EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 2
307-319
Year: 2016

# PHARMACEUTICALS IN THE AQUATIC ENVIRONMENT: A REVIEW ON THE ECOTOXICITY OF DICLOFENAC

**Prameela Devi Yalavarthy* Rohini Padma, Kola Ravi Kumar and Abdul Rasheed Md**

Environmental Bitechnology Lab, Department of Zoology, Kakatiya University.

***Author for Correspondence: Prof. Prameela Devi Yalavarthy**
Environmental Bitechnology Lab, Department of Zoology, Kakatiya University.

**Article Received on 03/12/2015** **Article Revised on 24/12/2015** **Article Accepted on 15/01/2016**

## ABSTRACT
Pharmaceuticals are the biologically active compounds that are used to treat various ailments in human and animals. These have made their entry into the aquatic systems through different pathways and are showing devastating effects on the biota. The issue of pharmaceuticals as the emerging pollutants and their impact on the environment has aroused the interest of researchers all around the world. They have been detected in the surface waters worldwide in the range of ng/l to µg/l. Diclofenac is a widely prescribed non –steroidal anti inflammatory drug which is used to reduce pain and inflammation in disorders like rheumatoid arthritis, osteoarthritis, ankylosing spondilytis, gout, dysmenorrhea, spondylarthritis etc,. It has been frequently found in surface waters worldwide. Many laboratory studies have shown its toxic effects on the aquatic biota like microorganisms, algae, crustaceans and fish. There are evidences of bioconcentration in tissues of fish and its bioaccumulation in the food chain. It has also shown cytotoxic, genotoxic, estrogenic and teratogenic effects in fish and frog. The transformation products were proven to be more toxic than parent compound**.** This article comprises of the occurrence, fate and toxicity of diclofenac. It also encompasses mechanism of its action, efficacy and potential side effects of the drug. The available literature warrants further research on all the above aspects.

**KEY WORDS:** pharmaceuticals, Diclofenac, toxic effects, bioconcentation.

## INTRODUCTION
Pharmaceuticals are a potent group of chemical components that are designed to exert specific biological effects at very low concentrations in human and animals.[1] The extensive use of both human and veterinary drugs has made their emergence into the aquatic environment through waste disposal.[2] The existence of drugs in surface waters, ground water and even marine systems has been confirmed at concentrations of high ng/l to µg/l.[3] Pharmaceuticals generate toxic effects even at low concentrations.[4] Most of the drug targets are conserved across various organisms on the evolutionary lines and they induce their effects even in non-target species.[5] There are ample experimental evidences indicating the harmful effects of pharmaceuticals, such as morphological, biochemical and sex alterations on aquatic species, selection of antibiotic resistance in pathogenic microorganisms and disruption of biodegradation activities in sewage treatment plants.[6] Consequently, pharmaceuticals have emerged as significant pollutants and have drawn the attention of researchers all over the globe.[7]

Diclofenac, 2-(2,6 dichloranilino) phenylacetic acid is a prominent emerging contaminant that has been frequently detected in surface waters worldwide.[8] It was first introduced in Japan in 1974.[9] It is a commonly prescribed non-steroidal anti inflammatory drug which has analgesic, anti-inflammatory and anti pyretic properties.[9] It is available in the form of tablets, capsules, suppositories, intravenous solutions and injections and is usually supplied in the form of either sodium or potassium salt. It is available with the common brand names as Voltaren, Pennsaid, Arthotec, Flector, Slaraze etc,. It is effective in reducing acute and chronic pain, and treating inflammation in some disorders like rheumatoid arthritis, osteoarthritis, ankylosing spondilytis, gout, dysmenorrhea, spondylarthritis etc.[10]

The annual consumption of the drug is in millions of tons all over the world.[8] The usage of veterinary drug diclofenac for livestock has led to the extinction of three vultures, *Gyps bengalensis*, *Gyps indicus*, *Gyps tenuirostris* in Indian subcontinent.[11] [12] [13] [14] The vultures when fed on the carcasses of cattle recently treated with diclofenac would die due to renal failure[12] The loss of vultures has led to the serious impact on the ecosystem and has also created socio-economic consequences.[15] The governments of India, Nepal and Pakistan have introduced a ban on the manufacture of veterinary diclofenac.[16] An alternative drug,

Meloxicam was introduced and proven to be harmless to vultures but effectively treated the cattle.[17]

**Occurrence**

Diclofenac has been detected in surface waters of rivers, lakes, seas, influents and effluents of waste water treatment plants, ground water, drinking water, soil and sediment world wide.[18] The determination of pharmaceuticals from different sources was conducted by using various technologies like liquid chromatography, gas chromatography, mass spectrometry, time of flight mass spectrometry, electrospray ionization, solid phase extraction, etc,.[19] Diclofenac has been detected in Switzerland [8][20] [21] [22], Europe [23] United kingdom, [24] [25] Brazil [26], South Korea [27], [28], [29] Spain [30], [31], Pakistan[32], Taiwan [33], India,[34] Italy [35] [36] [37] France [35] Canada [38]( [39] Germany [40], [41] [42] [43] [44], Greece [35] Sweden [35] [45], [46] Finland [47], [48] Malaysia [19] Mexico.[49] It is commonly found in wastewater at median concentration of 0.81µ g/L [40]) whereas the maximal concentration in wastewater and surface water is up to 2µg/L [26] [40]; [50] It was found in Germany in the range of 1.3-3.3µg/ml [43]

**Fate and Transformation**

Diclofenac enters into the aquatic environment through various pathways and undergoes many changes such as biodegradation, chemical degradation, photodegradation, adsorption, sedimentation, precipitation and volatilization. Biodegradation and sorption are two main processes through which pharmaceuticals are usually degraded.[[51]A study using high frequency ultrasound has reported that at neutral pH, diclofenac would completely convert into 45% of carbon, 30% of chlorine, and 25% of nitrogen.[52] Diclofenac could be removed from the waste water effluents through ozone treatment.[53,54] The ozonation process was found to be accelerated in the presence of activated carbons, particularly P110 Hydraffin, and the ecotoxicity was found to be reduced through ozonation but the catalytic ozonation increased the toxicity.[55] Photolysis was another efficient mechanism for the removal of diclofenac from surface waters.[8][56][57][35] [21[58] A study on photodegradation in aqueous solution by photofenton reaction has shown that diclofenac would undergo complete oxidation after 60 minutes and complete mineralization after 100 minutes.[59] A study on diclofenac degradation by $TiO_2$ photocatalysis has inferred that UV absorbance kinetics would reveal the transformation of intermediates easily.[60] $TiO_2$ catalysed photodegradation was found to be dependent on the geometry of the photoreactor, nature of water matrices and was an effiecient method for the removal of diclofenac from surface and drinking waters.[61] P- Benzoquinone imine of 5-hydroxydiclofenac was an important metabolite that was formed by the biodegradation of microorganisms found in the river sediments.[62] It was removed upto 39% and the lowest removal rate was attributed to disturbances in the activated sludge process.[63] A study has shown that molecularly imprinted polymer which was synthesized by using diclofenac as a template was found to be effective for removal from complex water.[64] There are evidences that conventional drinking water treatment along with granular activated carbon filtration has removed diclofenac upto 99.7%.[65] The nanofiltration technique was also utilised to remove diclofenac from drinking water resources [66] The degradation of diclofenac was studied using river biofilms in rotating annular reactors and about 10-25% of degradation was found to occur within 4 days.[67] The removal of diclofenac was also studied with the help a fungus *Phanerochaete sordida* YK-624. It was found that diclofenac degraded completely in 6 days and 4-hyroxy diclofenac , 5-hydroxy diclofenac and 4'5-dihydroxydidiclofenac were formed by fungal biotransformation.[68] A study has shown that Diclofenac would undergo photocyclisation into carbazole derivatives, initial decarboxylation and further oxidation of the alkyl chain during photolysis.[69] A recent study has shown that diclofenac undergoes phototransformation in the presence of sunlight and forms carbazoles as major metabolites which contain a ring with diketone function.[70] Diclofenac was found in higher concentrations in effluents than influents of sewage treatment plants and the rate of its removal was lowest with 26%±17%.[48]The solid phase extraction with prior methylation was used for the extraction of acidic pharmaceuticals from surface water , effluents from waste water plants and the optimized usage of gas chromatography and ion trap tandem mass spectrometry has resulted in the method detection limits of 1-1.0 ng/l.[71] Diclofenac removal from wastewater treatment plants was found to be low due to its poor biodegradation and sorption to sludge. However, the usage of membrane bioreactors, attached growth biomass process, enhancement of the hydraulic retention time and bioaugumentation would increase the elimination process.[72] A study has reported that calcined hydrotalcite was an efficient adsorbent for the removal of diclofenac through sorption.[73]

**Toxicity**

The primary sources of pharmaceuticals in the environment are human excretion [74] and disposal of unused products.[75] Diclofenac enters into the aquatic systems through discharge from domestic sewage systems, waste water treatment plant effluents and release from manufacturing units [76] [77] It shows negative consequences on the flora and fauna of the aquatic environment. This includes the microorganisms, phytoplankton, zooplankton, invertebrates and fishes.[4] Many experimental studies have been done on different aquatic organisms to detect its toxicity.

Studies with the use of different technologies on the lotic biofilms have indicated that DCF inhibits the growth of algae and bacteria.[67] A study on a fungus, *Candida albicans* has indicated that diclofenac sodium inhibited the hypha formation in both solid and liquid media [78] The bacterium, *Vibrio fischeri* has shown a 50%

reduction in bioluminescence with $EC_{50}$ for 30 min at 13.5 mg/L of diclofenac [30] Diclofenac has shown noteworthy effect on the population density of the marine phytoplankton, *Dunaliella tertiolecta* with the $EC_{50}$ value for 96 hour static algal bioassay protocol at 185690µg/l.[79] The acute and chronic toxicity of diclofenac was assessed by using standardized toxicity tests in *Daphnia magna, Pseudoinirikella subcapitiata,* and *Lemna minor* and the results were compared with the various biomarkers in Zebra mussel. The risk quotient value was calculated for diclofenac and was found above 1.[80] The studies have revealed that diclofenac can cause prominent change at environmentally relevant concentrations acting through oxidation pathways with destabilization of lysosomal membrane [81] Acute toxicity studies of diclofenac in algae and invertebrates have inferred that phytoplankton was more sensitive with lowest $EC_{50}$ for 96 h as 14.5 mg/L than zooplankton with lowest $EC_{50}$ for 96 h as 22.43 mg/L.[82] [83] A biochemical investigation using hsp70 level as a biomarker after 48hr in *Daphnia magna* has determined that hsp induction occurred at a lowest observed effect concentration of 30mg/l diclofenac along with 0.6mg/l dimethyl suphoxide and at a lowest observed effect concentration of 40mg/l diclofenac alone.[84] A study on rotifer, *Plationus patulus* and cladoceran, *Moina macrocopa* has shown that diclofenac would decrease their population growth at 1.56- 25mg/l.[85] A chronic study on freshwater crustaceans *Daphnia magna* and *Moina macrocopa* for three months has resulted in the decrease in their reproduction at 25mg/l and 50mg/l respectively.[86] A study on marine mussel, *Mytilus species* has indicated that diclofenac at environmentally relevant concentration of 1µg/l and elevated concentration of 1000µg/l after 96 h exposure would induce the lipid peroxidation indicating tissue damage.[87] Diclofenac has shown severe effects after exposure for four weeks on the survival, growth, reproduction and immunological proceeses of a soil arthropod, *Folsomia candida*.**[88]** . A study on a crab, *Carcinus maenas* has depicted that on exposure to diclofenac at near environmental concentrations would significantly alter the osmoregulatory function.[89]

There is inadequate data about the acute toxicity of fish. Diclofenac has shown highest acute toxicity to fish with lowest observed effect concentration often below 100mg/l.[90] The acute and chronic sub lethal toxicity of diclofenac was observed in fish and fern using mitochondrial activity, chlorophyll, lipid peroxidation and DNA content as biomarkers. The results have depicted that it would cause harmful effects in sensitive population.[91] Recent data on chronic toxicity has suggested that it had no adverse affect on zebra fish and rainbow trout upto 320µg/l.[92] There are evidences that diclofenac would bioconcentrate in certain tissues of fish and might accumulate in the food chain.[50] Some studies have evidenced that at environmental relevant concentrations, diclofenac could affect the multiple gene expression in the exposed fish.[93] Many chronic studies have reported that diclofenac would impair renal functions in fish.[94] [50] [95] Most of the earlier studies have demonstrated acute toxic effects on aquatic organisms at concentrations higher than environmental concentrations.[96][93][97][98] [99] [100] A subchronic toxicity study on common carp has indicated the lowest observed effect concentration at 0.03mg/l and no observed effect concentration at 0.015mg/l of diclofenac.[101] A chronic study in rainbow trout after 28 days of exposure has demonstrated histopathological effects at the LOEC of 5µg/L which include renal lesions (degeneration of tubular epithelia, interstitial nephritis) and alterations of the gills.[51] Another study in rainbow trout exposed to five different concentrations (1, 5, 29, 100, 500µg/l) has depicted cytological alterations in liver, kidney and gills at 1µg/L.[94] The eggs of zebrafish were exposed to six different concentrations of diclofenac (0, 1, 20, 100, 500, 1000, and 2000 $\mu g\ l^{-1}$) but had not shown any effect on the embryonic development, except delayed hatching at 1000 and 2000µg/l.[102] Fish embryo toxicity tests have shown effect on hatching, yolk sac formation and tail deformation at above 1.5mg/l of diclofenac.[103] A study on rainbow trout exposed to diclofenac intraperitoneally and through water at 1.7µg/l has reported the presence of the drug and its metabolites in bile.[104] The drug was observed in the bile of two wild fishes namely roach, *Rutilus rutilus at the concentration of 44-148ng/ml* and bream *Abramis brama at the concentration of 6-95ng/ml* from a lake which received treated municipal waste water.[105] The toxic effects of diclofenac were studied in *Clarius gariepinus* by acute and chronic static renewable bioassay. Acute exposure has caused abnormal behavior and mortality of some fish and with 96h $LC_{50}$ value as 25.12mg/l**.** Chronic exposure of the fish to different concentrations (1.57, 3.14 and 6.28mg/L) has shown significantly higher mean corpuscular concentration of the drug. Furthermore, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and glucose values have significantly increased while protein levels were reduced ($p$<0.05) in serum and gills throughout the 42-day exposure period.[106] Recent studies have indicated the presence of metabolites of diclofenac in the fish bile and the compound has undergone bioconcentration in fish bile showing the bioconcentration factor values upto 1000.[70] A study on Brown trout exposed to 0.5, 5 and 50µg/l of diclofenac for 7, 14 and 21 days, has revealed that diclofenac significantly reduced haematocrit levels after 7 and 14 days of exposure. After 21 days, there were reports of histopathological alterations like increased monocyte infiltration in the liver, telangiectasis in gills, and the occurrence of interstitial hyaline droplets, interstitial proteinaceous fluid and mild tubular necrosis in trunk kidney. Immunohistological analysis has revealed that there was an increase in the number of granulocytes and their accumulation in primary gill filaments, and increased major histocompatibility complex II expression in kidney, suggesting inflammatory process in these organs. The ability of diclofenac to hinder the

stimulation of prostaglandin E2 synthesis was shown in head kidney macrophages of brown trout in vitro.[95] There was an alteration in the feeding behavior of Japanese medaka fish on exposure to 1mg/l of diclofenac.[107] A subchronic exposure in rainbow trout has inferred that diclofenac and its metabolites were accumulated in the bile, interfered the biochemical functions by altering the expression of cyp1a1, cox1, cox 2 and p53 genes and hitopathological alterations were observed in intestine and kidney.[108] Long term exposure of *Oryzias latipes* for three months has reported that diclofenac would delay hatching as the concentrations increased from 0.001-10mg/l and no vitellogenin induction was observed in male fish.[109] Both short term and long term exposure of diclofenac on Indian major carp has shown significant changes in different enzymological parameters.[110] The expression of biomarker genes was related to cytotoxicity, genotoxicity and estrogenic effects in Japanese medaka exposed to 8mg/l and 1µg/l concentrations of DCF in fish [111] A subchronic exposure of fish to sublethal concentrations of DCF to 5, 15, 30 and 60mg/l for 28 days has caused decrease in the growth of fish at 30mg/l and 60mg/l.[112] A study on a fish, *Cyprinus carpio* has indicated that diclofenac would cause oxidative stress in liver, gill, brain and blood.[113] [114] Diclofenac has inhibited the enzyme ethoxyresorufin-O-deethylase (EROD) activity at sublethal concentrations in primary culture of rainbow trout hepatocytes [115] The bioconcentration factor values of diclofenac were reported in the fish rainbow trout in plasma as 4.02-0.75 l/kg [93] 7 l/kg [116] 4.9-5.7 l/kg [117] in bile as 509-657 l/kg [108] 476-797 l/kg [117] and in liver 2.54-0.36 l/kg.[93]

The comparision of fish blood plasma concentrations to the human therapeutic levels has indicated that Diclofenac has the highest risk for non-target species.[116] Studies on embryos of the frog, *Xenopus* have inferred that diclofenac causes morphological anamolies, but would not alter developmental tissue during embryogenesis.[118] A chronic exposure study on Swiss albino mice has revealed that diclofenac causes genotoxicity with induction of chromosomal aberrations and formation of micronucleipolychromatic erythrocytes in bone marrow and sperm abnormality in germ cells.[119]

Studies on Vulture mortality in Indian subcontinent was due to acute renal failure and visceral gout [12] and the occurrence of extensive deposits of uric acid on and within internal organs.[120] Diclofenac was proved to be toxic to the *Gyps fluvus* and *Gyps africanus* which were from Eurasia and Africa respectively.[121] The findings of a simulation model on Vulture population has supported that diclofenac poisoning was the cause of their rapid decline.[13]A comparative study in poultry birds using nimesulide and diclofenac sodium has inferred that it would cause histopathological lesions in liver and kidney.[11] The renal failure in oriental white backed vultures was found to be due to inhibition of the modulating effect of prostaglandin on angiotensin -2 mediated adrenergic stimulation.[122] A pathological and microbiological study on Vultures has indicated that death of birds was due to visceral gout and intra nuclear inclusions, perivascular lymphoid aggregates and haemolytic E.coli were also noticed in the tissues.[123] Diclofenac at higher doses has caused alterations in hepatobilliary, nephric and gastrointestinal systems in Swiss albino mice.[124] A major biliary metabolite of diclofenac, diclofenac acyl glucoronide has increased the ulceration in small intestine in rats.[125] A study on rats has revealed that intestinal bacteria play a significant role in the cause of hepatotoxicity by diclofenac.[126]

**Diclofenac in human**

Diclofenac can be administered either orally or topically into the human body. The World health organization has recommended a daily dose of 100mg for diclofenac.[72] It is rapidly absorbed from the gastrointestinal tract.[127,128] It is bound to the plasma proteins and thus its wider distribution to the extracellular spaces is reduced.[129,130] Diclofenac undergoes hepatic biotransformation involving aromatic hydroxylations and conjugations.[131,132] There are about five metabolites formed in the liver.[133] The drug is eliminated in the form of glucoroconjugates and sulphate metabolites to a greater extent and very little amount is excreted as parent drug through kidneys.[130] There are about six metabolites formed from diclofenac in human plasma namely 4' hydroxy diclofenac, 5'- hydroxydiclofenac, 3' hydroxy-diclofenac, 4',5- dihydroxy-diclofenac 4'-hydroxy-3-methoxy- diclofenac, 3'-hydroxy 4'-methoxy diclofenac.[134] [135,136] About 70% of the orally administered dose is excreted through urine and 30% in feces as the parent drug or its metabolites.[134] The mean terminal half life of the oral drug and intravenous doses in plasma were 1.8 hours and 1.1 hours respectively.[137]

**Mode of action**

It exerts its action by inhibiting the effect of cycloxygenase-1 (COX-1) and cyclooxygenase - 2 (COX-2) enzymes which are helpful in the biosynthesis of prostaglandins.[138] Prostaglandins are produced at the sites of injury or damage, and cause pain and inflammation.[139] Diclofenac by blocking the effect of COX enzymes, helps in the less production of prostaglandins, thus reducing pain and inflammation.[140] It may also interact with the lipoxygenase pathway with the release and reuptake of arachidonic acid.[9]

**Efficacy**

Diclofenac mouthwash was found to be helpful in reducing periodontal postoperative pain but was more effective in controlling pain when used along with other drugs. A study has found that diclofenac was more effective when used with vitamin B in the treatment of pain originated by lower limb fracture and surgery.[141] There was no clinical evidence that topical non steroidal anti inflammatory drugs were more effective in long duration.[142] A comparative study of diclofenac with other non steroidal anti-inflammatory drugs against

osteoarthritis and rheumatoid arthritis has found that the risk and safety depends on the individuals and should be taken into consideration before treatment.[143] A study has shown that fixed dose combination of diclofenac and tramadol was effective in reducing acute moderate to severe pain in various disorders.[144] Diclofenac when used along with muscle relaxant Thioclochocoside would decrease pain, stiffness and physical function of orthopedic patients than used alone.[145] Diclofenac in addition to its efficacy has acceptable tolerability profile in elderly patients with osteoarthritis.[146] It was effective in treating pain after conventional radiofrequency neurotomy with minimum cost of treatment.[147] Low dose submicron diclofenac was found as an effective agent for the treatment of chronic pain caused due to osteoarthritis.[148] Diclofenac was effective in treating acute shoulder pain.[149] Diclofenac epolamine topical patch 1.3% was effective in managing pains in soft tissue injuries as sprains, strains and contusions.[150,151] Diclofenac 100C topical formulation was found to be efficient in penetration into the local tissues without high systemic concentrations found in oral forms.[152] The usage of topical solution for the treatment of osteoarthritis was more effective and found to be safe with minimum side effects as local skin irritation.[153] [154] Diclofenac continues to be an effective pain management drug in osteoarthritis on par with other recent drugs.[155] Diclofenac along with Paracetomal was found to be effective in treating postoperative pain cesarean section against Meperdine.[156] It reduces the calcium loss in urine thus inhibiting the bone resorption in postmenopausal stage of women.[157] [158] The long term usage of diclofenac sodium gel was effective in treating knee osteoarthritis in elderly population with oral drug.[159] It was tested for antibacterial activity in vitro against 397 bacteria and resulted in inhibition at 50-100µg/100ml and the in vivo data was significant ($P<0.001$).[160] Diclofenac was found to be an effective antimicrobial agent inhibiting several antibiotic resistant bacteria.[161] Many strains of gram positive and gram negative bacteria were inhibited by diclofenac sodium due to the inhibition of DNA synthesis at the concentration 50-100mg/l.[162]

**Risks**

Diclofenac is reported to cause several side effects. It leads to gastrointestinal toxicity causing ulcers and bleeding.[128] The gastrointestinal toxicity and bleeding from the intestinal ulcers was believed to be due to the inhibition of the COX-1 gene and was proved in an invitro study.[163] Recently the COX -2 specific inhibitors have come into use to reduce the incidence of ulcers.[164] The excessive dosage of Diclofenac has reported the gastrointestinal damage with perforations.[165] [166] A clinical trial has shown that in a population of 268 patients, 0.3% were with gastrointestinal ulcers.[167] An ex-vivo study using precision-cut intestinal slices from jejunum of donors has indicated that Diclofenac can cause intestinal toxicity at the therapeutic concentrations independent of the metabolites formed in the liver or intestine.[168] There are evidences that diclofenac could cause hepatic toxicity related with higher rates of aminotransferase elevations.[169] A large clinical trial in arthritis patients treated with diclofenac has revealed that DCF caused aminotransferase elevations in 4-6 months of treatment and clinical liver injury might occur early or late in therapy.[170] There are evidences of hepatotoxicity in rat and human cell lines with the impairement of mitochondrial activity and futile consumption of NADPH.[171] [172] The high dose of diclofenac was found to be associated with a moderate increase in the risk of cardiovascular functions.[173] Diclofenac given intramuscularly in the left arm deltoid region has developed gangrene in the upper limb.[174] The intramuscular injection of diclofenac has caused Nicolau syndrome with symptoms of necrosis of skin, subcutaneous and muscle tissue.[175] [176] Sonographic studies of Nicolau syndrome after intramuscular injection of Diclofenac has shown diffuse thickening with increased echogenicity of the skin and subcutaneous fat layer, while MRI has revealed extensive edema involving gluteal and piriformis muscles and deep fascia, and fluid collection.[177] Diclofenac was associated with the increased risk of cardiovascular diseases even in low dose with short duration treatment and it was inferred that its usage in patients with myocardial infarction was more dangerous.[178] A comparative study of patient groups taking COX-2 inhibitor, Celecoxib and non steroidal anti-inflammatory drugs ibuprofen or Diclofenac has demonstrated that thromboembolic events like myocardial infarction, cardiovascular deaths, stroke and peripheral events were similar in all patient groups.[179] A systemic review of the observational studies has indicated that there is an increased risk of ischemic stroke with the use of rofecoxib and diclofenac.[180] A study in general population of Netherlands has inferred that there was a high risk of stroke with the use of both selective COX-2 and non-selective COX -2 non steroidal anti inflammatory drugs.[181]

## CONCLUSION

The availability of sophisticated instrumentation has made possible the detection of the pharmaceuticals from various sources. The fate and removal of pharmaceuticals has also been studied using different techniques. Further research is needed to understand the complete transformation and degradation of pharmaceuticals. The efficiency of sewage treatment plants should be improved to diminish the entry of pharmaceuticals into surface waters. The adverse impact of diclofenac on various organisms including human is not predictable with the available ecotoxicological data. The acute toxicity data of pharmaceuticals reveals that they are not of great concern to aquatic life at present environmental concentrations. However, chronic toxicity is anticipated as they are continuously released into the environment. There is a paucity of data on chronic toxicity studies in aquatic organisms, particularly in fish. Future research should be focussed on chronic toxicity

using novel approaches rather than conventional standard acute toxicity tests in all aquatic species. There is a need of more research on mixture toxicity of various drugs and their synergistic effects. In order to minimize the side effects of Diclofenac in human, it should be administered in combination with such compounds which do not alter the functional anatomy of human.

**REFERENCES**


1. Arnold KE, . Boxall ABA, Brown AR, . Cuthbert R J, Gaw S,. Hutchinson TH , Jobling S, Madden JC, Metcalfe CD, Naidoo V, Shore RF, . Smits J E , . Taggart MA, Thompson HM. Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. Biol Lett, 2013; 9(4): 20130492.
2. Peake BM, and Braund R. Environmental Aspects of the Disposal of Pharmaceuticals in New Zealand. Chem New Zealand, 2009; 73(2): 58-63.
3. Rodríguez I, Quintana JB, Carpinteiro J, Carro AM, Lorenzo RA, Cela R. Determination of acidic drugs in sewage water by gas chromatography-mass 16 spectrometry as tert.-butyldimethylsilyl derivatives. J Chromatogr A, 2003; 985: 265-274.
4. Hernando MD, Mezcua M, Fernández-Alba AR, Barceló D. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta,2006; 69( 2): 334–342.
5. Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environ Sci Technol , 2008; 42: 5807–5813
6. Bottoni P,Caroli S, Bama Caracciolo A. Pharmaceuticals as priority water contaminants. Toxicol Environ Chem 92(3) 549-565
7. Jones OAH, Voulvoulis N, Lester JN. The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilizing activated sludge treatment. Environ Pollut, 2007; 145: 738- 744
8. Buser HR, Poiger T , and Müller MD. Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake. Environ. Sci. Technol, 1998; 32(22): 3449- 3456.
9. Skoutakis VA, Carter CA, Mickle TR, Smith VH, Arkin CR, Alissandratos J, Petty DE. Review of diclofenac and evaluation of its place in therapy as a nonsteroidal antiinflammatory agent. Drug Intell Clin Pharm, 1988; 22(11): 850-859
10. Brogen RN, Pakes GE, Speight TM, Avery GS. Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. Drugs, 1980; 20:24-48.
11. Prakash V, Pain DJ, Cunningham AA, Donald PF, Prakash N, Verma A, Gargi R, Sivakumar S, Rahmani AR. Catastrophic collapse of Indian white-backed Gyps bengalensis and long-billed Gyps indicus vulture populations. Biol Cons, 2003; 109,: 381–390.
12. Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJ, Arshad M, Mahmood S,Ali A, Khan AA. Diclofenac residues as the cause of vulture population decline in Pakistan. Nature. 2004; 427(6975): 630-633.
13. Green RE, Newton I, Shultz S, Cunningham AA, Gilbert M , Pain DJ, Prakash V. Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. J Appl Ecol, 2004; 41( 5): 793–800.
14. Shultz S, Baral HS, Charman S, Cunningham AA, Das D, Ghalsasi GR, Goudar MS, Green RE, Jones A, Nighot P, Pain DJ, Prakash V. Diclofenac poisoning is widespread in declining vulture populations across the Indian subcontinent. Proc. Biol Sci, 2004; 271(6): 458-60.
15. Ogada DL, Keesing F, Virani MZ. Dropping dead: causes and consequences of vulture population declines worldwide. Ann. N.Y. Acad. Sci,. 2011; 1–15.
16. Ganguly S, Mukhopadhayay SK. Population threat to vultures in India. Int. J. Livest. Res, 2013; 3(1): 141-144.
17. Mahmood KT, Ashraf M, Ahmed MU. Eco- friendly Meloxicam replaces eco-damaging Diclofenac sodium in Veternary practice in South Asia. –A Review. J Pharm Sci Res, 2010; 2 (10): 672-685.
18. Nikolaou A, Sureyya Meric S, Fatta D. Occurrence patterns of pharmaceuticals in water and wastewater environments. Anal Bioanal Chem, 2007; 387(4): 1225-1234.
19. Al-Qaim FF, AbdullahMP, Mohamed Rozali Othman MR, Jalifah Latip J and Wan Mohamed Afiq WM. Development of Analytical Method for Detection of Some Pharmaceuticals in Surface Water. Trop J Pharm Res, 2013; 12 (4): 609-616.
20. Soulet B ,Taxue A, Tarradellas J. Analysis of acidic drugs in Swiss waste waters. Int J Environ Chem, 2002; 82: 659-667.
21. Tixier C, Singer HP , Oellers S , and Müller SR. Occurrence and Fate of Carbamazepine, Clofibric Acid, Diclofenac, Ibuprofen, Ketoprofen, and Naproxen in Surface Waters. Environ. Sci. Technol, 2003; 37(6): 1061–1068.
22. Tauxe-Wuersch A, De Alencastro LF, Grandjean D, Tarradellas J. Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. Water Res, 2005; 39: 1761-1772.
23. Sun Z, Schüssler W, Sengl M. Niessner R , Knopp D. Selective trace analysis of diclofenac in surface and wastewater samples using solid-phase extraction with a new molecularly imprinted polymer Analytica Chimica Acta, 2008; 620 (1–2): 73–81.
24. Roberts PH, Thomas KV. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ, 2006; 356,(1-3): 143–153.

25. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. The occurrence of pharmaceuticals, personal care products, endocrine disruptors in biosolids and illicit drugs in surface water in South Wales, UK. Water Res 2008. 42: 3498-3518.
26. Stumpf M, Ternes TA, Wilken RD, Rodrigues SV, Baumann W. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. Sci. Total Environ,1999; 225: 135–141.
27. Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water Res, 2007; 41(5), 1013–1021.
28. Choi K, Kim P, Park J. Pharmaceuticals in environment and their implication in environmental health. J. Environ. Health Sci, 2009; 35: 433–446.
29. Sim W J, Lee JW, Lee ES, Shin SK, Hwang SR, Oh JE. Occurrence and Distribution of Pharmaceuticals in Waste Water from Households, Livestock Farms, Hospitals and Pharmaceutical Manufactures, Chemosphere, 2011; 82: 179-86
30. Farre, M., Ferrer, I., Ginebreda, A., Figueras, M., Olivella, L., Tirapu, L., Vilanova, M., Barcelo, D. Determination of drugs in surface water and wastewater samples by liquid chromatography–mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fischeri. J. Chromatogr. A, 2001; 938: 187–197.
31. Gómez MJ, Martínez Bueno MJM, LacorteS, Fernández-Alba[a]AR, AgüeraAPilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere, 2007; 66(6): 993–1002.
32. Scheurell M, Franke S, Shah RM, Hühnerfuss H. Occurrence of diclofenac and its metabolites in surface water and effluent samples from Karachi, Pakistan. Chemosphere, 2009; 77(6): 870-6.
33. Lin AY , Tsai YT. Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. Sci. Total Environ, 2009; 407(12): 3793–3802.
34. Shanmugam G, Sampath S, Selvaraj KK, Larsson DG, Ramaswamy BR. Non-steroidal anti-inflammatory drugs in Indian rivers Environ Sci Pollut Res Int. 2014 Jan;21(2):921-31.
35. Andreozzi R, Raffaele M, Nicklas P. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere., 2003; 50(10): 1319-30.
36. Zuccato E, Castiglioni S, Fanelli R, Reitano G, Bagnati R, Chiabrando C, Pomati F, Rossetti C, Calamari D. Pharmaceuticals in the environment in Italy: causes, occurrence, effects and control. Environ Sci Pollut Res Int., 2006; 13(1):15-21.
37. Patrolecco L, Capri S, Ademollo N. Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters. Environ Sci Pollut Res, 2015; 22(8): 5864-5876.
38. Lishman L, Symth SA, Sarafin K, Kleywegt S, Toito J,Peart T, Lee B, Servos M, Beland M, Seto P. Occurrence and reductions of pharmaceuticals and personal care products estrogens by municipal wastewater treatment plants in Ontario, Canada. Sci Total Environ 367: 544-558.
39. Metcalfe CD, Miao XS, Koenig BGand Struger J. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada. Environ Toxicol Chem, 2003; 22(12): 2881–2889.
40. Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. Water Res, 1998; (32): 3245–3260.
41. Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett, 2002; 131, (1–2): 5–17.
42. Ashton D, Hilton M, Thomas KV.Investigating the environmental transport of human pharmaceuticals to streams in the united kingdom. Sci Total Environ, 2004; 333: 167-184.
43. Stülten D, Zühlke S, Lamshöft M, Spiteller M. Occurrence of diclofenac and selected metabolites in sewage effluents Sci Total Environ, 2008; 405(1-3): 310–316.
44. Zhang Y, Geißen S, Gal C. Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. Chemosphere, 2008; 73 (8): 1151–1161.
45. Bendz D, Paxéus NA, Ginn TR, Loge FJ. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Hörje River in Sweden. J Hazard Mater, 2005; 122: 195-204.
46. Zorita S, Mårtensson L, Mathiasson L. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. Sci Total Environ, 2009; 407: 2760–70.
47. Vieno NM, Tuhkanen T, Kronberg L. Seasonal variation in the occurrence of Pharmaceuticals in Effluents from a Sewage Treatment Plant in the Recipient Water. Environ Sci Technol 2005; 39 (21); 8220-8226.
48. Lindqvist N, Tuhkanen T, Kronberg L. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. Water Res, 2005; 39: 2219–2228.
49. Siemens J , Huschek G , Siebe C , Kaupenjohann M. Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City–Mezquital Valley. Water Res, 2008; 42(8–9): 2124–2134
50. Schwaiger J, Ferling H, Mallow U, Wintermayr H, Negele DR. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac Part I: Histopathological alterations and bioaccumulation in rainbow trout. Aquat Toxicol. 2004; 68:1 41–150.

51. Joss A., Keller E., Alder A., Göbel A., McArdell C.S., Ternes T., Siegrist H. Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res, 2005; 39: 3139 – 3152.
52. Yavas AZ, Dogan S, Cinar S A, Kidak R, Aviyente V, Ince NH. Sonochemical degradation of diclofenac: byproduct assessment, reaction mechanisms and environmental considerations. Environ Sci Pollut Res, 2014; 21(9): 2514-2517.
53. Wert EC , Rosario-Ortiz FL, Snyder SA. Effect of ozone exposure on the oxidation of trace organic contaminants in wastewater. Water Res, 2009; 43(4): 1005–1014.
54. Broseus R , Vincent S, Aboulfadl K, Daneshvar A, Sauvé S, Barbeau B, Prévost M. Ozone oxidation of pharmaceuticals, endocrine disruptors and pesticides during drinking water treatment. Water Res, 2009; 43(18): 4707–4717.
55. Beltrán FJ, Pocostales P, Alvarez P, Oropesa A. Diclofenac removal from water with ozone and activated carbon. J Hazard Mater. 2009;163(2):768-76.
56. Diniz MS , Salgado R , Pereira VJ, Carvalho G, Oehmen A, Reis MAM , Noronha JP Ecotoxicity of ketoprofen, diclofenac, atenolol and their photolysis byproducts in zebrafish (Danio rerio) Sci Total Environ, 2015; 505: 282–289.
57. Packer J L, Werner J J , Latch DE , McNeill K, Arnold WA. Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. Aquat Sci, 2003; 65 (4): 342-351.
58. Bartels P, von Tumpling W. Solar radiation influence on the decomposition process of diclofenac in surface waters. Sci Total Environ, 2007; 374(1): 143-55.
59. Pérez-Estrada LA , Malato S , Gernjak W , Agüera A , ThurmanEM , Ferrer I , and Fernández-Alba AR. Photo-Fenton Degradation of Diclofenac: Identification of Main Intermediates and Degradation Pathway. Environ. Sci. Technol, 2005; 39 (21): 8300–8306.
60. Rizzo L , Meric S, Kassinos D, Guida M, Russo F, Belgiorno V. Degradation of diclofenac by $TiO_2$ photocatalysis: UV absorbance kinetics and process evaluation through a set of toxicity bioassays. Water Res, 2009; 43(4): 979–988.
61. Kanakaraju D, Motti CA, Beverly D Glass BD, Oelgemoller M. Photolysis and Tio2 catalysed degradation of diclofenac in surface drinking water using circulating batch photoreactors. Environ Chem, 2014; 2(1): 51-62.
62. Groing J, Held C, Garten C , Claußnitzer U , Kaschabek SR, Schlömann M. Transformation of diclofenac by the indigenous microflora of river sediments and identification of a major intermediate. Chemosphere, 2007; 69 (4): 509–516.
63. Paxeus N. Removal of selected non-steroidal anti-inflammatory drugs (NSAIDs), gemfibrozil, carbamazepine, beta-blockers, trimethoprim and triclosan in conventional wastewater treatment plants in five EU countries and their discharge to the aquatic environment. Water Sci Technol, 2004; 50(5): 253-260.
64. Dai C, Geissen SU, Zhang Y, Zhang Y, Zhou X. Selective removal of diclofenac from contaminated water using molecularly imprinted polymer microspheres Environ Pollut, 2011; 159(6): 1660-1666.
65. Rigobello ES, Dantas AD, Di Bernardo L, Vieira EM. Removal of diclofenac by conventional drinking water treatment processes and granular activated carbonfiltration. Chemosphere, 2013; 92(2): 184-91.
66. Vergili I. Application of nanofiltration for the removal of carbamazepine, diclofenac and ibuprofen from drinking water sources. J Environ Manage, 2013; 127: 177–187
67. Paje M, Kuhlicke U, Winkler M, Neu T. Inhibition of lotic biofilms by Diclofenac. Appl Microbiol Biotechnol, 2002; 59 (4-5): 488-492.
68. Hata T , Kawai S, Okamura H, and Nishida T. Removal of diclofenac and mefenamic acid by the white rot fungus Phanerochaete sordida YK-624 and identification of their metabolites after fungal transformation. Biodegradation. 2010; 21(5): 681-9.
69. Agüera A, Pérez Estrada LA, Ferrer I, Thurman EM, Malato S and Fernández-Alba AR. Application of time-of-flight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight. J Mass Spectrom, 2005; 40 (7): 908–915.
70. Svanfelt J, Kallio JM, Eriksson J, Kronberg L. Environmental Fate and Hazards of the Pharmaceutical Diclofenac in Aquatic Environments. ACS Symp Ser, 2010; 1048: 243-255.
71. Verenitch SS, Lowe CJ, Mazumder A.Determination of acidic drugs and caffeine in municipal wastewaters and receiving waters by gas chromatography-ion trap tandem mass spectrometry. J Chromatogr A. 2006 26;1116(1-2):193-203.
72. Vieno N , Sillanpää M. Fate of diclofenac in municipal wastewater treatment plant — A review. Environ Int, 2014; 69: 28–39.
73. Khatem R, Miguel RO, Bakhti A. Use of synthetic clay for Removal of Diclofenac Anti-inflammatory Eurasian J of Soil Sci, 2015; 4(2): 76-143.
74. Williams RT. Human health pharmaceuticals in the environment—An introduction. In WilliamsRT, ed, Human Pharmaceuticals: Assessing the Impact on Aquatic Ecosystems. Society of Environmental Toxicology Chemistry, Pensacola, FL, USA, 2005. pp 1–46.

75. Bound JP and Voulvoulis N. Household Disposal of Pharmaceuticals as a Pathway for Aquatic Contamination in the United Kingdom. Environ Health Perspect, 2005; 113(12): 1705-1711.
76. Larsson DGJ, de Pedro C, Paxeus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J Hazard Mater, 2007; 148: 751–755.
77. Carlsson G, Örn S and Larsson DGJ. Effluent from bulk drug production is toxic to aquatic vertebrates. Environ Toxicol Chem, 2009; 28(12): 2656–2662.
78. Ghalehnoo ZR, Rashki A , Najimi M, Dominguez A. The role of diclofenac sodium in the dimorphic transition in Candida albicans. Microb Pathog, 2010; 48(3–4): 110–115.
79. DeLorenzo ME, Fleming J. Individual and mixture effects of selected pharmaceuticals and personal care products on the marine phytoplankton species Dunaliella tertiolecta. Arch Environ Contam Toxicol. 2008; 54(2): 203-10.
80. Grung M, Källqvist T, Sakshaug S, Skurtveit S, Thomas KV. Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. Ecotoxicol Environ Safe. 2008; 71(2):328-40.
81. Quinn B, Bound JP and Voulvoulis N. Household Disposal of Pharmaceuticals as a Pathway for Aquatic Contamination in the United Kingdom. Environ Health Perspect, 2005; 113(12): 1705-1711.
82. Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett, 2003; 142(3): 185–194.
83. Ferrari B, Mons R, Vollat B, Fraysse B, Paxeus N, Giudice RL, Pollio A, Garric J. Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment. Environ Toxicol Chem, 2004; 23:1344–1354.
84. Haap T, Triebskorn R, Köhler HR. Acute effects of diclofenac and DMSO to Daphnia magna: Immobilisation and hsp70-induction. Chemosphere, 2008; 73(3): 353–359.
85. Sarma SS, González-Pérez BK, Moreno-Gutiérrez RM, Nandini S. Effect of paracetamol and diclofenac on population growth of Plationus patulus and Moina macrocopa. J Environ Biol. 2013;35:119-26.
86. Lee J, Ji K, Kho YL, Kimc P, Choi K.Chronic exposure to diclofenac on two freshwater cladocerans and Japanese medaka. Ecotox Environl Safe, 2011; 74: 1216–1225.
87. Schmidt W, O'Rourke K , Hernan R, Quinn B. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (Mytilus spp.) and their comparison with standardized toxicity tests. Mar Pollut Bull, 2011; 62(7): 1389–1395.
88. Chen G, den Braver MW, van Gestel CAM, van Straalen NM , Roelofs D. Ecotoxicogenomic assessment of diclofenac toxicity in soil. Environ Pollut, 2015; 199: 253 – 260.
89. Eades C, Waring CP. The effects of diclofenac on the physiology of the green shore crab Carcinus maenas. Mar Environ Res, 2010; 69 (1): S46–S48.
90. Fent K,. Weston AA, Caminada D. Ecotoxicology of Pharmaceuticals. Aquat Toxicol, 2006; 76: 122–159.
91. Feito R, Valcárcel Y, Catalá M. Biomarker assessment of toxicity with miniaturised bioassays: diclofenac as a case study. Ecotoxicology. 2012 ;21(1): 289-96.
92. Memmert U, Peither A, Burri R, Weber K, Schmidt T, Sumpter JP, and Hartmann A. diclofenac: new data on chronic toxicity and bioconcentration in fish Environ Toxicol Chem, 2013; 32(2): 442–452.
93. Cuklev F, Kristiansson E, Fick J, Asker N, Förlin L, Larsson DGJ. Diclofenac in fish: Blood plasma levels similar to human therapeutic levels affect global hepatic gene expression. Environ Toxicol Chem, 2011; 30(9): 2126-2134.
94. Triebskorn R, Casper H, Heyd A, Eikemper R, Köhler HR, Schwaiger J. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part II: cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss). Aquat Toxicol, 2004; 68(2): 151-66.
95. Hoeger B, Kollner B, Dietrich DR, Hitzfeld B. Water-borne diclofenac affects kidney and gill integrity and selected immune parameters in brown trout (Salmo trutta f. fario). Aquat Toxicol, 2005; 75: 53-64.
96. Dietrich, D. R., & Prietz, A. (1999). Fish embryotoxicity and teratogenicity of pharmaceuticals, detergents and pesticides regularly detected in sewage treatment plant effluents and surface waters. Toxicologist, 1999; 48(1), 151.
97. Ferrari B, Paxéus N, Giudice RL, Pollio A, Garric J. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: Study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol Environ Safe. 2003;55:359–370
98. Halling-Sorensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F.,Holten Lu¨tzh^ft, H.C., Jorgensen, S.E., Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere, 1998; 36: 357–393.
99. Cleuvers M. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotox Environ Safe. 2004; 59: 309–315.
100. Han, G.H., Hur, H.G., Kim, S.D., 2006. Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: occurrence and toxicity to Daphnia magna. Environl Toxicol Chem, 2006; 25: 265–271.
101. Stepanova S, Praskova E, Chromcova L, Plhalova L, Prokes M, Blahova J, Svobodova Z. The effects

of diclofenac on early life stages of common carp (Cyprinus carpio). Environ Toxicol Pharmacol. 2013; 35(3): 454-60.
102. Hallare AV , Köhler HR, R. Triebskorn R. Developmental toxicity and stress protein responses in zebrafish embryos after exposure to diclofenac and its solvent, DMSO. Chemosphere, 2004; 56 (7): 659–666.
103. van den Brandhof EJ, Montforts M. Fish embryo toxicity of carbamazepine, diclofenac and metoprolol. Ecotoxicol Environ Safe, 2010; 73(8): 1862–1866.
104. Kallio JM , Lahti M , Oikari A , and Kronberg L. Metabolites of the Aquatic Pollutant Diclofenac in Fish Bile. Environ. Sci. Technol, 2010; 44 (19): 7213–7219.
105. Brozinski JM, Lahti M, Meierjohann A , Oikari A, and Kronberg L. The Anti-Inflammatory Drugs Diclofenac, Naproxen and Ibuprofen are found in the Bile of Wild Fish Caught Downstream of a Wastewater Treatment Plant. Environ. Sci. Technol, 2013; 47 (1): 342–348.
106. Ajima MNO, OA, Bala S. Audu, and Ugwoegbu KC. Chronic diclofenac (DCF) exposure alters both enzymatic and haematological profile of African catfish, Clarias gariepinus . Drug Cheml Toxicol, 2014; 30(4): 383-390.
107. Nassef M, Matsumoto S, Seki M, Khalil F, Kang IJ, Shimasaki Y, Oshima Y , Honjo T. Acute effects of triclosan, diclofenac and carbamazepine on feeding performance of Japanese medaka fish (Oryzias latipes). Chemosphere,2010; 80(9): 1095–1100.
108. Mehinto AC, Hill EM, Tyler CR. Uptake and Biological Effects of Environmentally Relevant Concentrations of the Nonsteroidal Anti-inflammatory Pharmaceutical Diclofenac in Rainbow Trout (Oncorhynchus mykiss). Environ. Sci. Technol, 2010; 44 (6): 2176–2182.
109. Lee J, Ji K, Kho YL, Kim P, Choi K. Chronic exposure to diclofenac on two freshwater cladocerans and Japanese medaka. Ecotoxicol Environ Safe, 2011;74(5):1216-25.
110. Saravanan M, Ramesh M, Petkam R. Alteration in certain enzymological parameters of an Indian major carp, Cirrhinus mrigala exposed to short- and long-term exposure of clofibric acid and diclofenac . Fish Physiol Biochem, 2013; 39(6): 1431-40.
111. Hong HN, Kim HN, Park KS, Lee SK, Gu MB. Analysis of theeffects diclofenac has on Japanese medaka (Oryzias latipes) using real-time PCR. Chemosphere, 2007; 67 (11): 2115–2121.
112. Praskova E, Plhalova L, Chromcova L, Stepanova S, Bedanova I , Blahova J, Hostovsky M, Skoric M, Maršálek P, Voslarova E, and Svobodova Z. Effects of Subchronic Exposure of Diclofenac on Growth, Histopathological Changes, and Oxidative Stress in Zebrafish (Danio rerio) The Scientific World Journal, 2014; (2014), Article ID 645737, 5 pages.
113. Hariz Islas-Flores a , Leobardo Manuel Go´mez-Oliva´n a,n , Marcela Galar-Martı´nez b , Arturo Colı´n-Cruz a , Nadia Neri-Cruz a , Sandra Garcı´a-Medina. Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (Cyprinus carpio). Ecotoxicol Environ Safe, 2013; 92: 32–38.
114. Saucedo-Vence K, Dublán-García O, López-Martínez LX, Valdes GM, Galar-Martínez M, Islas-Flores H, Gómez-Oliván LM. Short and long-term exposure to diclofenac alter oxidative stress status in common carp Cyprinus carpio. Ecotoxicology, 2015; 24( 3) : 527-539.
115. Laville, N., Aït-Aïssa, S., Gomez, E., Casellas, C., Porcher, J.M., 2004. Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes. Toxicology 196, 41-55.
116. Brown JN, Paxéus N, Förlin L , Larsson DGJ. Variations in bioconcentration of human pharmaceuticals from se wage effluents into fish blood plasma. Environ Toxicol Pharmacol, 2007; 24(3): 267–274
117. Lahti M., Brozinski J.-M., Jylhä A., Kronberg L. & Oikari A.. Uptake from water, biotransformation and biliary excretion of pharmaceuticals by rainbow trout. Environ Toxicol Chem, 2011; 30: 1403–1411.
118. Chae JP, Park MS, Hwang YS, Min BH, Kim SH, Lee HS, Park[MJ] Evaluation of developmental toxicity and teratogenicity of diclofenac using Xenopus embryos. Chemosphere, 2015; 120: 52–58.
119. Rina T, Pankaj T, Panncholi SS. Genotoxicity Studies of Diclofenac Sodium in the Bone marrow and germ cells of laboratory mice. Austin J Pharmacol Ther, 2014; 2(10): 8.
120. Gilbert, M., Virani, M. Z., Watson, R. T., Oaks, J. L., Benson, P. C., Khan, A. A.,Ahmed, S., Chaudhry, J., Arshad, M.,Mahmood, S. and Shah, Q. A. (2002) Breeding and mortality of Oriental Whitebacked Vulture Gyps bengalensis in Punjab province, Pakistan. Bird Conserv. Int. 12, 311–326.
121. AA, Duncan N, Meharg AA , Oaks JL, Parry-Jones J, Shultz S, Taggart MA, Verdoorn G, and Wolter K Toxicity of diclofenac to Gyps vultures. Biol Lett, 2006; 2(2): 279–282.
122. Meteyer CU, Rideout BA, Gilbert M, Shivaprasad HL, and Oaks JL. Pathology and proposed pathophysiology of diclofenac poisoning in free-living and experimentally exposed oriental white-backed vultures (Gyps bengalensis). J Wildl Dis, 2005; 41(4): 707-716.
123. Mishra SK, Prasad G, Malik MY, Mahajan NK , and Prakash V. Vulture mortality: pathological and microbiological investigations. Indian J Anim Sci, 2002; 72: 283-286.
124. Thanagari BS, Fefar DT, Prajapati KS, Jivani BM, Thakor KB, Patel JH, Ghodasara DJ,. Joshi BP, Undhad VV. Haemato-biochemical alterations

induced by Diclofenac sodium toxicity in Swiss albino mice. Vet World, 2012; 5(7): 417-419
125. Seitz S, Boelsterli UA. Diclofenac acyl glucuronide, a major biliary metabolite, is directly involved in small intestinal injury in rats. Gastroenterology, 1998; 115(6): 1476–1482.
126. Deng X, Liguori MJ, Sparkenbaugh EM, Waring JF, Blomme EAG, Ganey PE and Roth RA.Gene Expression Profiles in Livers from Diclofenac-Treated Rats Reveal Intestinal Bacteria-Dependent and -Independent Pathways Associated with Liver Injury. the J Pharmacol Exp Ther, 2008; 327 (3): 634-644.
127. Glieter CH, Antonin KH, Bieck P, Godbillon J, Scho nlebr W, Malchow H. Colonoscopy in the investigation of drug absorption in healthy volunteers. Gastrointest Endosc 1985; 31:71-73.
128. Carson J, Notis WM, Orris ES. Colonic ulceration a nd bleeding during diclofenac therapy. N Engl J Me d 1989; 323: 135-137.
129. Verbeeck RK, Blackburn JL, Loewen GR. Clinical pharmacokinetics of non-steroidal anti-inflammatory drugs. Clin Pharmacokinet, 1983; 8(4): 297-331.
130. Davies NM, Anderson KE. Clinical pharmacokinetics of diclofenac. Therapeutic insights and pitfalls. Clin Pharmacokinet, 1997; 33: 184–213.
131. Bort R, Mace K, Boobis A, Gomez-Lechon MJ, Pfeifer A, Castell J. Hepatic metabolism of diclofenac: Role of human CYP in the minor oxidative pathways. Biochem Pharmacol, 1999; 58: 787–796.
132. Kirchheiner J, Meineke I, Steinbach N, Meisel C, Roots I, Brockmo¨ller J. Pharmacokinetics of diclofenac and inhibition of cyclooxygenases 1 and 2: No relationship to the CYP2C9 genetic polymorphism in humans. Br J Clin Pharmacol, 2003; 55: 51–61.
133. John VA. The pharmacokinetics and metabolism of diclofenac sodium (Voltarol) in animals and man. Rheumatol Rehabil.,1978; 2: 22–37.
134. Stierlin H, Faigle JW. Biotransformation of Diclofenac Sodium (Voltaren®) in Animals and in Man.: II. Quantitative determination of the unchanged drug and principal phenolic metabolites, in urine and bile. Xenobiotica, 1979; 9(10): 611-621.
135. Faigle JW, BÖttcher I, Godbillon J, Kriemler HP, Schlumpf E, Schneider W, Schweizer A, Stierlin H, Winkler T. A new metabolite of diclofenac sodium in human plasma. Xenobiotica. 1988;18(10):1191-1197.
136. Blum W, Faigle JW, Pfaar U, Sallmann A. Characterization of a novel diclofenac metabolite in human urine by capillary gas chromatography-negative chemical ionization mass spectrometry. J Chromatogr B Biomed Appl. 1996; 685(2): 251-63.
137. Willis JV, Kendall MJ, Flinn RM, Thornhill DP, Welling PG. The pharmacokinetics of diclofenac sodium following intravenous and oral administration. J Clin Pharmacol, 1979; 16: 405–410.
138. Vane JR, Botting RM. 1998. Anti-inflammatory drugs and their mechanism of action. Inflamm. Res, 1998; 47: 78-87.
139. Ricciotti E, Fitz Gerald GA. Prostaglandins and Inflammation Arterioscler, Thromb Vasc Biol, 2011; 31: 986-1000.
140. Simmons DL, Botting RM, Hla T. Cyclooxygenase Isozymes: The Biology of Prostaglandin Synthesis and Inhibition. Pharmacol Rev, 2004; 56( 3): 387-437.
141. Ponce-Monter HA, . Ortiz MI, Garza-Hernández AF, Monroy-Maya R, Soto-Ríos MS, Carrillo-Alarcón L, Reyes-García G, Fernández-Martínez E. Effect of Diclofenac with B Vitamins on the Treatment of Acute Pain Originated by Lower-Limb Fracture and Surgery. Pain Research and Treatment, 2011,2012; 104782 .
142. Lin J, Zhang W, Jones A, Doherty. Efficacy of topical non-steroidal anti-inflammatory drugs in the treatment of osteoarthritis: meta-analysis of randomised controlled trials BMJ, 2004;329:324
143. Walsem AN, Pandhi S, Nixon RM, Guyot P, Karabis A and Moore RA. Relative benefit-risk comparing diclofenac to other traditional non-steroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors in patients with osteoarthritis or rheumatoid arthritis: a network meta-analysis. Arthritis Res Ther, 2015; 17: 66.
144. Chandanwale AS, Sundar S, Latchoumibady K, Biswas S, Gabhane M, Naik M, Patel K. Efficacy and safety profile of combination of tramadol-diclofenac versus tramadol-paracetamol in patients with acute musculoskeletal conditions, postoperative pain, and acute flare of osteoarthritis and rheumatoid arthritis: a Phase III, 5-day open-label. J Pain Res, 2014; 7:455-463.
145. Aswari R , Goutham R , Saanjay P, Nitin D. Comparative efficacy of combined use of Diclofenac and Thicolchicoside and Diclofenac alone in Orthopedic patients. Int Res J Pharm, 2013; 4 (2) 163-166.
146. Bakshi R, Darekar B, Langdon CG, and Rotman H. Efficacy and tolerability of diclofenac dispersible in elderly patients with osteoarthritis. Curr Med Res Opin,1991; 12(7 ) 459-465.
147. Ma K, Yiqun M, Wang TWW, Liu X, Huang X and Wang Y. Efficacy of Diclofenac Sodium in Pain Relief after Conventional Radiofrequency Denervation for Chronic Facet Joint Pain: A Double-Blind Randomized Controlled Trial. Pain Med, 2011; 12 (1): 27–35.
148. Gibofsky A, Hochberg MC, Jaros MJ, and Young CL. Efficacy and safety of low-dose submicron diclofenac for the treatment of osteoarthritis pain: a 12 week, phase 3 study. Curr Med Res Opin 2014, 30(9):1883-1893.
149. Dougados M, Henanff AL, Logeart I, Ravaud P. Short-Term Efficacy of Rofecoxib and Diclofenac in

Acute Shoulder Pain: A Placebo-Controlled Randomized Trial. PLoS Clin Trials, 2007; 2(3): e9.
150. Kuehl KS. Review of the efficacy and tolerability of the diclofenac epolamine topical patch 1.3% in patients with acute pain due to soft tissue injuries. Clin Ther. 2010; 32(6): 1001-14.
151. Kuehl K, Carr W, Yanchick J, Magelli M, Rovati S.Analgesic efficacy and safety of the diclofenac epolamine topical patch 1.3% (DETP) in minor soft tissue injury..Int J Sports Med. 2011; 32(8): 635-43.
152. Brunner M, Davies D, Martin W, Leuratti C, Lackner E, and Müller M. A new topical formulation enhances relative diclofenac bioavailability in healthy male subjects. Br J Clin Pharmacol, 2011; 71(6): 852–859.
153. Bookman AAM, Williams KSA, J. Zev Shainhouse Effect of a topical diclofenac solution for relieving symptoms of primary osteoarthritis of the knee: a randomized controlled trial CMAJ, 2004; 171( 4): 333-338.
154. Roth SH and Fuller P. Diclofenac topical solution compared with oral diclofenac: a pooled safety analysis. J Pain Res, 2011; 4: 159–167.
155. Pavelka K.A comparison of the therapeutic efficacy of diclofenac in osteoarthritis: a systematic review of randomised controlled trials. Curr Med Res Opin, 2012; 28(1): 163-78.
156. Darvish H, Ardestani BM, Shali SM, and Tajik A. Analgesic Efficacy of Diclofenac and Paracetamol vs. Meperidine in Cesarean SectionAnesth. Pain Med, 2014; 4(1): e9997.
157. Bell NH, Hollis BW, Shary JR, et al. Diclofenac sodium inhibits bone resorption in postmenopausal women. Am J Med, 1994; 96: 349–53.
158. Sharma S, Vaidyanathan S, Thind SK, et al. The effect of diclofenac sodium on urinary concentration of calcium, uric acid and glycosaminoglycans in traumatic paraplegics. Br J Urol 1991; 68: 240-242.
159. Peniston JH, Gold MS, Wieman MS, and Alwine LK. Long-term tolerability of topical diclofenac sodium 1% gel for osteoarthritis in seniors and patients with comorbidities. Clin Interv Aging. 2012; 7: 517–523.
160. Annadurai S, Basu S, Ray S, Dastidar SG, Chakrabarty AN. Antibacterial activity of the antiinflammatory agent diclofenac sodium. Indian J Exp Biol. 1998; 36(1): 86-90.
161. Dutta NK, Annadurai S, Mazumdar K, Dastidar SG, Kristiansen JE, Molnar J, Martins M, Amaral L. Potential management of resistant microbial infections with a novel non-antibiotic: the anti-inflammatory drug diclofenac sodium. Int J Antimicrob Agents, 2007; 30 (3): 242–249.
162. Dastidar SD , Ganguly K, Chaudhuri K, Chakrabarty AN. The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. Int J Antimicrob Agents, 2000; 14( 3): 249–251.
163. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA and Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis. Proc Natl Acad Sci USA, 1999; 96 (13): 7563–7568.
164. Buttgereit F, Burmester GR, Simon LS. Gastrointestinal toxic side effects of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2–specific inhibitors. Am J Med 2001; 110 : 3 ( 1) 13-17.
165. Deakin M. Small Bowel Perforation associated with an excessive dose of slow release diclofenac sodium. BMJ 1988. 488-489.
166. Allison MC, Howaston AG, Torrance CJ, Lee FD, Russell RI. Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs. N Engl J Med, 1992; 327(11): 749-54.
167. Rainsford KD. An analysis of the gastro-intestinal side-effects of non-steroidal anti-inflammatory drugs, with particular reference to comparative studies in man and laboratory species. Rheumatol Int, 1982; 2 (1): 1-10.
168. Niu X, de Graaf IA, Langelaar-Makkinje M, Horvatovich P, Groothuis GM. Diclofenac toxicity in human intestine ex vivo is not related to the formation of intestinal metabolites. Arch Toxicol 2015; 89(1): 107-19.
169. Rostom A, Goldkind L, Laine L. Nonsteroidal anti-inflammatory drugs and hepatic toxicity: a systematic review of randomized controlled trials in arthritis patients. Clin Gastroenterol Hepatol. 2005; 3(5): 489- 498.
170. Laine L, Goldkind L, Curtis SP, Connors LG, Yanqiong Z, Cannon CP. How Common Is Diclofenac-Associated Liver Injury? Analysis of 17,289 Arthritis Patients in a Long-Term Prospective Clinical Trial Diclofenac-Associated Liver Injury. The American Journal of Gastroenterology, 2009; 104: 356-362.
171. Bort R, Mace K, Boobis A, Gomez-Lechon MJ, Pfeifer A, Castell J. Hepatic metabolism of diclofenac: Role of human CYP in the minor oxidative pathways. Biochem Pharmacol, 1999; 58: 787–796.
172. Masubuchi Y, Nakayama S, Horie T. Role of mitochondrial permeability transition in diclofenac-induced hepatocyte injury in rats. Hepatology, 2002; 35(3): 544-51.
173. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials BMJ, 2006; 332(7553): 1302–1308.
174. Hajong R. Upper limb gangrene following intramuscular diclofenac: a rare side effect. J Surg case Rep, 2013; 2013(2): rjs039.
175. Lie C, Leung F, Chow SP Nicolau syndrome following intramuscular diclofenac administration: a

case report. J Orthop Surg (Hong Kong). 2006;14(1):104-7.
176. Panariello L, Ayala F. Nicolau syndrome following intramuscular diclofenac injection: a case report. Dermatol Ther, 2008; 21 (1): 10-12.
177. Park HJ, Kim MS, Park NH, Jung SW, Park SI, Park CS Sonographic findings in Nicolau syndrome following intramuscular diclofenac injection: a case report. J Clin Ultrasound, 2011; 39(2): 111-3.
178. Olsen AMS, Fosbøl EL, Lindhardsen J, Folke F, Charlot M, Selmer C, Lamberts M, Olesen JB, Køber L, Hansen PR, Torp-Pedersen C, Gislason GH, Health Services and Outcomes ResearchDuration of Treatment With Nonsteroidal Anti-Inflammatory Drugs and Impact on Risk of Death and Recurrent Myocardial Infarction in Patients With Prior Myocardial Infarction A Nationwide Cohort Study. Circulation, 2011; 123: 2226-2235.
179. White WB, Faich G, Whelton A, Maurath C , Ridge NJ, Verburg KM, Geis GS, Lefkowith JB. Comparison of thromboembolic events in patients treated withCelecoxib, a Cyclooxygenase-2 specific inhibitor,versus Ibuprofen or Diclofenac. Am J Cardiol, 2002; 89(4): 425–430.
180. Varas-Lorenzo C, Riera-Guardia N, Calingaert B, Castellsague J, Pariente A, Scotti L, Sturkenboom M, Perez-Gutthann S. Stroke risk and NSAIDs: a systematic review of observational studies. Pharmacoepidemiol Drug Saf, 2011; 20(12): 1225-36.
181. Haag MDM, Bos MJ, MD; Hofman A, Koudstaal PJ, Breteler MMB, Bruno H. C. Stricker BHC. Cyclooxygenase Selectivity of Nonsteroidal Anti-inflammatory Drugs and Risk of Stroke. Arch Intern Med, 2008; 168(11): 1219-1224.