Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
4(2):392-396
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

Diclofenac induced alterations in the phospholipid levels in different tissues of fresh water fish, *Channa punctatus*

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ABSTRACT

The extensive usage and improper disposal of pharmaceuticals have the potential to contaminate the water and sediments. Diclofenac is a widely prescribed non-steroidal anti-inflammatory drug that has been frequently detected in surface waters worldwide. There are evidences of its toxicity in aquatic organisms like phytoplankton, zooplankton and fish. The present study aims to investigate the biochemical alterations induced by Diclofenac in fresh water fish, *Channa punctatus*. Phospholipids, the major biochemical constituents of the cell membrane were taken as biomarkers. The acute toxicity assays were performed by exposing the fish to three different concentrations 5ppm, 25ppm and 50ppm of diclofenac. The effect was observed in different tissues like gill, liver, brain, muscle and kidney after 24h, 48h, 72h, and 96h of exposure with respect to control. Phospholipids were estimated by Zilversmidth and Davis method. Diclofenac exposure on fish at the set concentrations has caused reduction in the phospholipid level of different tissues when compared to control. The findings of the study assume that diclofenac causes biochemical alterations in fish which leads to the impairment of health status of the fish.

Key words: Diclofenac, acute toxicity, phospholipids, *Channa punctatus*

INTRODUCTION

The occurrence of pharmaceuticals in the aquatic environment is a growing concern throughout the world in recent times. Pharmaceuticals are the bioactive compounds that exhibit specific biological action in target molecules in human and animals (Arnold et al., 2013). But due to extensive usage and illegitimate disposal they have emerged as priority pollutants. The veterinary and human drugs enter into various water systems through different pathways. The drugs usually remain unchanged after excretion and contaminate the waters (Peake and Braund, 2009). They also enter into nearby waters through the disposal of the unused medication and effluents from manufacturing units (Larsson et al., 2007). They have been detected in surface waters and ground water at the concentrations ranged from ng/l to µg/l (Rodriguez et al., 2003). Pharmaceuticals have been found to show devastating effects in aquatic biota like algae, crustaceans and fish (Schwaiger et al., 2004).

Diclofenac, 2-(2,6 dichloranilino) phenylacetic acid, is one of the most frequently detected active pharmaceutical ingredient in surface waters worldwide (Buser et al 1998). It is a nonsteroidal anti-inflammatory drug that is used to treat inflammation in disorders like rheumatoid arthritis, osteoarthritis, polymyositis, spondylarthritis, gout attacks and dysmenorrhea. It is used as an analgesic to reduce pain certain conditions like post-operative pain, menstrual pain, endometriosis. (Skoutakis et al., 1988).

Fish is the best bioindicator for assessing the environmental risk caused due to various pollutants. (Chovanec 2003). Fish is the most sensitive animal to many toxicants and is a convenient model for toxicity evaluation (Adams and Ryon 1994). *Channa punctatus* was selected as the test animal due its wide

How to Site This Article:
Rohini Padma and Yalavarthy Prameela Devi (2016). Diclofenac induced alterations in the phospholipid levels in different tissues of fresh water fish, *Channa punctatus*. *Biolife*, 4(2), pp 392-396.
doi:10.17812/blj.2016.4229

Published online: 25 June, 2016

distribution, availability throughout the year and easy maintainance in the laboratory.

MATERIAL AND METHODS

The fresh water fish, *Channa punctatus* were collected from the local market of Hasanparthy village of Warangal district, Telangana, India. They were transported to laboratory in large plastic tank filled with water. The fish were acclimatized for a week in 25 litres capacity plastic tanks filled with dechlorinated water. They were washed with 1% potassium permanganate prior to acclimation to free from microbial infection. They were fed *ad libitum* with commercial feed during acclimation period. The water was renewed for every 12 hours and proper aeration was supplied. The fish were starved for one day before the experiment (Jones 1972). The dead animals were removed immediately during acclimation period to avoid water fouling.

Analytical grade of Diclofenac sodium (2- [(2-6 Dichlorphenyl) 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 at three different concentrations 5ppm, 25ppm and 50ppm with acetone. The fish were exposed for 24, 48, 72 and 96 hours to three different concentrations and observed with respect to control. The experiment was carried out in triplicate.

Phospholipid quantiation was done by Zilversmidth and Davis method (1950). The tissues were homogenized with 3 ml of 10% TCA and centrifuged at 1500 rpm for 10 min. The supernatants were discarded and to the residues 1 ml of 60% perchloric acid was added and digestion was continued on a heating mantle to obtain a clear and colourless solution. Then the contents were cooled and 5 ml of distilled water was added to each tube. 1 ml of 4% (w/v) ammonium molybdate was added to each sample. Finally 1 ml of ANSA (1-amino-2-naphthalo-4-sulphonic acid) reagent was added and the contents were made upto 10 ml with distilled water. The time of addition of ANSA reagent was noted. The colour developed was read at 660 nm against the blank within 6 min. Potassium dihydrogen phosphate was used as standard for phospholipid estimation. The results were tabulated in Table I

RESULTS

The calculated values of phospholipid level and percent changes in different concentrations of Diclofenac over control are given in Table-1 and are graphically represented in Fig-1. The phospholipid levels in muscle, liver, gill, brain and kidney of *Channa punctatus* exposed to three different concentrations (5ppm, 25ppm, 50ppm) of Diclofenac showed significant decrease when compared to the control fish. The phospholipid level in the control fish is in the order of Muscle > Liver > Gill > Brain > Kidney. The percent changes in the depletion of phospholipids varied in different tissues. The order of depletion in phospholipids levels is Liver> Muscle> Kidney> Brain> Gill. The maximum changes were observed in liver 19.52% in 5ppm, 39.98% in 25ppm, 74.36% in 50ppm of Diclofenac. The minimum changes were observed in gill 3.42% in 5ppm, 12.69% in 25ppm and 28.50% in 50ppm concentrations of Diclofenac.

Table-1. Concentration of phospholipid content (µg/100mg wet weight of tissue) and % changes over control in different tissues of *Channa punctatus* on exposure to 5ppm, 25ppm, and 50ppm of Diclofenac for 96 hrs.

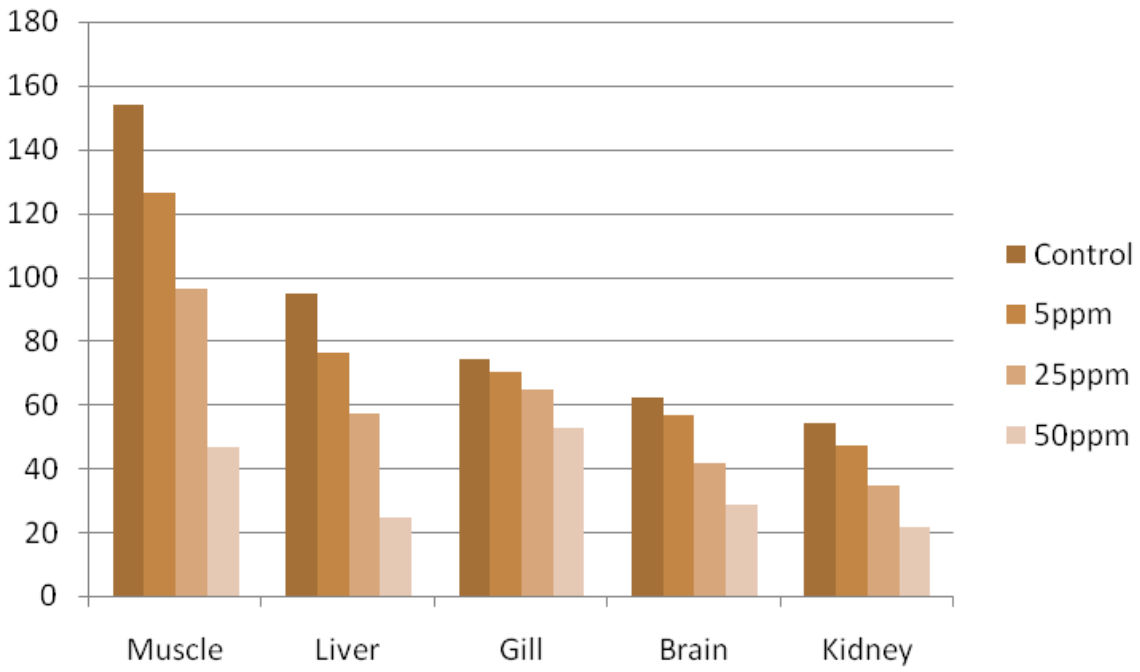
Tissues	Control	5ppm (%)	25ppm (%)	50ppm (%)
Muscle	153.92 ±0.07	126.57 ±0.04 (17.76)	96.34 ±0.05 (37.40)	46.53 ±0.03 (69.77)
Liver	94.88 ±0.09	76.35 ±0.08 (19.52)	56.94 ±0.07 (39.98)	24.32 ±0.11 (74.36)
Gill	73.96 ±0.04	70.42 ±0.18 (3.42)	64.57 ±0.06 (12.69)	52.88 ±0.05 (28.50)
Brain	62.40 ±0.11	56.48± 0.09 (9.48)	41.65 ±0.06 (33.25)	28.36 ±0.08 (54.55)
Kidney	54.08 ±0.06	46.92 ±0.03 (13.23)	34.36 ±0.05 (36.46)	21.46 ± 0.10 (60.31)

DISCUSSION

Phospholipids are the important class of biomolecules. They are the major building blocks of the biological cell membrane and maintain the structural integrity of the cell. Phospholipids uphold the membrane fluidity and function despite changes in water salinity and temperature (Bell *et al*., 1986).They function as secondary messengers in signal transduction. They act as surfactants particularly in the lungs. They are also a good source of energy and essential n-3 fatty acids (Fraser *et al*., 1988). Phospholipids constitute the major lipid fraction in the body tissue of fish (Mukhopadhyay *et al*., 2004). The total lipid content is highest in the muscle tissue of the fish (Keriko *et al*., 2010). The present study shows depletion in the phospholipid content of the tissues as the concentration of diclofenac increases. Several studies have reported decreasing trend in total lipid levels and phospholipd levels on exposure to various toxicants in different fish species.

There was a significant decline in total lipids on exposure to cadmium (Hammed and Muthukumaravel 2006) and cadmium chloride in fresh water fish, *Oreochromis mossambicus* and *Catla catla* respectively

Figure-1. Concentration of phospholipid content (µg/100mg wet weight of tissue) in different tissues of *Channa punctatus* on exposure to 5ppm, 25ppm, and 50ppm of Diclofenac for 96 hrs.



(Shoba *et al* ., 2007). Amudha et al (2002) have also reported decrease in the lipid level on exposure to dairy effluent in *Oreochromis mossambicus*. The similar results were observed on exposure to industrial effluents (Muley et al., 2007) and pesticide Monocrotophos in the tissues of *Labeo rohita (*Muthukumaravel *et al., 2013).* Stalin *et al.,* 2012 have reported maximum and minimum decrease of lipid levels in liver tissue of *Cirrhinus mrigala* on exposure for different periods. There was depletion in the lipid level on exposure to electroplating effluents in different tissues of *Cyprinus carpio* (Sudhasaravanan and Binukumari 2015). Diclofenac exposure has reduced lipid levels in the tissues and blood of *Cirrhinus mrigala* (Binukumari et al., 2016).

There was an increase in the phospholipid level in the gonads of Channa *punctatus* on exposure to two biocides fenitrothion and carbofuran (Saxena *et al*., 1986). The similar effect was observed in *Oreochromus niloticus* fingerlings on exposure to Propoxur (Ezymonoe *et al.,* 2014). The phospholipids were elevated in liver, reduced in kidney and the lipid contents were affected differently in different tissues (Bano and Hasan 1989). Javed and Usmani (2015) have reported increase in the phospholipid level on exposure to river water polluted by thermal power plant effluents. There was an elevation in the level of phospholipid on exposure to thiamethoxam in the serum of *Channa punctatus* (Anil 2010).

The results of the present investigation agree with that of Rao and Rao (1984) who reported a rapid decline in the level of phospholipid exposed to methyl parathion in *Oreochromis mossambicus*. The decreased level of phospholipids was observed on exposure to malathion and γ-BHC during different phases of life cycle in *Heteropneusteus fossilis* (Singh 1992). Singh and Singh (2006) have reported that endosulfan significantly decreased the phospholipid level in liver, ovary and plasma during different stages of reproductive life cycle. There was significant decrease in phospholipid levels in the testis on exposure to tannery effluent in *Channa striatus* (Sivachandran and Sulthana 2014).

The decrease in lipid level may be due to increased lipolysis for utilization at the cellular levels (Anusha et al., 1996). The depletion in lipid level may be due to rapid decline in the glycogen content of the tissues during the toxic stress conditions (Rao et al., 1985). Jha (1991) has reported that the loss of lipids may be due to mobilization of the stored lipids through β- oxidation or gradual unsaturation of the lipid molecules. The dercrease in lipids may also be due to its utilization in cell repair and tissue organization (Vutukuru, 2005).

Murthy et al., (1994) have reported that the depletion in total lipid level in liver may be due to their mobilization towards the blood or tissue metabolism. The decline in the phospholipid level may be due to increased utilization of the phospholipids to meet the energy demand under toxic stress conditions (Rao and Rao 1979). The loss of phospholipids may also be attributed to the inhibition of the enzymes required for lipid synthesis and mobilization of the stored lipid.

CONCLUSION

The biomarker employed in this study is useful in the assessment of the environmental impact of diclofenac on the aquatic species. The present study reveals that there is depletion in the phospholipid level of *Channa punctatus* on exposure to diclofenac. The pharmaceutical residues in the environment alter the biochemical composition of non-target organisms like fish. This leads to the impairment of the health condition of the fish.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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DOI: https://dx.doi.org/10.5281/zenodo.7318813
Received: 6 April 2016;
Accepted; 30 May 2016;
Available online : 8 June 2016