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Review Article

TOXIC EFFECT OF PHARMACEUTICALS WITH REFERENCE TO OXYTETRACYCLINE

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ABSTRACT

Manufacturing and release of pharmaceuticals are increasing in different countries of the world especially in developing countries. The discharge from wastewater plant contains a wide range of classes of pharmaceuticals. Rapidly growing economy, aquaculture, and livestock industry has led to increased incidences of diseases and has increased the release of pharmaceuticals into natural environment which is affecting the biodiversity of hotspots adversely. This review mainly focuses on antibiotic oxytetracycline, its use, structure, occurrence in aquatic environments and other environmental compartments, lastly an overview of toxicity to fish is provided. The threats posed by these pharmaceuticals are evident from the studies showing their toxic effect on different aquatic organisms.

Keywords: Pharmaceuticals, Oxytetracycline, Toxicity.

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INTRODUCTION

Concern about the effects of pharmaceuticals on the aquatic environment has been increasing in the last years. Antibiotics are of particular concern since they are extensively used in aquaculture where they may be applied directly into the water or be incorporated in the food [1]. In the aquatic ecosystem pharmaceuticals may cause deleterious effects on the aquatic organisms accidentally exposed to them. Antimicrobial agents are extensively used in human and veterinary medicine and in aquaculture. Worldwide estimation of antimicrobial agent's consumption lies between 100,000 and 200,000 tons/year [2]. According to the European Federation of Animal Health report, in the year 1999, 65% of antimicrobials were used in human medicine [3]. In the survey by the European medical agency, the sales of veterinary antimicrobial agents were compared among European countries, resulting from 18 to 188 mg kg^{-1} of antimicrobials per kilogram of biomass of food producing animals [4].

Between 2000 and 2015 antibiotic consumption in 76 countries around the world, expressed in defined daily doses (DDDs), increased 65% and, in 2015, reached 42 billion DDDs. Among high-income countries, the leading consumers of antibiotics in 2015 were the United States, France, and Italy. Leading consumers of antibiotics between low- and middle-income countries were India, China, and Pakistan [5]. Worldwide antibiotic use is expected to be 200% higher by 2030 than in 2015, with the greatest increase coming from low- and middle-income countries. Among antibiotics, tetracyclines were the most commonly used, followed by sulfonamides and macrolides that accounted for approximately 90% of the total antibiotics used in the UK and more than 50% in Korea and Denmark [6]. There are significant differences in trends in the antibiotic consumption in European countries. According to the Antimicrobial Consumption Annual Epidemiological Report for 2016 published by the European Center for Disease Prevention and Control (ECDC), a statistically significant trend of increasing antibiotics usage was observed for Greece and Spain from 2012 to 2016, while over the same time period a statistically significant decreasing antibiotics usage trends were observed for Finland, Luxembourg, Norway, and Sweden. The most prescribed categories of antibiotics in the United States and the European Union are penicillins, macrolides, cephalosporins, and fluoroquinolones (CDC Centers for Disease, 2015). More detailed information on the consumption of various antibiotics in some EU and US countries has been provided by Singer *et al*. [7].

In Indian economy, the pharmaceutical industries are one of the fastest growing segments. Nowadays, India is the world's leading exporter of generic drugs, which account for 75% of its market by volume [8]. Production of pharmaceuticals is rising at 14% per year. This may be due to excise duty free zone for pharmaceutical manufacturing done by the Central and State Governments of India leading to large scale production of pharmaceutical. Due to lack of strong and specific regulation in a country like India, pharmaceutical waste management has become a herculean task and leads to the problem of ever growing environmental pollution. The studies demonstrated that very high concentrations of antibiotics and antibiotics resistant bacteria were found in effluents from a sewage treatment plant in India. Particularly, high concentrations of terbinafine, citalopram, cetirizine, ciprofloxacin, and enoxacin were found in drinking water [9]. The main routes of antibiotics to reach in the aquatic environment are manufacturing industries, municipal effluent, ineffective sewage treatment plant, livestock, aquaculture, and agricultural activities [10].

Tetracyclines are useful against Gram-negative and Gram-positive bacteria, they were discovered in the 1940s, and they are a group of broad-spectrum antibiotics [11]. In veterinary and agriculture, the tetracyclines are one of the most widely used classes of broad spectrum antibiotics due to their oral administration, very low cost and few side effects.

Tetracyclines are formed of a four-ring core to which various side groups are attached. At the C4 carbon, dimethylamino group present, this shows the antimicrobial activity. 4-De-dimethylamino tetracyclines (Fig. 1), also called chemically modified tetracyclines (CMTs), due to the inability of the molecule to adapt a zwitterionic form (CMTs) and lacks antimicrobial activity *in vivo* [12]. The lower half of the molecule is oxygen-rich and binds with both eukaryotic and prokaryotic targets, and interference with this region reduces the effectiveness of the drug [13].

Oxytetracycline (OTC) is the broad spectrum antibiotics of tetracycline group and an antibacterial agent which is used as human and veterinary medicines for prophylactic and therapeutic purposes [14]. OTC inhibits the protein synthesis that interacts with 30 s ribosomal unit [15]. Oxytetracycline was first isolated from the bacteria *Streptomyces rimosus* in 1948 from soil and 2nd antibiotics to be discovered. OTC is used as animal and human medicine because it has low price and excellent antimicrobial activity with very less side effects [16].

Oxytetracycline is one of the main antibiotics used nowadays to defend bacterial disease in fish. Maximum of OTC is given to fishes through the feed, but fish have a very less absorption for OTC so 70–80% of OTC is excreted as waste material into the water [17]. It can be retained in sludge and bottom deposits of fish farms due to their lipophilic and non-degradable nature [18]. Its residue shows direct effect on fish and other consumers and has a relatively long half-life [19].

CHEMICAL STRUCTURE

Robert Woodward studied the chemical structure of the OTC (1953). From 1950–1980 several members of the tetracycline family are developed and they become very popular antibiotics in the United States [20]. OTC is in the form of hygroscopic yellow crystals and odorless. It has a bitter taste and decomposes at 180°C. It is insoluble in chloroform and ether, slightly soluble in ethanol and soluble in water [21].

Molecular formula: $C_{22}H_{24}N_2O_9$.

Like other tetracycline OTC is also formed of 4 core ring which contain many functional groups as seen in Fig. 2. OTC contains hydroxyl groups at C-3, C-5, C-6, C-10, C-12 position, Carbonyl group at C-1 and C-11, nitrogen atom at C-4 position, and caboxamide group at C-2 position of A-ring. In OTC both pharmacokinetic and antibacterial properties is influenced by the chelation with metal ions present in biological environment and food so it is a strong chelating agent [22].

OCCURRENCE

OTC is detected in soil, water bodies, in tissues of living organisms. Different studies have supported the presence of OTC in rivers, lakes, rainwater, ground water, sea, soil, and in the tissues of living organisms.

Kay [23] proved that most common antibiotics Oxytetracycline (OTC) is difficult to biodegrade. Residual OTC has been detected in different areas, for example, in British soil; the concentration of OTC was as high as 1691 μg/kg. Wang *et al*. [17] observed the gathering of OTC in muscles, kidney, blood, and liver tissues of black sea bream *Sparus macrocephalus* and perch *Lateolabras janopicus.* Boxall *et al*. [24] illustrated the presence of OTC in animal husbandry effluents (15.133 ng L^{-1}), in wastewater (1600 ng L^{-1}), and in surface water (340 ng L^{-1}), respectively. Bjorklund *et al*. [25] found that OTC can cause deleterious effects because it can persist for relatively long periods in fish tissues and sediments. Olatoye *et al*. [26] investigated that the OTC residues in the muscle and liver sample of a total of 160 fishes collected from fish farms and restaurants. Barani *et al*. [27] tested the OTC residues in the body of 138 trout sample and found its presence in 87 samples between 1.43 and 101.4 μg/kg. Baydon *et al*. [28] evaluated that the OTC residues in samples of fish *Oblada melanura and Mullus barbatus* in their natural habitat, caught in the vicinity of fish farms around Aegean Sea.

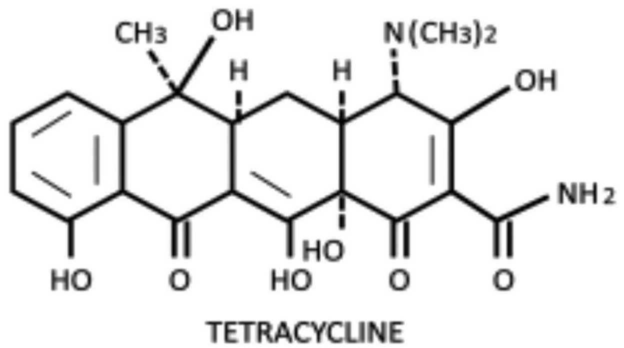

Fig. 1: Chemical structure of tetracycline

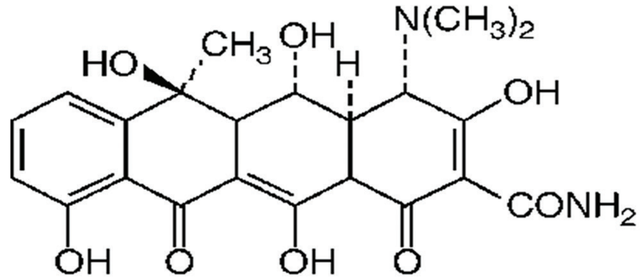

Fig. 2: Chemical structure of oxytetracycline

Kolpin *et al*. [29] conducted a study during 1999 and 2000 in 30 states, the concentration levels of oxytetracycline (OTC) ranging from 0.10 to 0.34 mg/L were found in streams. Thurman *et al*. [30] reported antibiotics in sewage from fish ponds, where the concentration of OTC ranged from 0.17 to 10 mg/L. Bebak-Williams *et al*. [31] observed that the higher OTC concentration is present in re-circulating water supplies than in adjoining surface waters. Samuelsen *et al*. [32] concluded that the primary fate of antibiotics is to deposition in the sediments in aquatic ecosystems. They measured the concentrations of OTC in sediments ranging from 0.4 to 495 mg/g. Capone *et al.* [33] studied the effect of OTC in non-target organisms such as clams, oysters, and crustaceans following discharge in the vicinity of aquaculture operations.

Coyne *et al*. [34] founded the OTC concentrations in oysters (*Crassostrea gigas*) and Dungeness crab, which is <0.1 mg/g, while in red rock crabs, the OTC concentration detected at levels of 0.8–3.8 mg/g. In soft tissue of mollusks, the concentration of OTC residues is up to 10.2 mg/g.

TC has been classified among the antibiotics frequently detected in sewage, domestic wastewaters, surface and groundwater resources, drinking water, and sludge (Panguha *et al*. 2011). Tetracyclines have the highest concentrations and are most frequently reported antibiotic residues in manure [35,36]. The concentrations of oxytetracycline and chlortetracycline in some agricultural lands may reach extremely high levels. Previously conducted studies showed that one of the most widely used antibiotics in animals is tetracycline [37]. Concentration of tetracycline was found to be between 170 and 850 ng L^{-1} in a study conducted in the United States of America in effluent from WMWTP [38]. In natural aquatic environments, OTC has been detected at micro concentration levels (μg/L) in surface water, wastewater, and treated wastewater [39,40]. Environmental monitoring studies have reported OTC as high as 68 μg/L in river water in Japan [41]. Javid *et al*. [42] found tetracycline levels in surface and ground water at nearby of animal farms in the vicinity of Fasha-foyab-dam to vary from 5.4 to 8.1 mg L^{-1} and found a maximum concentration at Yaft-Abad sampling station and found 9.3 ng L^{-1}, while TC concentration is found to be negligible in hospital wastewater treatment plant and the main reason TC is no longer being used among hospitalized patients. However, several studies reported their presence in coastal or estuarine marine systems [43,44]. Data are present in literature suggesting a long persistence of oxytetracycline in the aquatic sediments [39]. Oxytetracycline is very often detected in the marine environment [33,36].

India is among the top five world market of pharmaceuticals and expected turnover by 2020 will USD 45 billion/year [45]. In spite of bulk production around 80% is consumed in India only [8]. On the other hand, sewage treatment capacity in India is far below the generated by 1.3 billion populations. Therefore, different pharmaceuticals have been detected in various environmental compartments in India. Forty-three pharmaceuticals and 13 metabolites, including psychoactives, artificial sweetener, and illicit drugs in five waste treatment plants that treat domestic sewage in India have been reported by Subedi *et al*. [46]. Few studies on the presence of pharmaceuticals in hospital effluents have been conducted in India and found that hospital effluents contains a much higher concentration of these as compared to sewage treatment plant [47]. Similarly, different researchers have reported the presence of different pharmaceutical in different rivers of India [46-48].

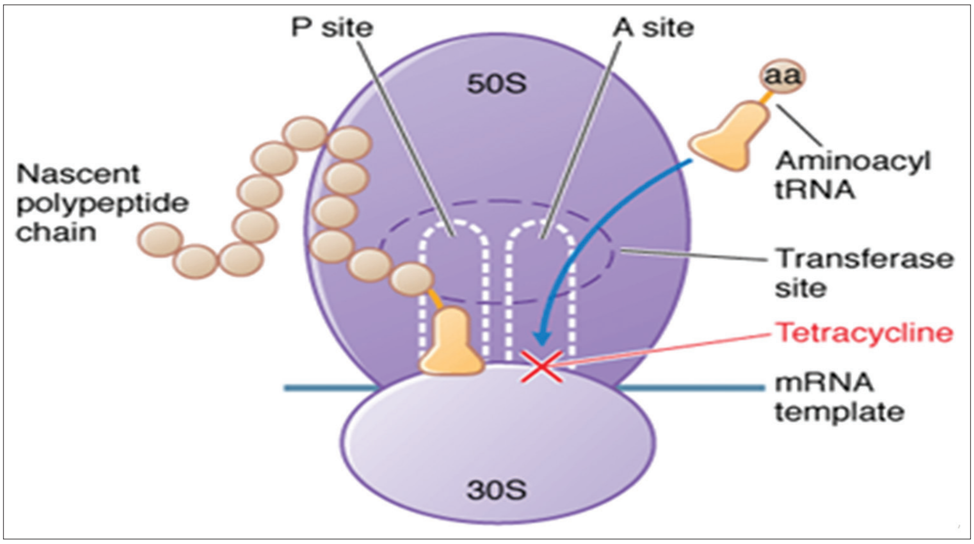

Fig. 3: Working of tetracycline as antibiotics

WORKING OF TETRACYCLINES AS ANTIBIOTICS

The survival and growth of bacterial cells are inhibited by the OTC; mostly it inhibits the production of bacterial proteins [49]. Tetracyclines also cause the outflow of genetic material and other complexes out of the cells by altering the bacterial membrane [49].

Hash *et al*. [50] suggested that the tetracyclines (OTC) primarily bind to the bacterial ribosome and inhibiting protein synthesis and exert their antibiotic effect. Semenkov *et al*. [51] studied that in bacterial ribosome (30S-50S subunit); many high-affinity binding site and low-affinity sites are present (Fig. 3). OTC gets bind with the 30S subunit and allosterically inhibit binding of the amino acyl-tRNA at the acceptor site (A-site), due to which protein synthesis ceases. Yamaguchi *et al.* [52] said that the use of OTC and other tetracyclines has reduced in recent decades due to the emergence of resistant strains of bacteria. Singh *et al*. [53] suggested that the use of OTC in aquaculture may increase the risk of transfer of antibiotic resistance to human pathogens and it became more dangerous to human.

TOXICITY OF OXYTETRACYCLINE

The excess amount of OTC causes various deleterious effects on living organisms. It gets stored in the tissues and muscle of fish and enters in to human when it is consumed by the them and shows deleterious effects on the health of human. Samanidou *et al*. [54] tested that the use of excessive amount of antibiotics (OTC) may lead to the presence of residual antibiotics in fish products and fish tissue.

Guardiola *et al*. [55] concluded that the OTC increases the cellular parameters in gilthead sea bream and interferes with humoral innate immune parameters. Hentschel *et al*. **[**56] elucidated that the side effects of long-term antibiotic like OTC use on fish can induce liver damage and nephrotoxicity. Zounkova *et al*. [15] reported that in aquatic ecosystems oxytetracycline may have both, the ecotoxic and genotoxic effects. Santos *et al.* **[**57] suggested that pharmaceuticals and drugs cause severe ecological disability, and they have tendency to interfere with various pathways of fish, such as Lionetto *et al*. [58] studied the neurotoxicity in fish and Ren *et al.* and Limbu *et al*. [59,60] studied the detoxification in fish. Micale *et al*. [61] visualized that the primary toxicity of antibiotics is exerted mainly at biochemical and molecular levels, later toxicity observed at higher levels of biological classification (cell, tissue, organ, individual, and population). Due to excess amount of antibiotics concentrations, the aquatic organisms undergoes in oxidative stress [62]**.** Several studies indicated that tetracyclines have an antioxidant activity [63]. Other studies suggested that it may be involved in reactive oxygen species formation. Khan *et al.* [64] have revealed that when tetracycline Cu (II) complex bind to DNA a methyl group is transferred by tetracycline to nitrogen base with generation of free radical. They pointed that the accumulation of abnormal proteins, lipid peroxidation products, and DNA damage (genotoxicity) all are the result of oxidative stress which occurs due to adverse environmental conditions. OTC is largely used as food additives in animal husbandry as their residues are found in several animal products such as milk, eggs, fish, or meat. Gunes *et al*. [19] revealed that the beekeepers use OTC against the bacterial diseases that affect honeybees, in apiculture and in the honey of treated bees OTC residues can be detected in trace levels which later pass into the body of consumers. Sara *et al*. [65] used (0.0002–200 μg/L) of erythromycin and 0.0004–400 μg/L of oxytetracycline on gilthead seabream (*Sparus aurata*) fish to study the histopathological effects in gills and liver. In both organs, several disorders such as circulatory, progressive, inflammatory, and regressive, are observed.

Botelho *et al*. [66] studied the genotoxic response in juvenile tilapia (*O. niloticus*) when exposed with 4, 8, 16, and 32 μg/L, concentration of OTC for 96 h. Genotoxic responses shown by the fish are higher DNA damage and nuclear abnormalities. Maximum damage is seen in the fish, exposed to higher concentrations. Ren *et al*. [59] exposed *P. trituberculatus* larvae with 30 μg/L of OTC and observed DNA damage in larvae. Khan *et al*. and Botelho *et al*. [64,66] observed the genotoxicity in fish *S. aurata* when exposed to OTC. Using comet assay they found that OTC has the potential to induce broken DNA strands. Genotoxicity can be an outcome of oxidative stress elicited by several pollutants. Limbu *et al*. [60] observed that the OTC stimulated neutrophils and macrophages in liver tissues when they exposed the *O. niloticus* with at 420 ng/l of OTC. Said *et al*. [67] visualized that in rodent species (guinea pig), tetracycline (OTC) is able to disturb the function of parasympathetic nervous system and can cause neuromuscular blockage and cranial nerve toxicity in humans. Rodrigues *et al*. and Khan *et al*. [62,64] investigated the genotoxicity of OTC in *O. mykiss*, they concluded that the OTC has high affinity to bind with DNA and form tetracycline – DNA (TC–DNA) binary complexes as a result it causes change in the secondary structure of DNA-duplex. Sharma [68] found OTC cytotoxic and genotoxic in fish *Cyprinus carpio.*

Limbu *et al*. and Nazeri *et al*. [60,69] demonstrated that the pathways of energy metabolism can affected by the exposure of OTC in treated fish. The enhanced gluconeogenesis, inhibition of aerobic glycolysis, β-oxidation of fatty acids in hepatic tissue and suppressed lipogenesis all are the effects caused by OTC. They also observed that these effects are due to oxidative stress. Oxidative stress is generated due to the ability of OTC to increase the generation of reactive oxygen species

(ROS) such as peroxides, oxygen ions, and free radicals. Various studies indicate that generation of ROS and nitrogen species can cause oxidative stress when erythromycin (ERY) and OTC are metabolized in tissues of various fish species, namely, *S. aurata* [55], *O. mykiss* [62,70], *C. auratus* [71], and *D. rerio* [72].

Wide range of pharmaceutical residues derived from domestic use, hospitals, ground water, sea, and estuaries. This review provides a wakeup call to the stakeholder including regulatory agencies in India to establish minimal permissible limit of pharmaceuticals in waste water to avoid its toxic effects.

AUTHORS' CONTRIBUTION

Dr. Madhu Sharma: Draft the review paper

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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