International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2018; 6 (1):260-264
ISSN:2320-7817(p) | 2320-964X(o)
UGC Approved Journal No 48951

Original Article Open Access

Effect of Nonylphenol (NP) LC-50 on Organic Metabolites in some Tissues of *Ophiocephalus punctatus* (Bloch, 1793)

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Manuscript details:

Received : 16.12.2017
Accepted : 12.03.2018
Published : 31.03.2018

Editor: Dr. Arvind Chavhan

Cite this article as:
Khandale DP, Khinchi PJ and Chilke AM (2018) Effect of Nonylphenol (NP) LC-50 on Organic Metabolites in some Tissues of *Ophiocephalus punctatus* (Bloch, 1793), *Int. J. of Life Sciences*, Volume 6(1): 260-264.

Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

In the present study the focus was made on the change in organic metabolites like total protein, cholesterol and glycogen in the tissues such as gills, liver, kidney and muscle of O. *punctatus* on exposure to NP LC-50 for short term duration. It was observed that the total protein and cholesterol significantly increased but glycogen decreased in all the tissues. It is concluded that the increased in total proteins and cholesterol may be due to enhanced metabolic activity, however decreased glycogen level in the tissues can be attributed to metabolic stress induced by NP and there may be the chances of inducing the enzymes that involve in the process of glycogenolysis.

Key words: - Nonylphenol, organic metabolites, LC-50, *Ophiocephalus punctatus*.

INTRODUCTION

Health of aquatic organisms cannot be measured directly. Instead, only indicators of health can be measured and in turn used to assess the "health" status. Effect of exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function (Hinton *et al*., 1992). Fish is a suitable indicator for monitoring environmental pollution because they concentrate pollutants in their tissues directly from water and also through their diet, thus enabling the assessment of transfer of pollutants though the trophic web (Fisk *et al*., 2001; Boon *et al*., 2002). Due to being exposed to pollutants, major structural damage may occur in their target organs and physiological stress may occur. This stress causes some changes in the metabolic functions. Gills are the first organs which come in contact with environmental pollutants. Absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is also rapid. Gills have frequently been used in the assessment of impact of aquatic pollutants

in marine as well as freshwater habitats (Athikesavan *et al*., 2006; Craig *et al*., 2007; Fernandes *et al*., 2007; Jimenez-Tenorio *et al*., 2007). Therefore, lesions in gill tissues can be the start of imbalance of the physiological and metabolic process of fish.

The liver plays a primary role in the metabolism and excretion of xenobiotic compounds on exposure to some toxic substances (Rocha and Moterio, 1999). In fish, as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen containing waste products from the metabolism such as ammonia, urea and creatinine. Hence, fish serve as an excellent bioassay animal for toxicological impact studies and has been widely used for this purpose. Muscle both the voluntary as well as involuntary give response to toxic material that results in structural, behavioural and biochemical changes.

METHODOLOGY

All the experimental fingerlings of *O. punctatus* selected for this present study were purchased from fisherman of Mulchera, District-Gachchiroli (M.S.) India. Fish were brought to the laboratory and bathed in 0.01% potassium permanganate solution for fifteen minutes for two subsequent days to kill the external infectious pathogenic microorganisms if any to avoid the possible mortality of fish due to microbial infections. After the treatment with disinfectant fingerlings were placed in a large glass aquarium for acclimatized for fifteen days.

During these period of acclimatization fish were fed alternate days with boiled egg albumen and dried minced prawn powder. Fingerlings were separated according their size and weight. Fish selected for the experiment had an average length 25±4 cm and weight 80±5 gm. Health of aquarium was monitored time to time for physiochemical parameters like pH, temperature, dissolved oxygen, conductivity; free carbon dioxide and total alkalinity.

Lethal concentration 50 for nonylphenol was evaluated (Finney, 1971) and found to be 15.51 ppm for in case of *O. punctatus* (Khandale *et al*., 2015). After the determination of LC-50, this concentration was employed on the fish *O. punctatus.* Six small glass aquarium having 30 liter of water holding capacity were set in the laboratory. One out of six was used as a control and remaining five for experimental. All the five tanks having 20 liter of dechlorinated water were diluted with working solution of NP until reaching lethal concentration 50, water mix properly after the addition of working solution of NP. Later fish were placed into the aquarium. Precautions were taken, if any fish found dead it was quickly removed from the aquarium. Fish during experiment were fed with albumen of boiled eggs and dried minced prawn powder in alternate days. This short term exposure of *O. punctatus* to NP LC-50 ran for four days. Tissue samples were collected every day at the interval of twenty four hours. The total protein was estimated using method of Lowry *et al.,* (1921), cholesterol concentration was determined according to the method of Zlatkis *et al.,* (1953) and total glycogen was estimated using method of Seifter *et al.,* (1950). For the estimation of these organic metabolites, spectrophotometer 'Labtronics India' was used.

Statistical analysis

All the statistical calculations were carried out by one way ANOVA method by using trial statistical software Prism Graph pad and Microsoft Excel- 2008.

RESULTS AND DISCUSSION

In the present work, the effect of p-nonylphenol at lethal concentration-50 was carried on tissues such as gills, liver, kidney and muscle of *O. punctatus*. The phenomenal changes were observed in the level of organic metabolites (total protein, cholesterol and glycogen). Environmental chemicals are largely taken up by organisms by passive diffusion and the primary sites of uptake include membranes of the gills, lungs and gastrointestinal tract in fishes (Hodgson, 2004).

Kapila and Raghothaman (1999) have suggested that the assessment of protein can be used as a diagnostic tool for determining the physiological phases of the cells. Liver is the main organ for detoxification of xenobiotics, including NP. Therefore, the changes in liver of aquatic fauna such as fish are reflective of aquatic pollution of their habitat (Moon *et al*., 2012). Being a metabolic centre it plays very important role in the metabolism of protein, lipid, cholesterol and carbohydrates. In the present study, it was observed that the total protein significantly increased in the liver, gill, kidney and muscle (Table-1 and Fig.1).

Table-1 Showing effect of NP LC-50 on Total Protein

Sr. No.	Tissues	Time in hours				
		Control	24-Hrs	48-Hrs	72-Hrs	96-Hrs
1.	Gills	0.38 ± 0.038	0.58 ± 0.131**	1.40 ± 0.212*	1.56 ± 0.233*	1.76 ± 0.159*
2.	Liver	1.46 ± 0.071	1.70 ± 0.053*	1.78 ± 0.175**	2.15 ± 0.026*	2.39 ± 0.025*
3.	Kidney	0.57 ± 0.086	0.82 ± 0.029*	1.49 ± 0.211*	1.80 ± 0.074*	2.26 ± 0.117*
4.	Muscle	0.85 ± 0.019	0.91 ± 0.043**	1.51 ± 0.179*	1.73 ± 0.079*	1.78 ± 0.081*

Note: * indicates p<0.01, ** indicates p<0.05 and without signed are statistically non-significant

Table-2 Showing effect of NP LC-50 on Cholesterol

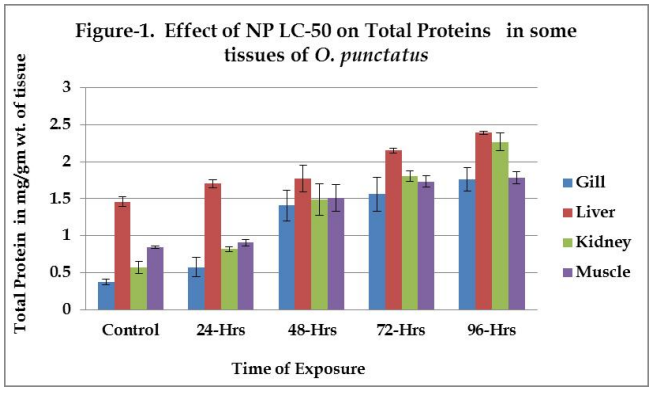
Sr. No.	Tissues	Time in hours				
		Control	24-Hrs	48-Hrs	72-Hrs	96-Hrs
1.	Gills	15.96 ± 0.507	33.37 ± 5.961*	46.29 ± 1.695*	47.12 ± 5.806*	50.43 ± 9.943*
2.	Liver	35.64 ± 1.576	45.14 ± 1.856*	47.85 ± 8.759**	53.97 ± 1.976*	57.59 ± 3.230*
3.	Kidney	17.61 ± 0.753	36.78 ± 5.671*	43.89 ± 4.058*	50.57 ± 1.909*	53.07 ± 3.674*
4.	Muscle	21.79 ± 1.947	32.97 ± 3.626*	34.97 ± 0.212*	37.29 ± 1.701*	39.86 ± 1.851*

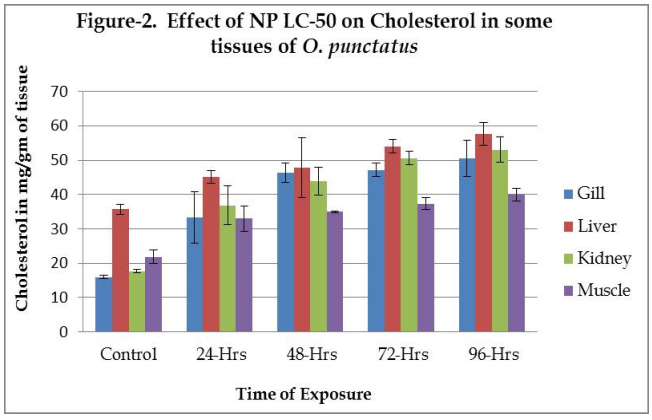
Note: * indicates p<0.01, ** indicates p<0.05 and without signed are statistically non-significant

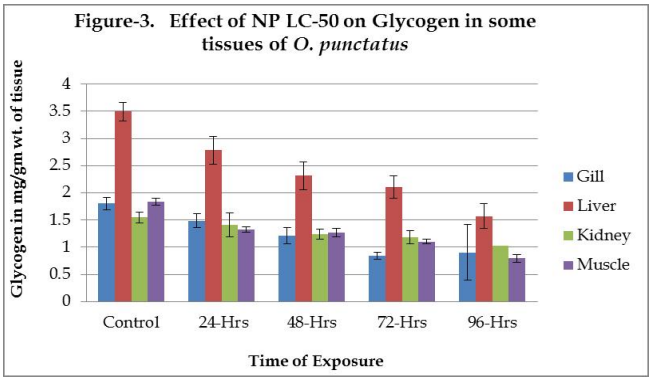
Table-3 Showing effect of NP LC-50 on Glycogen

Sr. No.	Tissues	Time in hours				
		Control	24-Hrs	48-Hrs	72-Hrs	96-Hrs
1.	Gills	1.80 ± 0.105	1.49 ± 0.129*	1.21 ± 0.152*	0.85 ± 0.068*	0.90 ± 0.509**
2.	Liver	3.49 ± 0.174	2.78 ± 0.250*	2.32 ± 0.264*	2.11 ± 0.209*	1.57 ± 0.235*
3.	Kidney	1.55 ± 0.097	1.41 ± 0.223	1.24 ± 0.099*	1.18 ± 0.099*	1.03 ± 0.116*
4.	Muscle	1.85 ± 0.063	1.33 ± 0.044*	1.27 ± 0.082*	1.11 ± 0.044*	0.79 ± 0.071*

Note: * indicates p<0.01, ** indicates p<0.05 and without signed are statistically non-significant







This increase reflects the increase in protein synthetic activity in all the tissues. These proteins may include the enzymes require for enhanced metabolic activity, membrane bound carrier proteins needed for transportation of variety of things across the membrane. Rajanna *et al.* (1981) have reported increase in protein content due to cadmium. Similarly, there are some reports that the cadmium stress induced the synthesis of total protein content in gill and liver of fish after a few days of cadmium exposure (Basha and Rani, 2003), contrary to above finding cadmium reported to reduce

the total protein in the kidney and muscle of *Catla catla* (Sobha *et al*., 2007).

Cholesterol is a chemical compounds that is naturally produced by the body, and is a combination of lipid (fat) and steroid. It is a building block for cell membranes and for sex hormones like estrogen and testosterone (Sayed and Moneeb, 2015), about 80% of the cholesterol is estimated to produce by the liver (Hasheesh *et al*., 2011). In the *O. punctatus,* on exposure to NP LC-50 from 24 hrs to 96 hrs significant increase in the level of cholesterol were observed in all the tissues such as gills, liver, kidney and muscle (Table-2 and Fig.2). Increased in the concentration of cholesterol in the tissues could not understood but may be for its use by the other demanding tissue to cope the deficiency of glucose if occurred. Wasserman *et al*. (1970), Gill and Pant (1983) and Nirmala and Eliza (2005) have reported enhanced catabolism of cholesterol with resultant hypercholestemia as the result of toxicity to insecticides and heavy metals.

Carbohydrates is stored in the form of glycogen as reserve food in organs like muscle and liver for the supply of energy that needs when there are hypoxic conditions, intensive stocking and a lack of food (Wendelaar-Bonga, 1997). Glycogen is also present in other tissues like gill and kidney in certain amount. In the present investigation it was observed that contrary to total protein and cholesterol, glycogen showed reverse phenomenon. The concentration of glycogen in the gill, liver, kidney and muscle consistently found decreased significantly right from the first day to fourth days of exposure (Table-3 and Fig.3). However decrease in the level of glycogen in the tissues may be due to glycogenolysis indicating that the rate of metabolic activity increased compared to fish under control. On exposure of silver carp, *Hypophthalmichthys molitrix* to cadmium, glycogen was reported to decrease in gills and this could be due to alteration in carbohydrate metabolism. This decrease was also reported in the liver of fish exposed to heavy metal (Sastry and Rao, 1984 and Naidu *et al*., 1984).

Conflict of interest- Authors stated that no conflict of interest.

Acknowledgement
All the authors are very thankful to Principal, Janata Mahavidyalaya, Chandrapur for providing the full research facilities to conduct the present work.

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