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Impacts of diclofenac sodium on zebra fish (*Danio rerio*): A comprehensive review

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Abstract
Diclofenac sodium, a widely used nonsteroidal anti-inflammatory drug (NSAID), is increasingly recognized as an environmental pollutant due to its persistence and bioaccumulation in aquatic ecosystems. This study accumulates the comprehensive studies on the impacts of diclofenac sodium on the model organism *Danio rerio* (Zebra fish), focusing on physiological, biochemical, and behavioral alterations. Different studies have suggested that significant hepatotoxicity, including altered liver morphology, increased lipid peroxidation, and elevated levels of stress enzymes such as catalase and superoxide dismutase. Behavioral analyses indicated reduced locomotion and erratic swimming patterns, suggesting neurotoxic effects. The findings underscore the ecotoxicological risks of diclofenac sodium to aquatic life and highlight the urgent need for stricter regulations on pharmaceutical discharges into water bodies. These study also establish zebrafish as a sensitive bioindicator for assessing pharmaceutical contamination in aquatic environments especially on fish population.

Keywords: Zebra fish, *Danio rerio***,** diclofenac, pharmacovigilance, ecotoxicity

Introduction

Drugs have been a blessing for humanity in controlling the ailments up to a great extent. However, they also impart some adverse reactions along with their beneficial effects. Dealing with this fact, a new branch of science called **"**Pharmacovigilance" was born in the year 1960. The discipline of pharmacovigilance became well accepted and practiced in developed world but it took a long time to reach the developing countries. At present, India has also begun to monitor the adverse effects of drugs and programs are run for the same. Unfortunately, the effect of drug use on environment at the international level remains unaddressed. Attention has been drawn to the environmental impact of chemicals used in small concentration, that is, drugs giving birth to the subject of eco-pharmacovigilance**,** which can be defined as science and activities concerning detection, assessment, understanding and prevention of adverse effects or other problems related to the presence of pharmaceuticals in the environment, which affect the biotic community.

The use of drugs for both the human and veterinary populations has been growing daily. Statistics show that antimicrobials are consumed at a rate of 100,000 tons annually. Additionally, the most industrialized nations take roughly 30 billion doses of nonsteroidal anti-inflammatory medications (NSAIDs) each year. There are two principal ways that drugs enter the environment (Figure 1): a) usual drug intake leaves the body either as metabolites or by excretion. Since they are frequently soluble in water, they ultimately find their way into the sewage; b) Drugs are introduced into the environment through industrial waste as well. Even though these enterprises utilise sewage treatment before disposal, the environment is still being contaminated by medicines due to their antiquated methods. Some medications are not completely eliminated by the treatment procedure, leaving remnants that end up in the environment's water supplies. Some examples are cocaine, oral contraceptives, carbamazepine, and iodine contrast agents. Thus, water gets tainted, although with a little amount of various medications. It results in the unintentional re-entry of medicines into humans when given out as drinking water. Another significant source of drug contamination in the environment is leftover medication.

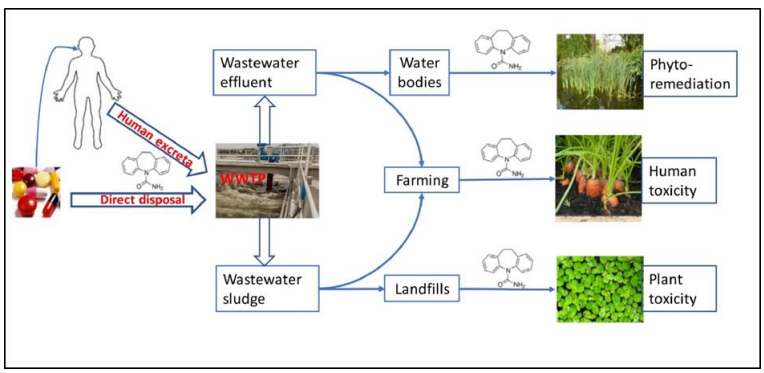


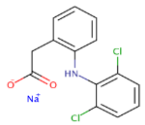
Fig 1: Different sources of pharmaceutical accumulation in environment

The environment is typically heavily contaminated when leftover medicine is disposed of improperly. The environment is becoming contaminated by drugs or their metabolites, which is a worrying development. The levels of

waste water found 150 times higher downstream from the Indian facilities than those found in the United States of America (Mason, 2009). Drugs can have a direct or indirect impact on people, plants, and animals when they are exposed to them through the environment. Microbial resistance is currently the main topic of discussion. Resistance may develop as a result of ongoing, low-dose antimicrobial exposure through drinking water. Pharmaceutical corporations' declining interest in creating novel antimicrobials in favour of creating "lifestyle" medications may exacerbate the issue (PGP Charles *et al.*, 2004). Even though the effects of very low doses from environmental cycling are unclear, certain groups of people, such as pregnant women, children, the elderly, and people with renal or hepatic insufficiencies, may still be more vulnerable to exposure (KM Lai *et al.*, 2002). In these groups, the pharmacokinetics is altered, and even very low doses may end up being toxic.

Since the 1970s, humans and domestic animals have utilised diclofenac, a nonsteroidal anti-inflammatory medication having pain-relieving effects (Caracciolo *et al.*, 2015; Barbosa *et al.*, 2016). Diclofenac has generally been introduced continuously through pharmaceutical companies, healthcare facilities, and household drainage (Table 1). Because diclofenac can pollute the environment either directly or through its metabolites (Cardoso *et al.*, 2014; Chiffre *et al.*, 2016; Lindholm-Lehto *et al.*, 2016; Meyer *et al.*, 2016; González-Alonso *et al.*, 2017; Koecka *et al.* According to Schmidt et al. (2018), the water and soil ecosystems absorb over 75% of the utilised diclofenac. Due to its hydrophilicity and stability, it is also more likely to survive in an aquatic environment (Madikizela *et al.*, 2017; Tiedeken *et al.*, 2017). Diclofenac might cause measurable amounts in drinking water throughout this expansion, even under treated settings (Loos *et al.*, 2017; Tröger *et al.*, 2018; Sharma *et al.*, 2019).

Table 1: Diclofenac sodium: Summary

Sl. No.	Particulars	Inference	Reference
1	Structure		https://sielc.com/diclofenac-sodium
2	Molecular Formula	$C_{14}H_{10}Cl_2NNaO_2$	PubChem (2024)
3	Synonyms	Diclofenac sodium QTG16297Q Voltaren Diclofenac sodium salt	PubChem (2024)
4	Molecular Weight	318.1 g/mol	PubChem (2024)

Additionally, current research has revealed that diclofenac accumulates in consumable fruits and vegetables (Bartrons and Peuelas, 2017; González *et al.*, 2018), which may have an immediate impact on human health. The concentration of diclofenac in the water cycle has increased due to a variety of factors, including general increases in the consumption of pharmaceuticals, the release of untreated wastewater into the environment, the inefficiency of wastewater treatment plants (WWTPs), and the deconjugation of diclofenac metabolites (Charuaud *et al.*, 2019a; Fekadu *et al.*, 2019; Williams *et al.*, 2019). Additionally, diclofenac is left behind during the treatment operations at WWTPs, resulting in record-high diclofenac concentrations that have a negative impact on inland aquatic habitats (Balakrishna *et al.*, 2017; Thelusmond *et al.*, 2018). It is well known that during the decontamination process, wastewater treatment plants (WWTPs) cannot entirely remove organics from wastewater effluents, which includes medicines (Figure 2). Accordingly, studies have demonstrated that the watering of crops with wastewater effluent that contains pharmaceutical elements may cause the roots of the plants to absorb the pharmaceuticals (Christou *et al.*, 2017). This might lead to the presence of drugs in food sources like vegetables, which could endanger the consumer's health. On the other side, plants' ability to absorb medications might be advantageous since it may help lower the amount of pollutants in surface water (Cui *et al.*, 2015).

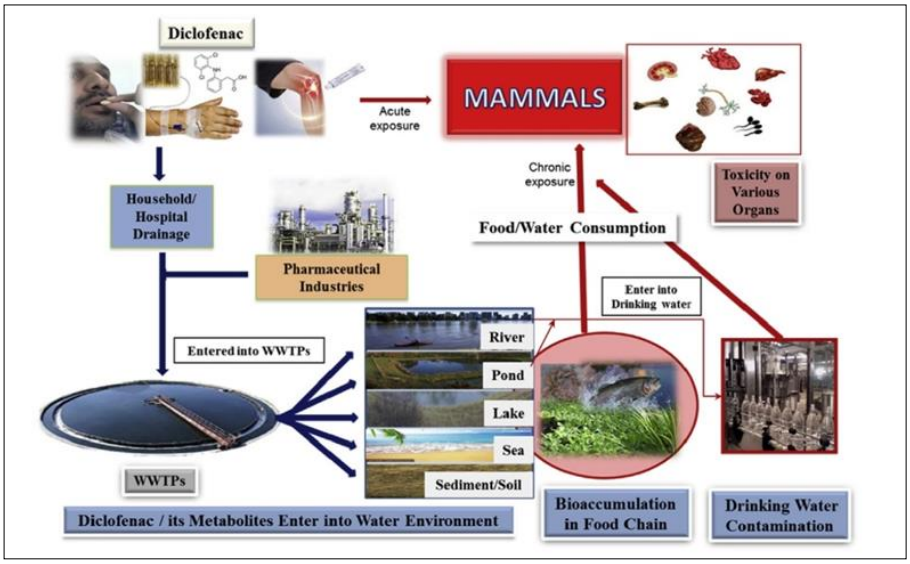


Fig 2: Environmental distribution, bioaccumulation, biomagnifications and ecological risk of diclofenac towards mammals.

In this communication, different effects of dichlofenac on zebra fish (*Danio rerio)* has been incorporated to provide a comprehensive knowledge about the hazardous effects of this widely used drug on fish population which eventually cause toxicity in wide range of animal groups.

***Danio rerio* (Zebra Fish) as a model organism**

Zebra fish (*Danio rerio*), a tropical freshwater fish native to South Asia, has emerged as a key model organism in biological research. Belonging to the family Cyprinidae, zebra fish were first used for scientific studies within the 1970s by George Streisinger on the college of Oregon, who recognized their ability for genetic research. unlike mammals which include mice, zebra fish are easier to control genetically, and their obvious embryos made them best for analyzing developmental techniques, especially the frightened machine. the use of zebra fish expanded within the 1990s whilst researchers like Christiane Nüsslein-Volhard, Wolfgang Driever, and Mark Fishman created genetic mutants in zebra fish, taking into consideration new insights into biological approaches. This genetic manipulation approach has due to the fact that grow to be a powerful method for analyzing various factors of biology, including disease mechanisms and gene functions (Santoriello *et al.,* 2012). [33] One of the primary motives zebra fish have become so famous in studies is their physiological and genetic similarities to humans. approximately 70% of human disease genes have practical counterparts in zebra fish, making them valuable for analyzing human illnesses (Carpio *et al.,* 2006). [8] Zebra fish share many key biological systems with human beings, which include the brain, digestive system, musculature, blood vessels, and immune system, permitting researchers to analyze these structures in a version organism with similar genetic makeup. Zebra fish embryos develop rapidly and are transparent, which allows for clean observation and manipulation. The fast improvement of zebra fish embryos makes them best for analyzing developmental biology. in just 24 hours, the embryo's number one organ structures are shaped, and through 72 hours, it hatches and begins to exhibit person-like conduct. This quick improvement, blended with their capacity to mature sexually in about 10 weeks and their excessive reproductive rate, similarly complements their value as a model for large-scale genetic research (Guyon *et al.,* 2007). [18]

Latest advances in zebra fish research have brought about extensive breakthroughs in several fields, such as developmental biology, toxicology, and ailment modeling. Zebra fish are specially beneficial in toxicological studies, as their sensitivity to environmental pollution makes them an first rate device for screening dangerous substances. Additionally, they may be extensively used in transgenic research to examine gene feature and in drug discovery due to their speedy improvement and excessive reproductive rate, which allows big-scale pharmaceutical screening (Weinstein, 2002). [41] Zebra fish have also made primary contributions to know-how human illnesses, inclusive of genetic disorders, most cancers, cardiovascular sicknesses, and neurological situations. Their genetic similarities to human beings make them an ideal model for studying disease mechanisms and trying out capability treatments (Lieschke *et al.,* 2001). [25] no matter the big progress in zebra fish studies, a great deal remains to be found. Ongoing studies keep to discover their potential in addressing complicated organic and scientific demanding situations. normal, zebra fish provide an invaluable aid for advancing studies in lots of scientific fields, mainly inside the expertise and remedy of human illnesses (Zhao *et al.,* 2015; Lele *et al.,* 1996). [43, 24]

Effects of diclofenac on *danio rerio* (zebra fish)

Prescribed drugs, specially non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac, are commonly used to deal with human and animal sicknesses, but their giant use has caused great nvironmental contamination, in particular in aquatic ecosystems (Fent, Weston, & Caminada, 2006; aus der Beek *et al.,* 2015; Brausch *et al.,* 2012). [15, 1, 3] Diclofenac, a widely fed on NSAID used to reduce pain and irritation, has been detected in water systems globally, together with in wastewater treatment plant life in nations like Germany, China, Iran, Spain, and Thailand (Stülten *et al.,*2008; Duan *et al.,* 2013; Eslami *et al.,* 2015; Carmona *et al.,* 2014; Tewari *et al.,* 2013). [37, 13, 14, 7, 38] The environmental presence of NSAIDs which include diclofenac is of growing situation because of their capability ecotoxicological results with studies showing toxic influences on numerous fish species (Brodin *et al.,* 2013; Chou *et al.,* 2010; Schwaiger *et al.,* 2004). [4, 10, 34] Diclofenac publicity has been connected to lethal effects and abnormalities in fish which includes zebra fish (*Danio rerio*) (Horie *et al.,* 2017; Ribeiro *et al.,* 2015), [20, 32] brown trout (Salmo trutta) (Schwarz *et al.,* 2017), [35] and not unusual carp (Cyprinus carpio) (Stepanova *et al.,* 2013). [36] additionally, it is able to set off developmental issues like morphological abnormalities and prevent growth and reproductive capabilities in species just like the Japanese medaka (Yokota *et al.,* 2017). [42] Zebra fish, due to their small size, brief technology time, and simplicity of use in toxicity trying out, are often used to evaluate environmental risks, together with the consequences of pharmaceuticals (Horie *et al.,* 2017). [20] Current research recommend that zebra fish embryos are sensitive to chemical publicity at decrease concentrations that may not purpose lethal outcomes but result in developmental disruptions (Horie *et al.,* 2017). [20] Notwithstanding this, there may be a lack of studies analyzing the relationship between morphological abnormalities at the embryo degree and subsequent lethal consequences after hatching. This research investigates the sub deadly and deadly results of diclofenac on zebra fish at some point of embryonic and adolescence levels, with a focal point at the correlation among discovered morphological defects and later mortality (OECD, 2013a). The take a look at investigates the sub lethal and deadly effects of diclofenac sodium on adolescence-stage zebra fish, using changed company for monetary Co-operation and improvement (OECD) check guiding principle No. 210. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is normally utilized in human and veterinary medication, however its environmental impact, particularly in aquatic ecosystems, has raised widespread worries due to its toxicity in aquatic organisms (Fent *et al.,* 2006; aus der Beek *et al.,* 2015). [1] The research goals to apprehend the toxicological results of diclofenac throughout zebra fish embryogenesis and early improvement, that specialize in hatching fulfilment, boom, survival, and the morphological effects ofpublicity.

Distribution of diclofenac within embryos of zebra fish (*Danio rerio*)

The distribution of diclofenac inside the embryo of zebra fish become also studied via fragmenting the embryos and measuring the attention of the drug in diverse booths. The outcomes confirmed that most people of diclofenac remained inside the extracellular aqueous solution, with much less than 5% of the drug sincerely associated with the embryo. Of the diclofenac related to the embryos, about 53% became adsorbed on the embryonic membranes, whilst the final fraction entered the cytoplasm. The study also discovered that as the publicity attention elevated, the amount of diclofenac sure to both the membranes and the cytoplasm expanded almost linearly, with partition coefficients (P) of 0.0016 and 0.00145 µL in line with embryo, respectively. This shows that higher concentrations of diclofenac result in extra uptake and association with the embryo's mobile systems (Zhang *et al.*, 2024). [9]

The experiments further explored the effects of environmental elements along with pH, ionic energy, and temperature on the association of diclofenac with embryos. It was observed that the quantity of diclofenac bound to the embryo expanded and reached a maximum because the ionic strength changed into raised from zero to 0.15 M. This trend will be attributed to the truth that accelerated ionic energy complements hydrophobic interactions, facilitating the binding of diclofenac to the embryo. But, whilst the attention of NaCl exceeded 0.15 M, the binding decreased, suggesting that high ionic strength beyond a positive point would possibly weaken the embryo's activity, thereby reducing the drug's binding potential. Temperature additionally had a giant impact on the binding manner, as better temperatures promoted an boom in the quantity of diclofenac associated with the embryo, probably due to improved metabolic pastime and quicker membrane go with the flow quotes. But, temperatures exceeding 40°C adversely affected embryo viability, main to a slight discount in the amount of diclofenac associated with the embryos. Ultimately, no steady fashion become determined in the amount of diclofenac sure to the embryos whilst the pH became numerous, suggesting that pH had minimum effect on diclofenac's binding behaviour (Zhang *et al.*, 2024). [9]

Effects on developmental parameters of zebra fish (*Danio rerio*)

The extended sensitivity of adolescence stages of fish to toxic substances, consisting of diclofenac, is properly documented inside the literature. Kovriznych and Urbancikova (2001) conducted a examine evaluating the LC_{50} values of eight different chemical substances in embryonic and juvenile tiers of *D. rerio*. They located big variations in sensitivity between these lifestyles stages, with embryos showing a better degree of toxicity to most materials. The better sensitivity of embryos is frequently attributed to several factors, which includes the underdevelopment of the enzymatic machine, variations in metabolic pathways, and the extraordinary processes with the aid of which chemicals are absorbed into the organism (Van Leeuwen *et al.*, 1985). [40]

In the case of diclofenac, even as low concentrations of the drug are generally found in environmental waters (within the ng/L variety), its sub-lethal outcomes on early developmental tiers of aquatic organisms have to not be disregarded. chronic exposure to even low concentrations of diclofenac should cause detrimental results on fish populations, doubtlessly affecting their increase, reproduction, and survival. consequently, further studies is wanted to assess the lengthy-term, sub-persistent effects of diclofenac exposure on aquatic ecosystems, in particular thinking about the massive presence of prescription drugs in floor waters (Mehinto *et al.*, 2010). [26]

At some point of the exposure to diclofenac, numerous developmental abnormalities were discovered in the zebra fish embryos. There was a delay inside the hatching time for embryos exposed to all concentrations, a finding that corroborates in advance research through Hallare *et al.* (2004), [19] who located a put off in hatching in zebra fish embryos uncovered to diclofenac concentrations ranging from 1 to 2000 µg/L. late-hatched embryos exhibited morphological deformities, such as signs of hydroedema, a condition that indicates water retention inside tissues, possibly due to impaired osmoregulation. that is regular with the findings of Van den Brandhof and Montforts (2010), [39] who reported comparable developmental delays and morphological abnormalities, such as tail and yolk sac deformations, in zebra fish embryos uncovered to diclofenac concentrations above 1.5 mg/L. in their study, the seventy two-hour effective attention (EC50) for diclofenac was determined to be 5.3 mg/L, further supporting the developmental toxicity found within the current have a look at.

The observe highlights the morphological abnormalities triggered by way of diclofenac exposure, specifically edema (swelling) and frame curvature, which were discovered at 2 days post-fertilization (dpf) in the handled embryos. These abnormalities have been not present within the manipulate institution, indicating that diclofenac causes awesome developmental disruptions at the embryo degree. The larvae exhibiting frame curvature additionally confirmed strange backbone improvement, further confirming that diclofenac interferes with skeletal formation. these effects had been in particular stated within the 7.0 mg/L publicity group, where about 90% of embryos showed unusual embryogenesis, characterised by edema and body curvature. No unusual embryogenesis became located in embryos exposed to lower concentrations of diclofenac (0, 0.4, 0.9, 1.8, and 3.5 mg/L). moreover, all larvae exhibiting morphological abnormalities died inside one week after hatching, further reinforcing the severity of the poisonous consequences brought on by using diclofenac publicity at some stage in embryogenesis.

Developmental toxicity of diclofenac on zebra fish embryos

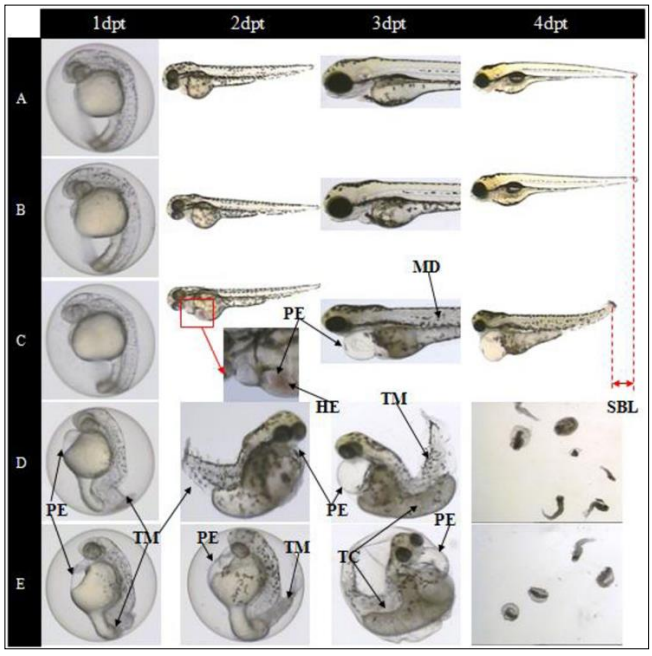
In comparison to preceding research, the findings in this look at showed better no-located-effect concentrations (NOECs) for diclofenac, suggesting that the edge for poisonous outcomes may additionally range relying on experimental situations. The NOEC for embryogenesis, survival, and growth on this look at changed into higher than those suggested by way of Memmert *et al.* (2013), [27] who discovered lower NOECs for diclofenac in zebra fish embryos and childhood stages. This difference can be attributed to versions in experimental techniques, consisting of the feeding protocols, stress variations, and exposure systems used. despite those differences, both research discovered similar traits, with diclofenac inducing deadly and sub-lethal consequences in zebra fish, inclusive of

atypical embryogenesis and reduced survival costs. Lawrence (2007) [23] also noted variability in survival costs for youth-level zebra fish, which can be motivated by factors along with feeding protocols and stress genetics. This reinforces the idea that experimental conditions play a vital position in determining the toxicity of chemical compounds like diclofenac.

A comparison with other research on diclofenac toxicity in aquatic organisms, including the work by way of Nassef *et al.* (2009), [28] affords useful context for the determined effects. Nassef *et al.* (2009) [28] assessed the 96-hour LC50 of diclofenac for mature jap killifish, which turned into determined to be 10.1 mg/L. This cost is corresponding to the 144-hour LC50 value of 6.11 mg/L obtained on this take a look at for zebra fish embryos, similarly helping the notion that diclofenac exerts its toxic effects across one-of-a-kind aquatic species at similar concentrations. Schwaiger *et al.* (2004) [34] performed histopathological investigations at the tissues of rainbow trout (Oncorhynchus mykiss) exposed to diclofenac concentrations starting from 1 µg/L to 500 µg/L over a 28-day length. They found considerable toxicity, mainly inside the kidneys, liver, and gills. in the kidneys, diclofenac exposure ended in excessive protein accumulation, macrophage infiltration, and structural damage to renal tubules. The liver confirmed symptoms of glycogen depletion and cellular compartment disintegrate, at the same time as the gills exhibited necrosis of pillar cells and hypertrophy of chloride cells. these findings highlight the capacity nephrotoxic and hepatotoxic effects of diclofenac, which may be exacerbated with persistent exposure, specially in aquatic organisms. Revai and Harmos (1999) [31] also examined the nephrotoxic outcomes of diclofenac in rainbow trout exposed to concentrations of seven-15 µg/L. Their take a look at located that diclofenac triggered tubular necrosis inside the kidneys and hyperplasia within the intestinal villi at these concentrations. The modern-day have a look at's findings on the developmental toxicity of diclofenac in zebra fish embryos endorse that early developmental tiers of fish are greater liable to the compound, potentially due to underdeveloped detoxing structures and extraordinary metabolic pathways in embryos compared to juvenile or grownup fish.

Ecotoxicological testing with zebra fish embryos

Zebra fish embryos are an increasing number of utilized in ecotoxicological trying out, especially to assess the impacts of environmental chemical substances like prescribed drugs on early development (european, 2010). The Fish Embryo Acute Toxicity test (OECD TG 236) has emerge as a broadly recommended technique to evaluate the extreme or deadly toxicity of chemical substances on zebrafish embryos. This test is considered an moral opportunity to standard fish toxicity testing, as it reduces using live animals in experiments (OECD, 2013b). the prevailing observe's findings contribute to the growing frame of research emphasizing the importance of assessing each sub-lethal and lethal results in early developmental levels, as those stages are specifically sensitive to chemical publicity. appreciably, the observe determined that diclofenac had awesome deadly results at distinct ranges, with embryos displaying large mortality at concentrations above 7 mg/L, even as adolescence tiers (after hatching) exhibited lethality at concentrations above 3.5 mg/L. The take a look at additionally discovered that diclofenac publicity caused atypical spinal improvement in larvae, especially in the ones exhibiting frame curvature. The atypical curvature become related to abnormal spinal improvement, that's regular with findings from different research on chemical-prompted spinal defects in zebra fish (Cao *et al.*, 2016; Chen *et al.*, 2014). [6, 9] The molecular mechanisms underlying these outcomes stay uncertain, however previous research has identified several genes vital for spine development in zebra fish, inclusive of kinesin member of the family 6 (kif6) and collagen kind VIII (col8a1a) (Buchan *et al.*, 2014; gray *et al.*, 2014). [5] The study shows that in addition studies is needed to explain the genetic and molecular pathways through which diclofenac and other environmental chemical substances result in spinal deformities and different developmental abnormalities in zebra fish. The observe's findings advise that morphological abnormalities in zebrafish embryos, consisting of edema and body curvature, should function reliable endpoints for predicting lethal results in later life levels. that is especially widespread as these abnormalities had been brought on at diclofenac concentrations decrease than those required to reason direct lethal results on the embryo degree. The combination of edema and strange spinal improvement in embryos suggests that those morphological markers could be indicative of developmental disruption that leads to post-hatching mortality. Di Paolo et al. (2015) [11] additionally located that sub-deadly outcomes, such as morphological abnormalities for the duration of embryogenesis, might also expect not on time deadly effects for the duration of later degrees of improvement. Their examine of PCB-126, as an example, validated that early sub-lethal outcomes should imply the capability for future mortality, even after the publicity was halted put up-hatching. This suggests that assessing atypical embryogenesis could be a valuable device for predicting the lengthy-time period toxicity of environmental chemical substances (Figure 3).



(*Source: Scientific Reports, ISSN: 2045-2322*)

Fig 3: Toxic effect of diclofenac on zebrafish during the exposure at 1–4 dpt: (A) Control group; (B) 1.01 µM exposure group; (C) 3.38 µM exposure group; (D) 10.13 µM exposure group; (E) 15.2 µM exposure group. HE: hemagglutination, MD: muscle degeneration, PE: pericardial edema, SBL: short body length, TC: trunk curvature, TM: tail malformation.

Gene Expression Changes Induced by Diclofenac

Similarly to developmental observations, the observe also assessed the impact of diclofenac on gene expression, specifically genes worried in developmental signaling. The expression of the reference gene β-actin remained stable during the experiment, confirming the consistency of RNA extraction and the reliability of the gene expression facts. At 1 dpt, no substantial changes inside the expression of Wnt3a or Wnt8a have been found. However, at 2 dpt, a huge reduction in Wnt3a expression was stated inside the 3.38 μM and 15.20 μM remedy businesses, suggesting that diclofenac can also intervene with the Wnt signaling pathway, which performs a critical function in embryonic improvement. However, the expression of Wnt8a become substantially extended in the 10.13 μM remedy institution. The expression of Gata4, a gene involved in early cardiac development, confirmed a trend closer to inhibition at higher diclofenac concentrations, with vast inhibition located at 15.20 μM at 1 dpt. The expression of Nkx2.5, every other vital developmental gene, remained stable at 1 dpt however changed into inhibited at 2 dpt within the 3.38 μM treatment. Interestingly, better concentrations caused an increase in Nkx2.5 expression, suggesting a attention-established modulation of gene expression (Zhang *et al.*, 2024). [9]

Conclusion

In end, the examine affords valuable insights into the sub-lethal and lethal effects of diclofenac sodium on zebra fish in the course of childhood tiers, specifically with regards to embryogenesis and developmental abnormalities. Diclofenac exposure led to morphological defects which includes edema, body curvature, and spinal deformities, which had been related to later mortality in the larvae. these findings underscore the importance of the usage of zebrafish embryos in ecotoxicological trying out and recommend that morphological abnormalities should serve as predictive markers for lethal effects in later levels of development. The observe also highlights the range in NOEC values for diclofenac, emphasizing the need for consistent experimental protocols and in addition research to recognize the mechanisms underlying the toxic effects of prescription drugs in aquatic environments. As diclofenac and other prescription drugs keep to pose risks to aquatic ecosystems, this studies contributes to the wider effort to evaluate and mitigate the ecological influences of these materials in the environment.

The study found out that diclofenac has substantial developmental poisonous effects on zebra fish embryos. As the concentration of diclofenac extended, there was a marked increase in mortality and excessive morphological deformities, together with tail malformations, pericardial edema, and muscle degeneration. Gene expression analysis further confirmed that diclofenac interferes with critical developmental pathways, inclusive of the Wnt signaling pathway, and inhibits the expression of genes like Gata4 and Nkx2.5. those findings spotlight the potential toxicity of diclofenac to early developmental tiers of vertebrates, suggesting that the environmental presence of diclofenac may want to pose dangers to aquatic life. future studies may want to awareness on in addition elucidating the molecular mechanisms underlying those effects and exploring the lengthy-term results of diclofenac publicity on embryonic improvement and health. The effects of the embryonic toxicity checks performed on *Danio rerio* display that diclofenac is enormously toxic to zebra fish embryos, with a extensively decrease LC_{50} fee for embryos in comparison to juvenile fish. The look at highlights the developmental toxicity of diclofenac, such as delays in hatching, morphological deformities, and multiplied mortality at higher concentrations. The findings also underscore the heightened sensitivity of formative years degrees of fish to toxic substances, a phenomenon that has been discovered in lots of other studies on environmental pollutants. Given the increasing degrees of prescription drugs consisting of diclofenac in aquatic environments, in addition studies into the continual effects of this compound is important for information its long-term ecological impact and for growing effective techniques to mitigate its harmful effects on aquatic organisms.

Conflict of interest

Authors declare there is no conflict of interest.

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