85	9.48 ± 1.59	81.52 ± 3.12	1.82 ± 0.35	1.55 ± 0.41	1.43 ± 0.14
II	3	M	988.83 ± 33.61	18.90 ± 1.83	8.67 ± 0.67	85.43 ± 0.79	1.70 ± 0.33	1.37 ± 0.35	1.53 ± 0.12
III	6	M	1358.67 ± 125.91	17.81 ± 1.75	9.85 ± 1.80	84.37 ± 2.19	1.63 ± 0.29	1.37 ± 0.31	1.50 ± 0.07
IV	9	M	887.50 ± 31.73*	19.15 ± 1.45	15.47 ± 1.26*	71.98 ± 1.54*	2.48 ± 0.51	1.22 ± 0.30	0.92 ± 0.07*
I	Control	F	1059.83 ± 45.55	15.57 ± 1.78	10.10 ± 1.35	82.97 ± 1.18	1.88 ± 0.28	2.42 ± 0.35	1.30 ± 0.12
II	3	F	1120.33 ± 96.14	16.74 ± 2.36	9.57 ± 1.14	83.37 ± 1.08	1.97 ± 0.24	2.33 ± 0.39	1.27 ± 0.08

III	**6**	**F**	1199.67 ± 49.05	18.62 ± 1.37	10.18 ± 1.05	82.85 ± 1.69	1.70 ± 0.21	2.73 ± 0.55	1.37 ± 0.11
IV	**9**	**F**	890.00 ± 22.94*	16.70 ± 1.84	18.27 ± 1.90*	73.45 ± 2.71*	3.18 ± 0.69	2.60 ± 0.37	0.92 ± 0.14

*: Significant ($p < 0.05$) & **: Highly significant ($p < 0.01$)

Table 2: Effect of daily intramuscular administration of ketoprofen for 28 days on various biochemical parameters in Wistar rats

Biochemical Parameters	**Sex**	**Groups**			
		I (Control)	**II (3 mg/kg)**	**III (6 mg/kg)**	**IV (9 mg/kg)**
		Mean ± SEM	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**
AST (IU/L)	**M**	136.70 ± 10.77	139.66 ± 10.81	160.87 ± 7.11	150.05 ± 12.39
ALT (IU/L)	**M**	48.80 ± 2.87	46.97 ± 1.70	49.02 ± 3.16	41.51 ± 3.73
ALP (IU/L)	**M**	176.00 ± 14.22	202.00 ± 10.56	158.33 ± 15.30	163.67 ± 13.04
Total Bilirubin (mg/dL)	**M**	0.104 ± 0.007	0.100 ± 0.006	0.088 ± 0.011	0.089 ± 0.005
Total Protein (g/dL)	**M**	8.04 ± 0.12	7.79 ± 0.23	7.21 ± 0.25*	6.44 ± 0.17**
Albumin (g/dL)	**M**	4.050 ± 0.043	4.004 ± 0.096	3.568 ± 0.140*	3.347 ± 0.153**
Globulin (g/dL)	**M**	4.00 ± 0.10	3.72 ± 0.17	3.63 ± 0.12*	3.07 ± 0.17**
Glucose (mg/dL)	**M**	83.00 ± 4.55	80.50 ± 4.42	72.17 ± 6.30	71.83 ± 2.52
Cholesterol (mg/dL)	**M**	58.17 ± 3.59	65.00 ± 2.80	64.50 ± 3.36	62.67 ± 6.23
Triglycerides (mg/dL)	**M**	89.67 ± 11.10	107.33 ± 6.89	82.83 ± 10.82	93.67 ± 2.76
Urea (mg/dL)	**M**	36.05 ± 3.14	42.02 ± 1.10	39.19 ± 1.34	42.53 ± 1.91
Creatinine (mg/dL)	**M**	0.502 ± 0.016	0.522 ± 0.011	0.515 ± 0.023	0.470 ± 0.012
AST (IU/L)	**F**	159.71 ± 0.08	143.21 ± 13.68	167.34 ± 10.65	134.24 ± 13.02
ALT (IU/L)	**F**	37.89 ± 3.57	43.48 ± 2.46	45.98 ± 2.52	30.27 ± 5.44
ALP (IU/L)	**F**	141.67 ± 13.17	145.83 ± 6.58	136.33 ± 13.72	135.50 ± 10.04
Total Bilirubin (mg/dL)	**F**	0.122 ± 0.013	0.126 ± 0.011	0.087 ± 0.011	0.076 ± 0.018
Total Protein (g/dL)	**F**	7.64 ± 0.26	8.03 ± 0.14	7.27 ± 0.28	5.65 ± 0.37**
Albumin (g/dL)	**F**	4.046 ± 0.123	4.147 ± 0.038	3.811 ± 0.119	2.869 ± 0.181**
Globulin (g/dL)	**F**	3.60 ± 0.16	3.87 ± 0.15	3.45 ± 0.17	2.77± 0.20*
Glucose (mg/dL)	**F**	70.67 ± 3.46	75.83 ± 4.06	74.50 ± 5.38	66.67 ± 2.87
Cholesterol (mg/dL)	**F**	78.17 ± 3.53	72.33 ± 3.84	74.33 ± 5.07	70.67 ± 1.89
Triglycerides (mg/dL)	**F**	89.00 ± 15.04	81.00 ± 7.29	84.67 ± 10.26	125.83 ± 4.22*
Urea (mg/dL)	**F**	37.88 ± 0.84	40.56 ± 1.18	44.66 ± 3.08	41.25 ± 2.99
Creatinine (mg/dL)	**F**	0.603 ± 0.022	0.602 ± 0.014	0.573 ± 0.024	0.562 ± 0.045

*: Significant ($p < 0.05$) & **: Highly significant ($p < 0.01$)

References

[1] Cashman J.N. (1996). The mechanisms of action of NSAIDs in analgesia. *Drugs*. **52** (5):13–23.

[2] Jain, N.C. (1986). Schalm's Veterinary Hematology, 4th Edn. Lea and Febiger, 600 Washington Square, Philadelphia; 19106-4198.

[3] Jelic Ivanovic, Z.; Spasic, S.; Majkic N. Singh and Todorovic, P. (1985). Effects of some anti-inflammatory drugs on 12 blood constituents: protocol for the study of in vivo effects of drugs. *Clin. Chem.* **31**: 1141-1143.

[4] Johnston, S.A. and Fox, S.M. (1997). Mechanisms of action of anti-inflammatory medications used for the treatment of osteoarthritis. *J. Am. Vet. Med. Assoc.* **210**(10):1486-92.

[5] Klasco, R.K. (2003). USP DI Drug information for the healthcare professional. Volume I. Greenwood Village, CO: Thomson MICROMEDEX, Inc.

[6] MacAllister, C.G.; Morgan, S.J.; Borne, A.T. and Pollet, R.A. (1993). Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. *J. Am. Vet. Med. Assoc.* **202**(1):71-7.

[7] Niemi, T.T.; Taxell, C. and Rosenberg, P.H. (1997). Comparison of the effect of intravenous ketoprofen, ketorolac and diclofenac on platelet function in volunteers. *Acta. Anaesthesiol. Scand.* **41**(10):1353-8.

[8] Peter, J.; Peter, B.; Rani, S.; Wendy, C.; Yoshihiko, E. and Nasir, M. (2003). Gastrointestinal and Renal Toxicity Associated with Loxoprofen Sodium in Dogs. *Priclinica.* **1**(3):107-113.

[9] Ramesh, N.; Jayakumar, K.; Honnegowda; Narayana, K. and Vijayasarathi, S.K. (2001). Nimesulide toxicity in dogs. *Indi. J. Pharmaco.* **33:**217-218.

[10] Sharma, A.B. (2002). Studies on the Efficacy of Certain Non-Steroidal Anti-inflammatory Drugs. M.V.Sc. Thesis, J.N.K.V.V., Jabalpur.

[11] Vane, J.R. and Botting, R.M. (1995). New insights into mode of action of anti-inflammatory drugs. *Inflammation research.* **44**:1-10.

[12] Velankar, S.S.; Sharma, R.K.; Reddy, A.G.; Shrivastava, P.N. and Tiwari, S. (1999). Effect of long term use of non-steroidal anti-inflammatory drugs on haematological parameters in rats: An experimental study. *Indian J. Toxicol.* **6**:9-12.