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Research Article

Behavioral responses and morphological changes to the drug Diclofenac in Channa punctatus

Rohini Padma

Department of Zoology, Government Degree College, Jammikunta, Telangana, India.

*Corresponding author:
E-mail: rohinipadma28@gmail.com

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ABSTRACT

Pharmaceuticals have emerged as priority pollutants of the aquatic environment in recent times. Diclofenac is a non-steroidal anti-inflammatory drug that has been usually detected in surface waters in the range of ng/l to µg/l. There are many experimental evidences on its toxicity in the aquatic flora and fauna. The objective of the present study is to study the behavioral responses and morphological changes in fresh water fish, *Channa punctatus* on exposure to various concentrations of diclofenac. The behavioral and morphological alterations were found to be significant and are dose dependent. This study emphasizes that morphological and behavioral alterations are the significant biomarkers in toxic studies. It also assumes that pharmaceutical residues in the water deteriorate the health of the fish..

1. Introduction

Pharmaceuticals are used for the diagnosis, treatment and prevention of various diseases in human and animals. But the pharmaceutical residues have become priority emerging pollutants in recent times. The increased consumption of these drugs has led to their discharge into the environment. A large number of pharmaceuticals both prescription and over the counter drugs have been detected in influents and effluents of wastewater, surface water, ground water and even in drinking water. They enter into water bodies through many routes and the foremost being excretion from treated patients either in the form of parent compound or its metabolites, direct release from manufacturing units, hospitals, disposal of unused drugs and leaching from terrestrial deposits.

Though pharmaceuticals have been detected in traces from ng/L to µg/L they show adverse effects on aquatic life. There are several experimental evidences on the toxicity of different classes of pharmaceuticals. They are known to cause behavioral alterations, changes in biochemical constituents, genotoxicity, endocrine disruption in non-target organisms like fish and selection of antibiotic resistance in pathogenic microorganisms.

Diclofenac is the widely prescribed drug for treating both acute and chronic pain in various disorders like rheumatoid arthritis, osteoarthritis, spondylitis, ocular inflammation, gout and dysmenorrhea (Skoutakis *et al*., 1988). It is available in the form of tablets, capsules, suppositories, intravenous solutions and injections. It is usually supplied in the form of either sodium or potassium salt. It is sold with the common brand names as Voltaren, Pennsaid, Arthrotec, Flector, Solaraze etc,. Diclofenac has been detected in surface waters of rivers, lakes, seas, influents and effluents of wastewater treatment plants, groundwater, drinking water, soil and sediment worldwide (Nikolaou *et al*., 2007). It was detected in the magnitude of high ng/L to µg/L in number of counties in the world including India. Diclofenac was found to induce many potential toxic effects in aquatic flora and fauna. The subchronic and chronic studies have reported the toxicity of diclofenac in aquatic flora and fauna. However, the acute toxicity data in fish is very scarce. There are no reports on acute exposure of diclofenac on behavioral and morphological parameters in fish. Therefore, these parameters have been taken up to find out acute toxicity of diclofenac.

2. Material and Methods

2.1 Preparation of samples:

The fresh water fish, *Channa punctatus* were collected from the waters of Hasanparthy village of Warangal district, Telangana, India. The experiments were performed according to the standard methods to determine the LC_{50} of *Channa punctatus.* The healthy fish weighing about 100-110g and 20±1.21cm in length were transported to laboratory in large plastic tanks and filled with water. The fish were washed in 1% potassium permanganate to free from microbial infections. The fishes were acclimatized in 50 litres capacity plastic tubs filled with dechlorinated water prior to experimentation. The fish were fed *ad libitum* with commercial feed rice bran and oil cake twice a day. Proper aeration was provided with the help of aerators. The fish were maintained in tanks under 12:12 hour light : dark period. The dead fish were removed immediately to keep the water afresh. During acclimatization and test period, water was renewed for every 12 hours followed by the addition of desired concentration of the test compound. The fish were starved one day before experimentation.

Analytical grade of Diclofenac sodium (2- [(2-6 Dichlorophenyl) amino] benzene acetic acid sodium salt, 99% pure (CAS 15307- 86-5) was purchased from Sara Exports, Ghaziabad, Uttar Pradesh, India. Diclofenac stock solution was prepared with acetone and ten different concentrations 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 35 ppm, 40 ppm, 45 ppm and 50 ppm were prepared from stock solution. The fish were exposed for 96 hours to ten different concentrations and the behavioral and morphological alterations were studied with respect to control. The experiment was carried out for six times.

2.2 Behavioral changes

Behavioral changes are the most sensitive indicators of environmental pollution. They help in screening the chemical pollutants and their toxic effect on the aquatic organisms. The alterations in the behavior of fish particularly non-migratory species provides an important sign for ecosystem assessment (Halappa and David, 2009). The behavioral responses are directly related to complex physiological response underlying in the animals and these have been used as a sensitive measure of stress (Mahurpawar, 2016).

The change in the behavior of fish can be directly related to poor quality of water which may affect the survival of fish (Olla *et al*., 1983). The change in biochemical composition and physiological function occurred due to pollution leads to the alteration of behavioral responses in fish. There is a limited literature on the behavioral and morphological alterations caused due to drug toxicity. In view of this, the effect of various concentrations of diclofenac on the general behaviour and morphology of fish was studied.

The response of different fish species to a toxicant varies extensively. The individuals of the same species also show variation in their behavioral responses to toxicants. The wide variation in sensitivity of different species or individuals within a species to different toxicants depends on various factors like age, sex, weight, physical status of the animal and presence or absence of enzyme system that can degrade the pollutants (Nagaratnamma and Ramamurthi, 1981).

3. Results and Discussion

3.1 Behavioural Changes

The fish in control group were very active and have shown well-coordinated movements. They have settled at the bottom, often reached the surface and moved normally throughout the tank. The fish have also maintained a dense schooling behavior.

The fish have exhibited a number of behavioral changes when they were exposed to different concentrations of Diclofenac. The behavioural changes noticed were listed in the Table-1. The abnormal behavior in the treated fish was characterized by erratic swimming, convulsions, jerky movements, frequent surfacing followed by sinking, loss of equilibrium, gulping of air, hyperactivity, aggressiveness, leaping out of the medium and gradual inception of inactivity, becoming lethargic and settling at the bottom. The fish in the lower concentrations of diclofenac fed actively while the fish in higher concentrations were sluggish and lazy in feeding.

Many investigations have reported behavioral alterations on exposure to various toxicants in fish. Prashant *et al*., (2011) have observed jumping movements, restlessness, turning upside down, excessive mucus secretion, loss of equilibrium, sluggishness with short jerky movements, frequent surfacing, gulping of air and erratic circular movements in *Labeo rohita* after exposure to Sodium cyanide. Ganeshwade *et al*., (2012) have reported behavioral changes such as irritation, fast opercular movements, violent actions of pelvic fin as well as spreading of the fins, loss of equilibrium and mucus covering in *Channa striatus* after treatment with endosulfan.

Sarma *et al*., (2013) have observed abnormal swimming pattern, increased jumping, fast opercular activity, erecting dorsal and ventral fin in *Channa punctatus* after exposure to Rogar. *Cyprinus carpio* exposed to lambda cyhalothrin has shown increased rate of gill operculum movements. with increased mucus secretion and the fish has also exhibited irregular, erratic and darting swimming movements followed by hanging vertically in water (Habeeba and David, 2016). Pawara and Patel (2016) have noticed lightening of the body colour, jumping movements, restlessness and loss of equilibrium in response to Endosulfan treatment. Reddy *et al*., (2016) have observed sluggishness, jerky movements, erratic opercula activity, shedding of scales and mucus secretion in *Cyprinus carpio* exposed to Chromium.

Dembele *et al*., (2000) have indicated that the alterations in fish behavior observed on exposure to organophosphate insecticides such as chlorfenvinphos, chlorpyrifos and diazinon were due to failure of energy production or the release of stored metabolic energy which caused severe stress leading to the death of the fish. Similar observations were found in *Ctenopharyngodon idella* on exposure to chlorantraniliprole.

There was a swift opercular movement in the fish exposed to lower concentrations of Diclofenac. The opercular movement was frequent in the lower concentrations and has gradually decreased as the concentration of diclofenac was increased. The decrease in opercular movement might have probably helped in reducing the absorption of toxicant through gills. The initial increase in gill opercular movement was to support enhanced physiological activities in stressful habitat, and later the decreased movements were possibly due to accumulation of mucus on gills (David *et al.,* 2002). The increased opercular movement may also be due to the increased demand for oxygen and energy due to altered physiological changes. The gradual decrease of the opercular movement appears to be an effort to reduce the contact of the gill epithelium with the toxicant.

There was an increase in erratic swimming, convulsions and jerky movements with increasing concentrations of diclofenac. The fish were swimming vertically and later they became restless and lethargic. Erratic swimming and jerky movements in swimming may be due to lack of nervous and muscular coordination which occurs as result of accumulation of acetylcholine in synaptic and neuromuscular junctions (Rao *et al*., 2005).

The hyperactivity of the fish and leaping out of the water was to evade from the effect of toxicant. The loss of equilibrium may be due to the effect of toxicant on the centre of brain associated with the maintenance of equilibrium (Mahurpawar,

Table: 1 Behavioral alterations in *Channa punctatus* on exposure to different concentrations of Diclofenac

Behavioral Changes	Control.	5 ppm	10 ppm	15 Ppm	20 ppm	25 ppm	30 ppm	35 ppm	40 ppm	45 ppm	50 ppm
Erratic swimming	-	-	+	+	+	++	++	++	++	+++	+++
Jerky movements	-	-	+	+	+	++	++	++	+++	+++	+++
Convulsions	-	+	+	+	+	++	++	++	+++	+++	+++
Leaping out of medium	-	-	+	+	+	++	++	++	++	++	+++
Settling at bottom	-	+	+	+	+	++	++	++	+++	+++	+++
Aggressiveness	-	-	-	+	+	++	++	++	++	++	+++
Gulping of air	-	-	+	+	+	++	++	++	+++	+++	+++
Opercular movement	-	+++	+++	+++	++	++	++	++	+	+	+
Surfacing	-	-	+	+	+	++	++	++	++	+++	+++
Lethargic	-	-	-	+	+	++	++	++	++	+++	+++

The increase or decrease in the level of behavioral parameter is shown by number of (+) sign. The (-) sign indicates normal behavior.

2016). The leaping out of the water shows the reduction of oxygen in the water due to chemical changes.

The frequent gulping of air and surfacing phenomenon was more in the fish treated with higher concentrations of Diclofenac. Gulping of air might be to avoid contact with the toxic medium and to ease respiratory stress (Halappa and David, 2009). Surfacing phenomenon in exposed groups may be due to higher demand for oxygen during the exposure period (Katja *et al*., 2005). Ray and Munshi (1987) have advocated that the change in rate of ventilation and surfacing frequencies which occur due to exposure of pesticides was to avoid contact with poison and fight against stress. The fish frequently swims to the surface in order to compensate for the stress caused due to hypoxic conditions created by toxicant.

The treated fish were very often seen lethargic at the end of exposure period. The lethargic condition in the fish may be due to loss of energy as a result of erratic swimming, leaping out of the medium and restlessness. The disruption of the schooling behavior in fish has indicated increased swimming activity and increased expenditure of energy (Murthy, 1987).

The hyperexcitability of diclofenac exposed fish may be due to the obstruction in the functioning of acetylcholinesterase enzyme in relation to nervous system. (Agarwal and Balakrishnan, 1989). It further leads to the accumulation of acetylcholine which is likely to cause prolonged excitatory postsynaptic potential which may first lead to stimulation and later cause a block in the cholinergic system.

Behavioural abnormalities have been attributed to nervous impairment due to blockage of transmission of impulses between the nervous system and various effector sites (Niragu, 1979). Behavioural anomalies evidenced right from the day of exposure to different concentrations of diclofenac were due to inhibition of AChE in brain. The inhibition of acetyl cholinesterase activity results in the accumulation of acetylcholine which is a sign of cholinergic toxicity. The disruption in sensory, hormonal, neurological and metabolic systems have great implications on the behavior of fish.

3.2. Morphological changes

The morphological alterations like secretion of mucus, lightening of the body color, shedding of scales and deformities in eye were observed in the exposed fish. These morphological manifestations were more pronounced in the higher concentrations of Diclofenac.

Several studies have reported similar changes on exposure to different concentrations of toxicants in different fish. Devi and Mishra (2013) have observed shedding of scales, discoloration, lesion of skin, split and necrosis of fins, eye deforities, scoliosis, damaged skull, lower lip extension and copious amount of mucus secretions in *Channa punctatus* after exposure to chlorpyrifos.

In the present study, a thick mucus coat was observed on the surface of the body of fish. The mucus coat might have been secreted to protect from the toxic effect of Diclofenac. The excess secretion of mucus may be due to nonspecific action of the toxicant thereby reducing the toxicant contact. The mucus forms a barrier between body and toxic medium so as to minimize its irritating effect or to cleanse through epidermal mucus (Venkatarathnamma and Nagaraju, 2013). The excessive mucous secretion over the gill might be to inhibit oxygen diffusion causing respiratory distress (David *et al*., 2003). Pandey *et al*., (1990) have stated that the secretion of mucus over the body was due to dysfunction of the endocrine gland under toxic stress causing changes in the number and area of mucus glands and chromatophores. The depigmentation and exudation of mucous could be attributed to the dysfunction of pitutary gland under stress causing changes in the number and area of mucous glands and chromatophores (Prathibabhanu, 2013).

Arillo and Melodia (1990) have explained that the mucus secretion in fishes has an ameliorating effect against the toxicant. Gopal *et al*., (1981) have stated that toxicants that enter through permeable gill epithelium might be excreted through mucus secretion. Dutta *et al*., (1995) and Dutta and Chaudhary (1996) have noticed heavy elution of mucous in fish as a defensive measure against toxicant. The increased mucus secretion may probably help in countering the irritating effect of toxicant in skin and mucus membrane (Singh, 2013). The high amount of mucus secretion in exposed fish definitely may be a defense mechanism to protect the body against negative consequences caused by the effect of Diclofenac.

4. Conclusion:

The present study has indicated that diclofenac is highly toxic to aquatic organisms like fish. Behavioral changes were clearly noticed in the fish exposed to diclofenac. It was clearly evidenced from the behavioral and morphological manifestations that diclofenac is harmful to fresh water fish and its discharge into the water bodies will be hazardous to the aquatic ecosystem.

Competing Interests

The authors have declared that no competing interests exist.

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