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DICLOFENAC: A Review on Its Synthesis, Mechanism of Action, Pharmacokinetics, Prospect and Environmental Impact

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REVIEW

DICLOFENAC: A Review on Its Synthesis, Mechanism of Action, Pharmacokinetics, Prospect and Environmental Impact

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ABSTRACT

Diclofenac, potent a nonsteroidal anti-inflammatory drug, used in the treatment of inflammation, pain and fever associated with a wide range of disease condition. This review article focuses the synthesis, mechanism of action, pharmacokinetics and derivatives of diclofenac and also highlights advances in the possible use of diclofenac in treating other condition outside its current clinical therapeutic use as an analgesic, anti-inflammatory and antipyretic agent as well the prospect of drug repurposing of diclofenac for treatment of disease like cancer. It further highlights the adverse effect of the drug as an emerging contaminant in the environment. Diclofenac acts by inhibition of COX enzymes and subsequently reduces the synthesis of prostaglandins, the predominant prostanoid produced in inflammatory processes. Diclofenac is primarily metabolized in the liver and is eliminated in the urine and bile as conjugates of diclofenac and its metabolites. Despite its effectiveness in management of pain, inflammation and fever, there are reports of adverse gastrointestinal, cardiovascular and renal effect resulting from its systemic use, suggesting the need for more research on its drug modification in order to reduce or eliminate the adverse effects. Research suggests that diclofenac has antimicrobial, anticancer and neuroprotective property. As an anticancer agent, research suggests diclofenac exhibit anti-proliferative and apoptosis mediating property. Diclofenac arrest cell cycle, thus preventing proliferation of neural stem cells, glioblastomas, ovarian cells, osteoblasts, human lymphatic endothelial cells, and vascular smooth muscle cells and induces apoptosis of neuroblastoma cell, this project this drug as a novel prospective anticancer agent. However, its adverse environmental effects as an emergent contaminant still remains a source of concern.

Keywords: Diclofenac, Analgelsic, Cyclooxygenase (COX), Property, Mechanism

1. Introduction

Pain and inflammation are common symptoms associated with a wide range of diseases and one of the most widely used medications to address this condition are nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are among the most often prescribed over-the-counter (OTC) drugs for lowering fever, pain, and inflammation; making up 5-10% of all prescription drugs and market size estimated at \$15.58 billion in 2019 and is expected to grow to about \$24.35 billion by 2027 globally [1]. NSAIDs

are a class of drugs that exercise their actions by inhibiting cyclooxygenases (COX) non-specifically [2]. COX is the enzyme that synthesizes prostaglandins, which mediate the process of pain and inflammation. The most often prescribed NSAID in the world for the treatment of pain and inflammation in a variety of conditions, such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis is diclofenac [3]. Additionally, diclofenac is approved for treating cataract extraction, ocular pain, and photophobia and applied topically to treat actinic keratosis; a skin disorder characterized by abnormal keratinocyte

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proliferation [4]. As the most widely used NSAID, it has a market share that is nearly equal to the sum of the next three most popular medications (ibuprofen, mefenamic acid, and naproxen). Adverse effects of diclofenac include cardiovascular (CV), kidney, and gastrointestinal injury and these are dose-dependent while prolonged usage at very high dose may result in gastrointestinal (GI) issues such as bleeding, ulcers, perforation, and enteropathy [5]. There are also reports of cases of cardiovascular conditions such as myocardial infarction, stroke, and thrombotic events. Patients may also experience liver damage, platelet inhibition, and renal failure, among other conditions [6].

This review article discusses the synthesis, mechanism of action, pharmacokinetics and derivatives of diclofenac and also highlights advances in the possible use of diclofenac in treating other conditions outside its traditional therapeutic use as an analgesic, anti-inflammatory and antipyretic agent as well as the prospect of drug repurposing of diclofenac for diseases treatment of disease like cancer. It also discusses the adverse effect of the drug on the environment.

2. Synthesis of diclofenac

Rudolf Pfister and Alfred Sallmann synthesized diclofenac for the first time in 1973 [7]. Diclofenac is a member of the phenylacetic group of NSAIDs. The monocarboxylic acid diclofenac is made up of phenylacetic acid with (2,6-dichlorophenyl) amino group at the second carbon atom. It is a weak acid with an acid constant of 4 and partially soluble in both water and nonpolar solvent with a partition coefficient of 13.4. From the structure (Fig. 1: Compound 1.5), it is also an amino acid as well as an aromatic amine. The

molecule's structural characteristics, which include a phenylacetic acid group, a secondary amino group and a phenyl ring with two chlorine atoms, allow the phenyl ring to twist as much as possible and fit well in the COX enzyme's substrate-binding site as a competitive inhibitor [8].

Diclofenac (2-[(2,6-dichlorophenyl)-amino]-phenylacetic acid) can be produced from 2,6-dichloroaniline and 2-chlorobenzoic acid (R). In the presence of sodium hydroxide and copper these compounds react to give N-(2,6-dichlorophenyl)anthranilic acid. Lithium aluminum hydride, a reducing agent then reduces the carboxylic group of this acid to give 2-[(2,6-dichlorophenyl)-amino]-benzyl alcohol which is then chlorinated further by thionyl chloride to form 2-[(2,6-dichlorophenyl)-amino]-benzyl chloride. 2-[(2,6-dichlorophenyl)-amino]-benzyl chloride reacts with sodium cyanide to give 2-[(2,6-dichlorophenyl)-amino]benzyl cyanide. Diclofenac is produced when the nitrile group of 2-[(2,6-dichlorophenyl)-amino]benzyl cyanide is hydrolyzed in the presence of sodium hydroxide Fig. 1.

2.1. Diclofenac sodium

Sodium o-dichloroanilinophenylacetate commonly known as diclofenac sodium, is a white, crystalline hygroscopic powder that is odorless, soluble in acetone, methanol, ethanol, and with a slight water solubility. It is the most widely used anti-inflammatory medication due to its good anti-inflammatory and analgesic effects as well as safety. It is used in the clinical treatment of various forms of arthritis and

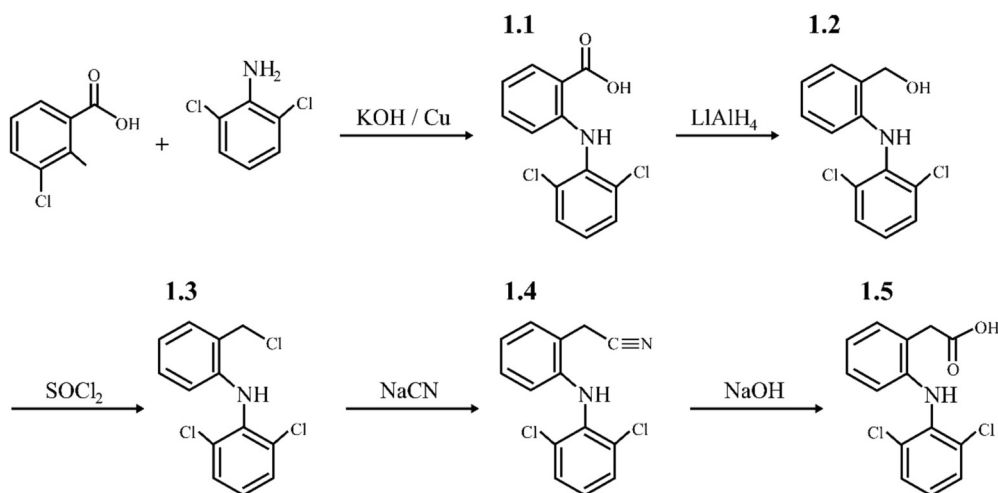


Fig. 1. Synthesis of diclofenac [136].

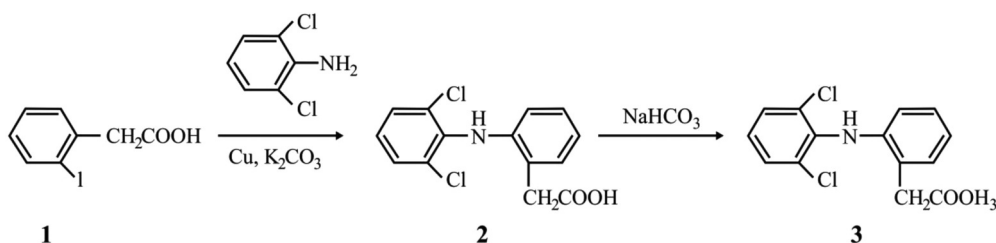


Fig. 2. Synthesis of diclofenac sodium from o-iodophenylacetic acid and 2,6-dichloroaniline [136].

other joint pain, neuralgia, pain throughout the body, and all types of inflammation caused by fever [9].

The first successful synthetic of diclofenac sodium was achieved in 1975; following this, numerous publications exist regarding the preparation techniques. The methods of synthesis include [10].

2.1.1. O-iodophenylacetic acid method

The method involves a condensation reaction between 2,6-dichloroaniline and o-iodophenylacetic acid using copper powder and potassium carbonate as a catalyst (Fig. 2). One limitation of this method is that the yield is low and silica gel column chromatography is required to separate it. The process is more complicated and high-efficiency catalysts and better reaction conditions are still required. The profit is better if the yield can be increased. However, this method is used by some pharmaceutical industries in China [11].

2.1.2. Cyclohexanone method

This method uses triphenylphosphine as a catalyst to first synthesize compound 2 from cyclohexanone in a carbon tetrachloride solution. (Fig. 3). Condensation reaction between Compound 2 and aniline using anhydrous titanium tetrachloride, as dehydrat-

ing agent produced Compound 3. By derivatization at 100°C, Compound 3 was dehydrochlorinated, and the reaction carried out in chlorobenzene to yield Compound 4. Compound 5 is the result of reacting excess chloroacetyl chloride with compound 4. Reaction between Compound 5 and anhydrous aluminum trichloride for two hours gave compound 6. In Finally to synthesize diclofenac sodium, compound 6 was hydrolyzed by ring-opening in an ethanol solution of sodium hydroxide [12]. The diclofenac sodium that is produced is highly pure, and the process is straightforward and easy to perform, although the catalyst triphenylphosphine is rather costly. Another limitation of this method is the synthesis of compound 3 requires the use of titanium tetrachloride, however the ratio of titanium tetrachloride affects compound 2's yield, and the yield of this method is poor [13].

Even though cyclohexanone is the starting material in the cyclohexanone method, 2,6-dichlorodiphenylamine can be synthesized with a relatively high purity; however, the three wastes are difficult to handle, the process is complex, and there is significant environmental pollution. Environmental protection agencies in some places have taken strict action to ban this process, and they have been gradually eradicated.

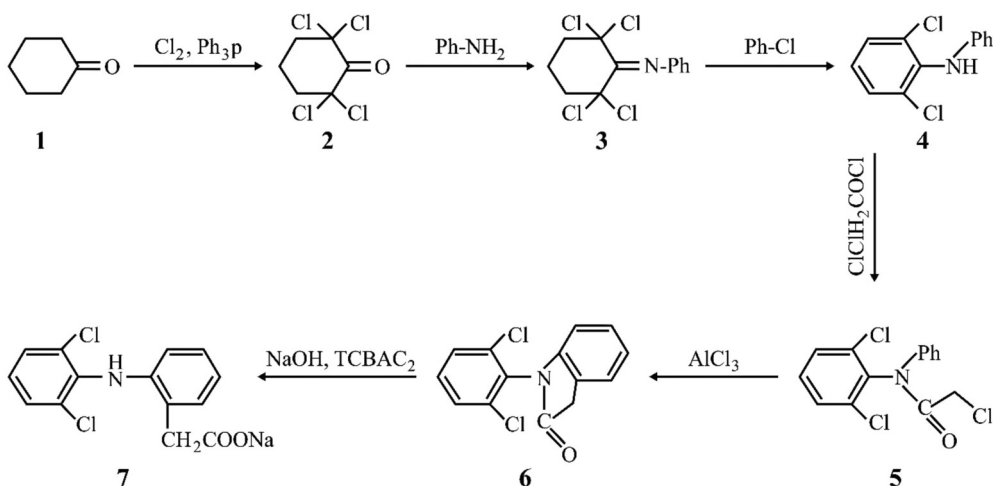


Fig. 3. Synthesis of diclofenac sodium from cyclohexanone [9].

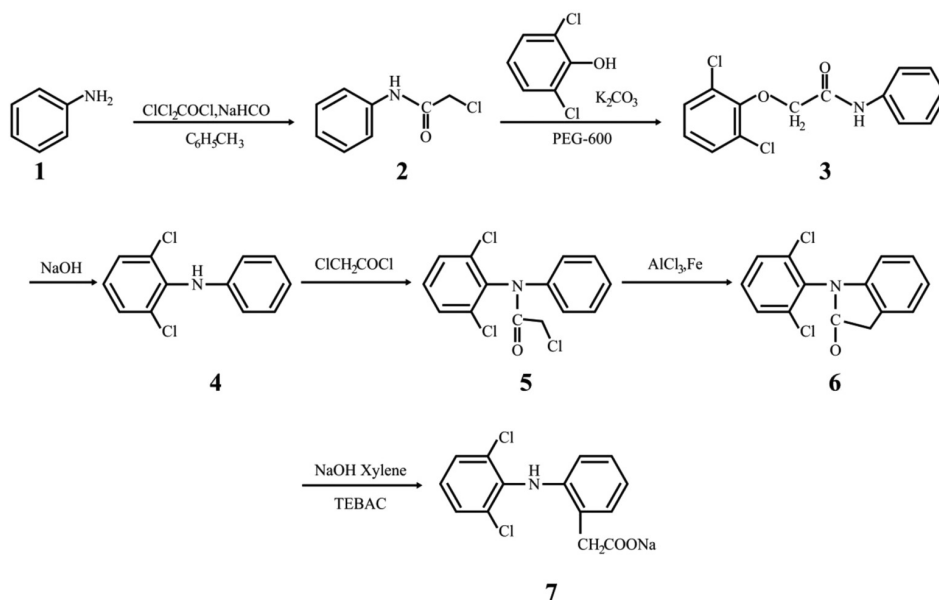


Fig. 4. Synthesis of diclofenac sodium from aniline [136].

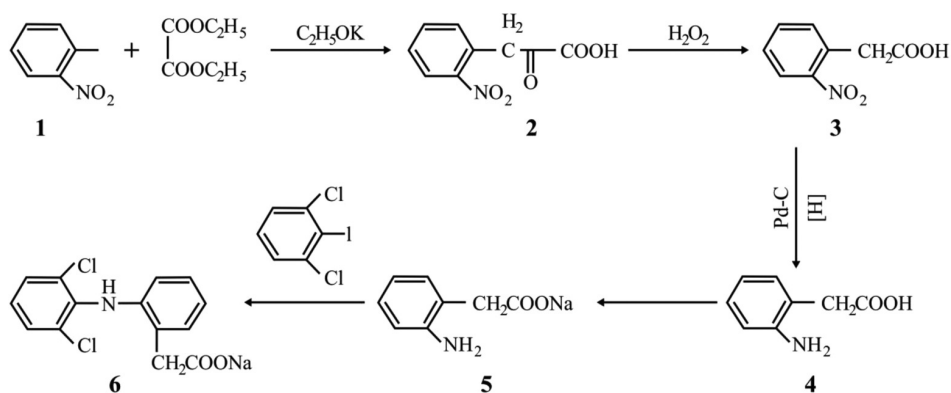


Fig. 5. Synthesis of diclofenac sodium from o-aminophenylacetic acid [137].

2.1.3. Aniline method

Through three stages of chloroacylation, condensation, and rearrangement reaction, the intermediate 2,6-dichlorodiphenylamine was synthesized using aniline as the starting material (Fig. 4). Following this, diclofenac sodium can be synthesized by cyclization, chloroacetylation and hydrolysis. Diclofenac sodium can be synthesized using this easy-to-understand method with a high yield that can exceed 80%. One limitation of the method is toluene is which is required as a solvent before compound can be synthesized has purchasing and management issues. If this solvent can be replaced, this synthesis technique will be perfect [14].

2.1.4. O-aminophenylacetic acid method

The initial step of this procedure require potassium ethoxide, hence it operates without the use of water

(Fig. 5). Contact hydrogenation is the process utilized for nitro reduction; compound 4 is unstable and prone to condensation and self-dehydration, which yields 2-hydroxyindole. In addition, the process of synthesizing dichloriodobenzene is a complex one. One limitation of this method is the process involves a lot of reaction steps and a stringent reaction process [15].

2.1.5. Indolone method

This is one of the best method since there is easy access to raw materials, easy reaction conditions, a straightforward operation as well as over a 50% yield. However, it requires Isatin (Compound 1 in Fig. 6), a dye with some toxicity that has been as a class 3 carcinogen by the World Health Organization's International Agency for Research on Cancer [16].

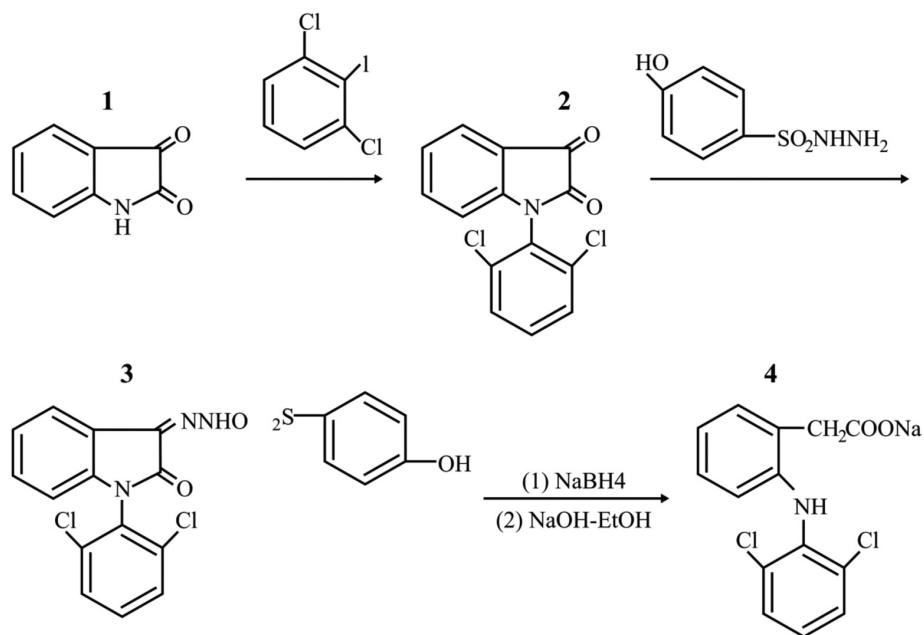


Fig. 6. Synthesis of diclofenac sodium using the indolone method [138].

2.1.6. Other method

This method's attributes include easy access to raw materials, low cost, straightforward operation, high yield, conditions are mild and a straightforward post-processing. However, the addition of a chlorinating agent to synthesize compound 3 (Fig. 7) during the procedure makes the result harder to purify [17].

3. Mechanism of action of diclofenac

Diclofenac acts by preventing the production of prostanoids such as prostaglandin-E2 (PGE2), prostacyclins, and thromboxanes, which are vital elements of the inflammatory response and sensations of pain, through the inhibition of the activities

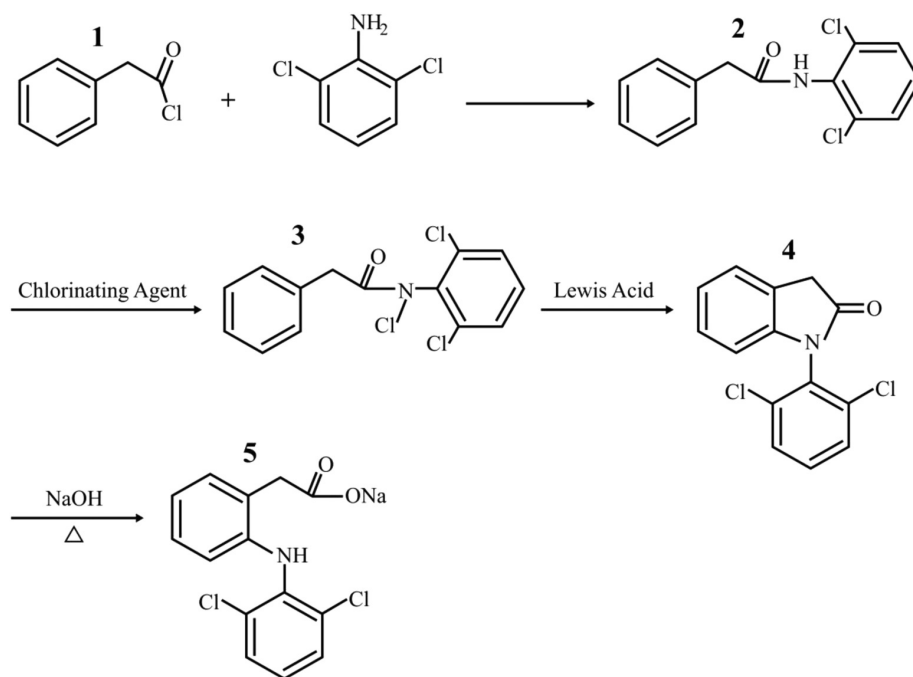


Fig. 7. Synthesis of diclofenac sodium from other method [136].

of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [18]. It prevents arachidonic acid from binding to COX-1 and COX-2 in a competitive manner. Because NSAIDs blocks PGE2 synthesis, the predominant prostanoid produced in inflammatory processes, it is thought that this is the primary mechanism underlying the drugs' strong analgesic and anti-inflammatory effects. Diclofenac is considered to be one of the most potent inhibitors of PGE2 synthesis [19].

Diclofenac inhibits COX-1 and COX-2 relatively similarly; however, unlike the majority of conventional NSAIDs, data suggests that it has preferential COX-2 inhibition, almost four fold that of COX-1 inhibition during in vitro studies. The level of COX-2 selectivity exhibited by diclofenac is similar to celecoxib [3]. Diclofenac inhibits COX-2 isoenzymes more strongly than COX-1 isoenzymes. In addition, it has been demonstrated that selectivity is sometimes dose dependent and that the estimated IC_{50} (concentration producing 50% inhibition of activity) values for COX-1 and COX-2 for various COX inhibitors vary between models [20].

Being a constitutively active enzyme, COX-1 is expressed almost everywhere in the human body. The concentration and activity of COX-1 are believed to be rather constant and it plays a role in a number of activities, including blood flow into renal tissues, the preservation of normal platelet function, and safeguarding of the gastrointestinal mucosa against damaging acidity [21]. Unlike CoX-1, COX-2 is an inducible enzyme and is overexpressed due to the presence of inflammatory mediators such as prostaglandins, thromboxanes, and leukotrienes that also have nociceptive characteristics and cause pain and also in situations involving tissue injury [22]. The inhibition of COX-2 that diclofenac produces appears to be mostly focused at the target tissues, such as joint capsules and synovial fluid. However, the suppression of COX enzymes in other tissues like the stomach may result in the loss of several protective compounds and, among other things, result to gastrointestinal discomfort [23].

Diclofenac's ability to down-regulate sensitized peripheral pain receptors is responsible for its peripheral analgesic effects. This appears to be achieved by activating ATP-sensitive potassium channels, which in turn stimulates the L-arginine nitric oxide cGMP pathway [24]. Furthermore, data indicates that diclofenac may also have an effect on lowering concentration of substance P, a pro-inflammatory neuropeptide with nociceptive action, which was previously elevated in the synovial fluid of rheumatoid arthritis patients [25].

Studies have shown that diclofenac triggers other action pathways that contribute to its analgesic effect, in addition to its recognized mode of action, which is to block prostaglandin synthesis and is shared by the other nonsteroidal analgesics. The involvement of endogenous opioids, serotonin, noradrenalin l- is seen in these pathways [26].

4. Pharmacokinetics of diclofenac

When diclofenac is administered orally, its systemic absorption is fast and in a dose-dependent manner. The drug content, time of administration in respect to food consumption, and salt form are other factors that can affect how quickly diclofenac is absorbed [10]. Analysis of systemic absorption of the drug using plots of diclofenac concentration vs time shows varying maximum plasma concentration (C_{max}) and time to taken to reach maximum plasma concentration (t_{max}), along with the existence of late or secondary plasma peaks, indicating inconsistency in absorption of the drug [27]. Depending on the dosage form such as enteric coated pills, solutions, etc and individual-based parameters such as stomach pH, the peak plasma concentration occurs between 10 minutes and 2 hours [28]. The partial precipitation of the dose in the stomach's acidic environment, variable timing of gastric emptying, variations in pH of gastrointestinal tract of individuals and enterohepatic circulation have all been suggested as causes of these inconsistency in observed in diclofenac absorption [29].

Diclofenac is transported bound mainly by the plasma transport protein albumin. It concentrates in systemic circulation as well as tissues with inflammations. In inflamed tissues, the weakly acidic environment decreases plasma protein binding resulting in the release of the free drug (unbound diclofenac) [30]. This increases the concentration of the free drug at these sites and promoting its diffusion into intracellular spaces, where it can have therapeutic effects. Concentration of diclofenac at the synovial fluid results in levels that eventually surpass plasma levels and this continues even after plasma levels have significantly dropped [31]. Following the administration of a 50-mg enteric-coated tablet and a 100-mg slow-release tablet, diclofenac delivered as the sodium salt was detectable in synovial fluid for up to 11 hours and up to 25 hours, respectively. The duration of diclofenac's therapeutic effect that lasts longer than its plasma half-life may be explained by its persistence at the site of inflammation and its inhibition of COX-2 enzymes in the inflammatory

cells. It is unknown whether diffusion into the synovial fluid of joints accounts for the therapeutic efficacy of diclofenac [26]. Nonetheless, the extended pharmacological half-life of diclofenac following high dose administration may potentially contribute to its protracted therapeutic effect. Diclofenac sodium treatment has been demonstrated in various studies to considerably reduce PGE2 levels in synovial fluid and also decreases levels of inflammatory cytokines such interleukin-6 and substance P [32].

First-pass metabolism allows for the systemic circulation to get about 60% of the intact diclofenac. The liver is the primary site of diclofenac metabolism, where it is conjugated with glucuronic acid. UDP glucuronosyltransferase-2b7 (UGT2B7) is the enzyme that catalyzes the conjugation to uronic acid [33]. Protein's sulfhydryl groups react with the resulting metabolite, acyl glucuronide. The enzyme cytochrome P4502C8 (CYP2C8) metabolizes acyl glucuronide to 4-hydroxy diclofenac acyl glucuronide, which generates benzoquinone imine and causes diclofenac to be activated by oxidation [34]. Along with two other minor metabolites, 3' hydroxyl metabolite and 5' hydroxyl metabolite, diclofenac is metabolized mostly into 4' hydroxyl metabolite. The 4' and 3' hydroxylation is catalyzed by the cytochrome P450 enzyme while the formation of the 5' hydroxyl metabolite is catalyzed by cytochrome P450 3A4 [35].

It is known that the primary metabolite, 4-hydroxydiclofenac, still has some weak analgesic and anti-inflammatory properties. Excretion of diclofenac in urine occurs after its biotransformation into glucuroconjugated and sulphate metabolites [26]. Diclofenac is removed by metabolism, which is followed by excretion in the urine and bile. Diclofenac and its oxidative metabolites are excreted through the biliary system after being glucuronidated or sulfated [36]. About 65% of the dosage is eliminated in the urine and 35% in the bile as conjugates of diclofenac and its metabolites.

Due to its rapid clearance rate (mean elimination half-life of 1.2–1.8 hours) and short biological half-life (2 h), diclofenac typically requires frequent administration to maintain its therapeutic dosage, which may raise the risk of side effects [37]. Nevertheless, since the C_{max} at therapeutic doses is higher than the threshold required to inhibit COX-2 by 80%, it is possible that efficacy can be attained at lower diclofenac doses, extending the very short pharmacological half-life of the drug [38].

5. Adverse effect of diclofenac

As earlier stated, diclofenac poses significant GI, CV, and renal adverse effects due to its COX en-

zyme inhibition and also. they are dose dependent, just like other NSAIDs. The decreased production of prostanoids associated with the drug causes reduction of the secretion of mucus by the epithelial cells of the gastric gland, bicarbonate and epithelial cell turnover, which ordinarily protect the stomach mucosa from harm [39]. This feature of the medication raises the risk of damage to gastric epithelial cells mediated by acid and reduces their capacity to multiply in the injured areas, which can result in GI injury ranging from moderate erosion to a conspicuous ulceration that can be seen with an endoscopy [40]. This result in the adverse effect of the gastrointestinal tract. Diclofenac has a low relative risk of complications with GIT, especially when taken at low doses (≤ 75 mg daily), which is consistent with the hypothesis that NSAIDs with the highest COX-1 selectivity are more likely to be associated with an increased risk of GI toxicity. Diclofenac appears to inhibit COX-2 more selectively, which increases the risk of cardiovascular events and decreases the risk of gastrointestinal events. High concentration of diclofenac in systemic circulation is necessary for this diclofenac's GI toxicity [41].

Vascular endothelial cells use prostacyclin (PGI_2), a primary product of COX-2-mediated metabolism of arachidonic acid, as a strong vasodilator and platelet inhibitor in physiological processes. Evidence from preclinical and clinical studies suggests that inhibiting PGI_2 production raises the risk of thrombosis and hypertension [42]. Observational investigations have reported a dose-related risk of thrombotic events, particularly with high doses of diclofenac precisely greater than 150 mg daily. When diclofenac is taken at doses higher than 150 mg per day, the estimated cardiovascular risk is similar to that of high-dosage ibuprofen, celecoxib, and rofecoxib [43]. The degree of COX-2 inhibition exhibited by NSAIDs that partially inhibit COX-1 is a major factor in the varied risk of myocardial infarction. Regardless of age or gender, diclofenac patients had a higher risk of cardiovascular events when compared to those taking paracetamol, ibuprofen, and naproxen, according to a study assessing the cardiovascular hazards of these drugs [3].

All NSAIDs increase the risk of myocardial infarction (MI), heart failure, stroke, and mortality, particularly the more selective COX-2 inhibitors. For patients who are predispose to cardiovascular disease, the risk of these events increases and with increasing dosage [44]. Accordingly, to treat patients with cardiovascular disease, it is imperative to utilize the lowest effective dose. Prostaglandin- I_2 (PGI_2), which has cardioprotective properties through prevention of thrombogenesis, hypertension, and the formation of atherosclerotic plaques, is synthesized less frequently when COX-2 is inhibited [41]. The

prothrombotic species thromboxane A₂ (TXA₂) is synthesized by COX-1, and the imbalance between TXA₂ and PGI₂ may be a factor in these elevated cardiovascular events. Patients with risk factors for the development of CV and GI adverse events may consider reducing their diclofenac dosage, as the incidence of adverse events is dose dependent [45].

Renal problems are also associated with long-term usage of NSAIDs. Protective prostaglandin production mechanisms are at play here, based on lowered PGE₂ and PGI₂ activity, which dilate renal blood arteries to provide adequate tissue perfusion. Acute kidney injury (AKI) and reduced renal perfusion are associated with decreased prostaglandin production. Patients having a history of renal injury and low perfusion pressure are more at risk [46].

There are also reports of liver injury of NSAIDs including diclofenac. NSAIDs, such as diclofenac, have the potential to raise liver transaminase levels and promote hepatic damage. Usually, these occurrences are short-lived and reversible [47]. Patients who take NSAIDs for an extended period of time run the risk of developing hepatitis and other potentially fatal adverse effects; however, this is not common. Patients taking diclofenac for rheumatoid arthritis on a long-term basis are more likely to experience these [48].

The use of NSAIDs has been linked to an increased risk of bleeding because of its ability to reduce platelet aggregation and adhesion through COX-1 inhibition. Additionally, patients may infrequently develop aplastic anemia and neutropenia [49].

The adverse effects highlighted result from systemic diclofenac exposure. There is less chance of systemic drug exposure with topical administration, therefore topical application has less adverse effects and are safer but limited to localized effect. Diclofenac applied topically may irritate the skin mildly to moderately where it is applied [50].

5.1. Drug-drug interaction

Low-dose aspirin (75–150 mg daily) is frequently used in the pharmacological prevention of cardiovascular disease because it inhibits platelet aggregation. When co-administered with NSAIDs in patients with CV disease, low-dose aspirin may have its antiplatelet actions interfered with, which is a significant therapeutic concern [51]. Aspirin inhibits platelet COX-1 activity irreversibly by first low-affinity anchoring to the COX channel's arginine-120 residue, a common docking site shared by other NSAIDs. Therefore, the antiplatelet effects of low dosage aspirin may be hampered by NSAIDs that inhibit platelet COX-1, such as ibuprofen or naproxen [52]. Aspirin's effect on platelets is not hindered by diclofenac or the selec-

tive COX2 inhibitors celecoxib and rofecoxib. Mass spectroscopy measurement of COX-1 acetylation in platelets may provide more insight into the molecular features of this therapeutically significant drug-drug interaction [53].

Angiotensin-converting enzyme (ACE) inhibitors and diclofenac in combination have been observed to elevate systolic blood pressure, which lessens the benefits of ACE inhibitors. It has been shown that using cyclosporine A as an immunosuppressant in conjunction with diclofenac increases the risk of nephrotoxic. It has been noted that the presence of diclofenac increases the convulsive effects of quinolones, such as ciprofloxacin. When diclofenac and methotrexate were administered together, there was a recorded incidence of renal failure that resulted in patient mortality [54]. Adverse drug-drug interactions have been documented when methotrexate is used with NSAIDs including diclofenac, ketoprofen, and naproxen because they lower methotrexate excretion. Also. It is not recommended to use fixed-dose combinations of tramadol and diclofenac in individuals with severe renal impairment [55].

6. Administration of diclofenac

Preparations of diclofenac is in the form salt of potassium, sodium, or epolamine diclofenac. Diclofenac sodium is available in tablet or suspension form for oral use, intramuscular and intravenous in solutions, transdermal as gel, and rectal routes as suppository. Diclofenac potassium comes in tablet and suspension forms that can be used orally while diclofenac epolamine as transdermal patch.

In order to attain a total daily dose of 100–150 mg, diclofenac sodium can be taken orally as 25–150 mg delayed-release or immediate-release tablets; the doses intended for rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. The formulation of diclofenac sodium salt, which is released gradually, dissolves more readily in the duodenum's high pH environment than it does in the stomach's low pH environment [56]. Diclofenac sodium gel formulations with doses ranging from 1 to 3% are available for topical use. Topical use of gel containing 1 to 2% diclofenac sodium is recommended for treating osteoarthritis in joints with monoarthritis up to 16 g per day and polyarthritic joints up to 32 g per day [32]. Actinic keratosis patients should use the 3% diclofenac sodium preparation, which should be applied twice a day as hybrid therapy. For acute moderate to severe pain, an intravenous bolus injection of 37.5 mg of diclofenac sodium can be given every 6 hours [57]. Intramuscular diclofenac solution (75

mg/3 mL solution) is administered by injection into large muscle such as the buttocks or thighs for the relief of moderate to severe pain. For the treatment of photophobia and eye pain, as well as after cataract surgery, ophthalmic preparations should be used in 1 to 2 drops per affected eye, four times/day.

Diclofenac potassium salt was created to speed up the drug's absorption, making it useful in situations requiring quick pain relief. Approximately 60% of intact diclofenac enters the bloodstream [58]. Diclofenac potassium is usually given in doses of 25 or 50 mg, one to four times a day, for a total of 50 to 200 mg per day; the recommended dosage for treatment of rheumatoid arthritis, osteoarthritis, migraines, primary dysmenorrhea and general pain. Transdermal patch inflammation.

7. Structural modification of diclofenac

One technique employed in discovering a better medication option of a drug is through structural modification. This is the simplest method for locating a better substance with the required targeted activity and minimal negative effects on the environment and human health [59]. The compound's physico-chemical, spectral, biological, and pharmacokinetic

properties are all significantly altered by this kind of structural modification, which will help choose some alternatives for additional research [60].

Diclofenac medications exhibit good analgesic and strong anti-inflammatory properties as well as fast action when administered on patients but has a number of adverse effect. Due to increase in the demand for the drug and need to reduce its adverse effects, a set of enhanced studies is required to screen for diclofenac derivatives with minimize unwanted reactions and to meet market demand [10]. Three method of synthesis of derivatives of diclofenac are discussed below.

7.1. Synthesis of diclofenac potassium

Potassium 2-[(2, 6-dichlorophenyl)amino] phenylacetate simply referred to as diclofenac potassium (Compound 7: Fig. 8) is an odorless, absorbent, yellowish white powder which is highly soluble in methanol but least soluble in chloroform. It is relatively soluble in ethanol and water. The dissociation constant (pKa) in water at 25°C is 4.0 and the partition coefficient in n-octanol is 15.45 at pH 5.2 and 13.4 at pH 7.4. The Biopharmaceutics Classification System (BCS) classifies diclofenac potassium (DP) in the class II drug group (high permeability, low

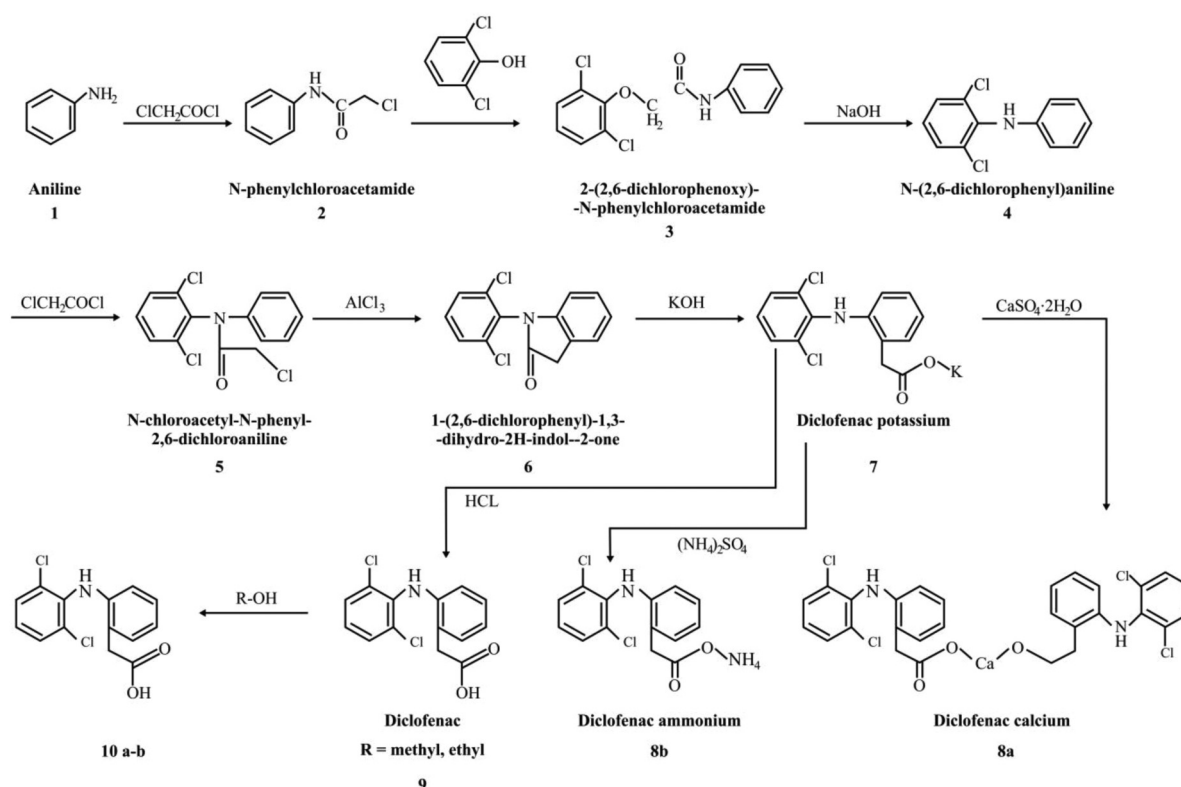


Fig. 8. Synthesis of diclofenac potassium [139].

solubility). It relieves pain quickly because of its high solubility in the stomach's acidic environment [61].

This drug was synthesized using aniline and chloroacetyl chloride as starting materials (R). Aniline was mixed with absolute toluene at 15°C-20°C and chloroacetyl chloride was added at 65–70°C and the mixture heated to give N-phenylchloroacetanilide (Fig. 8). N-phenylchloroacetanilide was combined with toluene PEG-400, 2, 6-dichlorophenol, and sodium carbonate in a stepwise manner while being stirred to yield 2(2,6-dichlorophenoxy)-N-phenylacetamide. This compound was reacted with sodium hydroxide to produce N-(2,6-dichlorophenyl)aniline. N-(2,6-dichlorophenyl)aniline was reacted with chloroacetyl chloride at 125°C to yield N-chloroacetyl-N-phenyl-2,6-dichloroaniline which was further reacted with aluminum chloride to produce 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one. This product was reacted with potassium hydroxide to produce diclofenac potassium.

7.2. Other diclofenac salt synthesis

Diclofenac sodium and either zinc or copper sulphate are reacted in a water bath at 80°C for 90 minutes to give the respective metallic diclofenac salt (Fig. 9). The yield is approximately 60%. The majority of the metal derivative of diclofenac produced are essential trace elements like copper and zinc that are needed by the human body as well as with less toxicity [62]. The human body needs copper, a trace element that is vital component of numerous metalloenzymes. Enzymes containing copper have vital physiological roles and a significant impact

on human health. Another important trace element in human health is zinc. In addition to aiding in the synthesis of several metalloenzymes within the human body, it also serves a number of critical physiological purposes, including sustaining a healthy body metabolism, stimulating growth and development, stimulating appetite, and supporting immunological function [63]. In comparison to other metals, copper and zinc are comparatively less poisonous. They can be readily coordinated with ligands consisting of oxygen and nitrogen to generate stable complexes that are inexpensive and simple to synthesize. Numerous copper complexes have the ability to stop the onset and spread of cancer, eliminate cancer cells and help scavenge reactive oxygen species and other free radicals [64].

Diclofenac calcium could be synthesized from the reaction between diclofenac potassium and calcium sulphate dehydrate with a yield of about 74% while diclofenac ammonium is synthesized from diclofenac potassium and ammonium sulphate with a 70% yield [65] (In Fig. 8).

7.3. Diclofenac choline synthesis

Literature review reveals diclofenac choline can be synthesized from diclofenac sodium. Didiclofenac sodium is acidified with hydrochloric acid to yield diclofenac before being added to choline [66]. Diclofenac choline is produced at 50°C; the yield of diclofenac is approximately 75%. In contrast to previous research, this process produces a product with excellent purity, good color, minimal industrial pollution, and ease of large-scale manufacturing while

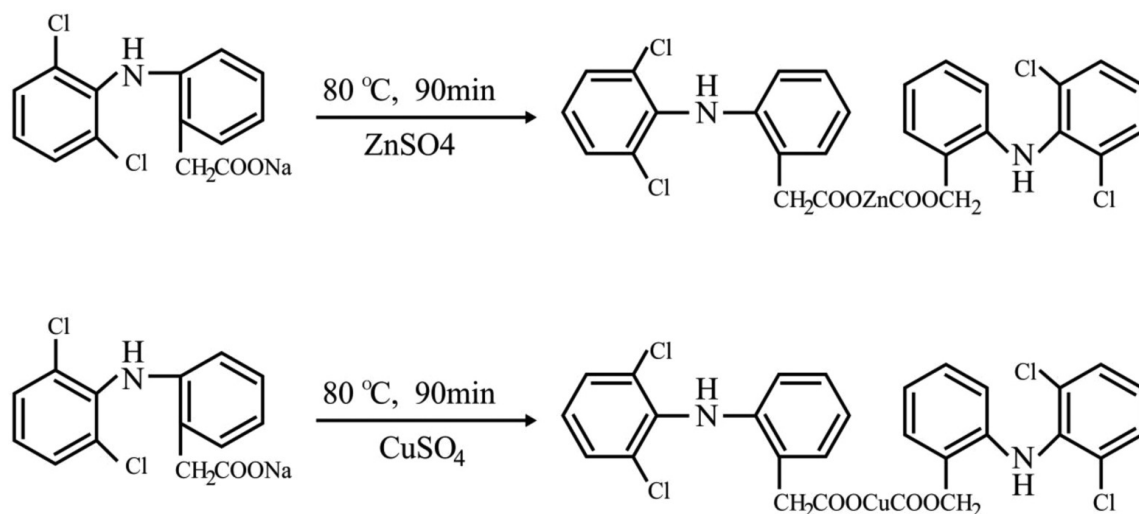


Fig. 9. Synthesis of diclofenac zinc and diclofenac copper respectively [140].

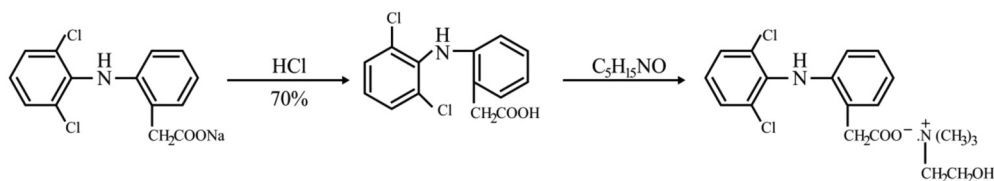


Fig. 10. Synthesis of diclofenac choline [141].

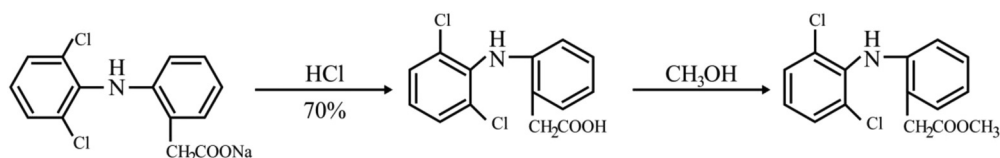


Fig. 11. Synthesis of diclofenac methyl ester [142].

saving a significant amount of reaction time and reagents [67].

7.4. Diclofenac esters synthesis

With aniline and 2,6-dichlorophenol as raw materials, three reactions are used to create the important intermediate 2,6-dichlorodiphenylamine was synthesized through a series of reactions involving chloroacylation, etherification and rearrangement. The intermediate 2,6-dichlorodiphenylamine is then subjected to acylation, cyclization, and hydrolysis reactions to synthesize diclofenac sodium. Diclofenac sodium can be acidified using hydrochloric acid. Subsequently, diclofenac reacted with methanol to produce diclofenac methyl ester (Fig. 10), which has a total yield of approximately 46% [68]. Literature review shows ethyl and n-butyl ester of diclofenac can be synthesized by reacting diclofenac with anhydrous ethanol and n-butanol respectively [69].

A research study of the antinociceptive and anti-inflammatory properties of diclofenac calcium, diclofenac ammonium, diclofenac methyl ester and diclofenac ethyl ester using oral administration of doses 16,32 and 64mg/Kg in kunming male mice while the standard group was administered 32mg/kg body weight potassium diclofenac showed diclofenac methyl ester had the best antinociceptive property at dose 32mg/kg body weight with approximately 62% inhibition rate. It had a higher analgesic activity than diclofenac potassium which is currently approved in clinical use, with an inhibition rate of 56%. Also, diclofenac methyl ester had the strongest anti-inflammatory activity at dose 32mg/kg body weight with an inhibition rate of 80% and higher than diclofenac potassium with an inhibition rate of 70% [26]. The research proposed diclofenac methyl ester to be an ungraded substitute of diclofenac potas-

sium due to its better analgesic and anti-inflammatory property. The researchers applied for a patent for the production and the use of diclofenac methyl ester as an analgesic and anti-inflammatory drug [70].

8. Potential applications of diclofenac beyond its anti-inflammatory property

Aside its classical analgesic, anti-inflammatory and antipyretic property, research indicates that diclofenac could possess other properties which could be exploited for therapeutic use. Diclofenac's off-target effects could potentially be leveraged for the creation of innovative drugs for treatments of conditions outside pain, inflammation and fever [26]. Researchers have shown that diclofenac and meclofenamate sodium function as a new voltage-gated potassium channel KCNQ2/KCNQ3 opener [71]. This action may be exploited therapeutically to treat conditions like epilepsy that are linked to neuronal hyperexcitability. Diclofenac has demonstrated positive findings in vivo in terms of its anticonvulsant properties. It has been reported that diclofenac can be used as a template to create new ion channel modulators [72]. Diclofenac also targets Kv1.3, a voltage-dependent potassium channel that mediates potassium-based current and is essential for the activation of immune cells: macrophages and lymphocytes [73]. Diclofenac has been shown to suppress immune response by interfering with Kv1.3 channels, which makes it a suitable starting compound for the development of drugs for treatment of autoimmune disorders [74].

Another target of diclofenac and NSAID is the acid-sensing ion channel-1. Diclofenac and NSAID which lowers the current this acid-sensing channel induces. In addition to the conventional prostaglandin inhibition by NSAIDs, this inhibition of current in sensory

neurons has been proposed as another mechanism of pain reduction [75]. Furthermore, diclofenac inhibits phospholipase A2, which is thought to be the fundamental mechanism by which this medication treats acute pancreatitis. However, it has been discovered that the best effective medication for acute pancreatitis is indomethacin, another NSAID that inhibits phospholipase A2 [26].

Diclofenac may prevent the production of transthyretin amyloid fibrils, according to a research and this may find useful application in management of familial amyloid polyneuropathy and senile systemic amyloidosis [76]. It has been discovered that one effective treatment approach for hormone-dependent breast and prostate cancers is inhibition of the AKR1C3 enzyme and it is interesting to note that one of the few known inhibitors of this enzyme is diclofenac [77].

While these targets may be useful for therapy, it is important to state that these targets should be considered as the sources of different side effects. Diclofenac's current drawbacks must therefore be addressed through meticulous structural and metabolic research in order to fully employ this medication in the creation of treatments for additional illnesses [26].

8.1. Antimicrobial property of diclofenac

In the drug-repositioning technique, a number of medications from various therapeutic classes are being evaluated as antimicrobials in an attempt to solve the challenges of antimicrobial resistance of some species of infectious microorganisms to antimicrobial agent and discover fresh, effective, low cost alternatives [78]. According to reports, certain non-antibiotic medications, such as NSAIDs, cytostatics, and psychotropics, as well as statins, have antibacterial qualities [79]. Recent studies indicate that some of these anti-inflammatory medications have antibacterial activity in addition to their primary function [80].

Diclofenac has been reported to have anti-bacterial and anti-mycotic properties [26]. Diclofenac has been reported as a powerful non-antibiotic antibacterial agent; inhibiting both gram-positive and gram-negative bacteria and also demonstrated synergism with various antibiotics [81].

Result from a research study showed that diclofenac inhibited the growth of *Enterococcus faecalis* invitro at a concentration of 50 $\mu\text{g}/\text{m}$ with a mean zone of microbial growth of 9.10 ± 2.02 mm. However antibacterial activity of diclofenac was lower than ibuprofen as well as two clinically used antibiotic amoxicillin and gentamycin [80]. According to [82]

the minimum inhibitory concentration (MIC) of diclofenac sodium against 45 mycobacterium strains is 10–25 $\mu\text{g}/\text{ml}$. This is significantly higher (5–6) than the MIC of the conventional anti-mycobacterial medications. Diclofenac exhibit antibacterial property by compromising membrane activity or preventing DNA production in bacteria [83]. It has been demonstrated that diclofenac prevents *Escherichia coli* and *Listeria monocytogenes* from replicating their DNA [84]. It will be interesting to find out if diclofenac functions as an anti-inflammatory, analgesic, antipyretic agent as well as antibacterial agent simultaneously in localized and systemic administration of the drug.

Pro-inflammatory drugs (NSAIDs) which block prostaglandin synthesis particularly diclofenac the most potent, may be crucial in affecting the metabolism of fungal prostaglandins, as several studies have demonstrated that pathogenic fungi can produce prostaglandins during biofilm adhesion and development. Also, recent research indicates that PGs may be crucial to the control of the eicosanoids pathway in fungal such as *Candida* spp. Due to a disruption in their metabolism, Diclofenac's inhibition of PG synthesis result in the fungal death. NSAIDs have been shown in numerous studies to be to exhibit both in vitro and in vivo antifungal property [85]. Biofilm diseases caused by *Candida* spp. and other invasive fungal strains can be reduced by combination therapy that combine NSAIDs with conventional antifungal medications. One potential way that NSAIDs reduce fungal growth, biofilm formation, and adherence in *Candida* species is through inhibiting the synthesis of fungal PG [86]. Repositioned medications combined with EOs that have established antimicrobial properties can be a useful strategy for quickly discovering novel treatments for acute infections [87].

8.2. Diclofenac's neuroprotective property

Research study indicates that diclofenac possesses neuroprotective qualities [26]. Diclofenac may have neuroprotective effects in chlorpromazine-induced catalepsy, which may have consequences for Parkinson's disease, according to a recent study [88]. In the past, diclofenac was demonstrated to similarly inhibit A β 1-42 oligomerization and fibrillation as celecoxib. Transthyretin amyloid has been discovered to be inhibited by diclofenac and its analogues [26]. In addition to type 2 diabetes, pancreatic amyloidosis is characterized by misfolding of the islet amyloid peptide (IAPP). It was found that diclofenac inhibited the islet amyloid peptide's (IAPP) oligomerization, which lessened the cytotoxic effects of the protein [89]. Remarkably, research has shown that diclofenac can prevent diseases caused by misfolded proteins,

such Alzheimer's. A frequent inflammatory process in the brain that includes chemokines, neurotoxins, and cytokines is known to be the activation of glial cells by COX enzymes [90]. Diclofenac's inhibition of COX enzymes is assumed to contribute to brain cell protection against inflammatory-induced toxicity. Reduced apoptosis and wound area appeared to be related to diclofenac's COX-2 inhibition-mediated protective action after an experimental localized penetrating traumatic brain injury in rats [91].

8.3. Prospect of diclofenac as an anticancer agent

Various naturally occurring and artificially produced compounds have been developed as anticancer drugs. While some anticancer medications like doxorubicin and epirubicin, were developed from a natural molecule, daunorubicin, which is frequently found in *Streptomyces* bacteria, some synthetic chemical compounds have been obtained from their natural analogs, such as topotecan from camptothecin [92]. The off-target consequences and our poor understanding of pharmacokinetic characteristics pose a significant challenge to the development of novel anticancer medications [93]. Repurposing drugs is one crucial tactic to address this challenge. Repurposing drugs entails using a well-known drug for different conditions. The practice of repurposing of drugs is becoming a more successful approach to reexamine well-known, old drugs for novel therapeutic uses, as well as lowering the cost and duration of de novo drug development [94]. For example, thalidomide which was first approved as a pregnancy-related nausea medication, is now used in combination with dexamethasone to treat multiple myeloma. Originally intended to treat Parkinson's disease, bromocriptine is now used to treat diabetes mellitus [26]. The potential therapeutic use of diclofenac in a number of other different illnesses has also been studied. Other advantageous uses of diclofenac, including its anti-convulsant and antibacterial properties, have been discovered [5]. Furthermore, research on diclofenac's potential as an anticancer agent has been reported. Diclofenac's anticancer qualities have been investigated in a variety of cancers, including pancreatic cancer, colorectal cancer, glioma, neuroblastoma, osteoblast, and fibrosarcoma [95]. Cell cycle and apoptosis, which are two important pathways in cells and therefore in the pathogenesis of cancers have been thoroughly examined to comprehend the manipulative function that diclofenac has in these pathways [96].

In several cell lines, namely neural stem cells, glioblastomas (GBM), ovarian cells, osteoblasts, human lymphatic endothelial cells (HLEC), and vascular

smooth muscle cells (VSMC), diclofenac has been shown to block their cell cycle, thus preventing cell proliferation which is characteristics cancer cells as shown in Fig. 12 [97]. Increased expression of the cell cycle inhibitor p21 was observed in glioma cells treated with diclofenac; this effect was linked to increased levels of 15-PGDH (15 hydroxy prostaglandin dehydrogenase) [98]. Additionally, vascular smooth muscle cells (VSMCs) treated with diclofenac showed higher levels of p21 and p27. These results were associated with diclofenac's inhibitory effect on the cell cycle in the G1 phase of VSMCs [99]. In a model of colon cancer, diclofenac also shown anti-carcinogenic properties. Diclofenac's anticarcinogenic action was linked to decreased levels of cyclin D1, cyclin E, CDK-4, and CDK-2 as well as increased telomerase activity. The transcription factor E2f1 is important for the development of cancer and chemoresistance because, when overexpressed, it promotes invasion and metastasis by triggering growth receptor signaling pathways [100]. Reduced expression of the transcription factor E2f1 may be the cause of diclofenac's anticarcinogenic effect in ovarian cancer cells. Enhanced cellular proliferation and apoptosis resistance in a variety of malignancies have been linked to elevated COX-2 levels. Remarkably, anticancer effects of diclofenac, an inhibitor of COX-2 of have been observed in rats with neuroblastoma [26].

Research revealed that the treatment of diclofenac resulted in the reduced expression of inducers of cell proliferation, such as cyclin D1 and the transcription factor E2F1, and the upregulation of cell cycle inhibitory proteins, such as p27, p21, and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) as depicted in Fig. 12. All these actions of diclofenac mitigate cell cycle and if such cells are prone to uncontrollable proliferation as observed in cancer cell, it implies that that diclofenac could be a possible anticancer agent [101].

It has also been reported that diclofenac promotes apoptosis and how diclofenac affects different apoptosis-inducing pathways has also been investigated. Diclofenac's apoptotic effect in neuroblastoma cell lines has been related to superoxide dismutase 2 (SOD2). SOD2, an antioxidant, shields cells against the oxidative activity of cell component by reactive oxygen species, which prevents cell damage or apoptosis [26]. Diclofenac inhibits SOD2's enzyme activity as well as its expression levels. It has been discovered that COX-2 inhibits the apoptotic pathway triggered by the Fas receptor, a member of the TNF family that, when in contact with its agonists, triggers apoptosis. Additionally, diclofenac may have an apoptotic impact because of its COX inhibitory characteristic, which inhibits prostaglandins

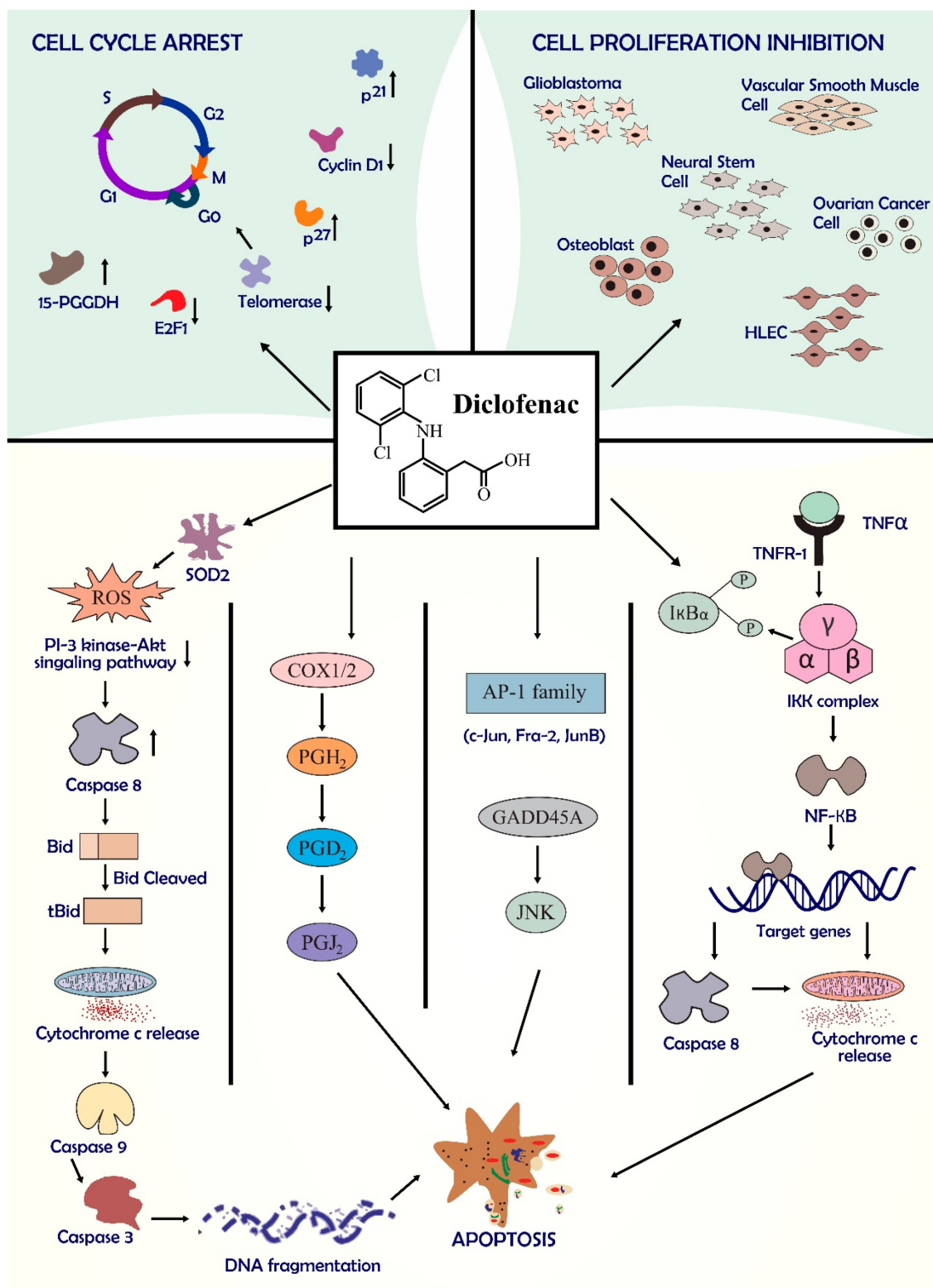


Fig. 12. Cell cycle inhibition and apoptosis induced by diclofenac: A schematic representation of its mechanism [26].

with anti-apoptotic actions such prostaglandin H2 (PGH2) [102]. In myeloid leukemia cells, diclofenac was shown to induce apoptosis through a mechanism that is dependent on activator protein-1. According to this study, increased amounts of the transcription factors activator protein-1, c-Jun, Jun-B, and Fra-2, led to enhanced levels of GADD45 α and, consequently, JNK, which in turn caused apoptosis. An additional investigation discovered that diclofenac sensitized hepatocytes to apoptosis by blocking TNF- α -mediated activation of NF- κ B transcription factor activity [103]. Diclofenac-induced apoptosis has been attributed to several downstream mechanisms, including: inhibition of NF- κ B activity, disruption of proteasome activity, induction of the JNK pathway via AP1 transcription factors, and ROS-induced downregulation of the PI 3-kinase/Akt signaling pathway [104]. The prospect of diclofenac to be employed as an anticancer treatment agent for different forms of cancer is supported by two important anticancer mechanisms in cells: a number of pro-apoptotic and cell cycle inhibition mechanism that have been report from various research investigations.

It is important to emphasize that majority of these investigations are in the preclinical stage. Notable among these research studies are NCT04091022 and NCT02636569. Important information regarding the potential use of diclofenac in cancer therapy may come from the results of trials like these, which are aimed at determining the impact of the medication on preventing or lowering the incidence of non-melanoma skin cancer [26]. A recent conclusion of a clinical experiment utilizing topical diclofenac on actinic keratosis patients indicated reprogramming of metabolism and immune cell infiltration in AK lesions [4]. A different ongoing clinical research is looking at diclofenac as part of TL-118, a four-drug combination that includes sulfasalazine, metronomic cyclophosphamide, cimetidine, and diclofenac [26]. This implies diclofenac, in combination with a variety of other medications, may play a significant role in cancer treatment and management.

While diclofenac has a promising prospect as an anticancer agent and in treatment of other ill health conditions, the negative effects of diclofenac are a drawback to using it. For example, recent study shows that using diclofenac as an adjuvant to curcumin can enhance its anti-Alzheimer benefits in rats. Therefore, research is needed to create innovative methods for minimizing the negative effects of diclofenac before using it or developing it in new therapies. A few of such research are stated in this review A substantial reduction in the risk of peptic ulcers has been found when proton pump inhibitors and diclofenac as combination therapy [105]. Furthermore, a naturally

occurring substance called thymoquinone lessens the kidney toxicity that diclofenac causes. A combination of vitamin B12 and diclofenac has shown that less diclofenac was required to have an analgesic effect [106]. This discovery is of great interest considering the fact that vitamin B 12 is a component of some food and also taken in the form of supplement with little or no potential of toxicity even at large doses [107]. The application of omega-3 fatty acids has also demonstrated anti-diclofenac induced hepatotoxicity benefits. Diclofenac and the curcuminoid complex combined together demonstrated improved knee osteoarthritis tolerability and functional capacity. A recent study suggested creating analogs of diclofenac with less hepatotoxicity [108].

Another technique to address the address the adverse effect of diclofenac use or in developing new therapy by exploiting new methods of delivering drugs can also help make the medicine more available at a specific location, which lowers the dosage. Increased ocular availability of diclofenac in rabbit eyes was observed in a study employing polymeric nanosuspensions as a drug carrier [26]. A study that delivered diclofenac locally using nanofibers derived from the polymer of poly (D, L-lactide-co-glycolide) revealed higher survival rates in a mouse model of oral cancer. Diclofenac nanocrystal suspensions were employed in another investigation to treat skin irritation, and the results showed that these formulations had better anti-inflammatory properties than the available commercial equivalents. This was suggested to be perhaps due to increase in the drug quantity or availability at the site of inflammation [109]. When critically considered collectively, these investigations could prove beneficial in creating new treatments that would successfully lessen the negative consequences of diclofenac

9. Environmental impact of diclofenac

Pharmaceuticals such as drugs, hormones and antibiotics are widely utilized in biotechnology, medicine, and agriculture [110]. In medicine, a wide variety of pharmaceuticals are used to treat different ill health conditions as well as maintain the health of human and animals. One of the notable families of pharmaceuticals are pharmaceutically active compounds (PAC), which enter the environment either as the parent component or as pharmacologically active metabolites through various means [111]. While many pharmaceutical substances are designed to have positive biological effect on the organism they are delivered to, they can also have unintended biological effects when they enter the environment. PAC

have been found in several different environments, and frequently, both the short and long term consequences may not be clearly understood [112].

The widespread presence of PAC and pharmaceuticals in aquatic environments has become a concern with unclear implications and in recent times has prompted environmentalists around the world to have interest in PAC [113]. Non-steroidal anti-inflammatory drugs (NSAIDs) have been reported at quantities ranging from low mg/L to ng/L in different environment. NSAIDs are available as over the counter drug in the majority of nations, there is a higher likelihood that they may be consumed and, consequently, exposed to the environment [48]. Due to its frequent presence in sources of drinking water and its possible detrimental effects on a wide range of organisms at considerable level, DCF has garnered interest in recent times. Animals with the same or similar target organs, tissues, cells, or biomolecules may experience similar effects from these pharmaceuticals when they enter the environment [113].

The source of diclofenac is the pharmaceutical drug and it finds its way through human and animal route into wastewater treatment plants or landfills as diclofenac or its metabolite. Diclofenac may also get into wastewater treatment facilities directly through wastes from pharmaceutical industry [114]. DCF can end up in surface water due to the ineffectiveness of the traditional treatment methods used in wastewater treatment plants, and there is the likelihood that DCF may seep into sources of drinking water. There is a relatively larger chance of DCF from landfills percolating into surface water [115].

DCF has been found in ng/L in surface water such as rivers, lakes and estuary, whereas it has been found in wastewater at concentrations as high as $\mu\text{g/L}$. Both physico-chemical processes in wastewater treatment facilities and natural processes including soil retention, biodegradation, and photo transformation contributed to the concentration's decline [111]. Instances of detection in drinking water and groundwater have also been reported. Although EU nations have accounted for the majority of detections, this does not mean that diclofenac was exclusively found in those regions. According to a report, Pakistan had the highest concentration in rivers (4900 mg/L), and one possible explanation for this could be that Asian nations lack sophisticated WWTPs [116]. Research from Canada revealed that WWTP effluents have a very high concentration of diclofenac of about 16 $\mu\text{g/L}$ [117]. Water bodies in Germany have also been severely contaminated by diclofenac; the highest concentration found in river water was 1030 ng/L. Residues of diclofenac have been found in the river delta of Spain. Of the over 30,000 surface water

samples that were taken in France between 2007 and 2018, 29% contained diclofenac. Treatment of wastewater reduces its concentration by only 20% to 50%. Nearly every nation in the European Union has DCF remnants. DCF has even been found in drinking water in the US and Germany, which is noteworthy [118]. Most cases of DCF have been found worldwide in freshwater environments.

Additionally, diclofenac in waste water treatment plants can act as ligand and form metal with metal presence in waste water since the structure of DCF contains active groups such amino, hydroxyl, carbonyl, and carboxyl groups through deprotonation of these functional group, facilitate by a variety of physicochemical factors of water treatment plants [119]. With the majority of metals, these groups can improve the metal complexation/binding characteristics. Thus, chelation-mediated organometallic complexation of pharmaceuticals is a potential outcome in WWTP [120]. The bactericidal properties of DCF complexes with Hg (II), Pb(II), and Sn(II) are well established, and the DCF Cu(II) complex has the ability to break DNA. DCF's characteristics can completely change when reacting with metal complexes to become a different kind of possible pollutant with the ability to destroy cells and have antibacterial property [121]. The metal complexes of DCF need to be managed carefully in light of its antibacterial qualities and should be regarded as an emerging contaminant with potential hazardous effects towards several organisms in the environment [113].

After treatment, WWTPs release water containing DCF into surface water with a range from a few hundred ng/L to thousands of ng/L [122]. There are no rigorous regulations to regulate the amount of DCF present in aquatic environments in Asian nations. It is evident from the few research on rivers that DCF is released into surface water without being properly treated. Appropriate treatment wastewater could lessen the burden of DCF entering the aquatic ecosystem in these parts of the world. For an efficient removal, it is recommended that these wastewater treatment plants should be provided basic treatment facilities from primary to secondary as well tertiary facilities.

A few instances of DCF detection in soil have been documented; it has been reported in Ontario, Canada and Israel. Diclofenac may find its way to farm lands through wastewater or the use of municipal sewage sludge as a source of nutrients in soil. When compared to other medications like sulfamethazine, DCF is less toxic to leguminous plants, and it has no negative effects on plant growth [123]. Earthworms and other soil organisms are not toxically affected by DCF in terms of behavior, weight change, or death. Research

studies on the application of wastewater sludge containing DCF to soil indicated moderate chances of dangers to soil microorganisms. It is not clearly understood how harmful DCF present in soil is to plants, microorganism and other soil organisms [124]. This is need for more research to be done in this area in order to ascertain the effect on various soil organisms. All that is known is that DCF degrades easily in soil and is highly absorbed by soil that is rich in organic matter. DCF appears to have a moderate level of persistence and lower toxicity. However, diclofenac becomes adsorbed to the soil and is resistance towards aerobic and anaerobic breakdown in soils rich in organic matter. It may also leak into groundwater, resulting in cumulative harmful consequences [125].

The toxicity of diclofenac to aquatic life has been the focus of several toxicity studies globally. One of the earliest investigations on the harmful effects of diclofenac on aquatic organism such as fish, bacteria, algae, and microcrustacean revealed comparatively less harmful effects even at environmental concentrations [126]. However, subsequent research exposed the possible environmental effects of diclofenac [127]. Studies on risk assessment indicated that there was a possibility of higher ecological risk associated with diclofenac in surface waters [128]. Aquatic species may be harmed by diclofenac from ecotoxicity investigations employing acute *Daphnia* and algae tests. Toxicity study indicated an increase in death rate in two species of crustaceans precisely *Daphnia magna* and *Ceriodaphnia dubia*. Diclofenac has been shown to cause fatal consequences in a variety of vertebrates, including fish, by causing damage to the kidney and gastrointestinal systems. Diclofenac may have long-term negative effects on fish populations according to an exposure assessment research [129]. Diclofenac negatively impacted the growth of the egg phase in Japanese medaka (*Oryzias latipes*) fish, which significantly reduced hatchability and delayed hatching. The gills, liver and kidneysAt environmental quantities, diclofenac disrupted the metabolic processes and caused tissue damage in rainbow trout [130]. Rainbow trout may store diclofenac in their liver, kidney, gills, and muscle tissues, which can result in cytological changes at a low concentration of 1 µg/L. Diclofenac elicited tissue specific biomarker responses that resulted in tissue damage at 250 ng L⁻¹, which was extremely near to the level in several German rivers [131]. Also, the growth and metabolism of the common Baltic Sea blue mussels were being negatively impacted by diclofenac. The majority of the research revealed that aquatic species may experience some negative consequences from ongoing exposure to diclofenac, even at very low doses. Diclofenac and its metabolites have been reported

to be present in fish bile [132]. At concentrations that may be close to those found in the environment, a number of photo-transformation products of diclofenac have the potential to be more hazardous than diclofenac.

The rapid death of vultures as a result of eating livestock remains containing diclofenac residues was the first publicly reported instance of a pharmaceutical causing significant effects on the ecosystem. These vultures depend on livestock such as goats and cows as their main food source. The animals were treated with diclofenac and Gyps vultures are particularly sensitive to very low doses of diclofenac. Although diclofenac was short-lived in these animals, it may still be highly present in the carcasses available to vultures. Research studies attributed the cause of death to renal portal vasoconstriction or accumulation of uric acid resulting from decreased uric acid excretion caused by consumption of diclofenac. The majority of the studies indicated that there is a likelihood that the ingestion of diclofenac could result in renal failure. Three Gyps vulture species; *Gyps bengalensis*, *Gyps indicus*, and *Gyps tenuirostris* were severely affected with a 98% decline in population in the Indian subcontinent putting numerous vulture species in danger of extinction [133]. As a result, they were added to the IUCN's (International Union for Conservation of Nature) list of "critically endangered" species. DCF had an impact on the ecosystem's community structure in addition to the vulture population. As keystone species, vultures' decrease affects culture, biodiversity, and a number of socioeconomic factors.

The vulture species in Africa are also under danger due to the veterinary use of diclofenac (R). A significant decrease in the number of vultures and other scavenger raptors from Kenya has been documented in certain instance and the cause has been linked to DCF. Additionally, DCF has been known to be lethal for eagles as of late, which increases the variety of raptors that DCF threatens [134]. According to recent studies, the prohibition of DCF for veterinary use in south Asian nations was a successful strategy, as evidenced by the current increase in vulture populations [135]. Thus, by outlawing its usage for veterinary purposes, legislative actions for DCF were successful in South Asia.

10. Conclusion

Diclofenac is a widely use and potent drug for treatment for treatment of inflammation, pain and fever associated with a variety of health conditions. Its mode of action stems from its competitive COX enzymes inhibition and subsequent decrease in

prostaglandins synthesis. Despite its potency as an anti-inflammatory, analgesic and antipyretic agent, its adverse gastrointestinal, cardiovascular and renal effect still remain a concern in the clinical use of this drug and serves as a limitation, suggesting the need for more research on its drug modification and delivery in order to reduce and if possible eliminate these adverse effects. However, this review reveals diclofenac methyl ester could become a better substitute of diclofenac potassium being a more potent anti-inflammatory and analgesic agent and possible reduced side effects. An application for patent for use as an anti-inflammatory and analgesic drug by the research has been made in China in 2022.

Research also projects diclofenac has a possible antimicrobial and anticancer agent and as well as having neuroprotective properties. Uncontrollable cell proliferation and evasion of apoptosis are characteristic features of cancer cell. Diclofenac arrest cell cycle, thus preventing proliferation of neural stem cells, glioblastomas, ovarian cells, osteoblasts, human lymphatic endothelial cells, and vascular smooth muscle cells and induces apoptosis of neuroblastoma cell, this projects this drug as a novel prospective anticancer agent. It will be interesting to find out if diclofenac can exhibit its classical anti-inflammatory and pain relieving drug along with its possible novel use as an anticancer and neuroprotective drug. Despite its current use and its promising prospect in treatment of a wide range of diseases, its adverse environmental impact from contamination from household and pharmaceutical waste water still remain a source of concern being a drug sold over the counter in most part of world and also its veterinary use. Diclofenac, its metabolites as well as its derivative that may be present in water waste, its primarily source of environment contamination constitute emerging environment contaminant. Therefore, waste water treatment initiative should encompass all.

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