

Review on Diclofenac Toxicities in Different Organs

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Abstract

Received in original form:
3 September 2023
Accepted in a final form:
12 December 2023

Introduction: Diclofenac is an over-the-counter (OTC) non-steroidal anti-inflammatory drug (NSAID) that works through non-selective inhibition of the cyclooxygenase (COX) enzyme, thereby inhibiting the release of prostaglandins. Known for its efficacy in alleviating inflammation, pain, and fever symptoms, concerns have arisen regarding the potential toxicity of Diclofenac. Its toxicity can cause a wide variety of adverse effects, ranging from self-limiting side effects that disappear after treatment cessation to an increased risk of death due to cardiovascular diseases and strokes. Determining the threshold dose that distinguishes between mild, self-limiting side effects and more severe adverse effects poses a major challenge in diclofenac toxicity research. **Method:** This article reviewed various doses and different organ toxicities observed in animal and human studies using diclofenac. **Aim:** Our objective is to define the toxicities in fourteen organs and organize them into different categories as animal toxicities, human toxicities, and teratogenicity through pathological changes. **Conclusion:** By searching different databases and collecting the observed toxicities, we have concluded that the primary affected organs are the stomach, liver, and kidney. However, there is limited knowledge on the toxic effects on the brain, heart, lungs, spleen, eye, bone, bone marrow and blood, and testes, while rare available data for the pancreas, bladder, skin, muscle, and uterus.

Key words

diclofenac, toxicity, experimental studies, rats, human

Introduction

Diclofenac is one of the non-steroidal anti-inflammatory drugs (NSAIDs) used to relieve inflammation, pain, and fever. It has been accessible to consumers as an over-the-counter (OTC) drug since its approval in 1988. Researchers designed diclofenac using rational drug design principles, based on the structures of three pre-existing NSAIDs: phenylbutazone, mefenamic acid, and indomethacin (fig 1). Adding two chlorine atoms to the phenyl ring in the ortho position by which it twists the ring as much as possible, and appears to make the drug to be more potent than others (Sallmann 1986).

Chemical and physical properties:

It is one of the phenylacetic acid derivatives. Its molecular formula is C₁₄H₁₀Cl₂NNaO₂, and its IUPAC name is 2-[2-[(2,6-chlorophenyl) amino] phenyl] acetic acid, with a molar weight of 318.1 g/mol, melting point is 283-285 °C, and water solubility is 2.37 mg/L at 25 °C.

Fig 1, skeletal structure of different NSAIDs (Sallmann 1986)

Pharmacological characteristics:

The USP36 pharmacopeia described it as a white to yellowish or off-white, hygroscopic, crystalline powder. It is highly soluble in water and methyl alcohol, slightly soluble in acetone, and practically insoluble in either ether or chloroform (Sweetman 2009). It has high lipid solubility, so it can pass the blood-brain barrier and enter the brain and spinal cord (Bartolomei et al. 2006). It is available on the market as a tablet, suspension, injection vial, suppository, gel, and eye drop.

Pharmacokinetic properties:

Diclofenac is fully absorbed from the digestive tract, but about 40% of the drug is metabolized through the first-pass effect before it reaches the bloodstream. Many topical formulations of diclofenac are absorbed through the skin and produce clinically significant plasma concentrations. Absorption of diclofenac is

proportional to the dose over the range of 25-150 mg. The time to peak plasma concentration (T_{max}) varies depending on the formulation, with the oral solution reaching peak plasma concentrations in 10-40 minutes, the enteric-coated tablet in 1.5-2 hours, and sustained- and extended-release formulations prolonging T_{max} even further. Administration of diclofenac with food does not significantly affect the amount of drug absorbed or area under the curve (AUC), but it does delay T_{max} to 2.5-12 hours (Whalen 2018).

Its total volume of distribution is 0.1-0.2 L/kg or 5-10 L with 0.04 L/kg volume of the central compartment. Its peak concentration in the synovial fluid is 2-4h after administration with a 2 h half-life, and plasma concentration is 8.22%. Doses of 50 mg delivered via intramuscular injection produced no detectable diclofenac concentrations in breast milk, however, metabolite concentrations were not investigated (Whalen 2018).

Many studies showed that diclofenac can cross the placenta in mice, rats, and humans (Siu et al. 2000). More than 99.7% of diclofenac in the blood can attach to proteins, mostly albumin. Most of the drug is removed through the urine and stool. Liver cells metabolized diclofenac by two main processes: oxidation and conjugation. Oxidation produces hydroxy metabolites, while conjugation produces glucuronic acid, sulfate, and taurine conjugates (fig 2). The main metabolites are 3'-hydroxy-diclofenac, 4'-hydroxy-diclofenac, 5'-hydroxy-diclofenac, diclofenac acyl glucuronides, diclofenac o-imine methane, diclofenac 2',3'-oxide, 2'-(glutathione-S-yl)-deschloro-diclofenac which produced from several subtypes of CYP450 (Whirl-Carrillo et al. 2021).

Mechanism of action:

Both mechanisms of action and toxicities of diclofenac depend on inhibiting prostaglandin production through nonselective cyclooxygenase (COX) enzyme inhibition, COX-1, and COX-2, with relatively equal potencies. It can also inhibit the production of leukotrienes and phospholipase A₂, therefore modulation the level of arachidonic acid. It is a safe drug when used according to the recommended dose, but patients should be aware that it might have some side effects (Gan et al. 2010).

Diclofenac, like other NSAIDs in general, induces self-limiting side effects that disappear after discontinued treatment. There may be pain with tissue damage at the site of injections, local irritation if used as suppositories, and a burning sensation with corneal damage when used as eye drops. However, the serious adverse effects that might require hospitalization are bleeding ulcers, GIT perforation, renal papillary necrosis, and hepatic tissue damage. So, some precautions should be considered during administration, especially for patients with a history of hemorrhage, renal disease, hepatic disease, or eye problems (Sweetman 2009). Its veterinary use was approved worldwide but should be replaced with meloxicam to protect vultures that might consume the diclofenac-treated carcasses (Shore et al. 2014).

Some concerns about diclofenac rose after several incidences of death due to heart attacks and strokes, especially among people who are at risk due to chronic pain, diabetes, high blood pressure, and high cholesterol levels (Schmidt et al. 2023). The healthcare system in the UK restricted oral preparations of diclofenac and reclassified it as a prescription-only drug.

The major problem in the research of diclofenac toxicities is that it is hard to identify the cut-off dose that splits between self-limiting side effects and serious adverse effects. In the literature reviews of several kinds of research, the doses of diclofenac used range from 1 mg/kg to 1300 mg/kg in animal studies and up to 3000 mg/kg in human studies, with discrepancies in the toxicities' sites and grades. Some showed that even a very low dose of diclofenac might produce severe toxicities.

In this review, we aimed to define these toxicities in several doses that are used in the literature and organize them to identify organ toxicities through pathological changes.

Methods

We searched different databases for primary and secondary studies using diclofenac as a drug and showed the differences between doses, routes of administration, and toxicities.

In this review, we will combine toxicities shown in studies on experimental animals as rats and mice, with clinical studies as clinical trials, case reports, case series, and reviews to figure out types, grades, and possible mechanisms of toxicities. We will talk about toxicity on each organ separately and show the variation of diclofenac doses used with toxicities reported.

In addition, we will search for studies in which authors focused on diclofenac during pregnancy to identify its teratogenic effect.

To the best of our knowledge, we figured out that the main organs affected by diclofenac were the stomach, liver, and kidney, with minimum knowledge of the brain, heart, spleen, lung, eye, bone marrow and blood, and testes, and rare data for the rest of the organs as the pancreas, bladder, bone, skin, muscle, and uterus.

Results

Diclofenac Toxicities

A deep look at a written review of worldwide clinical safety experience with diclofenac found that about 12% of people who took diclofenac had side effects. About 1.6% of people who had side effects stopped taking the drug. The most common side effects were stomach and intestinal problems, which occurred in 7.6% of people. Nervous system side effects occurred in 0.7%, and allergic reactions or local reactions occurred in 0.4% (Willkens 1985). This review with other studies accepted that side effects were usually mild and disappear after a short time, not related to the dose taken, and the incidence of side

effects in children is similar to that in adults (Standing et al. 2009).

Numerous experimental studies on animals and humans focus on stomach, liver, and kidney injuries due to diclofenac. They showed dissimilarities in the degree of these toxicities with high fluctuations in the diclofenac cumulative dose, duration of treatment, and routes of administration in their research and neglected the toxic effects on the rest of the organs.

Toxicities in experimental studies:

Stomach

In the first study, Aycaan et al. injected male Wistar rats with a diclofenac dose of 9 mg/kg intramuscularly (I.M) twice daily for consecutive 5 days, and showed toxicities as immediate ultrastructural gastric surface epithelial damage, obvious endoscopic gastroduodenal subepithelial hemorrhages, and erosions within several hours of ingestion. It is also associated with a progressive increase in epithelial permeability with multiple focal scattered erosions within the superficial epithelium and edema of the submucosal tissue, a marked increase in enteric gram-negative bacteria numbers, and intestinal ulceration (Fig 3) (Aycaan et al. 2018).

In another study in which Conforti et al. administered female Sprague-Dawley rats with diclofenac doses ranging from 3.5, 7, and 15 mg/kg through the oral route, induced ulcers in 30%, 62.5%, and 100% of the rats, respectively. Only at the highest dose was longitudinal and diffuse gastric ulcers observed. With the other two doses, small ulcers were evident in some animals, while the others showed wide hemorrhagic effusion (Conforti et al. 1993).

In a third study, Seo et al. used the gross ulcer index, damage area, and histological index to show acute gastric damage. They used 2 doses of diclofenac, 40 and 80 mg/kg per oral (P.O) for a single dose, and 3 ages of Male Sprague-Dawley rats: 7 and 25 weeks, and 1 year old. Gross findings showed a punctuate-type clean ulcer along with hemorrhage and a large gross ulcer index (fig 4) (Seo et al. 2012). The same toxicity indexes appeared in another study in which Peter et al. used female Wistar albino rats with a dose of 50 mg/kg intraperitoneal injection (I.P) for only 2 days (Peter et al. 2017).

Acute toxicity came across another study by Kim et al. studied the effect of increasing the dose of diclofenac on the stomach of Male Sprague Dawley rats. Gastric damage was directly proportional to the doses used. They were 2.5, 10, and 40 mg/kg I.P. per dose. Upon microscopic examination, they characterized the gastric mucosa by full-thickness necrosis of the mucosa reaching the muscularis mucosae (fig 5) (Kim et al. 2005).

Liver

Acute liver toxicity appeared as portal inflammation in a study in which Simon et al. used female Wistar albino rats and injected them with 50 mg/kg I.P. for 2 days (fig 6) (Simon et al. 2019). The same toxicity appeared different in another study as peri-vascular infiltration, and coagulative necrosis in

which Tomic et al. used the same rat species and diclofenac dose was 8 mg/kg I.P injections for 28 days. The lowest toxicity grade happened in a third study when authors administered diclofenac at 8 mg/kg P.O for just 2 days (Tomic et al. 2008).

Furthermore, two studies described an increase in lymphocytic infiltration with the same dose of 50 mg/kg diclofenac injections I.P for 2 days (fig 7) (Heidarian et al. 2021; Esmailzadeh et al. 2020). However, Peter et al. in a third study showed additional apoptotic changes, with periportal inflammation and pyknotic nuclei in the liver section with the same diclofenac dose, route, and duration (fig 8) (Peter et al. 2017).

Other group researchers from Alabi et al. showed dystrophic changes in the structure of the liver like sinusoidal congestion, perivenular zonal necrosis, ballooning degeneration, and mononuclear leukocytes and lymphocytes infiltrated by a microscopic examination in a study using male Wistar rats injected 10 mg/kg I.M for 7 days (fig 9) (Alabi et al. 2017).

Kidney

Three studies used male Wistar rats to evaluate the renal toxicity of diclofenac. In the first study, Aycaan et al. gave rats I.M. injections with 9 mg/kg diclofenac twice daily for 5 days. The results showed toxicities such as damage, dilation, atrophy, and vacuolization of the tubules with thickening of the basement membrane (fig 10) (Aycaan et al. 2018). In the second one, Anwar and Laila et al. injected rats I.P. with 10 mg/kg daily for 28 days. Histopathology results reported tissue atrophy and degeneration through kidney weight reduction and inflammation (fig 11) (Anwar et al. 2022). In the last one, Orabi et al. administered the rats a low dose of diclofenac 2 mg/kg P.O for 30 days and the toxicities appeared as swelling of Bowman's capsule with inflammatory cell infiltration, necrosis of the tubule lining epithelium, and the appearance of regeneration in the tubule (fig 12) (Orabi et al. 2020).

Instead of using Wistar male rats, another group by Efrati et al. used male Sprague—Dawley rats and injected them I.P with 15 mg/kg twice a day for 3 days. The consequences were acute visible damage to tubules with protein deposition, tubular obstruction, and a low percent of necrosis without any inflammatory cell infiltration (fig 13) (Efrati et al. 2007).

Other authors compared the outcome of the toxic effect of diclofenac between two doses, the first one was an I.M injection for rats with a 100 mg/kg single dose, and the other dose was a 100 mg/kg daily for consecutively 3 days (fig 14, fig 15) (Elshopakey et al. 2021; Ahmed et al. 2017). They appeared to have the same toxicity profile as the third study by Mousa et al., as the dose of diclofenac was 2.5 mg/kg P.O every other day for 8 weeks (fig 16) (Mousa et al. 2020). The general outcomes were hemorrhage in the interstitial tissue of the renal tubules, shrinkage of the glomerular tuft with swelling of the capsule, damage to the glomerular cells, necrosis of the proximal convoluted tubules with interstitial edema in the distal convoluted tubules, and fatty cell alterations inside the

epithelial cells of the glomerular tuft, renal cortex, and renal tubules.

In the last study, Hickey et al. used adult CD-1 mice and divided them into groups with a range of doses of 100, 200, and 300 mg/kg for only one oral dose. They showed damage to macula densa areas ranging from a slight injury to totally losing the region, and shrinkage, hyper-vacuolation, inflammatory cell infiltrations, and cellular necrosis in the areas of proximal and distal renal tubules (fig 17) (Hickey et al. 2001).

Brain

In only one study, Ilic et al. investigated the harmful effect of diclofenac on the brain. They used adult male Wistar albino rats and injected them I.P. with 12.5 mg/kg of diclofenac once a day for three days. They reported Brain edema and cyanosis to be more common in white matter than gray matter, particularly in the cerebral cortex and cerebellum. The cortex, cerebellar nuclei, Purkinje cells, and hippocampus neurons all showed expression of damaged or ballooned red neurons without any inflammation (fig 18) (Ilic et al. 2011).

Heart

Tan et al. also studied toxicity in the heart as researchers injected diclofenac 30 mg/kg I.P. one dose in ICR male mice 8 weeks old. They showed cardiac toxicity as vascular damage with congestion of the vessels of the myocardium with edematous focal areas and moderate inflammatory damage (fig 19) (Tan et al. 2013).

Lung

Tomic et al. conducted an experiment on mature mice of the Wistar strain. They divided them into groups, where a group of test mice received diclofenac at a dose of 8 mg/kg/day orally for 7 days, while another group received diclofenac I.P. at the same dose daily but for 28 days. They reported that the macroscopic and pathological examination of lung samples of the test groups revealed effusion and edema in the lung in addition to lung bleeding in some samples, as pathological changes, which were more frequent in the 7-day group (fig 20) (Tomic et al. 2008).

In another study by Al-Hayder et al., they injected Sprague Dawley mature rats with 200 mg/kg diclofenac as one I.P. single dose. They showed congested alveolar capillaries, bleeding alveolus, and infiltration of lung tissue with haemosiderin-laden macrophages (fig 21) (Al-Hayder et al. 2022).

Spleen

There is no experimental study confirming the presence of a toxic effect of diclofenac on the spleen. One study was published in 2013 confirming that diclofenac has no toxicity on the spleen in terms of histopathology. Tan et al. studied ICR male mice, 8–12 weeks old, and divided them into two groups. Group A, "the control group," was administered saline-only P.O, and Group B was administered diclofenac only as 30 mg/kg I.P. in one dose. The Diclofenac group showed no vascular or inflammation changes in the spleen

compared to the control group A (fig 22) (Tan et al. 2013).

Eye

No studies were done in experimental animals to evaluate and determine which doses might be toxic from the use of diclofenac. Despite that, Li et al. cultures of human corneal epithelial cells (HCEP) with diclofenac at doses ranging from 0.00625% to 0.1% for 28 hours showed significant cytotoxicity that appears in varying degrees dependent on dose and time, represented by cell atrophy, cytoplasmic vacuolization, and decreased viability (Li et al. 2021).

Bone

Krischak et al. made a study on male adult Wistar rats to investigate the effect of 10 mg/kg I.M. injections of diclofenac for 10 days on bone restructuring and healing. They found a severe reduction of osteoblast cell production and differentiation, therefore a reduction in the bone healing process (Krischak et al. 2007a).

In the same pattern, several researchers used a lower dose of diclofenac at 5mg/kg P.O for 10 to 21 days and they showed inhibition in the proliferation of osteoblast cells and delay in bone healing (Krischak et al. 2007b; Beck et al. 2003). Lower doses as 1-2 mg/kg for 10 days didn't show any change and did not affect bone healing (Akman et al. 2002).

Contrary another group of researchers studied the effect of 2mg/kg diclofenac daily for 5 days on bone implanted on the tibia of male Sprague-Dawley rats. They concluded that diclofenac with this low dose can delay the healing of bone and delay bone-to-implant contact (Pablos et al. 2008).

Bone marrow and blood

Chouhan and Sharma injected Balb-c adult male Swiss albino mice with diclofenac I.M. 10 mg/kg for 3 different durations 10 days, 20 days, and 30 days, and assessed the tibia and femur bones. They found that bone marrow toxicities increased by grade equivalent to dose and appeared as calcified cartilage, larger lacuna size, and disorganized lamellar pattern of bone matrix. Bone marrow elements were sparse, with fewer osteoblasts, with flatter lining on the endosteum. The epiphyseal region showed increased cartilage, thinned trabeculae, and fewer osteoclasts. On the other hand, the blood cells showed an abnormal myeloid to erythroid ratio as myeloid elements increased over the erythroid, which occurred after 10 days and the ratio did not change after 20 days or 30 days (Chouhan et al. 2012).

Reproductive System:

Testes

Many studies of diclofenac reported toxicities for testes only with no data for other male genitalia. Vyas et al. showed mild testes toxicities after oral administration of a very low dose of 1 mg/kg and showed a reduction in weights with the epididymis, ventral prostate, and seminal vesicles, with a reduction in sperm motility, degeneration of interstitial cells and spermatocytes, and shrinkages of seminiferous tubules (fig 22) (Vyas et al. 2019). The toxicities appeared

slightly more severe when researchers by Mousa et al. increased the diclofenac dose to 2.5 mg/kg four times per week for eight weeks P.O and showed sperm morphological anomalies with no changes in seminiferous tubules (fig 23) (Mousa et al. 2020). However, these toxicities appeared the same in another study in which authors increased diclofenac dose to 5 mg/kg every day for 3 weeks P.O (fig 24) (T et al. 2022).

Teratogenicity: Brain and CNS

In a study with prenatal exposure to diclofenac, Yurt et al. used 3.6 mg/kg I.P. injections during pregnancy for Wistar albino rats and noticed that the general shape of hippocampal tissue was getting worse. These changes include more vacuolization in the cells and the structures outside of the cells. Damaged cells often have dark spots on them (Yurt et al. 2018).

Chan et al. in another study showed that embryos of rats lost their caudal neural tube normal development when exposed to the high concentration of diclofenac starting from 7.5 microg/ml, without damaging effect for lower doses less than 5 microg/ml (Chan et al. 2001).

In addition to previous studies, Kudo et al. investigated the effects of NSAIDs on neural stem cells (NSCs). They found that diclofenac 10 microM killed NSCs and inhibited their proliferation and differentiation into neurons. The researchers found that diclofenac forces NSCs to undergo apoptosis (Kudo et al. 2003). In the same point of view, another researcher Ragbetli et al. studied 1 mg/kg diclofenac on Purkinje cells of a developing cerebellum and found that diclofenac reduced the development of them in offspring (Ragbetli et al. 2007).

Heart

In a study, Fikret et al. used 1 mg/kg I.M. injections and injected pregnant rats between the 5th and 20th days. They noticed a decrease in the volume of the heart ventricle wall in the experimental rats (Fikret et al. 2015).

Another study, Mokhtar et al. detailed the heart organogenic abnormalities. The authors showed that the hearts of the neonatal rats treated with diclofenac 1.5 mg/kg I.P. injections showed massive degenerative changes when compared to the control group of the same age. There were extensive areas of hemorrhage between the cardiac muscle fibers, as well as extravasated hemorrhage. The blood capillaries were also markedly dilated and congested. In addition, extensive cytoplasmic vacuolation with nuclear pyknosis of cardiomyocytes and extracellular edema was also observed (Mokhtar et al. 2023).

Lung

Ragbetli et al. injected I.P. pregnant rats with a low dose of diclofenac at 1mk/kg from the 5th to 19th days to investigate its effect on lung development. The results revealed that the lung tissues of the rats looked normal under the microscope, regardless of their age,

gender, or whether they were treated with diclofenac (Ragbetli et al. 2011).

In the same study of Mokhtar et al., the pulmonary blood vessels near the terminal bronchioles were markedly dilated and congested. They showed several vacuolar degenerative changes in the mesenchymal cells of the lung parenchyma and epithelium of the terminal bronchioles. They also showed detachment and disruption of the epithelial lining of the terminal bronchioles, edema, and hemorrhage in the mesenchymal tissue, dispersed blood spots between the mesenchymal tissues, vacuolated cells in the wall of the congested blood vessel next to the bronchiole (Mokhtar et al. 2023).

Liver

Mokhtar et al. also studied the livers of neonatal rats treated with diclofenac I.P injections with 1. mg/kg and showed extensive damage. They showed central vein congestions and dilatations with extensive cytoplasmic vacuolation in hepatocytes. There were also some hemorrhagic areas in the intrahepatic area, with distorted epithelial, necrosis of many hepatocytes. In the portal area, the portal vein was markedly dilated and the bile ductules were proliferated. The walls of the bile ductules were also thickened (Mokhtar et al. 2023).

Kidney

Examination of the kidney tissue of fetuses of CD-1 mice treated with diclofenac 1.5 mg/kg I.P injections for 8 days from day 7 to day 20 of gestation showed that the glomeruli were atrophic and the capsular spaces were widened. The renal convoluted tubular cells had vacuolated cytoplasm and pyknotic nuclei. Some proximal tubules showed disruption of their apical brush. The lumina of some proximal and distal tubules was occluded with hyaline casts (Sabry et al. 2014).

In another study, Mustafa et al. used adult Wistar albino rats and injected them with three doses of diclofenac 3.6, 9, and 18 mg/kg between days 15 and 21 of pregnancy. They developed a study to investigate the dose-dependent teratogenic effect of diclofenac, where tubular degeneration and widening, tubular necrosis, nuclear pyknosis, mononuclear cell infiltration, congested vessels, and fibrous tissue increased level of damage with increased injected dose (Mustafa et al. 2019).

Bone

In a study, Abd El-Rhaman et al. administered pregnant rats with either 15.4 mg/kg diclofenac P.O on the 5th to the 13th day of pregnancy, or on the 13th to the 19th day. They found that fetuses had incomplete or no ossification in different parts of their skeletons, including the skull, vertebral column, ribs, sternum, forelimbs, and hindlimbs (Abd El-Rhaman et al. 2019).

Testes

Medical researchers injected 3 groups of Wistar albino rat diclofenac I.P with a low dose of 1/25 of the LD50 dose, a medium dose of 1/10 of the LD50 dose, and a high 1/5 of the LD50 dose as 18 mg/kg/ day. The

researchers counted the total number of spermatogonia (immature sperm cells) and Sertoli cells (supporting cells in the testes) in the testes of male rats. By the end of the study, the total number of Sertoli cells was significantly decreased in the medium and high-dose groups, in a dose-dependent manner. This means that the higher the dose of diclofenac, the greater the decrease in Sertoli cells. Medium and high doses of diclofenac also reduced the total number of spermatogonia. They suggested that prenatal administration of diclofenac can cause harmful effects on the development of the testes, especially in high doses (**Arslan et al. 2016**).

Uterus

In a study, Güven et al. studied the organogenesis of the uterus during I.P. administration of 1 mg/kg diclofenac for 10 days during pregnancy. They revealed a thicker cortex with a larger medulla, a decrease in the mean volume of oocytes, and a decrease in the uterine horn. In addition, they appeared to atrophy in growing follicles, corpora lutea, and the columnar epithelium of the horns of the uterus with mitotic figures and apoptotic bodies (**Güven et al. 2013**).

Placenta

A group of researchers by Mokhtar et al. gave rats 1.5mg/kg diclofenac I.P injections daily between the 7th and 14th day of gestation. They investigated the effect of different diclofenac on placenta tissue and showed that the placenta of the diclofenac-treated rats had signs of severe damage, including reduced cell numbers, cell death, and bleeding. This damage could potentially interfere with the placenta's ability to function properly, which could have negative consequences for the fetus.

The placenta of the diclofenac-treated rats showed significant changes, including a marked reduction in the number of islands of glycogenic cells (cells that store sugar). Instead, there were large, merging cysts containing eosinophilic cellular debris (the remains of damaged cells). Some glycogenic cells migrated into the labyrinth zone. The spongiotrophoblast cells (cells that form the outer layer of the placenta) were also reduced in number, and some were necrotic or pyknotic. The trophoblastic giant cells (large cells that help to transport nutrients to the fetus) were also markedly decreased in number and size, and their nuclei were shrunken or disintegrated.

In addition, the blood vessels in the basal zone of the placenta were dilated and congested. In the labyrinth zone, there was extensive hemorrhage with rupture of nearby blood vessels. There was also marked vasodilatation and congestion of both the maternal sinusoids (blood vessels in the mother's part of the placenta) and the fetal capillaries. Many of the trophoblastic cells in the labyrinth zone degenerated when compared to the control group (**Mokhtar et al. 2023**).

Toxicities in human studies

GIT

Back in the 19th physicians, Gibson et al. reported several cases of women suffered from stomach and other GIT toxicities from diclofenac. Four of them took NSAIDs dose between 50 mg/kg and 357 mg/kg twice per day P.O for a maximum of 90 days and developed acute ulcerative colitis. Their symptoms, lab results, and colonoscopy findings were similar to those of people with inflammatory bowel disease as weight loss, diarrhea, tender abdomen, and occult blood in stool, with a marked reduction in hemoglobin, hypoalbuminemia, and high ESR (**Gibson et al. 1992**).

In another study, Baert et al. wrote a case report on a woman who took diclofenac P.O. for almost two years for the treatment of chronic pain. She had acute bloody diarrhea that required hospitalization, the colonoscopy showed deep sores in the middle part of the colon, redness, and shallow sores scattered throughout the rest of the colon. The biopsy showed many distorted crypts (the tiny pits in the colon lining) with bleeding and inflammation. In another biopsy from the deepest part of the ulcer, a large granuloma (a cluster of inflammatory cells) was seen. This suggests that the patient may induce Crohn's colitis, a type of inflammatory bowel disease, but in the fact that she was suffering from diclofenac toxicity (**Baert et al. 1995**).

In a recent study in 2018, physicians recorded videos from video capsules swallowed by patients (62.3% of them were women) treated with mixed NSAIDs for their rheumatic diseases. They found that half of the patients suffered from gastric and duodenal erosions with ulcers, and hemorrhages in the stomach, duodenum, small bowel, and upper and lower colon. The physicians agreed to link the toxicities with the NSAIDs (**Balabantseva et al. 2018**).

Liver

Helfgott et al. published a case report in the Journal of the American Medical Association (JAMA) in which physicians reported seven patients developed acute severe hepatitis, associated with one death. The toxicity developed after several weeks of diclofenac and continued for many weeks despite discontinuation of the drug. In addition, during the re-challenge of the drug, one patient developed another episode of acute hepatitis. At the end of their report, they advised careful monitoring of the liver functions during NSAID administration especially diclofenac (**Helfgott et al. 1990**).

In another case series, physicians reported three patients developed chronic hepatitis during their treatment with diclofenac. The symptoms were abdominal pain, excess fatigue, Jaundice, loss of appetite, and fever that all disappeared after cessation of the drug and reappeared with continuation. Surprisingly symptoms did not happen with another class of NSAID (**Scully et al. 1993**).

The most recent study authors evaluated the liver toxicity of diclofenac, in which they reported the NSAID liver toxicities for the Drug-Induced Liver Injury Network (DILIN). They collected both

retrospective and prospective data that represent features and outcomes of patients with NSAIDs' liver toxicities. They concluded that most liver damage caused by NSAIDs is hepatitis, and diclofenac is the most common NSAID to cause this type of damage. Because there are many other NSAIDs available, diclofenac should only be used for patients who have not responded to other NSAIDs and who are at high risk of liver damage (Schmeltzer et al. 2016).

Kidney

Several months after registration of diclofenac, two patients induced renal function changes, and their physicians wrote a case report to describe this new toxicity. They reported isolated G1 nephropathy associated with diclofenac. First patient took oral 100 mg/kg daily for two and half months then she developed anorexia, leg edema, hypertension, and hypoalbuminemia. For treatment, she took indomethacin instead of diclofenac and surprisingly the symptoms disappeared. The second patient developed interstitial nephritis with persistent proteinuria after 20 days of oral 150mg/kg diclofenac daily and signs gradually disappeared within 3 weeks of stopping diclofenac(Beun et al. 1987).

A few years later, physicians reported acute renal failure in a woman who took diclofenac for her urinary tract infection. She developed signs of renal impairment with hyperkalemia that were revealed after stopping diclofenac (O'Callaghan 1994).

Brain

It is relatively uncommon to indicate brain or CNS toxicity due to the use of diclofenac, but researchers and physicians reported some of these toxicities sporadically in literature. The first report showed that 14 patients developed aseptic meningitis during their treatment from systematic lupus erythematosus; the majority of them took ibuprofen or diclofenac tablets and the psychiatrists reported several episodes of depression, psychosis, and impaired cognitive functions (O'Brien et al. 1985).

Heart

Several reports discussed the probability of the toxic effect of diclofenac on the human heart and tried to know the lowest toxic dose and specific route of administration. Researchers recently linked NSAID use in the elderly with a higher risk of heart failure. In a case-control study, authors found that people who took an NSAID, a non-specific type, in the previous week were twice as likely to be hospitalized for heart failure. This risk was ten times higher for those with a history of heart disease. The study also suggested that taking higher doses of NSAIDs for longer periods might increase the risk of heart failure. It is important to note that this study is observational, which means that it cannot prove that NSAIDs cause heart failure. However, the findings suggest that NSAIDs may be a risk factor for heart failure, especially in elderly people with a history of heart disease (Page et al. 2000).

In another study, Cooper et al. reached out for evidence that diclofenac might increase the risk of myocardial infarction (MI). The researcher used a daily

dose of 150 mg of diclofenac and found an increased risk of MI within only seven days (Cooper et al. 2019).

In a meta-analysis of more than 50 randomized controlled studies that concerned about the relation between NSAIDs and elevated blood pressures, researchers found that patients who took any type of NSAID complied from cardiac problems and their physicians increased their antihypertensive doses to control the cardiac stress. They also advised to careful use of NSAIDs with any antihypertensive drugs (Johnson et al. 1994).

In the end, scientists in the multinational etoricoxib and diclofenac arthritis long-term (MEDAL) program analyzed three big trials. They randomized more than 30 thousand patients to take either diclofenac 150 mg daily or etoricoxib 90 mg daily for a long duration. They concluded that diclofenac is associated with an increased risk of thrombotic events as well as etoricoxib (Cannon et al. 2006).

Eye

The National Registry of Drug-Induced Ocular Side Effects in the United States analyzed several reports of possible adverse optic nerve reactions associated with NSAID use. There reported severe corneal toxicity associated with the use of some topical NSAIDs, such as diclofenac and ketorolac. NSAIDs are generally safe for most people to take, but they can cause eye problems in rare cases. These problems can include blurred vision, serious optic nerve reactions, and severe corneal toxicity (Vonkeman et al. 2010).

In a report, scientists identified a new term called ocular diclofenac or corneal melting, which indicated corneal toxicity as irritation, ulceration of conjunctiva, and perforation after using diclofenac eye drops after cataract surgery (Flach 2001).

Pancreas

A more recent study found that the risk of pancreatitis varies significantly between different NSAIDs. Diclofenac and ketoprofen have the highest risk followed by indomethacin and ibuprofen with odds ratios of 5.0 and 4.8, 3.6 and 1.5, respectively (Sorensen et al. 2006).

Researchers made a systematic review of the pancreatitis induced by NSAIDs. They found that patients who took diclofenac were at higher risk of pancreatitis than any other class of NSAIDs (Pezzilli et al. 2010).

Skin

Some people who take diclofenac may develop skin reactions such as rash or itching. These reactions are usually mild and go away on their own. In rare cases, more serious skin reactions can occur, such as bullous dermatitis and erythema multiforme (Gulin et al. 2016).

Physicians wrote a study on children attending a rheumatology clinic who received diclofenac and were more likely to have facial scars of unknown origin. The scars were more common in children with light skin and blue or green eyes. It is not clear whether this is a type of phototoxic reaction, but pseudoporphyria-like eruptions associated with diclofenac (Hollingworth 1993).

The Drug Database for Acute Porphyria, maintained by the Norwegian Porphyria Centre

(NAPOS) and the Porphyria Centre Sweden, classifies diclofenac as a drug that is likely to cause porphyria. It should only be prescribed when necessary, and precautions should be taken in all patients.

Bone

No specific study showed bone toxicity for diclofenac, but many showed for other NSAIDs as they slowed down bone healing and cartilage damage, especially with osteoarthritis (**Harder et al. 2003**). Other studies showed that many NSAIDs, including COX-2 inhibitors, could reduce bone healing in experimental conditions. There is also some concern that some NSAIDs, such as ketolac, may accelerate cartilage destruction in people with osteoarthritis (**Glassman et al. 1998**).

Bone marrow and blood

Back in the late 80s, a large-scale study found a strong link between diclofenac use and aplastic anemia, with a ten-fold increase in their risk. Other blood disorders linked to diclofenac include hemolytic anemia, thrombocytopenia, neutropenia, and agranulocytosis (**Levy 1986**). Additionally, diclofenac has been associated with localized spontaneous bleeding, bruising, reduced platelet aggregation, and prolonged bleeding time (**Ahrens et al. 2006**).

Fertility

A Case report of four women with severe arthritis who were on long-term diclofenac treatment for their chronic pain and undergoing extensive investigation for infertility and physicians advised them to stop diclofenac as a first step to the implantations succeed (**Mendonça et al. 2000**). NSAIDs may cause reversible infertility in women. Prostaglandins are hormones that play a role in ovulation, and NSAIDs can block the production of these prostaglandins. This may interfere with ovulation and make it difficult for women to get pregnant. Now physicians advise women who are trying to conceive should avoid taking NSAIDs.

Teratogenicity

A group of gynecological physicians designed a clinical trial to investigate if diclofenac injections can cross the placenta. They selected thirty women undergoing surgical termination of pregnancy between 8 and 12 weeks gestation and gave them two doses of diclofenac before the procedure. They collected samples from maternal serum, amniotic fluid, coelomic fluid, and fetal tissue and analyzed them for diclofenac levels. They found diclofenacin in all fetal tissue samples at concentrations similar to those found in maternal blood samples. These results confirm that diclofenac can readily cross the human placenta in the first trimester of pregnancy, and cannot exclude the potential teratogenic effects of diclofenac in embryos (**Siu et al. 2000**).

Studies in animals have shown that diclofenac can cause heart problems, underdeveloped lungs, and kidney problems in developing embryos. Scientists don't fully understand how diclofenac causes birth defects, but they believe it may be related to its ability to block prostaglandins. Prostaglandins are important signaling molecules that play a role in many aspects of

development, including the development of the heart, lungs, and kidneys. Although there haven't been many studies on the teratogenic effects of diclofenac in humans, some evidence suggests that it may be associated with an increased risk of birth defects. For example, one study found that women who took diclofenac during pregnancy were more likely to have babies with heart problems, low birth weight, a risk factor for developmental delays and chronic diseases (**Nezvalová-Henriksen et al. 2013**).

Due to the potential risks of diclofenac-induced teratogenicity, the FDA recommends that pregnant women avoid taking diclofenac, especially during the second and third trimesters of pregnancy. If diclofenac is necessary, physicians should prescribe it at the lowest possible dose for the shortest possible time.

Mechanism of toxicity

GIT

NSAIDs damage the gastric mucosa through both local and systemic effects. Both effects vary across the dependency on PH. The local effect depends on it but the systemic effect does not. It is clear that the local effect only occurs due to the oral route of administration, and the systemic effect occurs due to any route as oral, injections, or others as they are all involved in COX-1 inhibition. COX-1 prevents the production of prostaglandin E2 (PGE2), which helps to protect the stomach lining by increasing blood flow and mucus production. It protects the stomach from damage from hydrochloric acid (HCL). Without PGE2 to protect it, the stomach lining can become damaged, leading to erosions or ulcers (**Sohail et al. 2023**).

NSAID-induced enteropathy is a complex process that involves multiple steps. The first step is direct damage to the cells lining the intestine. The second step is mitochondrial injury. This leads to a cascade of events that includes disruption of the cell membrane, increased permeability of the intestine, and invasion of the intestine by harmful bacteria. These events can all lead to the clinical manifestations of NSAID-induced enteropathy, such as erosions, bleeding, ulceration, and protein loss.

A recent study showed another way to intestine injury, through suppressing the gastric acid which helps to kill harmful bacteria in the stomach. When gastric acid is suppressed, these bacteria can survive and multiply, which can lead to the development of small bowel ulcers (**Watanabe et al. 2020**).

Liver

To figure out the possible mechanism of toxicity, some researchers evaluated the potential of diclofenac to induce liver injury through the adverse outcome pathways (AOP) concept. They used two animal species mice and dogs and confirmed the same pattern of the immune cause of induction of hepatitis. They identified three molecular initiating events (MIEs) for the AOPs: reactive metabolites catalyzed by CYP of infiltrated neutrophils and specialized hepatic Kupffer cells, and the acyl glucuronides metabolite produced by uridine diphosphoglucuronosyl transferase. These reactive metabolites bind to proteins and act as neo-antigens, which are new antigens that can trigger an

immune response. Antigen-presenting cells then present the neo-antigens to B and T cells, which lead to the activation of the immune system (Selvaraj et al. 2020).

Also, they identified six different key events (KEs) at the cellular level and up to four KEs at the organ level for the AOP of immune-mediated hepatitis in mice. These KEs include cellular stress response, signaling of adipocytokine with interferon-gamma, and signaling of chemokine. Finally, they defined six different KEs at the cellular level and three KEs at the organ level for the AOP of diclofenac-induced hepatitis in dogs. These KEs include invalid programming of the immune responses, mast cell activation, and infiltration of mast cells into the liver parenchyma (Selvaraj et al. 2020).

Kidney

Primary, physicians described renal toxicity of NSAID through its effect as blocking the normal dilatation of the kidney blood vessels that is caused by prostaglandins. This widening is usually not a problem for healthy people, but it can be for those who are sick, especially if they are dehydrated or have something

that is causing their blood vessels to constrict. In other words, NSAIDs can reduce the blood flow to the kidneys, which can be dangerous for people who are already sick or dehydrated (O'Callaghan 1994).

Bone

To understand how NSAIDs might impair bone healing, it is important to examine the steps involved in the healing process itself. Bone healing involves three main stages: inflammation, bone resorption, and new bone formation. Several studies revealed that prostaglandins play a crucial role in all three stages. Prostaglandins help initiate and maintain inflammatory responses, stimulate osteoclasts to break down old bone, and boost osteoblast activity to form new bones. Given the integral role of prostaglandins in healing and the actuality that NSAIDs block their production, it becomes obvious that NSAIDs hinder bone repair. By inhibiting COX enzymes and thereby preventing prostaglandin synthesis, NSAIDs not only achieve their anti-inflammatory effects but also impede the increased prostaglandin production essential for bone healing (Harder et al. 2003).

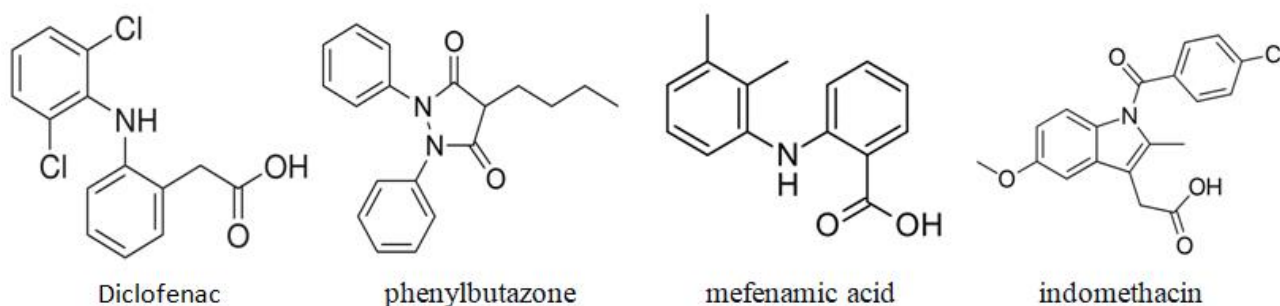


Figure (1): Skeletal structure of different NSAIDs (Sallmann 1986)

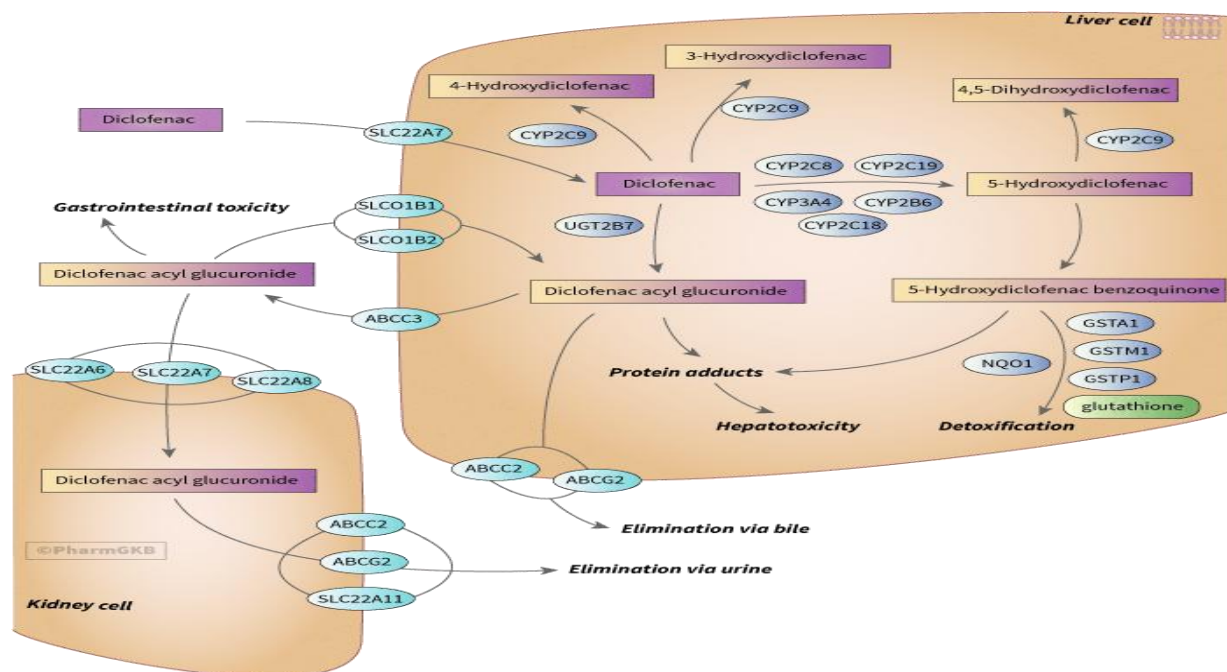


Figure (2): Metabolism and elimination of diclofenac inside liver and kidney cells (Whirl-Carrillo et al. 2021)

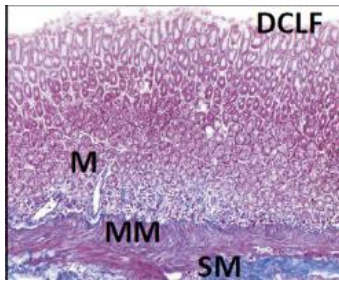


Fig (3): M, mucosa; SM, submucosa; MM, muscularis mucosa, DCLF, diclofenac treated. Using Masson trichrome staining, researchers assessed the severity of fibrosis in tissue samples. Weak blue staining indicated mild fibrosis, moderate blue staining indicated moderate fibrosis and strong blue staining indicated severe fibrosis. Groups treated with DCLF showed more fibrosis (Ayca et al. 2018).

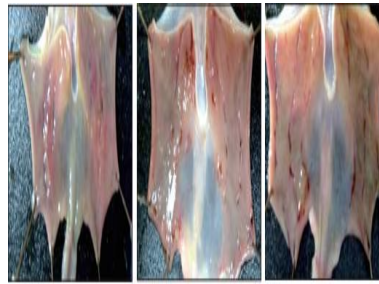


Fig (4): Effects of diclofenac on the stomach (40 mg/kg), 7 weeks, 25 weeks, and 1 year respectively (Seo et al. 2012).

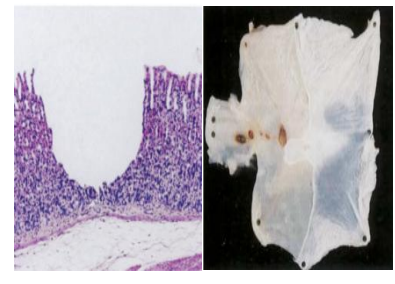


Fig (5): Microscopic and macroscopic appearance of gastric mucosa after repeated treatment of diclofenac. All drugs were administered orally to rats one time per day for 5 days. Stomachs were removed 8 h after the last 5th dose (Kim et al. 2005).

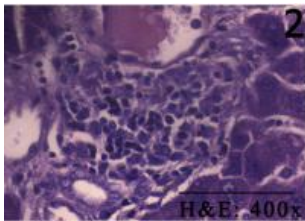


Fig 6, Liver histopathology (H&E: 400x) shows portal inflammation (Simon et al. 2019).

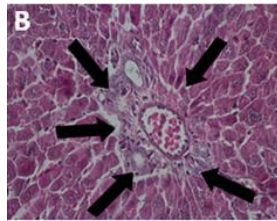


Fig 7, Black arrows show lymphocyte infiltration (Esmailzadeh et al. 2020).

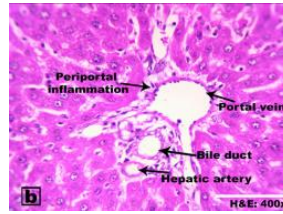


Fig 8, a Liver section with periportal inflammation and cells with pyknotic nuclei (Peter et al. 2017).

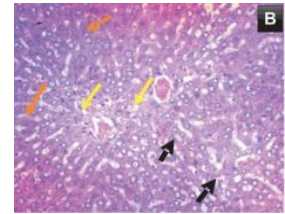


Fig 9, Dilated central vein, with paracentral, hepatocyte cell loss, prominent cell death with inflammation in the centrilobular areas (Alabi et al. 2017).

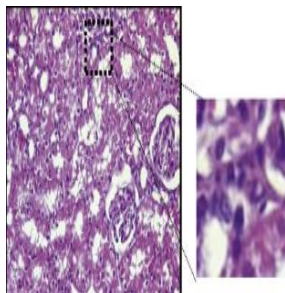


Fig (10): The kidney tubules were severely damaged, with some areas showing shrinkage (atrophy) and enlargement (dilatation). The cells lining the tubules were peeling off (desquamation) in some places, and there were vacuoles (fluid-filled sacs) in the cytoplasm of some cells. In some sections, there was a large number of inflammatory cells (infiltration) in the tissue between the tubules (Ayca et al. 2018).

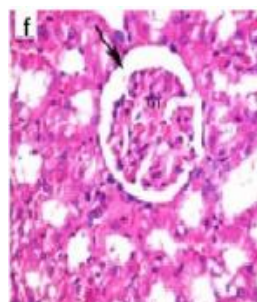


Fig (11): Proliferated Kupfer cells along with showing glomerular tuft (GT) with dilated congested blood capillaries, and dilated bowman's space (Arrow) (Anwar et al. 2022).

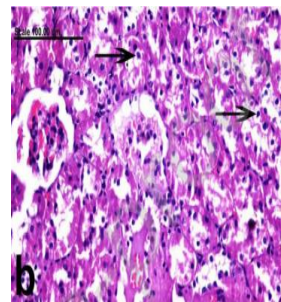


Fig (12): Bowman's space was enlarged and contained eosinophilic hyaline droplets. The epithelial cells lining the renal tubules were dead (necrosis), and their nuclei were either dead (necrotic) or dying (apoptotic). Mononuclear cells (inflammatory cells) had infiltrated the tissue between the tubules (Orabi et al. 2020).

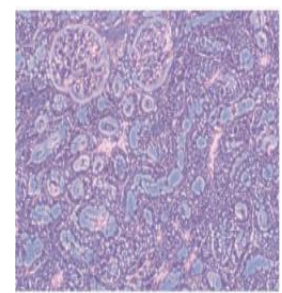


Fig (13): Tubuli containing protein and sloughed epithelial cells (Efrati et al. 2007).

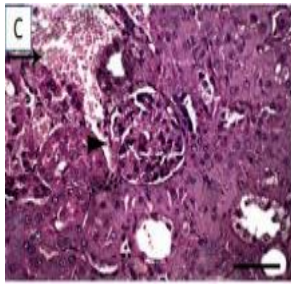


Fig 14, Proliferation of the renal glomeruli and severe hemorrhage in interstitial tissue displacing renal parenchyma (Elshopakey et al. 2021).

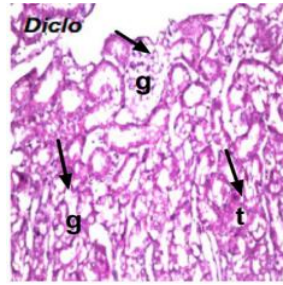


Fig 15, The endothelial cells lining the glomerular tuft were vacuolated (containing fluid-filled sacs), and the epithelial cells lining the tubules in the renal cortex showed severe fatty changes. Eosinophilic casts (plugs of protein) were present in the lumen of the tubules (Ahmed et al. 2017).

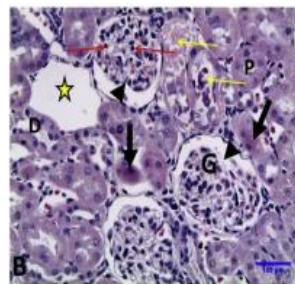


Fig 16, The glomerular tufts were shrunken, and Bowman's space was enlarged (as indicated by the arrows). Some glomerular cells were dead (necrosis), with shrunken nuclei (pyknotic nuclei). Some proximal convoluted tubules were also dead (coagulative necrosis). The distal convoluted tubules were damaged and contained hyaline and cellular casts (as indicated by the arrows). There was also fluid buildup (edema) in the tissue between the tubules (as indicated by the star) (Mousa et al. 2020).

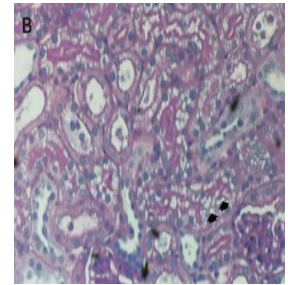


Fig 17, Double arrowhead indicates macula densa area of tubular cells (Hickey et al. 2001)

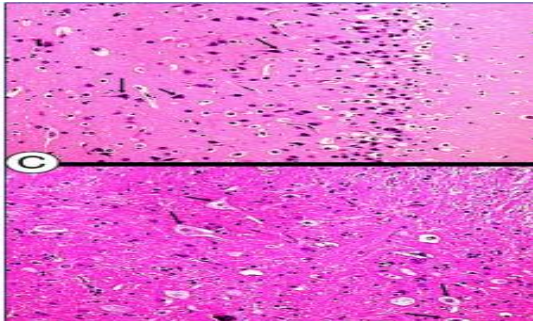


Fig (18): Red neurons in the cortex were severely damaged, without any signs of inflammation. Neurons in the dentate nucleus were also damaged, without any signs of inflammation (Ilic et al. 2011).

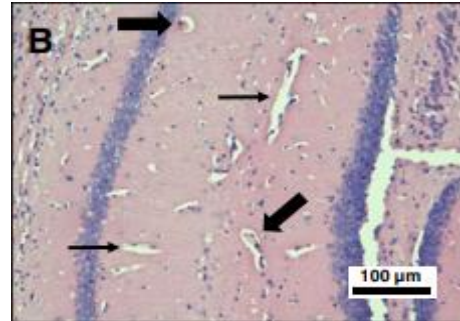


Fig (19): Blood vessels were widened (dilation) and congested (filled with blood, as indicated by the arrows) among normal neurons and glial tissue (Tan et al. 2013).

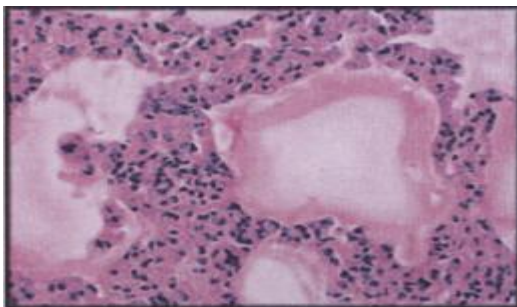


Fig (20): Lung edema (Tomic et al. 2008).

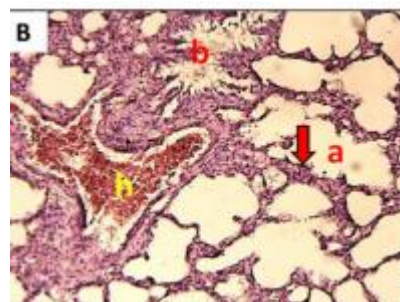


Fig (21): Normal bronchioles (b), thickened alveolar septa (red arrow), and intra-alveolar hemorrhage (h) (Al-Hayder et al. 2022).

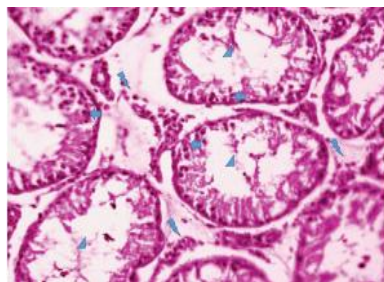


Fig (22): Increase in the fibrosis of interstitium and shrinkages in seminiferous tubules (Vyas et al. 2019)

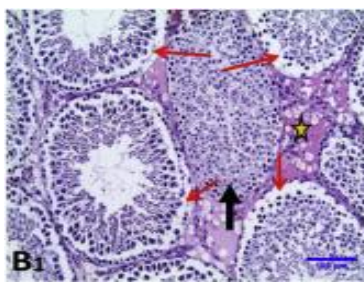


Fig (23): Some seminiferous tubules were damaged, with all of the germ cells shed into the lumen (desquamation) (as indicated by the thick arrow). Other seminiferous tubules had lost primary and secondary spermatocytes (as indicated by the thin arrow). There was also fluid buildup (edema) and vacuoles (fluid-filled sacs) in the tissue between the tubules (interstitial tissue) (as indicated by the star) (Mousa et al. 2020)

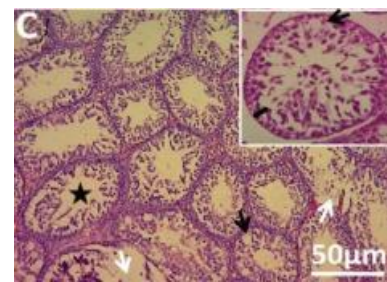


Fig (24): Some seminiferous tubules are damaged (degeneration, as indicated by the white arrow). The lumen of the seminiferous tubules is wide, and there are few sperms (as indicated by the asterisk). There are also clear vacuoles (fluid-filled sacs, as indicated by the black arrow) (T et al. 2022).

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مراجعة سمية الديكلوفيناك في الأعضاء المختلفة

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الملخص العربي

المقدمة: ديكلوفيناك هو دواء مضاد للالتهابات غير ستيرويدي (NSAID) متاح بدون وصفة طبية يعمل عن طريق تثبيط غير انتقائي لأنزيم سيكلوأوكسجيناز (COX)، وبالتالي يمنع تحرير البروستاجلاندين. يُعرف بفعاليته في تخفيف أعراض التهاب الألم والحمى، فقد أثرت مخاوف بشأن السمية المحتملة للديكلوفيناك. يمكن أن تسبب سميتها مجموعة واسعة من الآثار الضائرة، تتراوح من الآثار الجانبية المحدودة ذاتياً التي تختفي بعد إيقاف العلاج إلى زيادة خطر الوفاة بسبب أمراض القلب والأوعية الدموية والسكتات الدماغية. يعد تحديد الحد الأدنى للجرعة التي تميز بين الآثار الجانبية الخفيفة المحدودة ذاتياً والآثار الضائرة الأكثر شدة تحدياً كبيراً في أبحاث سمية الديكلوفيناك.

طريقة العمل: استعرضت هذه المقالة جرعات مختلفة وتسممات أعضاء مختلفة لوحظ في الدراسات الحيوانية والبشرية باستخدام الديكلوفيناك.

الهدف من هذه الدراسة: هدفنا هو تحديد سموم في أربعة عشر عضواً وتنظيمها في فئات مختلفة كسموم حيوانية وسموم بشرية وتشوهات خلقية من خلال التغيرات المرضية.

النتائج: من خلال البحث في قواعد البيانات المختلفة وجمع السموم المرصودة، خلصنا إلى أن الأعضاء المصابة بشكل أساسي هي المعدة والكبد والكلى. ومع ذلك، هناك معرفة محدودة بالتأثيرات السامة على الدماغ والقلب والرئتين والطحال والعين والعظام ونخاع العظام والدم والخضبتين، بينما تتوفر بيانات نادرة عن البنكرياس والمثانة والجلد والعضلات والرحم.

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