

BIOCHEMICAL ANALYSIS OF FLUBENDIAMIDE TOXICITY ON GILL OF *HETEROPNEUSTES FOSSILIS* (BLOCH)

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ABSTRACT: - Pollution of aquatic resources by insecticides, pesticides, heavy metals etc. has become a huge challenge and serious threats to the aquatic fauna. Recent years have witnessed significant attention being paid to the problem of an environmental contamination by a wide variety of chemical pollutants-toxicants; aquatic fauna like fish is badly affected. Not only gill get affected but blood, liver, kidney, intestine, gonads also affected / damaged seriously by the toxic action of flubendiamide. In the present piece of work gill of fresh water fish is the target organ for toxicant, flubendiamide. The biochemical analysis of glucose, protein and cholesterol level of fresh water fish, *Heteropneustes fossilis* (Bloch), after the exposure of flubendiamide for the period of 7, 14, 21 and 28 days.

KEYWORDS: Flubendiamide, *Heteropneustes fossilis*, Biochemical parameters

INTRODUCTION

The economy of India is largely depends upon the agricultural production. Better harvest requires the rigorous cultivation, irrigation and used fertilizers, pesticides, insecticides to protect the plants from the different pests and diseases. In modern world the urbanization and industrialization have boosted the man kind's economy through various means and ways. But at the same time pollution of aquatic sources has become the huge challenge and serious threats. Recent needs to pay attention to the problems of aquatic pollutions. Aquatic fauna like fish, living and cultivate in polluted water may accumulate toxicants via food chain (Hadson, 1998). Different environmental pollutants are like to affects the biological systems in different ways according to chemical properties. Thus by observing the effects of pollutants on a set of physiological along with biochemical parameters, it might be possible to establish specific response of that pollutant and may make it possible to identify a pollutant on the basis of its physiological effect pattern on the biochemical parameters.

The gills are not only for gaseous exchange in fish, they also perform several other physiological functions including osmoregulation and excretion. The fish gill is

the primary target organ of water contains toxicants. Gill damage is actually the direct cause of death in major situations of toxicity to fish. The compound was evaluated as a new compound by the 2010 JMPR for both residues and toxicological aspects. Information considered in this evaluation include metabolism of flubendiamide in animals and especially aquatic animals feed processing and animal feeding studies.

MATERIALS AND METHODS

Live specimens of fresh water fish, *Heteropneustes fossilis* was been selected for present work. The fish were obtained for the experimental purpose from the Adam dam, Taluka Karanja lad, Dist- Washim, (M.S.). They were treated with 0.5 % KMnO₄ for 5 minutes for dermal disinfection. The physicochemical parameter of the aged tap water was determined periodically as per standard method (APHA, 1998).

Pesticide, Flubendiamide was selected as a toxicant for the piece of investigation. 96h LC₅₀ and sub lethal concentration of flubendiamide for the fish, *Heteropneustes fossilis* was taken from literature. This was 0.5ml/L for 96h LC₅₀ value, approximately 5 times less concentration (0.1ml/L), i.e. sub lethal, used to expose the fish upto 28 days to study the gill-Biochemistry. The physico-chemical characters of water used also analysed by using standard method (APHA 1998). As the flubendiamide toxicant was of unknown toxicity, first literature survey was made and from that probable concentration was selected.

Prepared sub lethal concentration (0.1ml/L) of flubendiamide in the glass aquaria, up to 25 litres. The toxicant solution was added drop by drop with constant stirring and then acclimatized 10 fish were transfer to glass aquaria containing 25 litres of toxicant treated water. The fish were fed by boiled egg once in a day, particularly in morning hours. The experimental and control group of fish were sacrifices after the interval of 7th, 14th, 21st and 28th days.

Water Used: Throughout the experiment, the water used was aged tap water which was stored in a large overhead tank for about 10 days. As per the standard methods the physico-chemical parameters of used tap water were determined periodically (APHA, 1998).

The details of physico-chemical characteristic of water used are as follows:

1. Dissolved Oxygen = 6 ± 0.3 mg/L
2. Temperature = 29 ± 1 °C
3. Total hardness = 228 ± 2 mg/L
4. Total alkalinity = 243 ± 3 mg/L
5. Salinity = $1.02 \pm$ ppt
6. pH = 7.5 ± 0.6

Test Toxicant: The flubendiamide was selected as a toxicant for the present piece of investigation. The physico-chemical properties of flubendiamide are as below:

1. Common name : Flubendiamide
2. Chemical name : 3-iodo-N-phthalimide (2-methyl-1,1-dimethyl ethyl)-N-[4-(1,2,2,2-tetrafluoro-1-(tetrafluoromethyl) ethyl]-o-tolyl}phthalimide.
3. Molecular formula: C₂₃ H₂₂ F₇ N₂ O₄ S₄. Molecular Weight: 682.39
5. Appearance: Colourless crystal
6. Odour: No characteristic odour
7. Melting Point: 217.5 °C. Density (At 20.8 °C): 1.659 g/cm³

After sacrificing the fish, the gills were immediately removed, centrifuged at 2000 rpm for 10 minutes, after crushing. After centrifugation supernatant is extracted. Then after extraction of supernatant is prepared for the testing, then it is prior to immediate determination of biochemical parameters-total protein, glycogen and cholesterol.

Total protein was measured according to the procedure to the procedure of Lowry et al. (1951). Gill glycogen was estimated by using the methods of colorimeter for which Agar sugar estimation kit was used. The total cholesterol was estimated by using the method King and

Wolten (1959). The mean values of the various biochemical parameters for the control as well as experimental fish were analysed for significance of differences using test.

OBSERVATIONS

In present investigation, particular noticed that the decreased in gill tissue content total protein, glycogen and cholesterol after the exposure to the sub lethal concentration of flubendiamide upto 28 days when compared to the control fish (Table-1 and Fig. 1, 2 and 3).

The decrease in total protein content in *Heteropneustes fossilis* after the exposure to the experimental toxicant flubendiamide. The decline in gill protein content was observed as 138.80 % on 7th day, 136.40 % on 14th day, 135.30 % on 21st day and 130.20 on 28th day respectively (Fig.1).

The decrease in glycogen content in *Heteropneustes fossilis* after the exposure to the experimental toxicant flubendiamide. The depletion in gill was recorded as 16.08 % on 7th day, 14.40 % on 14th day, 13.03 % on 21st day and 10.40 on 28th day respectively, similar as compared to control fish (Fig.2).

Recorded values of cholesterol in the experimental fish, as compared to the control fish were found to be decline percentage as 26.89 % on 7th day, 25.30 % on 14th day, 26.02 % on 21st day and 27.01 % on 28th day (fig.3). Whereas decline of cholesterol content in *Heteropneustes fossilis* except on 14th days of exposure but there was a sudden decrease as 25.30 % on 14th day of exposure to sub lethal conc. of experimental toxicant (fig.3).

Table 1: Alterations in Total Protein, Glycogen and Cholesterol content (mg / 100gm wet weight) in gill of fish, *Heteropneustes fossilis* following exposure to sub lethal conc. of Flubendiamide

Parameters	Exposure Days				
	Sets-	7	14	21	28
Total Protein (mg/100ml)	Control	148.08	147.48	146.08	145.86
	Experimental	138.80	136.40	135.30	130.20
Glycogen (mg/100ml)	Control	17.20	17.30	17.48	18.00
	Experimental	16.08	14.40	13.03	10.40
Cholesterol (mf/100ml)	Control	23.12	22.80	22.60	21.90
	Experimental	26.80	25.30	26.02	27.01

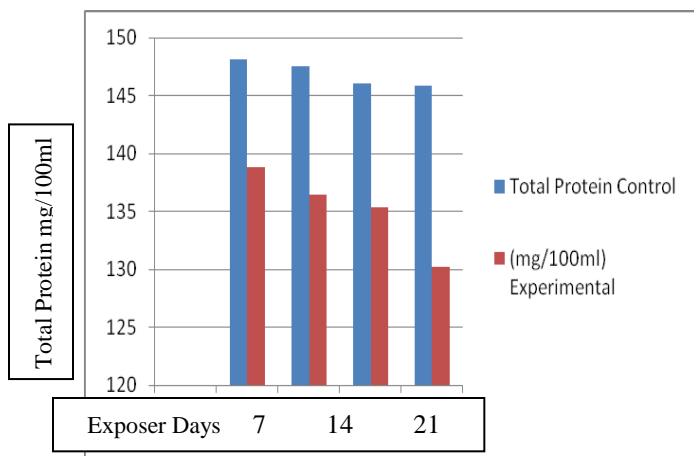


Figure 1- Changes in protein level in gills of fish, *Heteropneustes fossilis* exposed to sub lethal conc. of flubendiamide up to 28 days.

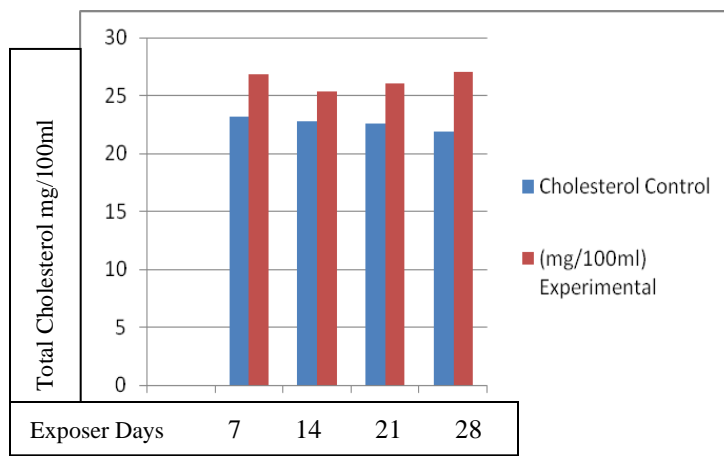


Figure 3- Changes in cholesterol level in gills of fish, *Heteropneustes fossilis* exposed to sub lethal conc. of flubendiamide up to 28 days.

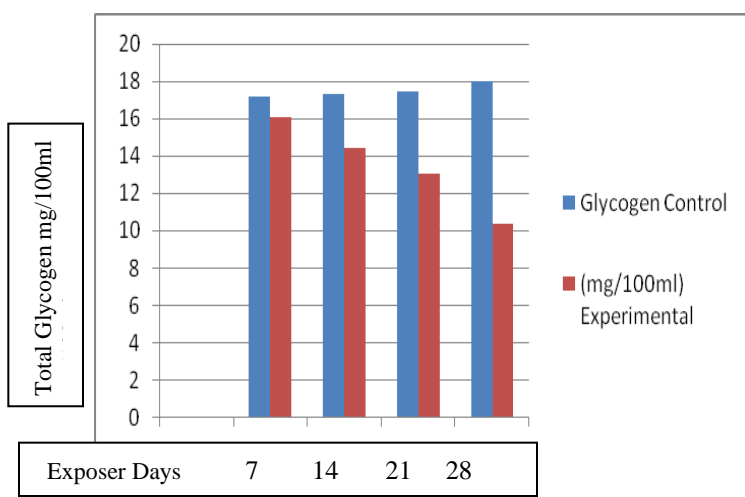


Figure 2- Changes in glycogen level in gills of fish, *Heteropneustes fossilis* exposed to sub lethal conc. of flubendiamide up to 28 days.

DISCUSSION

In present investigation the exposure test the impact of different conc. of experimental toxicant was observed that the gills biochemical parameters can be useful as diagnostic tool in toxicology to find their general health status and target organs affected by toxicants. Borah and Yadav (1995) have reported gradual decrease in protein and glycogen in gills of *Heteropneustes fossilis* under dimethoate toxicity.

Maximum decrease in glycogen content was observed in kidney followed by brain, gill, muscle, liver, testis and ovary (Power et al., 2009). Depletion of tissue proteins in fish, exposed to various toxicants has been reported by several investigators (Mcleay and Brown, 1974; Shakooriet al., 1976).

James and Sampath (1995), observed sub lethal effects of mixtures of copper and ammonia in biochemical parameters in *Heteropneustes fossilis* and showed concentration dependent significant reduction of protein and glycogen content in gill, liver and muscle.

Ramand Sathyanesan (1984) reported that mercuric chloride reduced the protein, lipid and cholesterol contents in various tissue and cholesterol contents in various tissue including gills of *Channa punctatus*.

The increased opercular activity may be due to shock received by the fish in new toxic environment for proper ventilation of the gills to cope with hypoxia, Joseph et al. 1987; Lata et al. 2001.

In the present peace of investigation the biochemical parameters like total protein and glycogenin the experimental fish, *H. Fossilis* expose to sub lethal conc. of Flubendiamide showed depletion in values mg/100ml there is similarities as above researchers. In case of cholesterol, experimental fish showed increase the values are the parallel observation as other investigators.

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