Impacts of Drugs in the Surface Waters on Fish Health

Bhawna Srivastava [1], Reddy P.B[2]*

[1]*Department of Zoology, DAV College, Kanpur, UP, India*; [2]*Government PG College, Ratlam, MP, India*

***Correspondence to:** Reddy PB, Department of Zoology, DAV College, Kanpur, UP, India, E-mail: reddysirr@gmail.com

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ABSTRACT

Human pharmaceuticals and its metabolites are known to affect the endocrine system and physiology of aquatic wildlife which is an important global ecological concern. The published data from open-source toxicology and worldwide web resources reveal that there are about 175 pharmaceuticals that can affect estrogen pathways to disrupt the endocrine system and metabolism. Such studies demand additional investigations on fish and wildlife. As predicted, the use of therapeutic drugs is expected to progressively increase over the coming years, following increased discharge in a freshwater environment. The widespread use and their incorrect dumping procedures have made these chemicals as contaminants of emerging concern (CEC). Particularly, the active pharmaceutical ingredients (APIs) are universally identified in surface water and soil, where they execute negative effects in living organisms. The associated presence of different drugs may undergo bioaccumulation which causes potential toxicological effects on behaviour, histopathological alterations, reproductive and immunotoxic responses in fish and wildlife. However, the results of published literature revealed that the intensity of impacts is mostly regulated by the concentration of active pharmaceutical compounds, time of exposure and some abiotic factors like photoperiod and nutrient availability. The response of species to these active pharmaceutical substances may be noticeably different from species type. Therefore, it is necessary to continue systematic research on active metabolites and drug detection methods, to check the great number of active pharmaceuticals in drinking water, surface and groundwater, and to assess the environmental risks arising from their increased presence in the freshwater environment.

Keywords: Freshwater; Fish health; Pharmaceuticals; Surface waters; Toxic responses

INTRODUCTION

All living beings are composed of water and it is very difficult to imagine life without water. The presence of several cocktails of contaminants including human pharmaceuticals and its metabolites, and other pollutants are of the major concerns of the world today [1,3]. A few numbers of these ecological pollutants are omnipresent and cannot be removed from water treatment plants. Indeed, such pollutants can bioaccumulate in the tissues of aquatic organisms and pose a number of health risks. Majority of pollutants undergo biotic and abiotic degradation in order to safeguard freshwater environments and their organisms. Wastewater usually contains a multifaceted mixture of pharmaceuticals, xenobiotics and microbes of human and animal origin. In the past, pharmaceuticals pollution was not considered a major issue of the release of drugs into the environment. But, the current modern research shows that certain pharmaceutical manufacture units can cause environmental

contamination at levels way above than standards [4]. The living organisms found around such ecosystems may experience adverse effects as reflections. Published literature evidently reveals that certain aquatic plants can absorb these pharmaceuticals and their metabolites and pass these harmful pollutants to all the organisms of at various levels of food chain [5,6].

Pharmaceutical contamination has become an inevitable global ecological issue. As roughly $800 billion worth of medicines sold globally more and more pharmaceuticals are being discharged into the environment [7]. Of the 175 potential drugs, around one-third were recognized as estrogen agonists and two-thirds were acknowledged as estrogen antagonists. The global use of medicines and drug use has continued to elevate, with Americans filling 5.8 billion recommendations in 2018 with an increase of 2.7% over the previous year. In 2018, growth on expenditures on pharmaceuticals in the United States bounced back to 4.5% on a net basis with an estimated $61 billion market. It is expected that in the next five years the net medicine expenses in the United States alone will enhance from $344 billion in 2018 to $420 billion in 2023 [8]. The sales growth was witnessed parallel with the advancement in medical technology and enhanced spending on health care.

The Indian sub-continent is the major contributor to generic drugs, bulk drugs, intermediates, drug formulations, and herbal products and surgical internationally. The country's pharmaceutical sector provides over 50 per cent of worldwide demand for various medicines and 40 per cent of generic demand in the US. The Indian pharmaceutical sector is projected to grow up to US$ 100 billion by 2025. The revenue of the nation's domestic pharmaceutical bazaar reached Rs 1.4 lakh crore (US$ 20.03 billion) in 2019 which is 9.8% raise compared from Rs 129,015 crore (US$ 18.12 billion) in 2018 [9].

The commonly found pharmaceuticals and their metabolites in freshwater environments reach water bodies through a variety of pathways. Direct dumping of household leftover drugs, excretion in faeces/urine after medicinal use, incomplete effluent treatment from the manufacturing units [10] handling and prophylaxis through the water in farms aquaculture farms [11], and livestock treatment and pet care [11] are found to be major pathways to reach the aquatic environment. In general, the detected levels of pharmaceuticals concentrations in the freshwater environment are comparatively low and usually lie between in the ng L−1 to μg L−1 range [12], but in some nations like India and China, these concentrations are comparatively high due feeble legislative regulations [13].

Potential risks associated with releases of pharmaceuticals into the environment have become an increasingly important issue for environmental regulators and the pharmaceuticals industries. This concern has been driven by prevalent exposure of pharmaceuticals in environmental samples as a result of improved analytical capabilities and the charging of focused field surveys. The pharmaceutical pollutants are biologically active substances intended to interact with a definite physiological pathway in the target organisms. Accordingly, they symbolize a class of emerging pollutants that are competent enough to affect particular physiological functions like growth, development, defence and reproduction at ecologically pertinent concentrations [14].

The long term studies of Grzesiuk *et al.*, [5] evidently discovered that the chronic exposure of pharmaceuticals (fluoxetine, ibuprofen, and propranolol) on *Acutodesmus obliquus* and *Nannochloropsis limnetica* for 30 generations exhibited decreased in cell number, increased carotenoid to chlorophyll ratio and affected their consumer feeding. Notably, the pharmaceuticals that are specifically designed to correct definite physiological function and this is different with most other pollutants entering the freshwater environment, where biological effects usually happen as an unintentional effect of their principal function [15]. Therefore, a number of pharmaceuticals which are designed to provoke a result in humans or livestock have a high possibility of being biologically active in wildlife species. Supporting this statement, the study of Gunnarssan *et al.* [16] conclude that zebrafish possesses orthologs to 86% of 1318 tested human gene-drug targets [17,18].

Besides, the biological target system in wildlife species is some extent different or is more susceptible to the effects of certain pharmaceuticals compared with that in livestock and humans or livestock animals [16]. For example, the diclofenac, a non-steroidal anti-inflammatory drug exhibited a severe effect on kidney function in *Gyps* vulture species and has resulted in their extensive decline and even extinctions [16]. One more example is that the β-blocker drug which mainly targets the adrenergic function is believed to be involved in melanophore function in fish are not observed in humans [19].

The most important adverse effects of pharmaceuticals include their capability to interfere with the endocrine system altering hormone secretion required for fundamental physiological functions. The most effective drugs are the synthetic steroid estrogens, for instance, 17β-estradiol (E2), estrone (E1), and 17 α-ethinylestradiol (EE2). A number of laboratory investigations offer confirmation that exposure to these compounds can lead to anomalous change or disruption of growth and reproduction in fish and aquatic wildlife. In a study, exposure to the pharmaceutical mixtures of Tb (1, 10, and 50 ng/L) and EE2 (2 and 5 ng/L) on juvenile zebrafish (*Danio rerio*) for 20 and 60 days caused the sexual disruption, masculinization effects and intersex condition [20]. Additionally, a reduction in reproductive function [21,22], reduced fecundity and/or fertility [23,24] and alterations to gonadal maturation are among the most frequently observed effects [25].

Pharmaceuticals are potentially competent to modify homeostasis and critical functions and mechanisms of an organism. In some cases, they may act as endocrine-disrupting chemicals and affect the hormone secretions. Altogether, the measurable effects of pharmaceuticals on physiological, biochemical, or behavioral modifications can be successfully applied as biomarkers in biomonitoring approaches [14]. The current appraisal could offer implications to advance the global pharmaceutical ecological management globally for effective monitoring systems in the release of pharmaceutical compounds in manufacturing units as well as from landfill effluent. Another area with a need for additional research is green chemistry which could decrease or even remove the probable hazards of pharmaceutical substances that enter the environment.

Fish and other aquatic animals are known to be more vulnerable to water pollution. The role of human pharmaceuticals to the endocrine disruption in the fish and the wild is very difficult to assess. In this document, we sincerely evaluate the probable biological impacts of pharmaceuticals discharged into the freshwater

environment in fish due to their ecological position and many resemblances with mammalian species in physiological processes. Besides, fish species are perhaps the most credible vertebrate species to be affected by pharmaceuticals in the freshwater environment. Therefore, this document positively assesses the potential for ecological impacts of pharmaceuticals in the freshwater environment on fish health, by drawing on comparisons between effects observed in the laboratory and concentrations measured in the field, and attempts to assess the scale of the (potential) problem.

The Food and Drug Administration (USFDA, 2017) has approved about 10,000 pharmaceuticals and 3,000 ingredients for the treatment and are regular human applications. However, much amount of pharmaceutical waste is generated by the improper disposal by patients, veterinary drug additives, and agrarian wastes which ultimately released into the freshwater environment. The extensive usage and inappropriate dumping of pharmaceutical drugs cause spreading of multi-drug resistant pathogens. As there are no specific guidelines on the antibiotic residue discharge limits, the effluents produced from these industries are treated as per the guidelines prescribed by the Central Pollution Control Board (CPCB) on wastewater which unfortunately do not include antibiotic residues. As a consequence, they are not monitored in the pharmaceutical industry effluents. Besides, we advocate that pharmaceutical industries should utilize the best available and advanced technology to tackle the pharmaceutical contamination in India.

MATERIALS AND METHODOLOGY

The present study is based on a systematic survey of the literature. The literature survey was executed on publications acquired for the data related to impacts of pharmaceuticals on fish health and ecotoxicity. Web searches began with Google Scholar and Web of Science and using key terms “pharmaceuticals,” “drugs in the water” “aquaculture,” and “fish health.” Supplementary data were obtained from the reference sections of each article. Information was also acquired from news media and periodicals. We reviewed and included the most recent peer-reviewed academic literature for the present study and a total of 66 key sources are cited in manuscript to demonstrate key issues and emphasize key research gaps still awaiting attention in future studies.

RESULTS AND DISCUSSIONS

Pharmaceuticals **in** *Freshwater aquatic system*: Globally, every year, several tons of pharmaceutical chemical substances are produced and used. Pharmaceuticals are an important class of emerging environmental micropollutants and their occurrence in the freshwater environment is an increasing environmental concern. Nevertheless, the majority of these drugs may undergo biotic or abiotic degradation and finally get accumulate in the tissues of fish and other aquatic organisms to induce potential toxicological effects like behaviour, histopathology, reproductive disruption and immunotoxic responses.

Behavioral responses

Behavioural alterations are the reflections of early toxicity signs, as they offer integrative actions of neurotoxicity. [27] Several aquatic organisms in general exposed to various pharmaceutical wastewater. Fish and other organisms potentially exhibit defence behaviour to combat any overall effect. Species may exhibit asymmetrical behavioral effects to pharmaceutical contamination which vary with the concentration of the drug [28]. Many pharmaceuticals and their metabolites can accumulate in aquatic food webs and alter innate and vital behaviors in fish [29-32]. However, the behavioral effects of drugs in freshwater ecosystems depend on species-specific interactions and abiotic interactions [30]. For example, in an experiment, the fish *Lepomis gibbosus* (pumpkin-seed sunfish) exposed to three anti-anticonvulsant drugs (diazepam, carbamazepine, and phenytoin) exhibited a significant increase in time spent in motion behaviour [33]. Another laboratory study of McCallum *et al.* [34] demonstrated reduced aggressive behaviour and misplace their nesting site which is an important behaviour in round goby (*Neogobius melanostomus*) exposed to wastewater with pharmaceutically active compounds. The study of Lagesson [30] confirmed that the anxiety drug (Oxazepam) has the potential to alter the growth and behaviour in *Perca fluviatilis (perch) but not in Esox lucius (pike)* in the presence of temperature and predators as additional stressors. The experiment of Melvin [35] evidently confirmed that a mixture of SSRI and SNRI (antidepressant drugs) are capable of altering circadian rhythms (feeding, predation and competition behaviour) in male mosquito fish (*Gambusia holbrooki*) at concentrations approaching1g/L. The study of Saaristo *et al.* [31] had shown altered and abnormal behaviour upon the exposure to psychoactive pharmaceutical drugs with other stressors likes temperature and predation in European perch (*Perca fluviatilis*). The 35-day experiment of Martin *et al.* [36] showed that wild male mosquito fish (*Gambusia holbrooki*) exhibited severe behavioral impacts like boldness, exploration and mating behaviour upon the exposure of antidepressant fluoxetine at two ecologically relevant concentrations (31 and 374 ng/L). The study of Ziegler *et al.* [37] has shown that the brown trout (*Salmo trutta* f. *fario*) exposed to an antidepressant drug (citalopram) at two different life stages exhibited severe alterations in behaviour (increased swimming behaviour with reduced anxiety) bioaccumulation and growth. The study also confirms that behavioral changes were stronger in early life stages, which are linked with the chronic exposure time in larvae compared to juvenile fish.

On the basis of the above literature, it is evident that various pharmaceuticals and their metabolites can induce behavioural alterations in different species. Nevertheless both drug- and species-specific effects were also obvious. It is clear from the above literature that antidepressants, psychiatric drugs (benzodiazepines) and antihistamines can induce behavioural changes in fish at environmentally relevant concentrations. However, as such, the data is difficult to forecast the direct effects using solitary species and under static laboratory conditions. More investigations should evaluate the adverse effects of pharmaceuticals and their metabolites in freshwater ecosystems under more complex conditions to achieve an enhanced perception. Even though the existing literature advocates that the environmental effects of pharmaceuticals may occur in freshwater ecosystems dominated by wastewater effluent and effects of pharmaceuticals were seen only at higher concentrations but not at ecologically relevant concentrations. Therefore, the scarcity of experiments using behavioural endpoints on wildlife makes it tough to depict any universal conclusions on the subject of the ecological impact of pharmaceuticals found in freshwater ecosystems. Further, the behavioural outcomes produce even more complicated data to understand, synthesize, and extrapolate as fish and other aquatic biota living in polluted

environments is exposed to a wide range of pharmaceuticals. For that reason, apart from the regular behavioural endpoints, research on the effects of pharmaceutical mixtures are deeply required to acquire a better perception of biological effects of exposed wildlife.

Impacts of residual pharmaceuticals on the health of fish and wildlife:

India is the home for more than 10 per cent (9.06 million metric tons) of global fish productivity and ranked in the second position at global level [38]. The huge productivity is achieved by rigorous farming at elevated mass which has resulted in an increased vulnerability of fish to infections caused by microorganisms [39]. In order to enhance the fish productivity, farmers use excessive chemicals and antibiotics to prevent the infections and diseases which enter into the aquatic environment in significant quantities. The introduction of antibiotic drugs in various segments including aquaculture is threatening the effectiveness of antibiotics [39]. The harmful impacts of leftover pharmaceuticals in the freshwater ecosystem and human health are a big concern. Published literature reveals that drug circulation and bioaccumulation in the fish is mainly influenced by species and specific substances [40]. The laboratory investigations of Triebskorn et al. [41] have confirmed harmful effects of human pharmaceuticals (carbamazepine, clofibric acid, metoprolol, and diclofenac) in liver, gills and kidney of the in rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) at environmentally relevant concentrations. The study found severe glomerulonephritis with hyaline droplet degeneration of proximal renal tubule as a major pathological effect. The findings of Gürcü *et al.* [42] showed that the accumulation of a common antibiotic Metranidazole (MTZ) in fish (*Oncorhynchus mykiss*) intestine can affect histopathological organization of the intestine in dose-dependent and time-dependent manner. The alterations include necrosis, edema, inflammation and small tears in villi. For instance, when the juvenile fish rainbow trout (*Oncorhynchus mykiss*) exposed to the common antibiotic oxytetracycline (OTC) had shown the interference of the drug in different biochemical pathways and metabolic processes with increased histological damage like leucocyte infiltrations, hypertrophy, vacuolization and pyknotic nucleus in the tissues of liver and gills [43]. In another experiment, Rodrigues *et al.* [44] exposed the fish rainbow trout (*Oncorhynchus mykiss*) with a common antibiotic Erythromycin (ERY) and is frequently prescribed for humans and livestock medicine is frequently found in the aquatic environment. In an experiment Rodrigues, S., et al. [43] exposed the juveniles of rainbow trout (*Oncorhynchus mykiss*) exposed to environmentally relevant concentrations of Erythromycin (ERY) exhibited considerable progressive (hypertrophy of mucous cells and hyperplasia) and regressive tissue damage (cytoplasmic vacuolization, pyknotic nucleus and cellular degeneration, hemorrhage and increase of sinusoidal space) in gills and liver.

The study of Alimba *et al.* [45] found cytogenotoxic, hematological and histopathological alterations in *Clarias gariepinus exposed to* pharmaceutical effluents. Pharmaceutical effluent caused a major time-dependent increase in the frequency of micronucleus (MN) and irregular erythrocytes, decline in total erythrocyte counts, hemoglobin and haematocrit values and increase in total leucocyte number and lymphocytes compared to the control fish. The fish exposed to pharmaceutical effluent also exhibited pathological injuries on gills, liver and kidneys. Akinpelu *et al.*, [46] found histological alterations like infiltration of secondary lamellae, oedema, congestion of the blood vessels, epithelial thickening and lifting, and necrosis in the gill tissues of *Clarias gariepinus u*pon exposure to sub-

lethal concentrations of pharmaceutical effluents in dose-dependent manner. dos Santos et al. [47] found neurotoxic and respiratory effects in a Neotropical fish species, *Phalloceros harpagos* exposed to two common human used drugs namely paracetamol (analgesic and antipyretic) and propranolol. In a recent study, Santos et al., [48] confirmed that chronic environmental concentrations of methamphetamine can lead to health issues in aquatic organisms. The study found bioaccumulation of methamphetamine (psychostimulant) in various tissues (kidney > liver > brain > muscle > plasma) to induce degenerative symptoms (apoptosis) in liver and heart of the brown trout (*Salmo trutta fario*) exposed to environmentally relevant concentrations for 35 days. The recent study of Hubená *et al.*, [49] showed varied effects of four psychoactive compounds on food intake and brain function in juvenile chub (*Squalius cephalus*) exposed to environmentally relevant concentrations.

From the above literature of review, it is clear that pharmaceuticals in wastewater interfered with histopathological changes in the liver, gill, kidney and gut tissues of various fish species exposed to wastewater with pharmaceuticals. Results reveal that pharmaceuticals and their metabolites, even at ecologically relevant concentrations, can interfere to exert harmful effects on the histopathology of important organs like liver, gills and kidney and obstruct proper functioning of these organs. Bioaccumulation of pharmaceuticals via food webs in fish tissues like plasma, brain and muscle samples are slightest or negligible compared to liver, gills and kidney to induce degenerative symptoms like vacuolization, pyknotic nucleus and cellular degeneration, hemorrhage and infiltration of secondary lamellae and lifting in gills and liver while severe glomerulonephritis with hyaline droplet degeneration of proximal renal tubule as a major pathological effect in the kidney. The gut exhibited necrosis, edema, inflammation and small tears in villi. Results reveal that the interference of the pharmaceuticals in different biochemical pathways and metabolic processes caused increased histological damages in tissues in dose-dependent and time-dependent manner. These outcomes of the presented work enlightening a prospective for additional studies on metabolic pathways of pharmaceuticals and its metabolomics in fish exposed under natural conditions.

Impacts of residual pharmaceuticals on fish reproduction: Reproduction is a biological process which depends on the synchronizing activities of the neuroendocrine system. A number of human and veterinary pharmaceuticals are released into urban wastewater and upon exposure such contaminants can induce detrimental effects in aquatic organisms and fish. Runnalls *et al.* [50] found a decline in sperm parameters when adult fathead minnow exposed to clofibricacid (human pharmaceutical). The *in situ* studies of Sanchez *et al.* [51] confirmed the alterations in the enzyme activities, neurotoxicity, intersex, vitellogenin induction in fish exposed to pharmaceutical effluent at downstream of the Dore river (France). The study of Galus *et al.* [52] confirmed the incidence of developmental abnormalities like decreased embryo production, atretic oocytes and altered ovarian and kidney histology in adult zebrafish (*Danio rerio*) exposed to a pharmaceutical mixture of acetaminophen, carbamazepine, gemfibrozil and venlafaxine and to diluted wastewater effluent for 6 weeks. Based on the results of a laboratory study of Margiotta-Casaluci *et al.* [21] addressed the mode of action of human pharmaceuticals in fish species. Their study confirmed that the pharmaceutical drug (dutasteride) which used to cure benign prostatic hyperplasia in human, may inhibit the activities of both isoforms of 5α-reductase (5αR), the enzyme that converts

testosterone into dihydrotestosterone and cause detrimental effects in the fish, fathead minnow (*Pimephales promelas*).

The study of Prasad et al. [53] proven that certain pharmaceutical contaminants present in the natural environment may influence the fish reproductive process by means of the serotonin system. The *in vitro* studies of Yokota et al. [54] found severe antiovulatory activities of non-steroidal anti-inflammatory medicines in comparison with in vivo reproductive studies in medaka (*Oryzias latipes*). Aguirre-Martínez *et al.* [55] demonstrated significant changes in neurotoxic responses, inflammation, reduced gametogenic activity and energy status in fish *Corbicula fluminea* exposed to environmentally relevant concentrations of human drugs for 21 days (caffeine, CAF, ibuprofen, IBU, carbamazepine, CBZ, novobiocin, NOV and tamoxifen, TMX). The laboratory experiments of Martin et al. [36] and Fursdon et al. [22] confirm that the antidepressant drug (fluoxetine) can alter the reproductive behaviour and sperm traits in fish. Fraz et al. 56 found transgenerational impacts like reduced reproductive function, courtship and aggressive behaviors, and sperm speed and morphology for four generations in zebrafish exposed to a common drug residue (carbamazepine (CBZ). Constantine *et al.*, 57 found a decline in fecundity and fertility, vitellogenin and hatching success rate in zebrafish (*Danio rerio*) exposed to ibuprofen at environmentally relevant concentrations. de Lima et al. [58] found that certain diets which used to reduce oxidative stress in the human population can interfere with reproductive process, and growth in female Nile tilapia (*Oreochromis niloticus).* Godoi et al. [59] found a significant reduction in reproductive activities by reducing 17β- Estradiol (E_2), and testosterone levels in male fish (*Astyanax altiparanae) exposed to common drugs* (Diclofenac (DCF) and caffeine (CAF) at normal levels. But in contrast, the study of Thoré et al. [60] demonstrated increased mating, enhanced fecundity and alteration in social behaviour in the short-lived kill fish (*Nothobranchius furzeri*) exposed to antidepressants. The results of a recent laboratory study of Liang et al. [61] showed reduced plasma 11-ketotestosterone levels in males, increased reproductive toxicity, decreased spermatogenesis and anti-androgenicity in Japanese medaka (*Oryzias latipes*) upon the exposure of 3-(4-Methylbenzylidene) camphor (4-MBC) at environmentally relevant concentrations.

The above literature survey clearly revealed that pharmaceuticals in the aquatic environment can induce negative effects on fish reproductive function by affecting on sperm parameters, alterations in the enzyme activities, intersex, and vitellogenin induction in fish exposed to pharmaceutical substances. Some reports revealed decreased embryo production, atretic oocytes and altered ovarian histology in exposed fish to even at environmentally relevant concentrations of human drugs. Few studies reported transgenerational impacts like reduced reproductive function, courtship and aggressive behaviors, and sperm speed and morphology in exposed fish species. But in contrast, few authors reported better mating, improved fecundity and alteration in social behaviour in fish exposed to drugs in the laboratory.

Immuno toxic effects: The immune system in fish and other organisms possibly affected directly by toxic compounds. The detection of pharmaceutical remains of various drugs in surface waters has provoked concerns about probable adverse effects on freshwater biota. Several ecological pollutants including pharmaceuticals are identified as immune modulators in fish and wildlife species [62] which make the organism more vulnerable to pathogen infections. The research analysis of Milla et al. [63] had shown the interactions of the immune system

with synthetic steroids (estrogenic and androgenic) in fish. The review analysis of Burgos-Aceves et al. [64] demonstrated the immunomodulation functions in fish are regulated by both natural and synthetic estrogens which act through estrogen receptors and regulate definite target genes, and also through microRNAs (miRNAs). The experimental findings of Ribas et al. [65] revealed significant immunosuppressive effects by declining in RBC, WBC, haemoglobin and haematocrit values in the fish (*Hoplias malabaricus)* exposed to common drugs like diclofenac and dexamethasone. Kleinert et al. [66] observed a significant reduction in the functional activities of the immune system by decreasing lymphocyte transformation and G_0/G_1 phase of the cell cycle in the harbor seal (*Phoca vitulina) exposed to* 17α-ethinyl estradiol and 25,000 µg/L naproxen as mixtures. Khoei et al. [67] observed reduced aggressions, and IgM levels in fish Siamese fighting fish (*Betta splendens*) exposed to immunosuppressant drug tacrolimus. The guaranteed immunotoxic effects of pharmaceuticals have been found to be related with the defense mechanism of both host and microbiota [68].

The laboratory findings of Liang et al. [69] demonstrated decreased hatching rate and body length and increased abnormality and mortality in the early life stages of zebrafish *(Danio rerio)* upon exposure of Norfloxacin nicotinate (an antibacterial fluoroquinolones, NOR-N). The results also confirmed increased antioxidant system (MDA, SOD, CAT, and GPx) and expression of TNFα, IFN, IL-1β, IL-8, CXCL-clc, CC-chemokine, Lzy and C3 genes in exposed fish. Another recent work of Li et al. [70] established immunotoxic effects in association with hepatic damage (hepatic vacuolization and nuclei pyknosis and inflammation) in juvenile zebrafish (*Danio rerio*) exposed to Benzotriazole ultraviolet stabilizers for 28 days. The experimental findings of Bera et al. [71] established that triclosan suppressed both cellular mediated and humoral immune responses with a decrease in respiratory burst activity (RBA), myeloperoxidase activity (MPO), phagocytic activity (PA) in triclosan exposed catfish, *Pangasianodon hypophthalmus*). In an experiment, Rehberger et al. [72] demonstrated that the chronic exposure to low ethinylestradiol (EE2) concentrations combined with parasite infection in juvenile rainbow trout (*Oncorhynchus mykiss*) can induce endocrine disruptive and immunomodulating activities.

In conclusion, it is clear that the majority of pharmaceutical substances and their metabolites mimic hormone structure and interfere in endocrine function to exhibit immunotoxic actions. Accordingly, immunomodulation in affected fish may be a result of such exposure. Several ecological pollutants including pharmaceuticals are identified as immune modulators in fish and wildlife species. Several authors reported significant immunosuppressive effects like declining in RBC, WBC, haemoglobin, haematocrit values, decreasing lymphocyte transformation and G_0/G_1 phase of the cell cycle in harbor, reduced aggression, and IgM levels in fish exposed to pharmaceutical pollution. Few authors also reported the association of immunotoxic responses with hepatic tissue damage. Therefore, fish immuno-toxicity studies may be used as valuable biomarkers of pharmaceutical contamination. However, to standardize our understanding of these mechanisms, care should be taken when considering the intrinsic and extrinsic factors related to the fish and the contamination model.

CONCLUSIONS

The presence of persistent drugs in surface waters is a big global concern today. Literature is still scanty regarding how cocktails of pharmaceutical residues in surface water affect the environment or fish health. Until now, the published literature showed bioaccumulation and negative effects on fish and other aquatic organisms. As there is

no specific legislation on pharmaceutical waste management, the drug contamination in water is increasing globally year by year which effectively forming an epidemiological experiment with the public and freshwater ecosystem. The present study reviewed and analyzed the impacts of pharmaceutical substances on various health aspects of fish. Based on an inclusive assessment of the data and analysis, it is well established that drugs in water can affect the fish health by the accumulation in specific tissues which induce negative effects in histopathology, oxidative stress, haematological indices, reproductive function, gene expression and immune toxic effects. However, the degree of severity depends on species, sex, and phase of the life cycle, dose and duration of the substances. The study advocates framing and implementing rigid guidelines for treatment and monitoring of waste discharged from municipal wastewater and pharmaceutical industries for controlling pharmaceutical pollution in wastewater.

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