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TOXICOPATHOLOGICAL STUDIES OF EXPERIMENTALLY INDUCED MELOXICAM TOXICITY IN WISTAR RATS (*Rattus norvegicus*)

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The present study was performed to determine the hemato-biochemical parameters and pathological alterations induced by meloxicam toxicity in Wistar rats. Thirty adult Wistar rats were divided uniformly into three equal groups viz. Group A, Group B and Group C. Group A rats received only 0.5% Sodium Carboxy Methyl Cellulose (CMC) and it served as vehicle control. Group B (Low dose) and Group C (High dose) rats were given meloxicam in 0.5% sodium CMC @ 4.2 mg/kg b.wt. ($1/20^{th}$ of LD_{50}), 8.4 mg/kg b.wt. ($1/10^{th}$ of LD_{50}) respectively orally by gavage for 28 days. The LD_{50} of meloxicam is 84 mg/kg b.wt. Meloxicam treated animals showed weakness, lethargy and distended abdomen. A dose dependent significant reduction in Hb, PCV, TEC, MCH, and MCHC were observed while value of TLC and neutrophils count were increased in all treatment groups. Biochemically dose dependent significant rise in alkaline phosphatase and decrease in total protein, albumin and globulin were observed. Histopathological sections of stomach, intestine, liver & kidney revealed varying degrees of haemorrhage, degeneration, necrosis and ulcer in rats of different treatment groups.Meloxicam is responsible to produce the serious gastrointestinal toxicity, such as inflammation, bleeding, ulceration can occur at any time, with or without warning symptoms.

Key words: Hematology, Biochemistry, Pathology, Meloxicam and Rat.

Meloxicam is an anti-inflammatory drug and is a member of the oxicam family of NSAIDs (Vane and Botting, 1997). The advantage of this drug over the traditional NSAIDs is that it has greater in vitro and in vivo inhibitory action against the inducible COX–2 isoform. Meloxicam inhibits COX-2 about 12 times more selectively than COX-1 (Ogino *et al*., 1996). Meloxicam as NSAID either alone or with antimicrobial drugs, is indicated for use in ruminants for the treatment of pneumonia, pleuritis, laminitis, myositis, sprain, mastitis, prolapse of uterus, premature labour etc. (Vane and Botting, 1997 and Samad and Gaikwad, 2000). Meloxicam produces serious gastrointestinal toxicity, such as inflammation, bleeding, ulceration, and perforation of the stomach in laboratory animals. There are many toxicological studies carried out on meloxicam but very limited information is available on detailed pathomorphological changes in meloxicam toxicity. Hence looking to the paucity of information, the present investigation was undertaken to study the clinical symptoms, hematological changes, biochemical variation, pathomorphological alteration that occurs due to various doses of meloxicam.

MATERIALS AND METHODS

Experimental animals: The study was conducted on thirty colony bred Albino Wistar strain rats of both sexes, which were procured from Cadilla Pharmaceuticals, Dholka, Ahmadabad , Gujarat, India and were maintained under standard management conditions. Males and females were separated and all the animals were quarantined and acclimatized to the laboratory conditions of 12 hours day and 12

hours night. The animals were provided with standard pelleted food and water *ad lib.* The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol vide letter No. SDAU/DVC/VSR/IAEC/ 7992-8001/08 dated 6th June 2008. The experimental protocol met the national guidelines as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Chemicals: Meloxicam (99 % pure) obtained obtained from Cadila Healthcare Limited, Ankleshwar, Gujarat, India, was used for inducing toxicity in rats. All the other chemicals used in the study were of standard analytical grade.

Experimental design: Thirty adult Wistar rats were divided uniformly into three equal groups viz. Group A, Group B and Group C. Group A rats received only 0.5% Sodium Carboxy Methyl Cellulose (CMC) and it served as vehicle control. Group B (Low dose) and Group C (High dose) rats were given meloxicam in 0.5% sodium CMC @ 4.2 mg/kg b.wt. (1/20th of LD50), 8.4 mg/kg b.wt. (1/10th of LD50) respectively orally by gavage for 28 days. The LD50 of meloxicam is 84 mg/kg b.wt.

Collection of Blood: Rats were anesthetized by using diethyl ether and blood was collected from all experimental groups on 28th day of experiment from retro-orbital plexus with the help of capillary tube in a heparinised vial (10 IU) for biochemical estimations where as in K_3EDTA vial for Hematological analysis. Plasma was separated from heparinised blood for biochemical estimations. (Why did not you sacrifice them on the same (28th) day? Because it was a 28 days (sub acute) study. The effect of Meloxicam on rats at 28th day was also measured. So that we were sacrificed them on the next day.)

Collection of tissues: Rats were sacrificed on 29th day of post-treatment and tissues from various organs were collected in 10 % neutral buffered formalin. Tissue samples *viz.*, liver, lung, kidney, stomach, intestine, spleen, thymus, skin, heart, brain and testis were collected for histopathological study.

Biochemical assay: All the biochemical parameters were analysed using Mercks Kits (Mercks Specialities Private Ltd., Mumbai-400018, India) by Clinical Analyzer (Systronics, Ahmedabad).

Pathomorphology: After recording the gross lesions, the tissues from affected stomach, intestine, lung liver, brain, heart, spleen, kidney and testis were collected from sacrificed animals and subsequently preserved in 10 % neutral buffered formalin for at least 24-48 hours. Further these tissues were processed by routine method of dehydration in graded alcohol, clearing in xylene and embedding in paraffin. Sections of 5-6 µ thicknesses were prepared and processed by routine Hematoxylene and Eosin method to study the general histopathological alterations (Luna, 1968).

Statistical analysis: The statistical analysis of data generated on various parameters was performed using completely randomized design (CRD) (Snedecor and Cochran, 1980) and using CD values compared the treatment means. Since, the CD permits comparison of two consecutive treatment mean after arranging treatment mean in ascending or descending order, it was thought worthwhile to compare treatment mean with all other treatment mean (Overall comparison). Hence, Duncan's New Multiple Range Test (DNMRT (Steel and Torrie, 1984) was used for the same.

RESULTS

In meloxicam treated animals, clinical signs like weakness, lethargy and distended abdomen where observed in Group C while in lower dose group B same clinical symptoms were observed with mild intensity. Significant reduction in body weight gain and body weight was recorded in male and female of group C. Effect of Meloxicam on Hb, PCV, TEC, MCH, MCHC (Mean ± S.E,) of different experimental group rats were shown in Table 1. Significant reduction in erythron (Hb, PCV, TEC, MCH, MCHC) was recorded in high dose group while value of TLC and neutrophils count were increased. The details of mean ± S.E. values of total leucocyte count and differential leucocyte count of all the treatment groups were summarized and presented in Table 2.

Table 1: Effect of Meloxicam on Hb, PCV, TEC, MCH, MCHC (Mean ± S.E,) of different experimental group rats (n = 10).

GROUP	A Control	B	C
Hb (g/dl)	14.00 ±0.098 [a]	11.97±0.176 [b]	10.93±0.164 [c]
PCV	40.88±0.283 [a]	37.35±2.036 [b]	34.56±2.568 [c]
TEC	7.80±0.186 [a]	7.14±0.188 [b]	6.14±0.301 [c]
MCV	53.20±0.826 [a]	55.19±0.906 [b]	56.62±0.508 [c]
MCH	18.21±0.155 [a]	16.21±0.508 [b]	14.98 ±0.559 [c]
MCHC	34.33±0.362 [a]	32.83 ±0.486 [b]	31.99 ±0.136 [c]

- Superscripts are to be read column wise for mean comparison
- Mean with similar superscripts in column do not differ significantly ($P < 0.05$).

Table 2: Effect of Meloxicam on total leukocyte count and Differential leukocyte count (Mean ± S.E, thousands / cumm) of different experimental group rats (n =10).

GROUP	A Control	B	C
TLC	17.94±0.426 [a]	18.98±0.209 [b]	28.45±0.447 [c]
Neutrophil	18.10±0.406 [a]	19.40±0.305 [b]	28.20±0.442 [c]
Eosinophil	2.00±0.149 [a]	1.70±0.213 [a]	1.30±0.252 [a]
Basophil	0.90±0.099 [a]	0.80±0.133 [a]	0.90±0.099 [a]
Lymphocyte	78.20±0.243 [a]	74.10±0.673 [b]	62.60±0.968 [c]
Monocyte	2.10±0.179 [a]	2.30±0.399 [a]	3.30±0.399 [a]

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- Mean with similar superscripts in column do not differ significantly ($P < 0.05$).

Clinical biochemistry reveled, alkaline phosphatase (AP) concentration was increased in plasma while decreased in total protein, albumin and globulin were noticed. No significance differences were observed in plasma alanine aminotransferase (ALT) and plasma aspartate aminotransferase (AST) throughout study when compared with Group A control. The details of mean ± S.E. value of biochemical parameters of all the treatment groups were presented in table 3.

In the present study, the pathomorphological lesions in all the Meloxicam treated groups were almost uniform and variation was dose dependent. Pathomorphologically, stomach and intestine was observed as the most affected organ followed by liver, kidney, lung, brain, heart, testes and spleen.

At the time of necropsy, stomach and intestine showed moderate erosion with hemorrhages and ulcer in Group C. While a mild serosal congestion was seen in Group B. Macroscopic changes in heart includes focal pale area of necrosis and mild congestion on auriculo-ventricular groove receiving higher dose (Group C) of Meloxicam whereas, only mild to moderate serosal congestion was observed in Group B. Other organs did not revealed any test article related changes.

Microscopic examination of stomach reveled multifocal congestion in both treated groups. In high dose group, stomach revealed severe necrosis and sloughing of glandular portion with sub mucosal hemorrhage. Multifocal necrosis and sloughing of epithelium was also found in intestine of Group C animals. Intestine of group B revealed glandular degeneration with mild congestion and moderate atrophy of villi. Microscopic examination of liver revealed mild congestion in Group B whereas Group C revealed severe kupffer cell proliferation with moderate fatty changes and hepatic necrosis in central vein. Microscopic

Table 3: Effect of Meloxicam on plasma AP, total protein, Albumin, Globulin, AST and ALT activity (Mean ± S.E) of different experimental group rats (n =10).

GROUP	A Control	B	C
AP	159.89±6.172 [a]	159.33±5.598 [a]	213.83±8.775 [b]
total protein	7.30±0.041 [a]	7.17±0.087 [b]	5.63±0.144 [c]
Albumin	4.56±0.144 [a]	3.80±0.185 [b]	3.33±0.174 [c]
Globulin	2.77±0.188 [a]	2.17±0.777 [b]	1.85±0.083 [c]
AST	124.14±3.961 [a]	118.67±1.967 [a]	125.82±3.831 [a]
ALT	49.48±2.399 [a]	46.30±0.870 [a]	43.56±0.527 [a]

- Superscripts are to be read column wise for mean comparison.
- Mean with similar superscripts in column do not differ significantly ($P < 0.05$).

examination of kidney revealed shrinkage and atrophy of glomeruli in Group B whereas Group C revealed tubular degeneration with hemorrhage and oedema. Microscopic examination of lung revealed Pulmonary congestion in Group B whereas Group C revealed interalvolar moderate haemorrhage, severe pulmonary oedema.

DISCUSSION

In meloxicam treated animals, weakness and decrease in body weight gain occur due to direct or indirect effect of on gastrointestinal tract resulting in decreased appetite and absorption (Taiwo *et al., 2008*). Similar clinical signs observed in monkey treated with lornoxicam (Atzpodien *et al., 1997*) and rats treated with meloxicam (Bhadja, 2007).

Decrease in Hb, PCV, TEC values as observed in present study may be due to the effect of meloxicam which causes injury to haematopoietic stem cells thus decreasing blood cells in rats by inhibiting the bone marrow activity (Merchant, 2004). Similar finding were reported in dog administered with NSAIDS, Meloxicam, Loxoproxen sodium (Sharma *et al.,* 2002; Marilac *et al.,* 2003; Peter *et al.,* 2003), mice administered with aspirin (Merchant *et al.,* 2004) and monkey treated with lornoxicam (Atzpodien *et al.,* 1997). The decrease in MCH and MCHC in rats is indicative of anemia (Merchant *et al.,* 2004). Similar result observed in rats treated with meloxicam (Bhadja, 2007) and mice treated with aspirin (Merchant *et al.,* 2004). Significant increase in TLC values may be due to enhanced bone marrow effect of meloxicam on leucopoiesis (Merchant, 2004). Similar finding were observed in dog administered with NSAID (Sharma *et al.,* 2002) and buffalo calves treated with Meloxicam (Magarwadiya *et al.,* 2002).

The increase in alkaline phosphatase might be due hepatotoxic effect of meloxicam (Abatan *et al.,* 2006). Similar values were also reported in dogs treated with nimesulide (Ramesh *et al.,* 2001). The decrease total protein values could be attributed to the disruption of lysosomal membranes under the effect of NSAID toxicity leading to the liberation of their hydrolytic enzymes in the cytoplasm resulting in marked lysis and dissolution of the target material (Ebaid *et al.,* 2007). Similar value also reported in monkey treated with lornoxicam (Atzpodien *et al.,* 1997), rats treated with meloxicam (Bhadja, 2007), mice treated with piroxicam (Ebaid *et al.,* 2007).

Ulcer and haemorrhage seen in stomach may be due to the inhibitory effect of meloxicam on synthesis of prostaglandins, which have cytoprotective effects on gastric mucosa (Taiwo *et al.,* 2008). Meloxicam has been shown to uncouple oxidative phosphorylation at supratherapeutic concentrations and depresses the biosynthesis of mucopolysaccharides. Mucopolysaccharides are secreted into intestinal mucosae by goblet cells where they act as surface protective substances against the very alkaline nature of the intestines. Lack of these substances will expose these mucosae to alkali leading to necrosis, erosions and ulceration (Taiwo *et*

al., 2008). The lesion observed in stomach and intestine were in conformity with in monkeys treated with lornoxicam (Atzpodien *et al.,* 1997), dog treated with nimesulide (Ramesh *et al.,* 2001), in buffalo calves treated with meloxicam (Marilac *et al.,* 2003) and rats treated with meloxicam (Bhadja, 2007).

Liver function which interferes with the secretion of plasma proteins. This leads to decreased blood osmotic pressure, with subsequent decreased drainage of tissue fluids, which explains the congestion observed. (Ebaid *et al.,* 2007). Similar finding had also been reported in dogs treated with nimesulide (Ramesh *et al.,* 2001) and mice treated with piroxicam (Ebaid *et al.,* 2007).

CONCLUSION

Stomach and intestines were found as the most affected target organs of meloxicam intoxication in rats. Meloxicam produce the serious gastrointestinal toxicity, such as inflammation, bleeding and ulceration can occur at any time, with or without warning symptoms in rats.

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