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Hepato-renal and Hematological Effect of Diclofenac in Sheep

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Abstract

Diclofenac is a non-steriodal anti-inflammatory drug that is used as an antipyretic and analgesic agent in various animal species. This study aimed to assess the side effects of diclofenac on the liver, kidney, and blood components. The study was conducted on six local breed sheep, whose blood was collected from the jugular vein before treatment (pre-treatment group). Diclofenac was administered intramuscularly at a dose of 2 mg/kg body weight for three consecutive days. Twenty-four hour post last dose blood was collected and considered as a post-treatment group. Biochemical and blood analyses were performed. Biochemical parameters showed a significant increase in the serum levels of alanine aminotransferase, aspartate aminotransferase, and creatinine, with a significant decrease in sodium and potassium ion concentrations. Blood parameters result showed a significant decrease in mean corpuscular volume, with a significant increase in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total number of white blood cells. The study concluded that diclofenac within the dose and treatment period had a negative effect on the liver and kidney functions and blood components of sheep.

Keywords:

Blood analysis, Diclofenac, Kidney, Liver, Sheep.

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Introduction

Diclofenac is a Phenyl acetic acid derivative that is a nonselective cyclooxygenase inhibitor, it is used in various animals by mouth or injection as an antipyretic, analgesic, and anti-inflammatory agent (Chikkamath, Hampannavar and Palkar, 2019). Diclofenac is rapidly absorbed, significantly binds to proteins, has a short excretion half-life, and is exposed to a first-pass effect; thus, approximately 50% is available in the body after oral administration and accumulates in the synovial fluid, which explains the length of its effect compared to its concentration in blood plasma (Moore, 2007). Despite its beneficial effects, numerous studies have indicated that diclofenac has harmful effects on the liver and kidneys (Orinya, Adenkola and Ogbe, 2016). Diclofenac may induce hepatotoxicity and may be related to the formation of drug metabolites and, thus, hepatitis in some individuals (Aithal, 2004). The aim of this study was to evaluate the effects of diclofenac on the liver, kidneys, and blood profile of sheep within the dose and treatment period.

Material and methods

Ethical approval

The animals were handled as directed by the College of Veterinary Medicine's animal ethics committee. The study's protocol (no.977) was evaluated and approved by the Scientific Committee of the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, University of Mosul.

Experimental animals and Experimental protocol:

Six clinically healthy, male domestic sheep were used in this study. Animals were 10-11months old and weighed between 33-44 kg at the beginning of the experiment. The experiment was conducted in the farm of one of the breeders in Kokjali district, Mosul, Iraq. After weighing with a digital scale, the diclofenac sodium (Diclovap, 25 mg/1 mL, Vabco, Jordan) dose was calculated for each animal at the rate of 2 mg/kg of body weight. The calculated dose was administered by intramuscular (i.m.) injection once daily for three consecutive days.

Before treatment, five ml blood samples were withdrawn from the jugular vein after site sterilization with Ethyl alcohol 70% (Control group) using plastic syrings. Each 5 ml sample was further subdivided into to 2 parts each of 2,5ml, the first part was collected in clean dry sterile test tube without anticoagulant to separate blood serum for serobiochemical analysis. The second part was collected in clean dry sterile test tube with EDTA anticoagulant for hematological analysis. Immediately after completing the blood drawing from sheep, diclofenac at a dose of 2 mg/kg body weight was injected intramuscularly, and the experiment continued for three consecutive days as a single dose per day. Twenty-four hours post last diclofenac injection, blood samples were collected and divided into 2 parts as previously described (Post treatment group).

Biochemical and blood tests were performed on the same day of blood drawn from both groups, the blood serum was separated in a centrifuge and after separation the blood serum was transferred to plastic tubes and biochemical tests were performed for the parameters (ALT, AST, Creatinine, Urea, Sodium., Potassium and Chloride) using a Dri-ChemNX500 auto analyzer (Fujifilm Corporation, Japan).Whole blood was used to measure blood parameters; red blood cells (RBCs) count, Hemoglobin (HGB) concentration, Hematocrit (HCT), mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), white blood cell (WBCs) count, and total platelet

count) using an Orphee Mythic 18 Hematology Analyzer (Orphee Medical Corporation, South Australia).

Statistical analyses

The results were analyzed statistically using (SPSS Version 17.0) and a Paired-Samples *t* test. The level of significant difference was at (*p*) probability level less than 0.05 ($p < 0.05$).

Results

Table (1) shows the results of the biochemical parameters in sheep pre and post treatment with diclofenac at a dose (2 mg/kg, intramuscularly), there were a significant increase in the activity of ALT and AST observed in the serum in the post-treatment group, (29.83±1.70 and 165.83±13.92) units/liter compared to the pre-treatment group (18.33±0.55 and 89.83±8.57) units/liter, respectively, There was also a significant increase in serum creatinine concentration in the post-treatment group (55.33 ± 2.72) µmol/L compared to the pre-treatment group (44.67 ±2.49) µmol/L, as well as a significant decrease in the concentration of sodium and potassium ions in the serum of the post-treatment group (145.83 ± 0.70 and 3.88 ± 0.16) mEq/L compared with the pre-treatment group (147.33 ±0.49 and 4.58 ± 0.19) mEq/L, respectively. There was no significant difference in the concentration of urea and chloride ion in the post-treatment group compared to the pre-treatment group.

Table (2) shows the results of the hematological parameters. A non-significant decrease in the total number of red blood cells, hemoglobin, and the packed cell volume was observed in the post-treatment group compared to the pre-treatment group, and a significant decrease in the mean corpuscular volume was observed in the post-treatment group (27.11 ± 0.29) μm^2 compared with the pre-treatment group (27.38 ± 0.27) μm^2, A significant increase in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was observed in the post-treatment group (11.41 ± 0.25 pg and 42.13 ± 1.25 g/dL) compared with the pre-treatment group (11.13 ± 0.30 pg and 40.63 ± 1.32 pg/dL), respectively. And a significant increase in the total number of white blood cells was observed in the post-treatment group (6.82 ± 0.66) 10^9 / liter compared with the pre-treatment group (5.73 ± 0.84) 10^9 / liter, and an insignificant decrease was noted in the total number of platelets in the post-treatment group compared with the pre-treatment group.

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Table 1: Biochemical parameters in in sheep pre-and post-treatment with diclofenac (2 mg/kg, i. m.) for three consecutive days.

Parameters	Pre-treatment	Post-treatment	Change %
ALT (U/L)	18.33±0.55	29.83±1.70*	+62.73
AST (U/L)	89.83±8.57	165.83±13.92*	+84.60
Creatinine (Umol/L)	44.67±2.49	55.33±2.72*	+23.86
Urea (Mmol/L)	5.20±0.66	4.12±0.16	–20.76
Sodium ions (Meq/L)	147.33±0.49	145.83±0.70*	–1.01
Potassium ions (Meq/L)	4.58±0.19	3.88±0.16*	–15.28
Chloride ions (Meq/L)	101.51±0.95	101.83±0.83	+0.31

* Significantly different in compare with Pre-treatment value, P<0.05.

Table 2: Hematological parameters in sheep pre-and post-treatment with diclofenac (2 mg/kg, i. m.) for three consecutive days.

Parameters	Pre-treatment	Post-treatment	Change %
Red blood cells (10^{12}/L)	9.69±0.57	9.41±0.47	–2.88
Hemoglobin conc. (g/dl)	10.73±0.51	10.71±0.47	–0.18
Hematocrit (%)	26.60±1.66	25.56±1.40	–3.90
Mean corpuscular vol. (Um^3)	27.38±0.27	27.11±0.29*	–0.98
Mean corpuscular Hemoglobin(pg)	11.13±0.30	11.41±0.25*	+2.51
Mean corpuscular Hemoglobin Conc.(g/dl)	40.63±1.32	42.13±1.25*	+3.69
White blood cells (10^9/L)	5.73±0.84	6.82±0.66*	+19.02
Platelet(10^9/L)	347.83±34.75	385.66±32.94	+10.87

* Significantly different in compare with Pre-treatment value, P<0.05.

Discussion

In this study, diclofenac significantly increased the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum, which is an indicator of liver damage. This result is consistent with several studies conducted in rams (Er et al., 2013), goats (Ahmad et al., 2012), rats (EO et al., 2018), mice (Mohan and Sharma, 2017) and broiler chickens (Ramzan et al., 2015).

Aspartate aminotransferase (AST), also known as Glutamic Oxaloacetic Transaminase (GOT), is present in the liver, heart, muscles, brain, and kidneys and is therefore considered an indicator of general toxicity (Dufour et al., 2000). Alanine aminotransferase (ALT), also known as (Glutamic Pyruvic Transaminase) (GPT), is mainly found in the liver and is thus considered an indicator of hepatotoxicity (Williamson, Okpako and Evans, 1996). AST and ALT are present in the liver cells. These enzymes are intracellular and are located in the mitochondria, cytoplasm, or both. When cell function is altered or damaged, the enzyme is attracted to the blood (O'connor, Dargan and Jones, 2003).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been implicated in liver damage and diclofenac is usually associated with hepatotoxicity (Unzueta and Vargas, 2013). Liver injury was observed in mice treated with diclofenac, which is characterized by cellular degeneration, necrosis, and vasodilatation, with congestion and accumulation of mixed inflammatory cells in the area of dead hepatocytes (Lar, Patel and Pandanaboina, 2016). Oxidative stress plays an important role in pathophysiology and, thus, damage to body tissues, and it has been shown that diclofenac causes oxidative stress in the liver (Islas-Flores et al., 2013). Bioactivation of diclofenac intermediate metabolites 4-OH and 5-OH occurs after metabolism by CYP2C9 and CYP3A4 enzymes (Lim et al., 2006). It has the potential to induce apoptosis in the liver (Gómez-Lechón et al., 2003). Diclofenac-induced hepatocyte injury causes membrane damage to lysosomes, resulting in the release of proteolytic enzymes, and cathepsins (B, D, L) induce mitochondrial permeability transition (MPT) pore opening, release of cytochrome C, and initiation of caspase-3-activating events. Therefore, programmed cell death occurs (Pourahmad et al., 2011).

Literature indicates that diclofenac has adverse effects on the kidneys, ranging from impaired renal function to renal failure. This is what the researcher Aydin and his group indicated that diclofenac caused kidney damage in a dose-dependent manner, and this effect was observed in rats (Aydin et al., 2003). The kidneys are highly active in the synthesis and metabolism of prostaglandins, which play an important role in many vital processes, including the autoregulation of renal blood flow, glomerular filtration, control of renin release, and transport of ions and water in the renal tubules. Unsurprisingly, inhibition of prostaglandin synthesis disturbs the physiological processes of the kidneys, leading to renal impairment caused by diclofenac (Yasmeen, Qureshi and Perveen, 2007).

NSAIDs inhibit the synthesis of prostaglandins by inhibiting cyclooxygenase (COX). There are two homologues of this enzyme: COX-1 and COX-2. COX-1 controls the glomerular filtration rate (GFR) and renal hemodynamics, while COX-2 controls the excretion of salts and water (Weir, 2002). Creatinine and urea levels are indicators of kidney function, but the creatinine test is more sensitive than urea (Vasudevan and Sreekumari, 2007). Based on the results of the study, a significant increase in serum creatinine concentration was observed. Creatinine is excreted in the urine after filtration by the renal glomeruli. It is not excreted or absorbed by the renal tubules to any degree and can be used as an indicator of glomerular filtration rate (Sahu et al., 2009). Any defect in the glomerular filtration rate (GFR) leads to an increase in serum creatinine levels (Talwar and Srivastava, 2002). the increase in creatinine concentration may be attributed to the inhibition of the enzyme COX-1, and thus the decrease in the synthesis of prostaglandins, which leads to a decrease in the glomerular filtration rate and blood perfusion to the kidneys, and then a decrease in the excretion of creatinine, which is reflected in the increase in its concentration in the serum.

There was an insignificant decrease in the concentration of urea in the serum, and we can attribute this decrease to the decrease in the protein content as a result of the animals' lack of appetite after treatment with diclofenac, as well as the liver damage observed in the study, which may affect the synthesis of urea. In addition to disturbances in kidney function, it may also affect the excretion and reabsorption of urea (Bolat and Selcuk, 2013).

From the results of the study, we noticed a significant decrease in the concentration of sodium and potassium ions in the serum. This can be attributed to a disturbance in the regulatory functions of the kidneys, which leads to a decrease in the levels of these ions, which we referred to earlier because of the mechanism of action of diclofenac, which inhibits prostaglandin synthesis.

The results of our study showed a non-significant decrease in the total number of red blood cells, hemoglobin concentration, and packed cell volume. Gomaa indicated that administration of diclofenac to rats at a dose of (14.8 mg / kg) caused a decrease in the number of red blood cells, concentration of hemoglobin (Hb), and packed cell volume, with no effect of diclofenac at a dose (7.4 mg/kg), indicating that the effect of diclofenac is dose-dependent (Gomaa, 2017). This supports our study that dose and treatment period have an important and influential role.

Several studies have indicated that diclofenac decreases the total number of red blood cells, hemoglobin concentration, and packed cell volume in rats (Orinya, Adenkola and Ogbe, 2016), goats (Ahmad et al., 2012), fish (Saravanan et al., 2011),

chickens (Akter and Sarker, 2015), and mice (Thanagari et al., 2012).Among the results of the study, a significant decrease in the mean corpuscular volume and a significant increase in the mean corpuscular Hemoglobin and Mean corpuscular hemoglobin concentrations were observed, indicating hyperchromic microcytic anemia. Hyperchromic microcytic anemia is found in various red blood cell disorders including immune hemolytic anemia (Ballas and Kocher, 1988).This may be attributed to the fact that diclofenac induces immune hemolysis as a result of the broad spectrum of diclofenac/RBC antibodies. The 4'-OH- diclofenac metabolite plays a role in stimulating the immune system. The serum contains a mixture of antibodies that recognize many epitopes. These epitopes consist of different drug metabolites and target proteins on the surface of blood cells. Therefore, diclofenac is a neoantigen of erythrocytes that may stimulate antibody production and drug-dependent antibodies (Sachs et al., 2004).We attribute the presence of hyperchromic microcytic cells to defects in kidney function and the disturbance observed in our study by increasing the excretion of potassium and sodium, thus increasing the excretion of water. This leads to an imbalance in the osmotic balance and the escape of potassium and water from red blood cells, which leads to a small size and hemoglobin concentration.

We noticed a significant increase in the total number of white blood cells, and this result is consistent with (Qureshi et al., 2013) who noted that in goats treated with diclofenac at a dose of (2.5 mg / kg) there was a significant increase in the number of white blood cells at 6, 12, and 24 h, and by 96 h, the values of white blood cells returned to the normal range. It is possible to explain the increase in the total number of white blood cells in the blood to the mechanism of action of diclofenac, as it inhibits the prostaglandin synthesis chain from arachidonic acid by inhibiting cyclooxygenase, which leads to the availability of the precursor substance arachidonic acid for lipoxygenase, leading to increased leukotriene production. As it is known that leukotriene are strong chemical attractants that stimulate the movement of white blood cells (Paino et al., 2005).

Conclusion

This study concluded that diclofenac, within the dose and treatment period, has harmful effects on the liver, represented by an increase in the levels of alanine aminotransferase and aspartate aminotransferase, and that these effects are due to the oxidative stress of the drug on hepatocytes. In addition, its effect on the kidney was represented by an increase in the level of creatinine and a decrease in the level of sodium and potassium ions in the blood serum, which indicates a disorder in the regulatory function of the kidney. The effects of the drug on blood components were also manifested in microcytic hyperchromic anemia, which may be due to the possibility of the drug causing immune hemolysis.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Ahmad I, Qureshi TA, Khan FA, Mughal SAK, Sadique U, Shah Z, Khan

S, Hassan ZU (2012). Evaluation of biochemical effects of diclofenac sodium in goats', J Anim Plant Sci, 22(2):. 1–4.

Aithal GP (2004). Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity', Expert Opinion on Drug Safety. Taylor & Francis, 519–523.

Akter R and Sarker M (2015). 'Effect of Diclofenac Sodium in Broilers', Bangladesh Journal of Veterinary Medicine, 13(1): 19–24.https://doi.org/10.3329/bjvm.v13i1.23710.

Aydin G, Gökçimen A, Öncü M, Çiçek E, Karahan N and Gökalp O. (2003). Histopathologic Changes in Liver and Renal Tissues Induced by Different Doses of Diclofenac Sodium in Rats', Turkish Journal of Veterinary and Animal Sciences, 27(5): 1131–1140.

Ballas SK and Kocher W (1988). Erythrocytes in Hb SC disease are microcytic and hyperchromic', American Journal of Hematology, 28(1): 37–39.

Bolat D and Selcuk ML (2013). Evaluation Of Diclofenac Induced Acute Nephrotoxicity In Rats 291 Material and Methods', Revue Méd. Vét, 164(6): 290–294.

Chikkamath MK, Hampannavar GA and Palkar MB (2019). 'Design, synthesis, and biological evaluation of novel diclofenac analogs as promising anti-inflammatory agents', Indian Journal of Health Sciences and Biomedical Research (KLEU), 12(1): 35.

Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS and Seeff LB (2000). Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests, Clinical chemistry, 46(12): 2027–2049.

Eo O, Oa O, Oi O, Mab O. and Od, A. (2018). Antioxidant and Hepatoprotective Activities of Carica papaya (Papaw Leaf) and Loranthus bengwensis (Cocoa Mistletoes) against Diclofenac Induced Hepatotoxicity in Rats', International Journal of Life-Sciences Scientific Research, 4(5): 1974–1982. Available at: https://doi.org/10.21276/ijlssr.2018.4.5.3.

Er A, Dik B, Corum O and Cetin G (2013). 'Cardiac Safety of Diclofenac at a Single Dose in Ram', The Scientific World Journal, 2013, 1–4. Available at: https://doi.org/10.1155/2013/808731.

Gomaa S (2017). Immunomodulatory and hematological effects induced by diclofenac, ibuprofen or paracetamol toxicity in Swiss albino mice, European Journal of Biological Research, 7(4): 348–359.

Gómez-Lechón MJ, Ponsoda X, O'connor E, Donato T, Castell JV and Jover R (2003). 'Diclofenac induces apoptosis in hepatocytes by alteration of mitochondrial function and generation of ROS', Biochemical pharmacology, 66(11): 2155–2167.

Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, Colín-Cruz A, Neri-Cruz N, García-Medina S (2013). Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (Cyprinus

carpio), Ecotoxicology and environmental safety, 92: 32–38.

Lar S, Patel DS, Pandanaboina CS (2016). Hepato-Protective Effects of Blue-Green Alga Spirulina platensis on Diclofenac-Induced Liver Injury in Rats, Malaysian Journal of Nutrition, 22(2).

Lim MS, Lim PLK, Gupta R, Boelsterli UA (2006) Critical role of free cytosolic calcium, but not uncoupling, in mitochondrial permeability transition and cell death induced by diclofenac oxidative metabolites in immortalized human hepatocytes, Toxicology and applied pharmacology, 217(3): 322–331.

Mohan D and Sharma S. (2017) Biochemical Changes in the Liver of Mice after Exposure to Different Doses of Diclofenac Sodium, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 2(5).

Moore N (2007). Diclofenac potassium 12.5 mg tablets for mild to moderate pain and fever, Clinical drug investigation, 27(3): 163–195.

O'connor N, Dargan PI, Jones AL (2003). Hepatocellular damage from non-steroidal anti-inflammatory drugs, Qjm, 96(11): 787–791.

Orinya OA, Adenkola AY, Ogbe RJ (2016). Haematological and biochemical studies on the effect of diclofenac sodium on Wistar Rattus norvegicus, International Journal of Biological and Chemical Sciences, 10(5), pp. 2231–2242.

Paino IMM, Ximenes VF, da Fonseca LM, Kanegae MPP, Khalil NM, Brunetti IL (2005). Effect of therapeutic plasma concentrations of non-steroidal anti-inflammatory drugs on the production of reactive oxygen species by activated rat neutrophils, Brazilian Journal of Medical and Biological Research, 38(4): 543–551. https://doi.org/10.1590/S0100-879X2005000400007.

Pourahmad J, Mortada Y, Eskandari MR, Shahraki J (2011). Involvement of lysosomal labilisation and lysosomal/mitochondrial cross-talk in diclofenac induced hepatotoxicity, Iranian journal of pharmaceutical research: IJPR, 10(4): 877.

Qureshi TA, Sadique U, Khan SA, Ahmed S, Rehman ZU, Bahadar S, Mushtaq M (2013). Hematological effects of diclofenac sodium in goat, 23: 103–107.

Ramzan M, Ashraf M, Hashmi HA, Iqbal Z, Anjum AA (2015). Evaluation of diclofenac sodium toxicity at different concentrations in relation to time using broiler chicken model, Journal of Animal and Plant Sciences, 25(2): 357–365.

Sachs UJH, Santoso S, Röder L, Smart E, Bein G, Kroll H (2004). Diclofenac-induced antibodies against red blood cells are heterogeneous and recognize different epitopes, Transfusion, 44(8): 1226–1230.

Sahu U, Jain T, Roy S, Tiwari S, Ghosh R, Koley K, Vadlamudi V (2009). Diclofenac-induced biochemical and histopathological changes in white leghorn birds (Gallus

domesticus), Indian Journal of Pharmacology, 41(5): 237. https://doi.org/10.4103/0253-7613.58515.

Saravanan M, Karthika S, Malarvizhi A, Ramesh M (2011). Ecotoxicological impacts of clofibric acid and diclofenac in common carp (Cyprinus carpio) fingerlings: Hematological, biochemical, ionoregulatory and enzymological responses, Journal of Hazardous Materials, 195: 188–194. https://doi.org/10.1016/j.jhazmat.2011.08.029.

Talwar GP and Srivastava LM (2002). Textbook of biochemistry and human biology. PHI Learning Pvt. Ltd.

Thanagari BS, Fefar DT, Prajapati KS, Jivani BM, Thakor KB, Patel JH, Ghodasara DJ, Joshi BP and Undhad VV (2012). Haemato-biochemical alterations induced by diclofenac sodium toxicity in Swiss albino mice, Vet World, 5: 417–419.

Unzueta A and Vargas HE (2013). Nonsteroidal Anti-Inflammatory Drug–Induced Hepatoxicity', Clinics in liver disease, 17(4): 643–656.

Vasudevan DM and Sreekumari S (2007). Textbook of biochemistry for medicinal students 5th Edn, New Delhi: Jaypee Brothers Medical publishers (P) Ltd, 535.

Weir M (2002). Renal effects of nonselective NSAIDs and coxibs, Cleveland Clinic journal of medicine, 69(1): 53–58.

Williamson EM, Okpako DT, Evans FJ (1996). Selection, preparation and pharmacological evaluation of plant material. John Wiley & Sons.

Yasmeen T, Qureshi GS, Perveen S (2007). Adverse effects of Diclofenac sodium on renal parenchyma of adult albino rats, Journal of the Pakistan Medical Association, 57(7): 349–351.