Section A -Research paper

TOXICOLOGICAL EFFECTS OF DICLOFENAC: REVIEW ARTICLE

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Abstract:

Background: Diclofenac is the most often prescribed non-steroidal anti-inflammatory drug worldwide. It is a phenylacetic acid derivative that works by inhibiting cyclooxygenase enzyme and has analgesic, antipyretic and anti-inflammatory activities. Like other non-steroidal anti-inflammatory drugs, it is sold over the counter without any control over the use or the dose, and although it is an effective drug with many useful effects, with its inhibition of prostaglandin biosynthesis, harmful effects occur in both animals and humans.

Aim: The current paper aims to gather information on diclofenac regarding its chemical composition, mechanism of toxicity and harmful effects.

Conclusion: In summary, research proved that diclofenac has multiple toxicological effects affecting many organs and it should be used in a controlled and supervised manner.

Keywords: diclofenac, free radicals, NSAIDS.

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Introduction:

Diclofenac, which is used to treat a variety of inflammatory conditions, is the most often prescribed non-steroidal anti-inflammatory medicine in the world. It lessens the prostaglandin manufacture from arachidonic acid because it is a strong cyclooxygenase inhibitor **(Izak-Shirian**

et al., 2022). Although it is classified as a non-selective COX inhibitor, in-vitro studies showed that diclofenac inhibits COX-2 more than COX-1. It is most commonly prescribed to relief pain **(Thomas and Richards, 2021).**

Millions of individuals worldwide use diclofenac mainly for the treatment of pain, inflammation, degenerative joint disease, rheumatoid arthritis, dysmenorrhea, and trauma **(Ahmed et al., 2020)**. Also, its market size is expected to raise revenue of 5.64 billion dollars by 2025, with a growth rate of 3.87% during 2020–2025 **(Sathishkumar et al., 2021)**.

It is mostly available in the form of Diclofenac sodium **(Thanagari et al., 2012)**, and its yearly global consumption has been estimated to be approximately 940 tons and although it is an effective drug, with its inhibition of prostaglandin biosynthesis, harmful effects occur in both animals and humans **(Owumi et al., 2020)**. It is the commonest offender in drug induced liver injury occurrence in an Egyptian cohort study **(Alhaddad et al., 2020)**.

Chemical and physical properties:

Diclofenac is a phenylacetic acid derivative (2-[2, 6-dichloranilino]phenylacetic acid) that is available in oral formulations in sodium, potassium, or sodium/misoprostol forms **(Gan, 2010)**.

The diclofenac molecule's main structural elements are a phenylacetic acid group, a secondary amino group, and a phenyl ring with two chlorine atoms in the ortho position to force maximum twisting of the phenyl ring in relation to the rest of the molecule **(Skoutakis et al., 1988)**.

Diclofenac has an acidity constant of 4.0 and a partition coefficient of 13.4 **(Sallmann, 1986).** In its unionized state, it is an acidic compound (p*K*a 3.80 at 25 °C) with very low water solubility (6×10−5 M at 25 °C) **(O'Connor and Corrigan, 2001).**

Mechanism of action:

Diclofenac is a member of a class of NSAIDs that blocks both COX-1 and COX-2 enzymes and it is more potent in inhibiting COX-2 than COX-1. The synthesis of prostanoids (i.e., prostaglandin [PG]-E2, PGD2, PGF2, prostacyclin [PGI2], and thromboxane [TX] A2) is impeded by diclofenac's binding to COX isozymes **(Altman et al., 2015).**

In addition, diclofenac inhibits phospholipase A2 both in vitro and in vivo **(Mäkelä et al., 1997)**. Phospholipase A2 generates arachidonic acid and lysophospholipids that generate pro-inflammatory eicosanoids and platelet activating factor **(Nanda et al., 2007).**

A more recently discovered mechanism involves Gamma peroxisome proliferator-activated receptors (PPARγ), which are receptors involved in fatty acid metabolism, macrophage differentiation, and inhibition of tumour cell proliferation. Diclofenac's affinity for PPARγ is 50 times higher than any NSAIDs. In the synovial cells of rheumatoid arthritis patients, diclofenac increases PPARγ activity, which inhibits cell proliferation by lowering cell viability and triggering apoptosis. Additionally, PPARγ ligands have anti-inflammatory properties **(Atzeni et al., 2018)**.

Toxicokinetics:

Commonly used routes of diclofenac administration are oral, intramuscular, intravenous, transdermal and rectal **(Singh et al., 2011).**

Diclofenac preparations pair the drug with a salt such as sodium, potassium, or epolamine salt. Diclofenac sodium can be administered orally as a tablet or suspension, intramuscular in solution, intravenous in solution, transdermal in gel, or rectal routes as a suppository. Diclofenac potassium is available for oral administration in oral tablet or suspension forms. Diclofenac epolamine is available as a transdermal patch **(Alfaro and Davis, 2020).**

Following oral administration, systemic diclofenac absorption is often quick and directly proportionate to the dose. The potassium salt of diclofenac is more rapidly absorbed **(Altman et al., 2015)**.

The drug has a short elimination half-life of 2 - 3 hours in most species including humans, with an average of 1.5 hours in plasma but accumulates at the site of inflammation and tends to persist in synovial fluid **(Orinya et al., 2016)**.

Diclofenac exhibits a high level of plasma protein binding **(Auler Jr et al., 1997)**, and goes through extensive hepatic metabolism with 4'-hydroxy-diclofenac being the main metabolite **(Skoutakis et al., 1988)**.

Diclofenac is metabolized in the liver to 4-hydroxydiclofenac by CYP2C9 and a minor metabolite, 5-hydroxydiclofenac, by CYP 3A417, 18 and other hydroxylated forms. These metabolites undergo phase II reaction such as glucuronidation and sulfation prior to excretion in the urine (65%) and bile (35%) **(Owumi and Dim, 2019).**

Mechanism of toxicity:

The mechanism of diclofenac induced toxicity involves mitochondrial dysfunction and elevated oxidative stress **(Aycan et al., 2018).** Oxidative stress and genomic DNA fragmentation are caused by reactive oxygen species generated during the metabolism of diclofenac **(Mousa et al., 2020)**.

Diclofenac releases cytochrome c from the mitochondrial permeability transition pores, which causes the production of an excessive amount of free radicals, that in turn stimulate the caspase cascade and lipid peroxidation, which leads to cellular apoptosis **(Izak-Shirian et al., 2022)**.

The mechanism also includes the reaction between diclofenac reactive metabolites and cellular macromolecules, which changes the integrity of proteins. The binding of diclofenac to macromolecules and significant liver proteins is amplified by low intracellular levels of nicotinamide adenine dinucleotide (NADH), reduced glutathione (GSH), nicotinamide adenine dinucleotide phosphate (NADPH), and other reducing agents **(Owumi et al., 2019)**.

It also induces lipid peroxidation and depletion of GSH and Adenosine triphosphate (ATP) **(Orabi et al., 2020),** and has been reported of causing accumulation of cellular polyunsaturated fatty acids (PUFAs) which seems to be ultimately harmful **(Aslan et al., 2020)**.

Toxicological effects of diclofenac:

After therapeutic usage in humans and pharmacological investigations on lab animals like rats and dogs, a number of unfavorable diclofenac side effects have been reported **(Schwaiger et al., 2004).**

1) Gastrointestinal effects:

The NSAIDs' gastrointestinal (GI) side effects, which affect 10–60% of patients, are a defining characteristic of this pharmacological family. The side effects can include a wide range of symptoms ranging from mild dyspepsia with pyrosis to fully established stomach or intestinal ulcers. A life-threatening condition might arise when the ulcerations become compounded by acute bleeding and perforation **(Vostinaru, 2017)**.

Diclofenac has more specificity to COX-2 than COX-1 and is associated with a relatively low level of GI toxicity compared with the other NSAIDs **(Gan, 2010).** The GI adverse events occur as a result of decreased prostanoids synthesis, which limits mucus and bicarbonate secretion, which typically protects the gastric mucosa from injury **(Atzeni et al., 2018).**

Diclofenac leads to bleeding of gastric mucosa, deficiency in gastric blood flow and apoptosis. Diclofenac injected rats showed a significant increase in GI ulcer count, indicating severe GI damage as evidenced by histopathological changes which included abnormal histological structure of the gastric mucosa as was manifested by sloughing of superficial secreting cells along with inflammation and necrosis of gastric glands. Intestinal sections showed distorted histological structure of the villi **(Mostafa et al., 2020).**

2) Renal effects:

Although Diclofenac is the most prescribed global NSAID, its harmful effect on the kidney represents a real obstacle to its clinical application **(Izak-Shirian et al., 2022)**.

According to **Abdou et al. (2021),** diclofenac resulted in significant renal impairment as evidenced by marked rise in serum levels of creatinine and urea, which are markers of renal dysfunction, and also marked rise in serum kidney injury molecule-1 (KIM-1) levels, which is a major biomarker for the diagnosis of proximal renal tubular injury and a sensitive indicator of acute kidney injury (AKI).

Diclofenac causes degenerative alterations, brush border damage, and finally a reduction in the quantity of both proximal and distal convoluted tubules in the kidneys of rats through its inhibitory effects on renal prostaglandins **(Orabi et al., 2020)**.

3) Hepatic effect:

According to **Alabi and Akomolafe (2020)**, the plasma levels of liver enzymes in rats treated with diclofenac, such as alanine transaminase (ALT) and aspartate transaminase (AST), and alkaline phosphatase (ALP) were significantly increased. Superoxide dismutase (SOD), catalase (CAT), and GSH were significantly decreased while the levels of Malondialdehyde (MDA) increased.

Diclofenac also leads to histopathological changes, such as liver degeneration and lymphocytic cell infiltration **(Nouri et al., 2019)**.

4) Effect on male fertility:

Diclofenac causes adverse changes at the levels of seminiferous tubules, and germ, Leydig, and Sertoli cells. This leads to disruption of androgen biosynthetic activity and the capacity of the testis to nourish the sperms **(Waly et al., 2022).**

According to **Tella et al. (2022)**, diclofenac caused significant decrease in sperm count and motility. It also caused interstitial edema of the testis.

5) Cardiovascular effect:

According to **Dolanbay et al. (2021)**, Cardiac tissue of rats treated with diclofenac showed histopathological damage. The electrocardiography (ECG) data showed a significant prolongation in the QT interval, a significant increase in heart rate, and a significant widening of the QRS complex. This can be explained by either a delay in action potential time or a slowing of conduction as a result of the cardiac conduction pathway being affected.

Diclofenac also causes hypertension and heart failure as a result of fluid retention **(Al-Saady et al., 2011)**. Compared with other NSAIDs, it causes more cardiovascular health risks and its use may increase atrial fibrillation/flutter, ischaemic stroke, heart failure, myocardial infarction, and cardiac death **(Aljuhani et al., 2019)**.

6) Central nervous system:

In the developing mammalian brain, neural stem cells (NSCs) can differentiate into neurons, astrocytes, and oligodendrocytes and are thought to be a key source of neurons. Diclofenac caused the death of NSCs, also inhibited their differentiation into neurons. It could disturb embryonic development and have a teratogenic effect on the CNS. Since NSCs are present in the adult brain, diclofenac may interfere with neurogenesis in the adult brain as well as the developing brain. **(Kudo et al., 2003).**

7) Teratogenic effect:

The United States Food and medication Administration (FDA) has determined that diclofenac is a pregnancy risk class C medication **(Chae et al., 2015).**

Diclofenac treatment during pregnancy greatly increases the apoptosis of fetal neural cells in rats **(Gokcimen et al., 2007).** Furthermore, pregnant rodents given diclofenac had fetuses with significant morphological anomalies, such as limb and palate deformities and ductus arteriosus **(Chan et al., 2002).**

8) Hematological effect

According to **Orinya et al. (2016),** there were significant alterations in hematological parameters of rats treated with diclofenac including significant reduction in packed cell volume, red blood cells and hemoglobin values that may indicate excessive destruction of red blood cells resulting in anemia. It may also be due to loss of erythrocytes as a result of GI bleeding.

In diclofenac treated rats there was also an increase in white blood cells and lymphocytes count but reduced neutrophils and eosinophil level **(Owumi et al., 2019)**.

Salama and Mueller-Eckhardt (1991) declared that auto antibodies and drug or metabolites dependent antibodies have been found in patients with diclofenac induced immune hemolytic anemia.

Alabi and Akomolafe (2020) reported that there was an increase in the numbers of platelets in the blood of rats chronically treated with diclofenac. The increase in platelets count

might be due to tissue injury and inflammatory response caused by the toxic metabolites of diclofenac.

9) Toxic effect on lungs:

Aljuhani et al. (2019) stated that diclofenac acute toxic overdose in rats produced substantial congestion of alveolar capillaries, alveolar haemorrhages, thick edematous alveolar walls, edema fluid exudates in the alveoli, and infiltration of lung tissue by inflammatory cells and haemosedrine laden macrophages.

10) Allergic reaction:

Allergic reactions, such as anaphylactic and anaphylactoid reactions, can occur with diclofenac like the rest of the NSAIDs family, even without earlier exposure to the drug **(Pejcic et al., 2023)**. Severe allergic reactions to diclofenac include anaphylactic reaction, anaphylactic shock, and Kounis syndrome **(Sen et al., 2001)**. Even fatal allergic reactions have been reported **(Alkhawajah et al., 1993).**

Intravenous diclofenac can cause an anaphylactic reaction that mimics pulmonary embolism **(Singh, 2011).**

Conclusion: In summary, research proved that diclofenac in spite of its multiple beneficial effects has several toxicological effects on different organs.

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