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A Comparative Patho-Physiological Study of Diclofenac and Meloxicam Induced Toxicity In *Gallus Domestics*

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ABSTRACT

The present study was conducted to evaluate comparative toxicity of two widely used non-steroidal anti-inflammatory drugs (NSAIDs) i.e. Diclofenac and meloxicam in *Gallus domesticus*. Diclofenac is claimed to be a major responsible cause of vulture population declination and considered as most devastating environmental toxicant. Today, it is replaced by meloxicam which is believed to be a safer drug than diclofenac. The whole experiment was divided in three comparative groups consisting of seven adult healthy broilers in each group. After the completion of experiments, the animals were autopsied as per standard protocols and blood was collected directly from cardiac puncture whereas vital organs were fixed in formalin for histopathological investigations. The results of serum biochemistry, hematology and histo-pathology revealed significant alterations in comparison to vehicle control. The levels of SGOT and SGPT were significantly ($P \leq 0.001$) increased by diclofenac treatment as compared to meloxicam. The levels of uric acid, creatinine, alkaline phosphatase, bilirubin, albumin, globulin and total proteins were indicated abnormalities in renal and hepatic functions in the diclofenac treated birds. Histopathology of the renal and hepatic tissues showed different degrees of degeneration like pyknosis, apoptosis and necrosis by diclofenac treatment as well as meloxicam when compared with vehicle control. Although the hematology parameters were not altered significantly. Therefore, the results of pathophysiology and biochemistry indicate that meloxicam shows less toxicity in comparison to diclofenac at same dose and duration in the experimental model *Gallus domesticus*.

Keywords: Diclofenac, meloxicam, Pathophysiology, Toxicity, Environmental Pollutant, Vulture.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAID) i.e. diclofenac emerges as a threat for vulture population. It came as a burning issue in 2004 when diclofenac use was linked with vulture population declination and meloxicam was considered as a safe alternative [1,2] Oaks and other researchers (2004) claimed that diclofenac residue is the main cause of decline in vulture population. Even some researchers also proposed molecular hypothesis of diclofenac poisoning [1,3]. Although, this drug has an inhibitory effect on prostaglandin synthesis which is used as an initial therapy for inflammatory and degenerative rheumatic diseases as well as pain conditions such as musculoskeletal, post-operative pains, acute occurrences of gout and ureteric colic [3,4]. Whereas, the global consumption of diclofenac is estimated at 940 tons per year in the form of capsules, suppositories, tablets, intravenous drugs and ointments [4,5]**.** But lately, meloxicam came as a new potent non-steroidal anti-inflammatory drug (NSAID), which shown in animal tests as a potent antiarthritic accomplishment and has a broader spectrum of anti-inflammatory action than presently existing NSAIDs (Lehmann et al.,1996). Toxicological testing of meloxicam in animals proposes that acute oral over dosage is questionable to cause severe toxicity in human being. When compared with other NSAIDs, meloxicam has a comparatively non-significant effect on gastric acid secretion and on ulceration in the rat stomach. However utmost NSAIDs can cause parenchymal renal impairment in animals at low plasma levels and over relatively little periods, whereas, meloxicam only induces such injury in the rat over the longer term. The mechanism of action of meloxicam following differently as inhibits cyclooxygenase-2 (COX-2), rather than cyclooxygenase-1 (COX-l), which may elucidate its good gastric and renal tolerability (Lehmann et al.,1996; Swarup et al., 2007). In this context, Jodhpur and western Rajasthan is considered as safe and potential habitat for vultures and avian fauna. But, this region is not safe from diclofenac and other NSAIDs contamination in the environment due to mismanagement of municipal discharge, hospital discharges and unauthenticated veterinary uses of diclofenac. Therefore, this study designed to evaluate toxicity of diclofenac in comparison to meloxicam at low dose regime in domestic poultry breed animal model as it is phylogenically near to vulture.

MATERIALS AND METHOD

Experimental animals

Six week broilers (*Gallus domesticus*) were equally divided into three groups with consisting of seven birds to each. Diet and water supply was made as per recommendations of poultry keeping

guidelines. (Reg. no: 1646/GO/ERe/S/12/CPCSEA).

Experimental Design

Adult and healthy animals were randomly divided into three groups consisting of seven broilers to each group. Experiments were carried out in duplicates. Diclofenac and meloxicam were administered intra-muscular for a period of 30 days.

- Group 1: Vehicle control received distilled water.
- Group 2: Diclofenac treatment (0.1 mg/kg BW) intramuscular for 30 days.
- Group 2: Meloxicam treatment (0.1 mg/kg BW) intramuscular for 30 days.

Serum Biochemistry

Serum Glucose

Serum glucose was estimated by using commercially available test kit by standard method. In this method, the enzyme peroxides catalyzes the oxidative coupling of 4-aminoantipyrine with phenol to yield a colored quinonemine complex, with absorbance proportional to the concentration of glucose in the sample [8].

Serum urea

Urea level was measured by using diacetyl monoxime standard method kit. The enzyme methodology working as urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The product of reaction measuring by the rate of decrease in absorbance at 340 nm as NADH is converted to NAD [9].

Serum creatinine

Serum creatinine level was considered by standard method test kit. The product of reaction considered by orange-yellow colour which is directly proportional to creatinine concentration and is shown photometrically at 490-510 nm [10].

Total Bilirubin:

Total bilirubin is calculated by indirect and direct measurement of bilirubin which is yielded by degradation of hemoglobin. Modified method of Pearlman & Lee in which a surfactant is used as a solubilizer was used. Bilirubin glucoronate reacts directly with sulphodiazonium salt and forms colored imitative azobilirubin [11].

Albumin and Globulin estimation:

Albumin and globulin were estimated by commercially available test kits. In this method, albumin binds with Bromo Cresol Green (BCG) at pH 4.2 producing a shift in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin,

when calculated photometrically between 540–630 nm with maximum absorbance at 625 nm. However globulin was measured by subtracting albumin from total protein (Ponder and Ponder, 1960; Zeng et al., 1995).

SGOT and SGPT

The measurement of SGOT and SGPT were carried out by using standard enzymatic method kits. The yield of reaction is monitored by determining the rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD (Reitman and Frankel, 1957).

Similarly, in case of SGPT test the SGPT present in the sample from alanine to the carbon atom of 2-oxoglutarate yields pyruvate and L-glutamate which was monitored by absorbance at 340 nm(Reitman and Frankel, 1957).

Alkaline Phosphatase

The alkaline phosphatase activities were measured by using standard method(Chauhan and Sharma, 2011).

Total Protein

Serum total protein was measured by following Lowry method by using commercial kits. This method following as the peptide bonds of protein react with copper II ions in alkaline solution to form a blue-violet coloured complex as each copper ion forms complex with 5 or 6 peptide bonds. The color formed is proportional to the protein concentration and is measured at 546 nm [17].

Hematology

Blood samples were collected by direct cardiac puncture and hematological assessments were performed. The measurements included RBC count, WBC count, platelet counts, HGB concentration and HCT by following the standard protocols (Merchant and Modi, 2004; Ramesh et al., 2001).

Histology:

The kidney and liver tissues were fixed in formalin and proceed for paraffin wax embedding after dehydration and clearing by xylene. The tissues sectioning were made at 5 µ and mounted on glass slide by DPX [21].

Statistical analysis:

All values of were expressed as mean ± standard error of mean (S.E.M.) and analyzed by one-way ANOVA.

RESULTS AND DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDs) are mainly inhibit to cyclo-oxygenase enzymes, which are involved in the formation of prostaglandins. But, there are obvious differences between drugs in their selective inhibition of the two subtypes of cyclo-oxygenase i.e. COX-1 and COX-2 [1,3,22]. It is the latter being involved with the modulation of inflammation-mediated responses and pain, whereas the earlier modulates blood flow to the kidneys. The nitrogenous metabolites filtration in living being by kidney categories the toxicity of NSAIDs and further pathogenicity of toxicants [23]. In this study, results of diclofenac and meloxicam treatment shown different levels of toxicities as assessed by results of body and organs weights, serum biochemistry of hepatic and renal functions parameters, hematology and histopathology.

The overall body weight of the chicks was not significantly altered in treatment of diclofenac and meloxicam for 30 days as compared to counted. Whereas, groups 2 and 3 exhibited a significant increase in the weights of liver and kidney. (Table.1). A significant increase in weights of the liver and kidney were occurred in diclofenac treated chicks corresponded with the swelling and increased size of these organs. Similar kind of gross abrasions have been reported in other avian species suffering from diclofenac toxicity[24,25]. However, an inconsistent change in the relative weight of the liver and a decreased relative weight of kidneys in meloxicam treatment did not correlate with the gross lesions.

Table 1: Effects on Body and organs weights of diclofenac and melaxicam toxicity in *Gallus domesticus*

Treatment groups	Body weight (gm)		heart	kidney	liver
	Initial	Final	gm/100gm BW		
Control (Gr. 1)	211.3 ±38.2	237±39.51	279.7±7.4	315.5±16.2	515±25.3
30 days treatment of diclofenac (Gr. 2)	283 ±20.12	300 $^{c, g}$±29.12	226 $^{d, h}$ ±5.76	363$^{d, h}$ ±11.12	757$^{c, g}$ ±17.36
30 days treatment of meloxicam group (Gr. 3)	280 ±22.13	305$^{b, h}$ ±31.67	236 $^{d, h}$ ±6.12	329 $^{d, h}$ ±12.63	603$^{c, g}$ ±20.12

Data are means ± S.E.M. (??=7) and c, ?? ≤ 0.001 for group 2 as compared to the respective control values and g, ?? ≤ 0.001 value for treatment group 3 compared to the respective values of the control.

The results of serum biochemistry of toxicity profiles exposed different spectra of toxicity levels. Toxicity profile of the treated birds (creatinine, urea, uric acid, SGOT, SGPT, bilirubin, albumin, globulin, albumin/globulin ratio, total protein and glucose) was shown significant alterations in groups 2 and 3 by treatment of diclofenac and meloxicam. Creatinine, albumin/globulin ratio and

uric acid were significantly ($P \leq 0.001$) increased in group 2 as compared to group 3 and group 1. Concurrently, SGOT and SGPT were also altered significantly ($P \leq 0.001$) in treated groups 2 when compared with control group 3 and group 1 (Figure A and B). Simultaneously, serum biochemistry revealed abnormal renal and hepatic function parameters due to toxicity of diclofenac and meloxicam. This may be indication of possible hepatocellular damage due to toxic effects exerted by diclofenac and meloxicam. Accordingly, total proteins, bilirubin, alkaline phosphatase and albumin - globulin ratio were also abnormally altered. This kind of hepato-toxicity of diclofenac and meloxicam was also reported by other researchers explained though drugs interactions and basic approaches[26, 27]. The treatments of diclofenac and meloxicam significantly altered renal function parameters as illustrated by several studies [1,22,28]. Consequently, the uric acid and creatinine levels were also show abnormalities of excretion and renal function. The renal functions and excretion abnormalities connected with impact of toxicants directly and indirectly up to cellular levels [28].

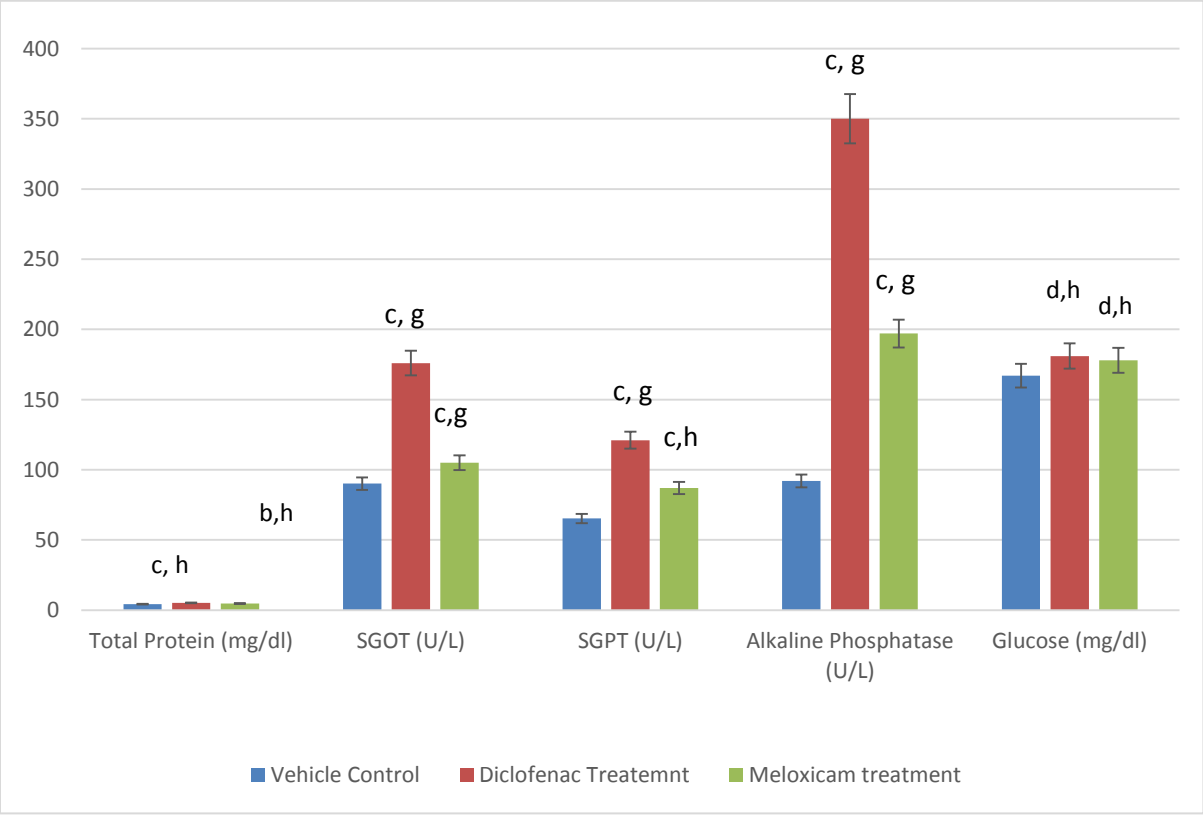

Figure A: Effects on Serum biochemistry of diclofenac and meloxicam toxicity in *Gallus domesticus.*

Data are means ±S.E.M. (n=7), c, P ≤ 0.001, g, P ≤ 0.001for group 2 as compared to the respective control values and g, P ≤ 0.001 value for treatment group 3 compared to the respective values of the control.

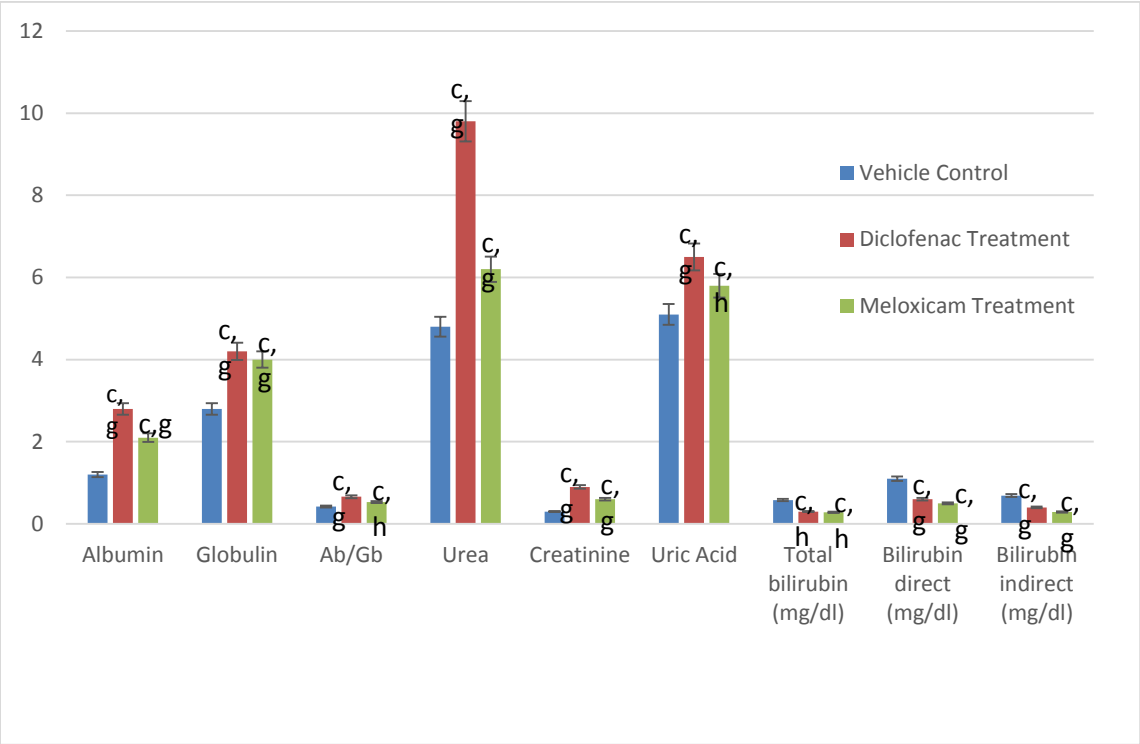

Figure B: Effects on Serum biochemistry of diclofenac and meloxicam toxicity in *Gallus domesticus.*

Data are means ±S.E.M. (n=7), c, P ≤ 0.001, g, P ≤ 0.001for group 2 as compared to the respective control values and g, P ≤ 0.001 value for treatment group 3 compared to the respective values of the control.

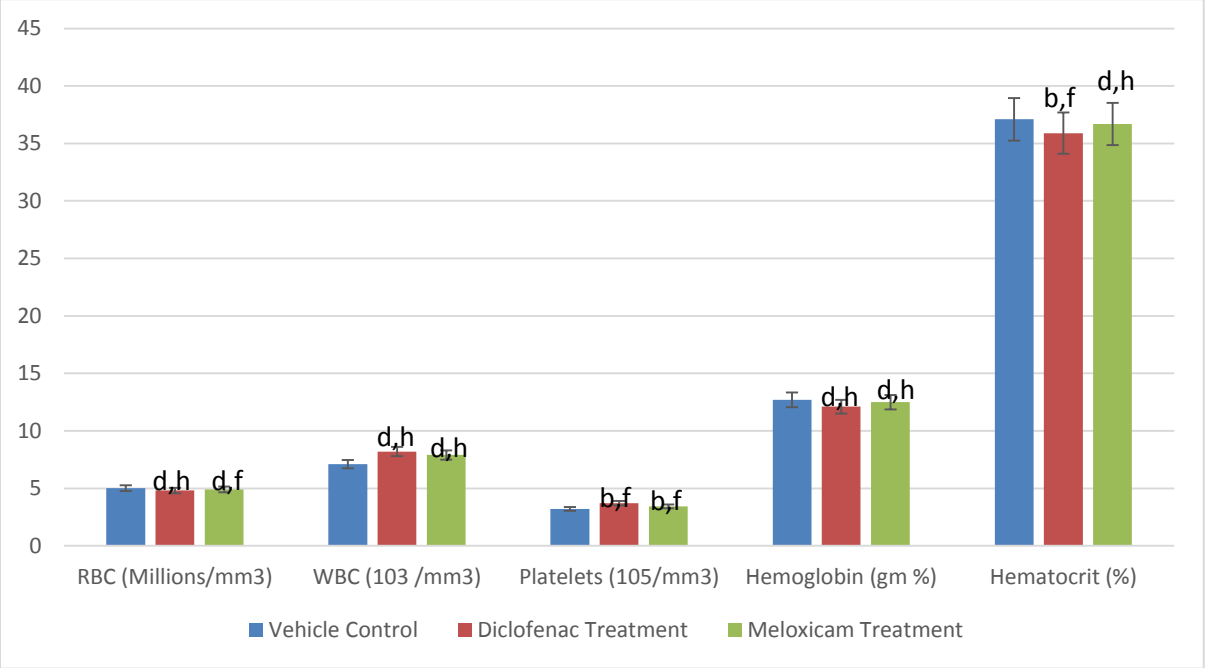

Figure C: Hematology of diclofenac and meloxicam treatments in *Gallus domesticus.*

Data are means ±S.E.M. (n=7), c, $P \leq 0.001$, g, $P \leq 0.001$for group 2 as compared to the respective control values and g, $P \leq 0.001$ value for treatment group 3 compared to the respective values of the control.

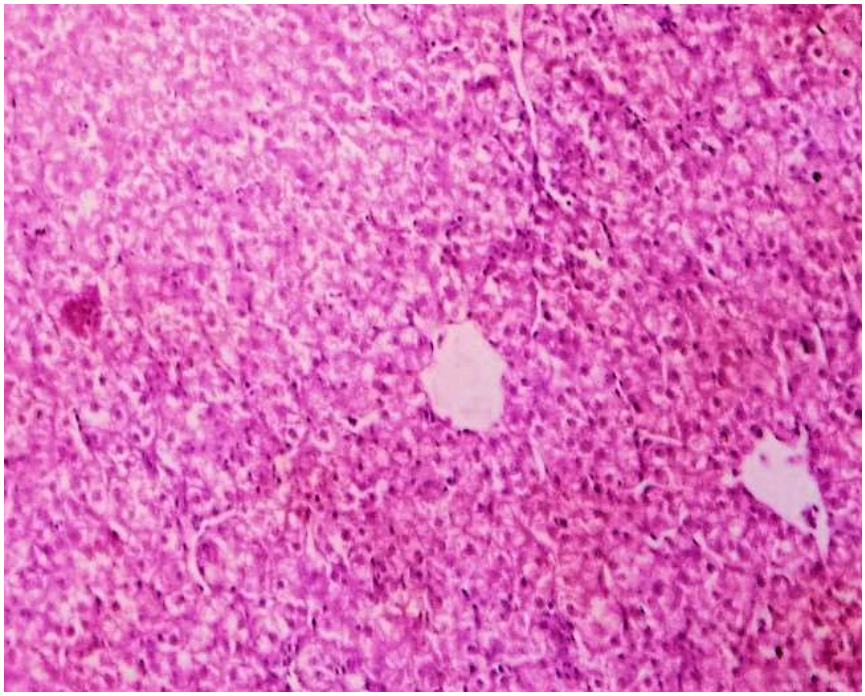

Figure 1: Vehicle control liver histo-architecture (100x, H & E) - The histo-architecture showing a proper composition of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in

trabeculae consecutively radiant from the central vein and are separated by sinusoids containing Kupffer's cells.

Hematological assessments shown different kind of alterations. The treatment of diclofenac and meloxicam caused non-significant changes in hematology ($P \leq 0.5$) in comparison to group 1. Whereas comparison between parameters of group 1 and 2 shown son significant changes with non-functional alterations (Figure C). Although hematological parameters were not altered significantly it indicates less interferences of toxicities at hematopoietic tissues [29].

Subsequently, the histopathology of kidney, liver, heart and small intestine shown functional and structural alterations in histo-architectures by treatments of diclofenac and meloxicam. Histopathology of hepatic tissue of vehicle control exhibits proper shape of hepatocytes along with blood vessels and bile ducts (Figure 1). In the liver tissue of groups no. 3 and 4, higher degree of degeneration and necrosis were observed by diclofenac treatment in comparison to meloxicam. Additionally, cellular morphometric abnormalities were also seen in the diclofenac treatment group (Figure 2 and 3). In parallel, the kidney of vehicle control exhibits glomerulus with Bowman's capsule as normal structure of proximal and distal convoluted tubules (PCT and DCT) (Figure 4). The treatment of diclofenac and meloxicam show different degrees of degenerations like pyknosis, necrosis and cellular disarrangements. The shrinkages with cytoplasmic abnormalities in PCT and DCT were also observed in different manner in groups 2 and 3 (Figure 5 and 6).

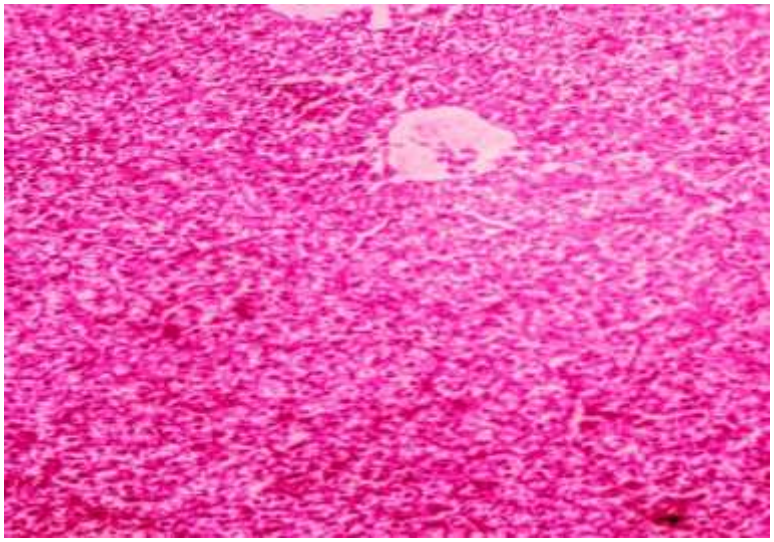

Figure 2: Histo-architecture of diclofenac treated liver (H&E, 100X) - The trabecular structure of the liver is distorted. The hepatocyte cytoplasm is light, foamy and filled with vacuoles; cell sizes are enlarged, nuclear chromatin is more compact, slightly smaller nucleoli are not conspicuous. Necrosis of hepatocytes-nuclei are constricted and pycnotic with

abbreviated chromatin. Accumulation of mononuclear cells in the vicinity of sinusoids. The sinusoid walls exhibit numerous Kupffer's cells.

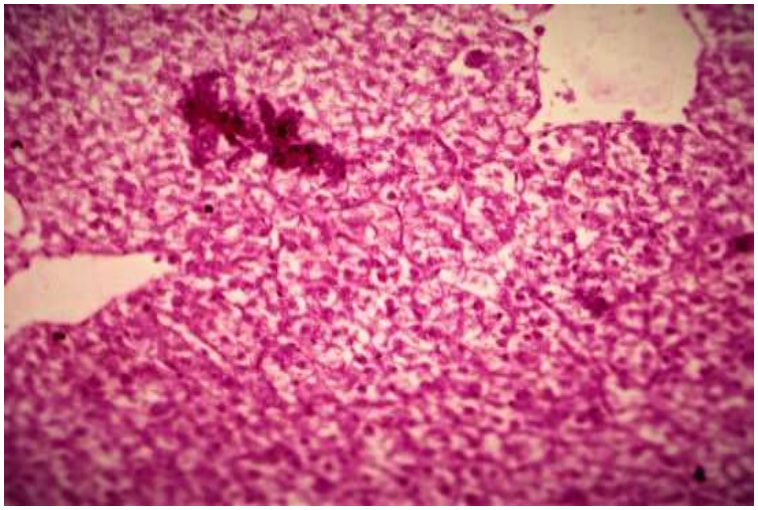

Figure 3: Meloxicam treated liver (100X, H& E) - The trabecular structure of the lobules having some degrees of distortion. The cytoplasm of some hepatocytes having some degrees of enlargement, light, with vacuoles. The hepatocytes performing the structure of nuclei having some degrees of abnormalities up to pyknosis. Additionally, some number of erythrocytes are observed in the lobular sinusoid lumen.

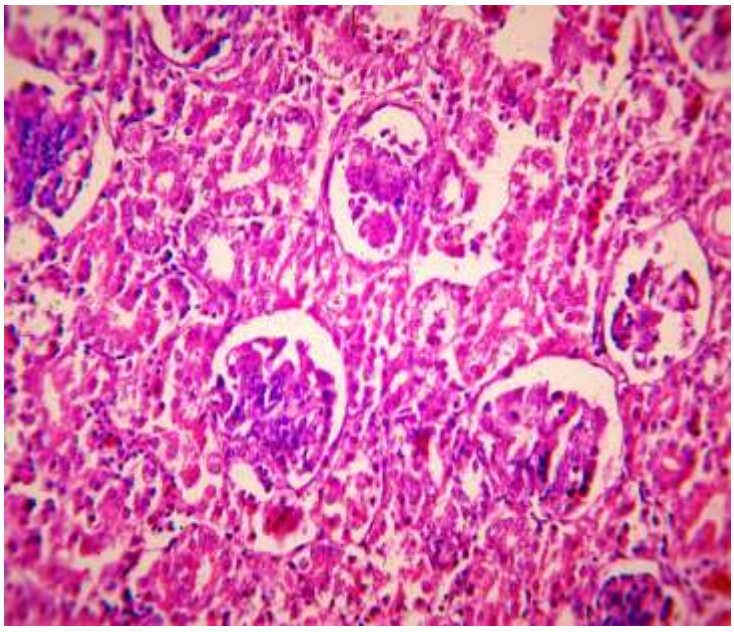

Figure 4: Histo-architecture of vehicle control kidney (100X, H & E) - Renal glomeruli showing normal structure with proper arrangement of Bauman's capsular tissue. The renal tubules are lined with typical thick cubic epithelium. Tubules having a regular occurrence of distinct lumen. The organization of lobule having proper arrangement of the glomerule and a flat epithelium lining the glomerular capsule can be seen.

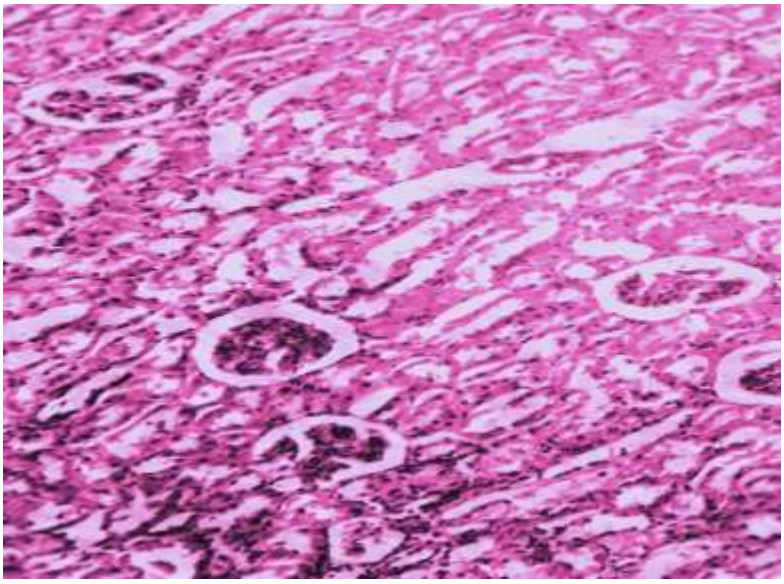

Figure 5: Diclofenac treated kidney (100x, H & E) - Glomeruli showing distortion with abnormal filling of the Bowmann's capsule. Nuclei of Some cells performing some degrees of degenerations up to pycnosis and cytoplasmic abnormalities. Capillaries are filled with blood cells; some tubules contain single desquamated cells. PCT (Proximal convoluted tubules) and DCT (Distal convoluted tubules) also performing hypertrophy.

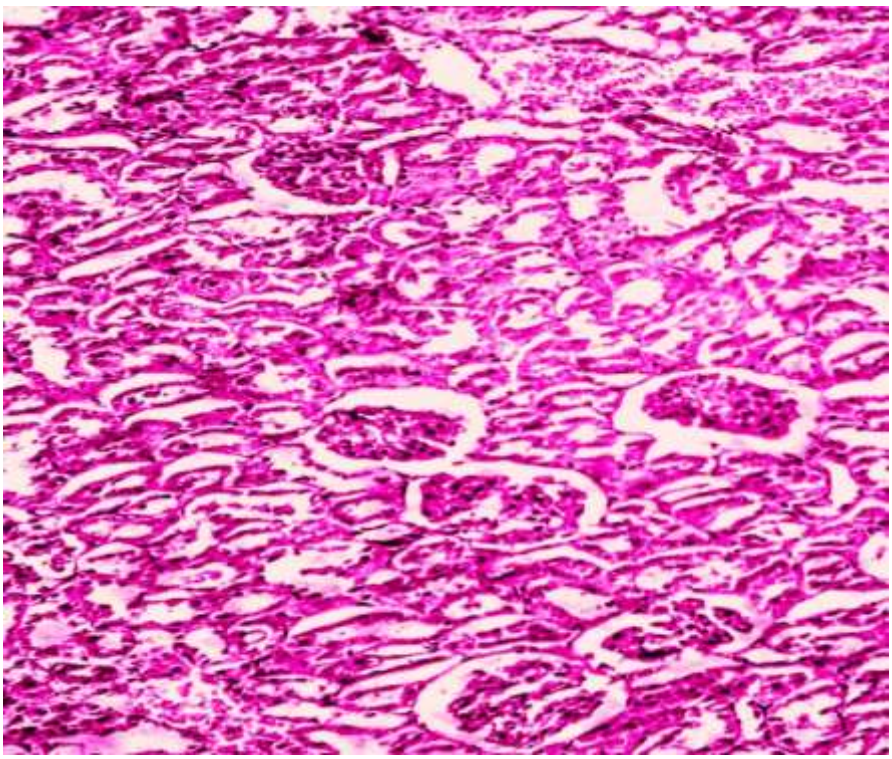

Figure 6: Meloxicam treated kidney (100x, H&E) - Glomeruli showing some degrees of disarrangements and fragmentations. The pycnotic level degree of degeneration also showing by nuclei. Vasculation pattern of capillaries having disarrangements and some tubules contain

desquamated cells. Some levels of hypertrophy performing by PCT (Proximal convoluted tubules) and DCT.

Both of the treatments of diclofenac and meloxicam both caused abnormal histo-architectures in renal and hepatic tissues but varying in degrees of degenerations. Whereas, the treatment of diclofenac caused severe alteration in histology of kidney and liver as compared to meloxicam. Supportively, results of histopathology of renal and hepatic tissues indicate different degrees of necrosis, pyknosis and apoptosis through treatments of diclofenac and meloxicam. These kinds of degenerative changes in renal and hepatic tissues governed at both levels of cytoplasmic as well as nucleoplasmic interferences of toxicants [30–32].

CONCLUSION

It is concluded that diclofenac shows more toxicity in chick animal model in comparison to meloxicam on same dose and duration. This is also supported and revealed by results of histopathology of renal and hepatic tissues, abnormal serum biochemistry, body and organs weights and hematology. Therefore, it can be illustrated that meloxicam is safer to use than diclofenac.

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