Effect of Biochemical Parameters of Intestine of Freshwater fish, *Heteropneustes fossilis*(Bloch), when exposed to Flubendiamide

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ABSTRACT

In recent years, the high rate of increase in human population and rapid pace of industrialization have created problem of disposal of waste water. Use of pesticides and insecticides during regular agricultural activity, is known to cause serious environmental problem, especially in the dry season, because during this period the dilution capacity of the water systems is low, thus increasing the risk of high concentrations of toxic chemicals. Moreover, the dry season is often the critical period for many animals, especially fish and birds. Contamination of water by pesticides and insecticides either directly or indirectly can leads to fish kills or reduced fish productivity of fish including local edible fish too. Owing to undesirable toxicants mixed in water body the living edible fish systems, tissue can affects the health of human eating these fish as food.
In this research work, I concentrate on the toxic effect on flubendiamide, due to its toxicity produce biochemical changes in the organs of aquatic animals.The organs of the fish, Heteropneustes fossilis such as Intestine, Liver, kidney, Gills etc. get changes as in biochemical parameters, especially concerned with the Intestine-glycogen, protein and cholesterol.

Keywords: *Heteropneustes fossilis, Flubendiamide, Intestine*

Introduction

Water pollution is a major problem over the entire world. During last decade an attention has been paid to the protection of aquatic environment against pollution both nationally and internationally. Because the insecticides, pesticides, toxic heavy metal compounds including industrial effluents, domestic sewage etc. are common pollutant of aquatic environment because their persistence and tendency to concentrate in aquatic organisms (Hoo*et al.*,2004; Shrivastava and Verma,2009).The contamination of water by such toxicant may be detrimental to aquatic food chain reaching to human. Acute concentration of toxicant in water causes mortality to aquatic fauna including fish, the sub-acute condition render the fish unsuitable for human consumption.

Fish are widely use to evaluate the health of aquatic ecosystem because pollutants built up in the food chain and are responsible for adverse effects and death in the aquatic system (Farkas*et al.*, 2002).

These heavy metal toxicants are accumulated in the fish through the general body surface which affects severally their life support system at molecular, biochemical levels. Once these toxic substance enters into the body they damage and weaker the mechanism concerned leading to physiological, pathological and biochemical disorders (Arasta*et al.*, 1999).

Intestine is my target organ for the research work, as according to my work the particular toxicant;flubendiamide can affect the digestive organs such as intestine and by the activity of flubendiamidethe biochemical parameters of the organ can be changed.

Materials and Methods

The fresh water fish, *Heteropneustes fossilis*was been selected for present work. The fish were obtained for the experimental purpose from the Adan dam TalukaKaranja lad, Dist- Washim,(M.S.). They were treated with 0.5 % $KMnO_4$ 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 LC_{50} and sub lethal concentration of flubendiamide for the fish, *Heteropneustes fossilis*was taken from literature. This was 0.5ml/L for 96h LC_{50} value, approximately 5 times less concentration (0.1ml/L), i.e. sublethal, used to expose the fish upto 28 days to study the Blood- 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 7^{th}, 14 th 21^{st} and 28^{th} 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 physic-chemical parameters of used tap water were determined periodically (APHA, 1998).

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

1. Dissolved Oxygen = 6 ± 0.3 mg/L
2. Temperature = $29^oC \pm 1^oC$
3. Total hardness = 228 ± 2mg/L
4. Total alkalinity = 243 ± 3mg/L

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

1. Common name : Flubendiamide

2.Chemical name:3-iodo-N, phthalamide (2-mesyl-l,l-dimethyl ethyl)-N-{4-[1,2,2,2 tetrafluro-l-(tetrafluromethyl) ethyl]-o-tolyl}phthalamide.

3. Molecular formula: C_{23} H_{22} F_7 lN_2 O_4S
4. Molecular Weight : 682.39
5. Appearance: Colourless crystal
6. Odour : No characteristic odour
7. Melting Point : 217.5^oC
8. Density (At 20.8^oC) :1.659g/cm^3

Biochemical study: For the study of biochemical parameters of theintestine of the control and experimental fish, the intestine was collected and immediately 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 glucose, total cholesterol and total protein.Intestine glycogen was estimated by using the colorimeter for which Agape sugar estimation kit was used. For measuring of standard sugar solution RI, 1000ml and 10 µ of standard solution and kept it incubation for 15 minutes. Pink colour is obtained and after that solution is run for the test on the calorimeter. The standard value of kit solution was 99-100% to confirm standard values. Total cholesterol was estimated by using the method of King and Wolten (1959),total protein was measured according to the procedure of Lowery*et al.*(1951),and total glucose was estimated by the method of Nelson and Somogyi (1952).

The mean values of the various biological parameters of the intestine for the control and experimental fish were analysed for significance of differences using the various test.

Result and Discussion

In the present investigation,many significant changes were observed in the value of biochemical parametersof digestive organs like intestine of the fish, *Heteropneustes fossilis(*Bloch), the experimental fish

exposed to sub lethal concentrations of flubendiamide (Table-1) where remarkable higher as compared to control fish, it may be due to its more accumulation in this organ.

The properties of the flubendiamidehave been affected the organ and shown in its harmful effects on the specimen. Freshwater catfish,*Heteropneustes fossilis (*Bloch),werestudied on the fish on the basis of intestine effects. The changing in biochemical parameters occurred in the tissues of intestine of this fish treated with flubendiamide toxicant. The observed intestine changes were as a result of various factors mentioned in a table. The actual effect is seen into biochemical parameters of intestine glycogen, totalprotein and cholesterol level get fluctuate due to the activity offlubendiamide as a toxicant. The changing biochemical parameters of the intestine of the specimen, *Heteropneustesfossilis*are given in the table.

The glycogen level in *Heteropneustes fossilis* due to exposure of sub lethal concentration (0.1ml/L) are shown in table 1. The glycogen content of control fish was 17.20 mg,17.30 mg, 17.48 mg and 18.00 mg respectively. After the treatment of flubendiamide on fish, the glycogenwas recorded to be 16.08 mg on 7^{th} day, 14.40 mg on 14^{th} day, 13.03 mg on 21^{st} day and 10.40 mg on 28^{th} day respectively. Total glycogen level was decreased, noticed during experimental workas compared to the experimental fish after 7^{th}, 14^{th}, 21^{st} and 28^{th} day exposure to sub lethal concentrations of flubendiamide (Table-1).

Total protein content of control fish was 148.08 mg, 147.48 mg, 146.08 mg and 145.86 mg, on the period of exposure 7^{th}, 14^{th}, 21^{st} and 28^{th} days respectively. The decrease in total protein content was recorded after the exposure to the experimental toxicant flubendiamide. The decline in totalprotein content was observed as 138.80 mg on 7^{th} day, 136.40 mg on 14^{th} day, 135.30 mg on 21^{st} day and 130.20 mg on 28^{th} day respectively as compared to control fish.

The cholesterol content of control fish was 23.12 mg, 22.80 mg, 22.60 mg and 21.90 mg respectively. Whereas, increased in cholesterol content in experimental fish in all exposure days as 26.80 mg on 7^{th} day, 25.30 mg on 14^{th} day, 26.02 mg on 21^{st} day and 27.01 mg on 28^{th} day respectively was observed as compared to the control fish (Table-1).

Table-1 Alterations in Glycogen, Protein andCholesterol content (mg/100gm. wet weight) in intestine of fish, *Heteropneustes fossilis*(Bloch) following exposure to sub lethal concentrations of flubendiamide.

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

In this flubendiamide toxicology effect experiment, noticed that the glycogen and total protein level in the intestine of fresh water fish, Heteropneustes fossilis was continues declines. The cholesterol level was increased constantly, when experimental fish exposed up to 28 days.

The intestine is an organ through which toxicants are transported either directly by drinking and feeding or indirectly through the blood circulation. Along with digested food materials certain amount of toxicants can also enter through it. It is likely to serve as sensitive index for the study of the action of the toxicants. In this investigation, the epithelial cells get blocking and formed breakage in microvilli of the intestine was observed in fish exposed to flubendiamide. Breakage of cell wall was observed after 21 days of exposure to sub lethal concentration (0.1ml/L) of flubendiamide.

Drysdale (1968) suggest that specific response elicited by heavy metals can be used as probes for studying protein introduction in higher organisms. In present experiments, such introduction could be observed in both the tissue of stomach and intestine after treatment with $ZnSO_4$and $CuSO_4$ separately. Both salts severely affect the protein contents of these two tissues.

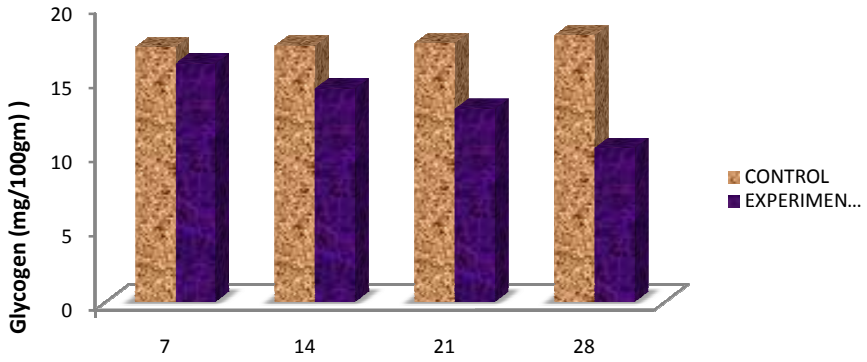

Fig. 1: Changes in the glycogen level in intestine of fish, *Heteropneustes fossilis* exposed to subletaha concentration of Flubendiamide upto 28 days.

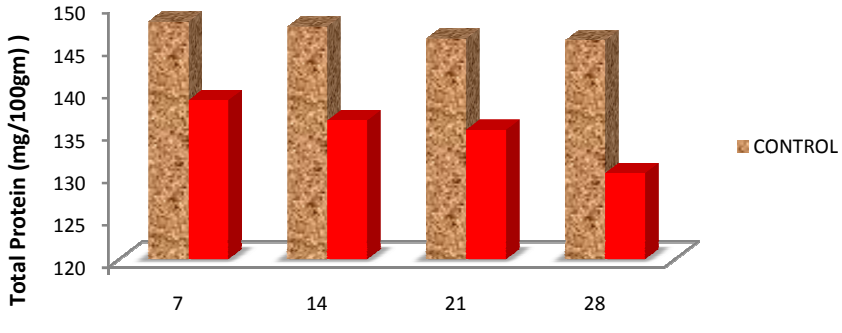

Fig. 2: Changes in the Total protine level in intestine of fish, Heteropneustes fossilis exposed to subletaha concentration of Flubendiamide upto 28 days.

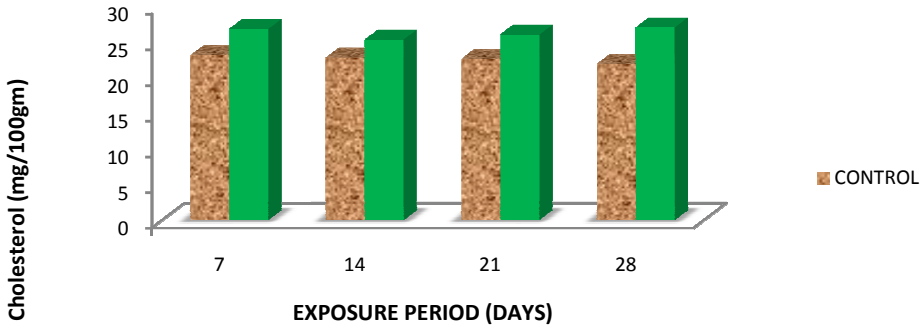

Fig. 3: Changes in the Cholesterol level in intestine of fish, Heteropneustes fossilis exposed to subletaha concentration of Flubendiamide upto 28 days.

The hypoglycaemia observed after 20 and 30 days of exposure to zinc sulphate may also due to the depleting glycogen reserves resulting from continued glycogenesis without a compensatory glycogenesis or owing to the irregular functioning or damage of the gastro-intestinal tract, liver or enzyme involved in the carbohydrate metabolism as reported by Srivastava (1982) in *Heteropneustes fossilis*.

Borah and Yadav (1995) have reported gradual decreased in protein and glycogen of intestine in *Heteropneustes fossilis* under dimethoate toxicity.According to Nanda *et al*. (2000) also reported gradual and significant reduction in the protein contents in the tissue like muscle, intestine, kidney, liver and gill of the fish, *Heteropneustes fossilis* after chronic exposure to nickel.

Gautam and Thakur (2003) suggested an estimation of proteins in these tissues and comparison with controls show that protein contents are severally affected by these heavy metals. Copper sulphate ranks in effecting the protein contents.

Significant decrease in the cholesterol was observed in the experimental fish after acute exposure which may be due to utilization of cholesterol by the fish in intermediary metabolism when under chronic stress as observed by Herpet*et al.* (1977);Rao and Rao (1979); Gupta (1987); Baskaran*et al.* (1989); Patil (1995).

Thus the resultsof the present investigations with the effect of insecticide flubendiamide on the biochemical parameters of intestine of *Heteropneustes fossilis* (Bloch) are almost identical to those of above eminent investigators.

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