Research in: Agricultural & Veterinary Sciences
Vol.8, No.3, 2024, 153-165
https://doi.org/10.62476/ravs83153

TOXICOLOGICAL IMPACT OF DICLOFENAC SODIUM: HEMATOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN MALE RATS

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Abstract. *Background:* Long-term administration of diclofenac sodium can lead to harmful effects on body tissues, causing cellular injuries that include changes in kidney function and contributing to the development of immune mechanisms against kidney tissues. This study aimed to evaluate the toxic effects of diclofenac sodium on hematological and biochemical parameters, as well as histopathological changes in the kidneys of experimental animals. *Methods:* Forty male Albino-Aster mice were divided into four groups: control and three groups administered diclofenac sodium orally at doses of 20.5, 45 and 56.25 mg/kg body weight for 20 days. Blood samples were collected for hematological and biochemical analyses and kidney tissues were examined histopathologically. *Results:* Diclofenac sodium administration caused a significant decrease in red blood cells, hemoglobin, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin concentration, as well as a significant increase in platelets and total white blood cells. Serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase were significantly elevated. Histopathological examination revealed congestion, inflammation, glomerular abnormalities, tubular necrosis and vascular changes in the kidney tissues, with the severity increasing with higher doses. *Conclusion:* Diclofenac sodium administration at different doses can cause significant alterations in hematological and biochemical parameters, as well as histopathological changes in the kidneys of male rats, indicating potential adverse effects on animal tissues. Caution is recommended in the clinical use of diclofenac sodium and the lowest effective dose and duration should be used to achieve the desired therapeutic effect.

Keywords: *Diclofenac sodium, hematological parameters, histopathological changes, kidney tissues.*

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Received: *13 August 2024;* ***Accepted:*** *18 October 2024;* ***Published:*** *9 December 2024.*

1. Introduction

Diclofenac (Voltaren) is a non-steroidal anti-inflammatory drug (NSAID) belonging to the acetic acid family, which includes etodolac, indomethacin, ketorolac, nabumetone, sulindac and tolmetin (Sharef *et al*., 2020; Abdel-Rahman & Abdel-Baky, 2021). Diclofenac is widely used in the treatment of musculoskeletal diseases due to its anti-inflammatory and analgesic properties (Sharef *et al*., 2020; Abdel-Rahman & Abdel-

How to cite (APA):
Mraisel, A.C., Wale, M.M. & Saeid, H.H. (2024). Toxicological impact of diclofenac sodium: Hematological, biochemical and histopathological changes in male rats. *Research in Agricultural & Veterinary Sciences, 8*(3), 153-165 https://doi.org/10.62476/ravs83153

Baky, 2021). Diclofenac is believed to have chemopreventive effects by inhibiting cyclooxygenase, the enzyme that converts arachidonic acid to prostaglandins (Alabi *et al*., 2017). By inhibiting prostaglandins, diclofenac may indirectly enhance immune responses and upregulate the expression of major histocompatibility complex antigens (Alabi *et al*., 2017).

The suppression of prostaglandin synthesis by inhibiting cyclooxygenase (COX), which has two isoforms (COX-1 and COX-2), is the primary mechanism of action of diclofenac (Alabi & Akomolafe, 2020). COX-1 functions mainly in the control of renal hemodynamics and glomerular filtration rate, while COX-2 primarily affects salt and water excretion (Alabi & Akomolafe, 2020).

However, the administration of diclofenac sodium for long periods can lead to adverse effects on body tissues or cause cellular injuries, long-term chronic disorders and alterations in renal function, blood pressure, hepatic injury, platelet inhibition, gastrointestinal and cardiovascular disorders (Allain *et al*., 1974).

Oxidative stress can disturb the oxidant/antioxidant balance in cells, leading to a significant rise in reactive oxygen species and a decline in antioxidant levels, which can affect cellular structural macromolecules such as protein, lipid, carbohydrate and DNA (Thanagari *et al*., 2012).

The toxicity of diclofenac is believed to occur through the mechanism of damaging the mitochondrial transmembrane, resulting in the reduction of glutathione conjugation, the production of toxic metabolites and decreased antioxidant activity, leading to peroxidative damage to cell membranes, necrosis and diminished ATP production (Crofford, 2013).

Diclofenac can also induce hepatorenal changes and cause many alterations in organs such as the kidney, heart and stomach (Dhanvijay *et al*., 2013). Higher doses of diclofenac sodium administered for long periods can lead to hepato-nephron changes, bone marrow toxicity and enteropathy, resulting in gastrointestinal bleeding, ulceration, fulminant hepatic failure, aplastic anemia and acute kidney injury (El-Maddawy & El-Ashmawy, 2013). The use of diclofenac for long periods can also cause nephrotoxicity and contribute to the development of various immune mechanisms leading to nephrotoxicity (Alabi *et al*., 2017). Some studies have shown thickening of the glomerular basement membranes with mild focal tubular necrosis, interstitial nephritis, lipid peroxidation and papillary necrosis, as well as clinical features such as hematuria and proteinuria associated with the use of diclofenac for long periods (Enendu *et al*., 2010).

Therefore, this study was conducted to evaluate the toxic effects of diclofenac sodium on hematological and biochemical parameters, as well as histopathological changes in the kidneys of experimental animals.

2. Material and Methods

Diclofenac sodium (DS) tablets (diclac, 100 mg/kg), manufactured by MICRO LABS, Bangalore-560058, India, were used in this study. Diclofenac sodium was administered orally at three different doses: 20.5 mg/kg body weight, 45 mg/kg body weight and 56.25 mg/kg body weight, for 20 days. These doses were calculated based on the equivalent therapeutic dosages of human-rat conversion factor (Hardman & Limbird, 1995).

Experimental animals: Forty male Albino-Aster rats weighing 200-250 g were obtained from the animal house of the Faculty of Medicine, Alexandria University. The animals were handled in accordance with the principles of laboratory animal care as contained in the NIH guide for laboratory animal welfare and the experimental protocol was approved by the Local Ethics Committee and Animals Research. The rats were divided into four groups (10 rats per group) and maintained at a temperature of 22 ± 2°C, relative humidity of 40-60% and a 12 h/12 h light/dark cycle, with free access to food and water. The test substances were administered to the animals according to the following experimental protocol:

- Group I (Control): Rats were fed a basal diet and given tap water as drinking water daily for 20 days.
- Group II: Rats were given diclofenac sodium orally at a dose of 20.5 mg/kg body weight, dissolved in 5 ml of normal saline (0.9%), daily for 20 days.
- Group III: Rats were given diclofenac sodium orally at a dose of 45 mg/kg body weight, dissolved in 5 ml of normal saline (0.9%), daily for 20 days.
- Group IV: Rats were given diclofenac sodium orally at a dose of 56.25 mg/kg body weight, dissolved in 5 ml of normal saline (0.9%), daily for 20 days.

At the end of the experimental period, the rats were starved overnight, euthanized and dissected. Blood samples were collected from the inferior vena cava and placed in clean tubes with EDTA anticoagulant. Complete blood pictures (CBC) were analyzed using an automated hematology analyzer (Celltac X kx 021n, Japan CARE Co, Ltd.), which included hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), platelets and packed cell volume (PCV).

Serum samples were separated by centrifugation at 3000 rpm for 15 minutes and stored at -18°C for the estimation of alkaline phosphatase, creatinine, urea, cholesterol and total protein. Alkaline phosphatase activity was detected according to the method of Principato et al. (1985), creatinine and urea concentrations in serum were assayed using commercial kits (Diamond Company, Egypt) according to the methods of Henry et al. (1975) and Patton and Crouch (1977), respectively. Serum total cholesterol was determined according to the method of Allain et al. (1974) using kits from Linear Chemicals, S.L. (Spain) and serum total protein was determined by the method of Lowry et al. (1951).

Kidney tissues from each rat were immediately removed, fixed in 10% neutral buffer formalin and processed for histopathological examination according to the Luna (1968) method. The fixed tissues were dehydrated, embedded in paraffin, sectioned and stained with Hematoxylin-Eosin.

Statistical analysis: The study results were expressed as mean ± standard error (SE) and analyzed using one-way analysis of variance (ANOVA) with SPSS 17 software. Significant differences between groups were determined at $P<0.05$.

3. Results

The median lethal dose (LD50) of diclofenac sodium was estimated to be 53 mg/kg body weight by the oral route of administration.

Hematological findings: The hematological results showed a significant ($P<0.05$) decrease in red blood cells (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin

concentration (MCHC) in all groups after the administration of diclofenac sodium, compared to the control group (Figures 1-5).

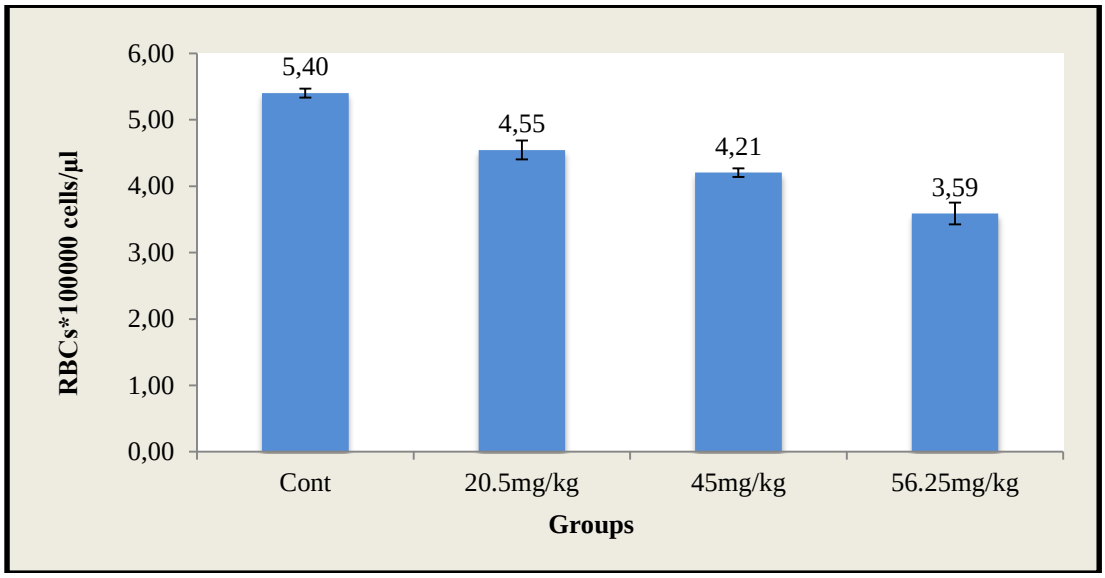

Figure 1. Changes in red blood cells (RBCs) count in male rats after administration different doses of Diclofenac sodium Values are expressed as means±SD of per group

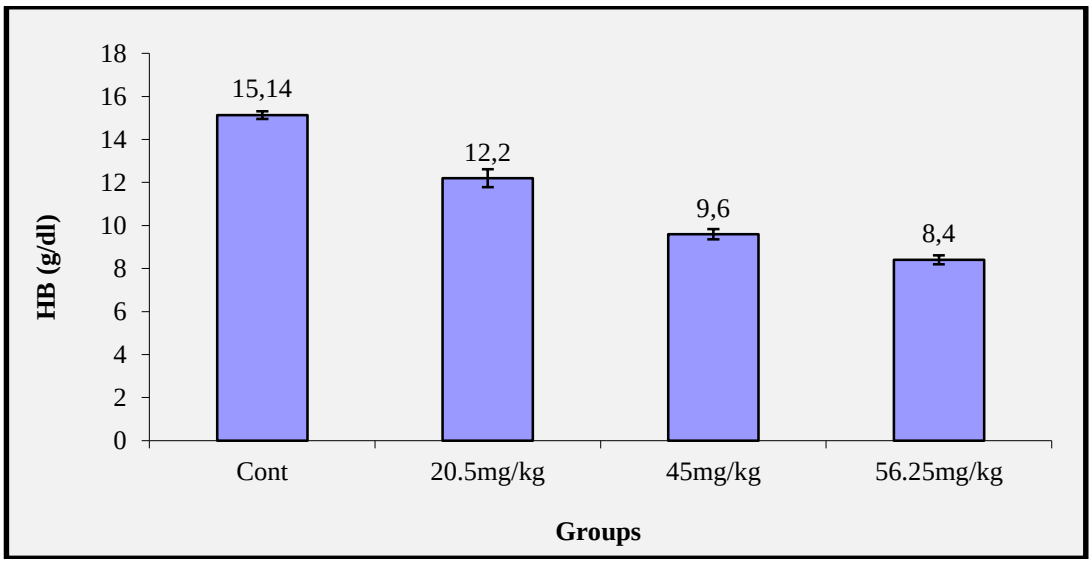

Figure 2. Changes in Hemoglobin concentration (Hb) in male rats after administration different doses of Diclofenac sodium. Values are expressed as means±SD of per group

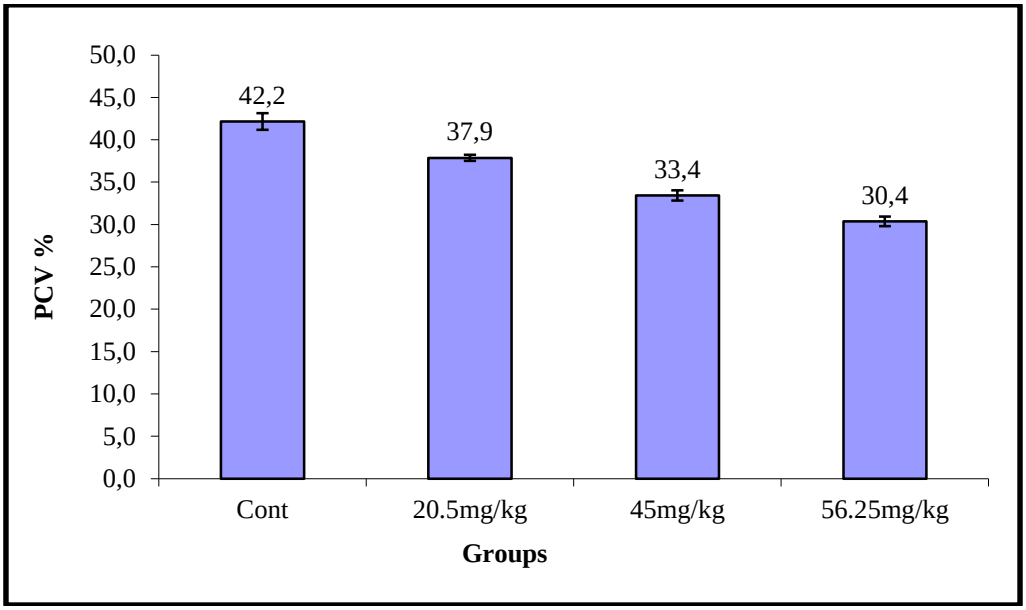

Figure 3. Changes in percentage of packed cells volum (PCV) in male rats after administration different doses of Diclofenac sodium. Values are expressed as means±SD of per group

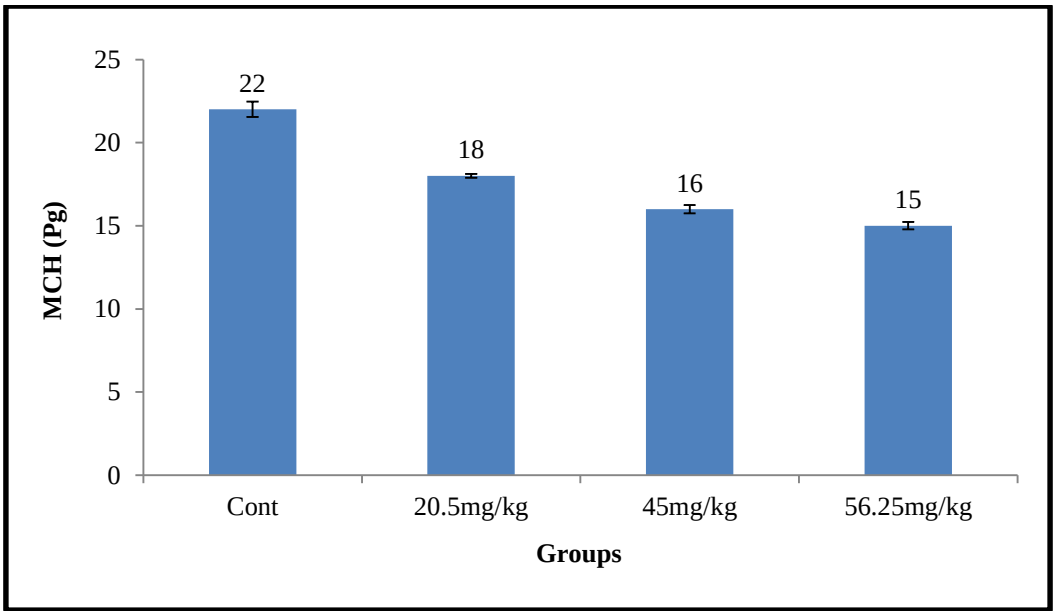

Figure 4. Changes in platelets (MCH) level in male rats after administration different doses of Diclofenac sodium. Values are expressed as means±SD of per group

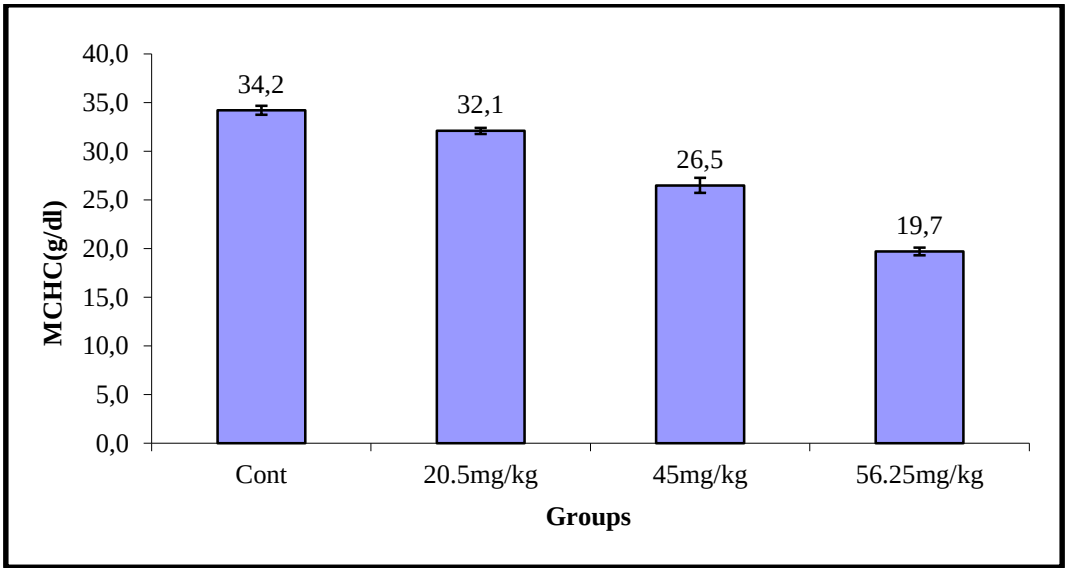

Figure 5. Changes in platelets (MCHC) level in male rats after administration different doses of Diclofenac sodium. Values are expressed as means±SD of per group

On the other hand, the results showed a significant (P<0.05) increase in platelet counts (Plt) in all groups after the administration of diclofenac sodium, compared to the control group (Figure 6).

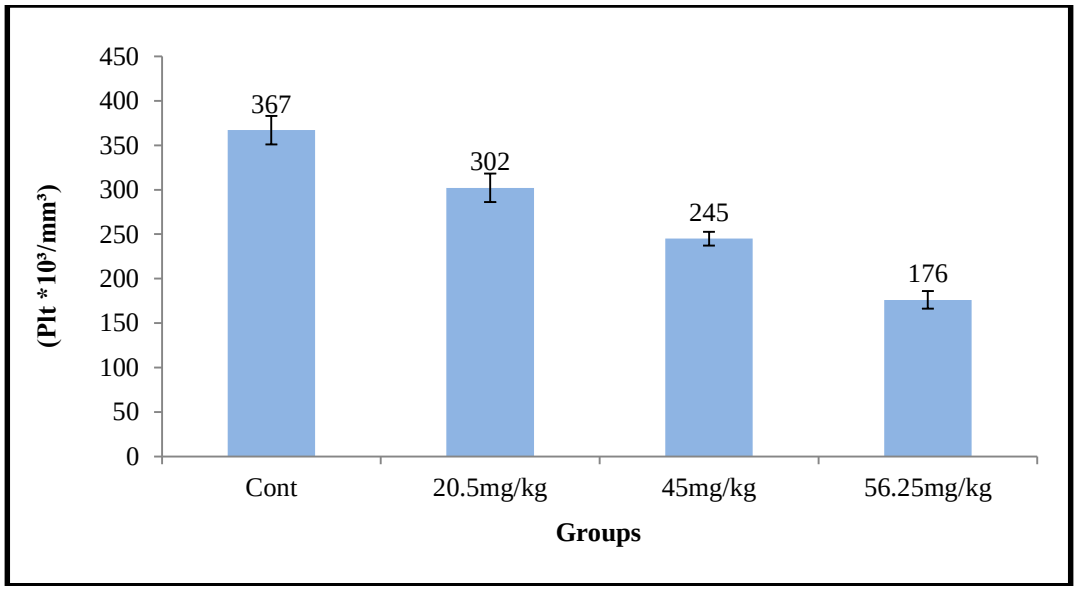

Figure 6. Changes in platelets (Plts) level in male rats after administration different doses of Diclofenac sodium. Values are expressed as means±SD of per group

The results also showed a significant (P<0.05) increase in the total white blood cells (WBCs) in the groups treated with the higher doses of diclofenac sodium (45 mg/kg and 56.25 mg/kg) compared to the control group (Figure 7).

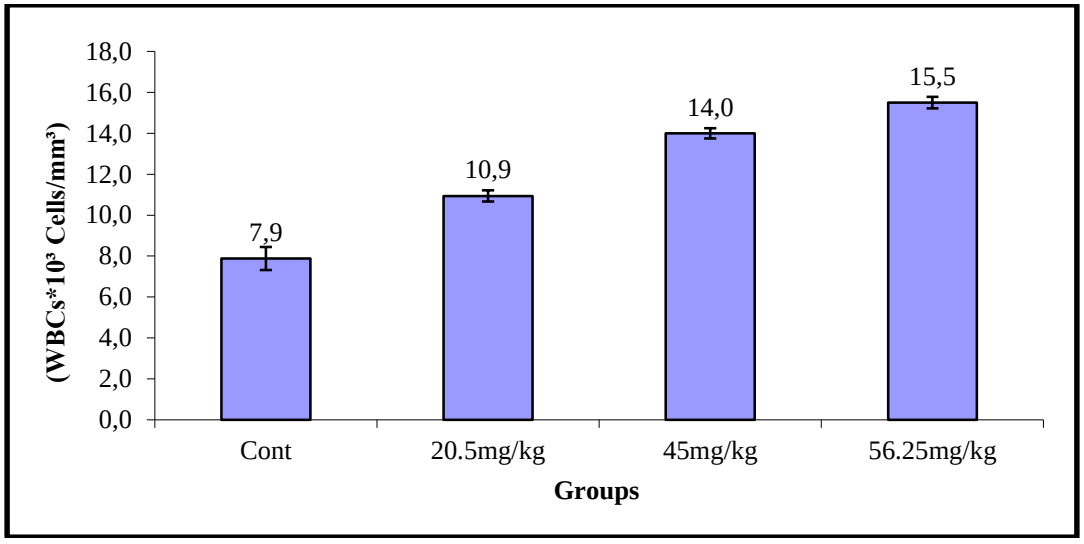

Figure 7. Changes in White blood cells (WBCs) level in male rats after administration different doses of Diclofenac sodium. Values are expressed as means±SD of per group

Biochemical findings: The oral administration of diclofenac sodium at doses of 20.5, 45 and 56.25 mg/kg body weight to the male rats resulted in a significant ($P<0.05$) increase in serum alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to the control group. The highest activity of these enzymes was observed in the group receiving the 56.25 mg/kg body weight dose (Figure 8).

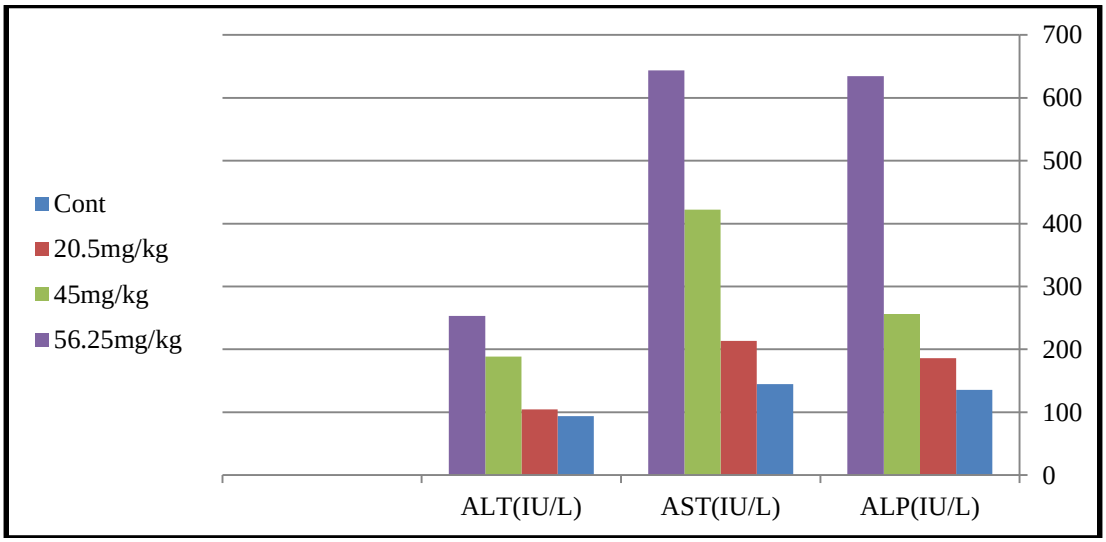

Figure 8. Changes in alkaline phosphatase (ALP), AST and ALT activity in serum of male rats adminstrated different doses of of Diclofenac sodium. Values are expressed as means±SD of per group

The results also showed a significant increase in serum total cholesterol, creatinine and urea concentrations, as well as a significant ($P<0.05$) decrease in total protein in the groups treated with diclofenac sodium, compared to the control group (Figure 9).

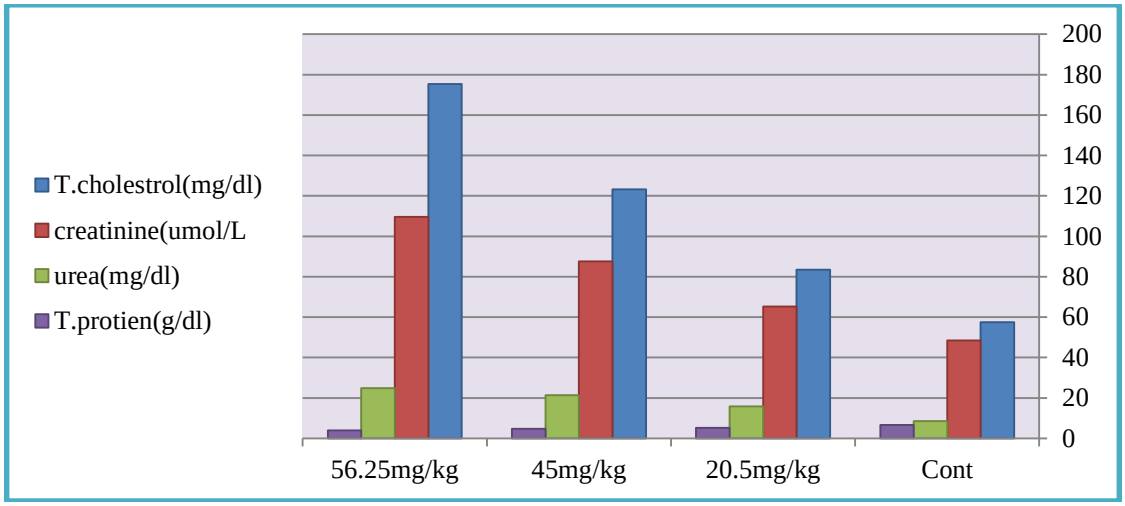

Figure 9. Changes in serum Total cholestrol (T.ch), creatinine, urea and Total protien of male rats adminstrated different doses of Diclofenac sodium. Values are expressed as means±SD of per group

Histopathological findings: Microscopic examination of kidney tissue sections in the control group (Group I) showed a normal architecture with a normal appearance of the glomerular tuft of blood capillaries surrounded by Bowman's capsule and the proximal and distal convoluted tubules lined by cuboidal cells appeared normal in size with their nuclei (Figure 10).

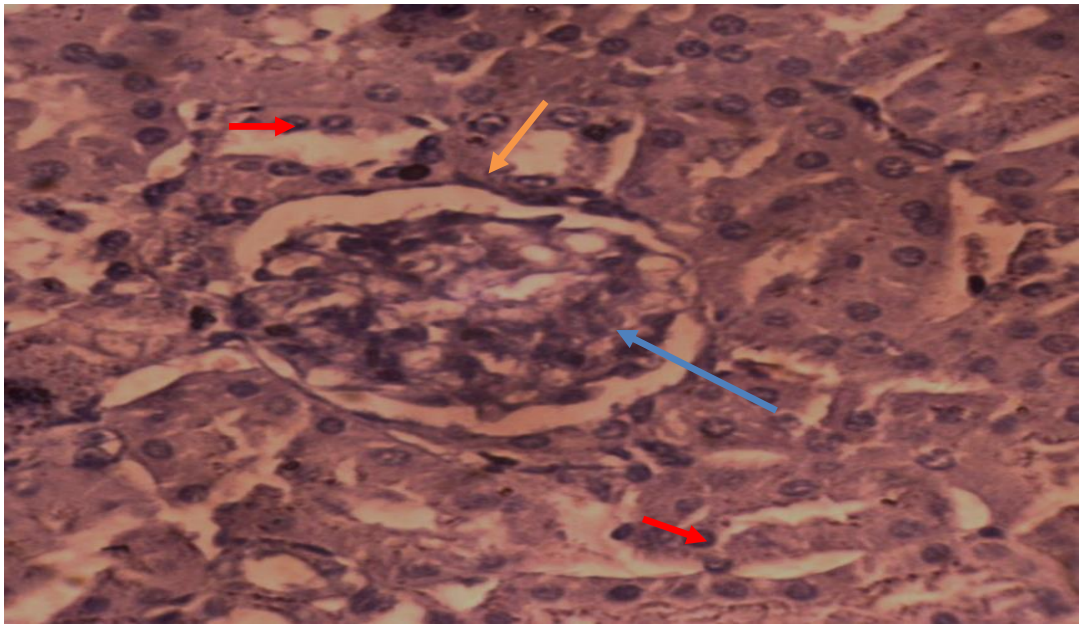

Figure 10. Photomicrographs control group (G1) for mice kidney tissue sections stained with Haematoxylin & Eosin (HE.400): Observed normal tissue architecture with normal glumerular tuft of blood capillaries → surrounded by Bowmann`s capsule → the proxiamal and distal convoluted tubules and lined by cuboidal cells appear in normal size with their nuclei →

In the group treated with diclofenac sodium at a dose of 20.5 mg/kg body weight (Group II), the kidney tissue sections showed a mild to moderate degree of congestion and focal interstitial inflammation, with mild abnormalities and atrophy in the glomeruli

and mild to moderate abnormal changes in the architecture of the renal tubules, with cytoplasmic degeneration of cells in some of the renal tubules (Figure 11).

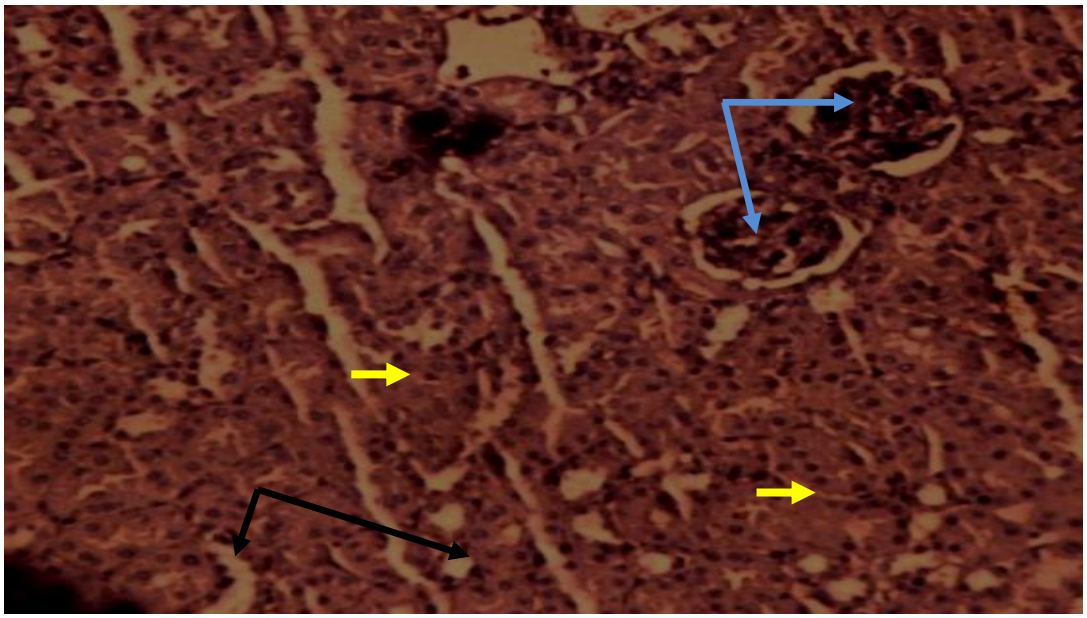

Figure 11. Photomicrographs (G2) for mice kidney tissue sections stained with Haematoxylin & Eosin (HE.400): Observed mild focal inflammation and atrophy → in the glumerulous, mild abnormal changes in renal tubules with decrease in lumen → with cytoplasm degeneration of cells in some of renal tubules →

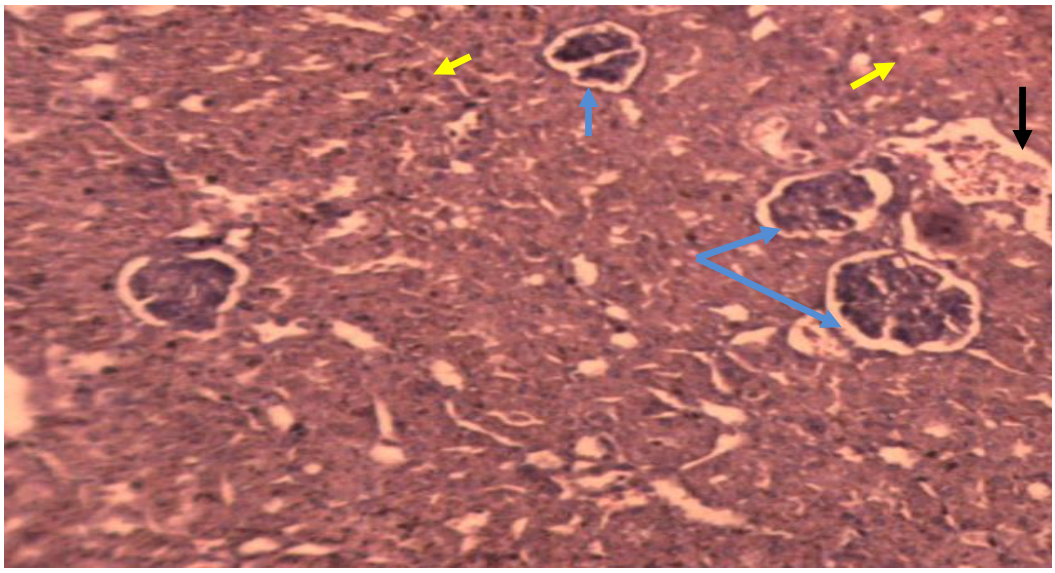

Figure 12. Photomicrographs (G3) for mice kidney tissue sections stained with Haematoxylin & Eosin (HE.400): Observed shrinking and degeneration of the glumerulous and necrosis in the renal tubules → dilatation and congestion in the renal blood vessels →, infiltration of the inflammatory cells →

The kidney tissue sections from the group treated with diclofenac sodium at a dose of 45 mg/kg body weight (Group III) exhibited shrinking and degeneration of the glomerular blood vessels, necrosis in the proximal and distal convoluted renal tubules,

multiple foci of hemorrhage, dilatation and congestion of the blood vessels and infiltration of inflammatory cells (Figure 12).

In the group treated with diclofenac sodium at a dose of 56.25 mg/kg body weight (Group IV), the kidney tissue sections showed severe degeneration and damage of the glomeruli, severe coagulative necrosis in the renal tubules, with infiltration of inflammatory cells, multiple foci of hemorrhage, dilatation and congestion of the blood vessels with coagulated red blood cells (Figure 13).

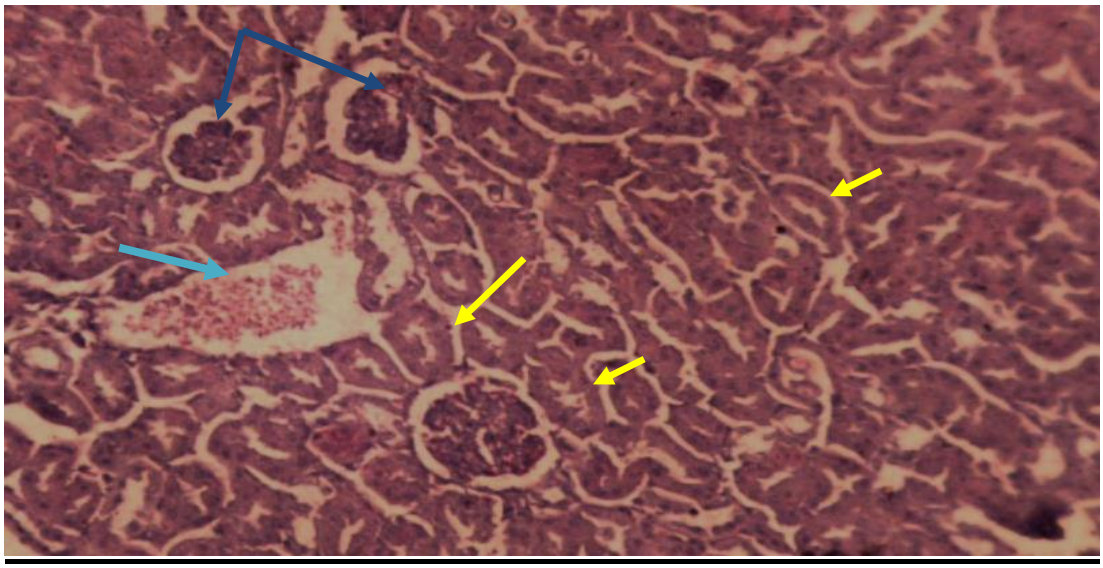

Figure 13. Photomicrographs (G4) for mice kidney tissue sections stained with Haematoxylin & Eosin (HE.100): observed sever degeneration and damage of the glumerulous → coaggulative necrosis in the renal tubules → , dilatation and congestion of the blood vessels with coagulated RBCs →

4. Discussion

The results of this study showed significant alterations in the hematological parameters, including a decrease in red blood cells (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), as well as an increase in platelets (Plt) and total white blood cells (WBCs) after the oral administration of different doses of diclofenac sodium in the experimental groups, compared to the control group.

The hematotoxicity effect of diclofenac sodium can cause excessive destruction of red blood cells and loss of erythrocytes as a result of gastrointestinal bleeding, leading to anemia (Lowry *et al*., 1951; Luna, 1968; Makki *et al*., 2014). The increase in platelets may be due to tissue injury and inflammatory response induced by the toxic metabolites of diclofenac sodium (Abdel-Rahman & Abdel-Baky, 2021; Ong & Seymour, 2007; Orinya *et al*., 2016). The increase in total white blood cells could be attributed to the direct toxicity of diclofenac sodium to the myeloid cells and the formation of toxic metabolites, which may cause severe neutropenia (Owumi & Dim, 2019a; 2019b).

The results also showed a significant increase in serum alkaline phosphatase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the groups treated with diclofenac sodium, indicating hepatic and renal impairments (Paget & Barnes, 1964; Patton & Crouch, 1977). The significant increase in total cholesterol, creatinine and urea, as well as the decrease in total protein, suggest the adverse effects of

diclofenac sodium on lipid metabolism and renal function (Paget & Barnes, 1964; Patton & Crouch, 1977; Peter & Prince, 2018; Principato *et al*., 1985).

The histopathological examination of the kidney tissues revealed a dose-dependent pattern of changes, ranging from mild congestion and inflammation to severe degeneration and necrosis of the glomeruli and renal tubules, with infiltration of inflammatory cells, dilatation and congestion of blood vessels (Pontikoglou & Papadaki, 2010; Sabry *et al*., 2014; Subramanian, 2009; Kasem *et al*., 2022). These histopathological alterations may be attributed to the oxidative stress and the generation of reactive oxygen species induced by diclofenac sodium, leading to cellular damage and impaired renal function (Thanagari *et al*., 2012; Syed *et al*., 2012).

5. Conclusion

In conclusion, the findings of this study have shown that the administration of different doses of diclofenac sodium can cause significant alterations in hematological and biochemical parameters, as well as histopathological changes in the kidneys of male rats, indicating potential adverse effects on animal tissues. Caution should be exercised in the clinical use of diclofenac sodium and the lowest effective dose and duration should be used to achieve the desired therapeutic effect.

References

Abdel-Rahman, O.N., Abdel-Baky, E.S. (2021). Hematological and renoprotective effects of folic acid and lentil extract in diclofenac sodium exposed rats. *Brazilian Journal of Biology*, 83, e247360.

Alabi, Q.K., Akomolafe, R.O. (2020). Kolaviron diminishes diclofenac-induced liver and kidney toxicity in wistar rats via suppressing inflammatory events, upregulating antioxidant defenses and improving hematological indices. *Dose-Response, 18*(1), 1559325819899256. https://doi.org/10.1177/1559325819899256

Alabi, Q.K., Akomolafe, R.O., Olukiran, O.S., Adeyemi, W.J., Nafiu, A.O., Adefisayo, M.A. & Odujoko, O.O. (2017). The Garcinia kola biflavonoid kolaviron attenuates experimental hepatotoxicity induced by diclofenac. *Pathophysiology, 24*(4), 281-290.

Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. & Fu, P.C. (1974). Enzymatic colorimetric method for cholesterol. *Clinical Chemistry, 20*(4), 470-475.

Crofford, L.J. (2013). Use of NSAIDs in treating patients with arthritis. *Arthritis Research & Therapy*, 15, 1-10.

Dhanvijay, P., Misra, A.K. & Varma, S.K. (2013). Diclofenac induced acute renal failure in a decompensated elderly patient. *Journal of Pharmacology and Pharmacotherapeutics, 4*(2), 155-157. https://doi.org/10.4103/0976-500X.110916

El-Maddawy, Z.K., El-Ashmawy, I. (2013). Hepato-renal and hematological effects of diclofenac sodium in rats. *Global Journal of Pharmacology, 7*(2), 123-132. https://doi.org/10.5829/idosi.gjp.2013.7.2.72171

Enendu, A.C., Unekwe, P.C., Esimone, C.O., Obi, E. & Chilaka, K.C. (2016). Protective effect of phenylalanine and glycine on chloramphenicol-induced bone marrow toxicity in albino rats infected with Klebsiella Pneumoniae. *International Journal of Biological and Chemical Sciences, 10*(1), 369-383.

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.

Hardman, J.G., Limbird, L.E. (1995). *Goodman & Gilman's: The Pharmacological Basis of Therapeutics*, 9th edition. New York: McGraw Hill.

Henry, R.J., Cannon, D.C. & Winkelman, J.W. (1974). *Clinical Chemistry Principles and Techniques*, 11th edition. New York: Harper & Row.

Hesham, N.M., Isinsu, A, Ömür, G.D., Berrin, Z.A., Ebru, A. & Suleyman, K. (2019). A study on the toxic effect of different doses of diclofenac sodium on the development of the kidney in the postnatal period. *International Journal of Morphology*, *37*(3), 877-884.

Hickey, E.J., Raje, R.R., Reid, V.E., Gross, S.M. & Ray, S.D. (2001). Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. *Free Radical Biology and Medicine, 31*(2), 139-152.

Hörl, W.H. (2010). Nonsteroidal anti-inflammatory drugs and the kidney. *Pharmaceuticals, 3*(7), 2291-2321.

Iftikhar, A., Hasan, I.J., Sarfraz, M., Jafri, L. & Ashraf, M.A. (2015). Nephroprotective effect of the leaves of Aloe barbadensis (Aloe Vera) against toxicity induced by diclofenac sodium in albino rabbits. *The West Indian Medical Journal, 64*(5), 462.

Kasem, S.E., Helal, M., Mahmoud, S., Salama, E., Elkady, A. & Elsayed, R.M. (2022). Ameliorative effect of N acetyl cysteine against diclofenac-induced oxidative stress, hematological and splenic toxicity in male rats. *Egyptian Society of Clinical Toxicology Journal, 10*(1), 1-13.

Konyalioglu, S., Er, A., Uney, K., Elmas, M. & Yazar, E. (2007). Efekti flunixin meglumina na antioksidativni status miševa sa endotoksemijom. *Acta Veterinaria, 57*(2-3), 241-246. https://doi.org/10.2298/AVB0703241K

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry 193*(1), 265-275.

Luna, L.G. (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd edition. McGrow-Hill.

Makki, M.J.A., Umran, H.J. & Jawad, A.M. (2014). The effect of diclofenac sodium given alone or in combination with paracetamol in treatment of patients with type-2 diabetes mellitus. *The Medical Journal of Basrah University, 32*(1), 22-29.

Ong, K.S., Seymour, R.A. (2007). An evidence-based update on non-steroidal anti-inflammatory drugs and COX-2 inhibitors. *Clinical Medicine, 5*(1), 19-34. https://doi.org/10.3121/cmr.2007.698

Orinya, O.A., Adenkola, A.Y. & Ogbe, R.J. (2016). Haematological and biochemical studies on the effect of diclofenac sodium on Wistar Rattus norvegicus. *International Journal of Biological and Chemical Sciences, 10*(5), 2231-2242.

Owumi, S.E., Dim, U.J. (2019a). Biochemical alterations in diclofenac-treated rats: Effect of selenium on oxidative stress, inflammation and hematological changes. *Toxicology Research and Application*, 3, 2397847319874359.

Owumi, S.E., Dim, U.J. (2019b). Manganese suppresses oxidative stress, inflammation and caspase-3 activation in rats exposed to chlorpyrifos. *Toxicology Reports*, 6, 202-209.

Paget, G.E., Barnes, J.M. (1964). Toxicity tests. *Evaluation of Drug Activities: Pharmacometrics*, 1, 135-165.

Patton, C.J., Crouch, S.R. (1977). Enzymatic determination of urea. *Analytical Chemistry*, 49, 464-469.

Peter, J.S., Prince, S.E. (2018). Diclofenac-induced renal toxicity in female Wistar albino rats is protected by the pre-treatment of aqueous leaves extract of Madhuca longifolia through suppression of inflammation, oxidative stress and cytokine formation. *Biomedicine & Pharmacotherapy*, 98, 45-51.

Pontikoglou, C., Papadaki, H.A. (2010). Idiosyncratic drug-induced agranulocytosis: The paradigm of deferiprone. *Hemoglobin, 34*(3), 291-304.

Principato, G.B., Aisa, M.C., Talesa, V., Rosi, G. & Giovannini, E. (1985). Characterization of the soluble alkaline phosphatase from hepatopancreas of Squilla mantis L. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 80*(4), 801-804.

Sabry, S.A., Sakr, S.M. & Shahin, M.A. (2014). Histological and ultrastructural studies on the effect of diclofenac sodium on the renal cortex of fetuses of albino mice. *Global Journal of Pharmacology, 8*(3), 369-377.

Sharef, A.Y., Hamdi, B.A. & Alnajar, Z.A. (2020). Histopathological aspects of co-administration of dexamethasone and diclofenac sodium on male albino rats. *Zanco Journal of Medical Sciences, 24*(2), 236-245.

Subramanian, S. (2009). Diclofenac induced toxic manifestations on adjuvant induced arthritic rats pheripheral and reproductive organ of male wistar rats rattus norvegicus. *Journal of Toxicology and Environmental Health, 1*(1), 12-21.

Syed, N.I., Zehra, F., Syed, A.A., Karim, S. & Khan, F.S. (2012). Comparing the effects of salts of diclofenac and almioprofen with aspirin on serum electrolytes, creatinine and urea levels in rabbits. *Pakistan Journal of Pharmaceutical Sciences, 25*(4), 777-782.

Thanagari, B.S., Fefar, D.T., Prajapati, K.S., Jivani, B.M., Thakor, K.B., Patel, J.H. & Undhad, V.V. (2012). Haemato-biochemical alterations induced by diclofenac sodium toxicity in Swiss albino mice. *Vet World, 5*(7), 417-419.