International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 3, Issue 1, pp: 110-115, 2018 **http://www.ijzab.com**

Research Article

TOXICOLOGICAL PROFILE AND BIOCHEMICAL ANALYSIS OF ATRAZINE EXPOSED FISH *CHANNA PUNCTATUS*: BLOOD SERUM INVESTIGATION AT SERIAL DILUTION

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Article History: Received 23rd January 2018; Accepted 15th February 2018; Published 20th February 2018

ABSTRACT

Biomagnifications of aqueous bodies as a result of discharged herbicides from agricultural and industrial sources is counted among serious health hazards for the water inhabiting organisms. In due fascination in this context scientific community is eager to know the related toxic reactions in aquatic biota especially fish. In the present study, an attempt has been made to examine the sub-lethal and lethal toxic effects of Atrazine, an organophosphate herbicide, on biochemical parameters *viz*, glucose, protein and cholesterol in *Channa punctatus*, a commonly known fish in Jabalpur, Madhya Pradesh-India. A set of twelve fishes of this class were exposed to 0, 25, 30, 35, 40, 45 and 50 mg/L concentration of Atrazine. The lethal concentration (LC_{50}) value of Atrazine was 40 mg/L for 96 hours of exposure. *C. punctatus* were exposed to three sub lethal concentrations of Atrazine (5 mg/L, 10 mg/L, 15 mg/L) for 15 and 30 days respectively. The serum protein and cholesterol level was observed to be declined, while serum glucose level was detected to be inclined after Atrazine treatment. From the study, it has been established that the increasing level of glucose and decline in other biochemical parameters are apparently indicators of fish's response to the stress caused by the pollutant (target herbicide).

Keywords: Serum protein, Serum cholesterol, Serum glucose, *Channa punctatus*, Atrazine.

INTRODUCTION

Herbicides are commonly used in agricultural purposes. They drift into the aquatic environment, where it acts as a toxicant for aquatic organisms. These toxicants produce several biochemical and physiological responses. The herbicides effect on biochemical properties has been studied previously by many workers (Wild, 1975; Murty and Devi, 1982). Atrazine (2-chloro-4-thylamino-6-isopropylaminostriazine) is one of the most commonly used herbicide in India and U.S.A. Its utilization is controversial worldwide, as it is currently banned in Europe (Dong *et al*., 2009). It imparts toxic effects on aquatic non-target organisms. Fishes are important source of food and play an important role in the food chain. Snake headed fish *Channa punctatus* is popular edible fish in Jabalpur (M.P.) region. It is supplementary diet for protein than meat. It is collected by the Fishermen in several lakes viz, Hanumantal lake, Robertson lake, Mahanadi lake and river Narmada etc in Jabalpur region and sold in local market regularly so it has great economic importance. The purpose of present investigation is to determine the LC_{50} value of Atrazine and effects of various concentrations of Atrazine on biochemical parameters viz. glucose, total protein and cholesterol of fish *C. punctatus*. Biomolecules are important constituents of blood serum which play vital role in physiology of living organisms.

MATERIALS AND METHODS

Chemicals: The required herbicide Atrazine was purchased from local market Jabalpur in one kilogram bags and the

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herbicide Atrazine is Altra-50 Herbicide (50% WP) manufactured by Green Cross Agro Chemicals (P) Ltd.

Animal collection and maintenance

For studies, healthy and disease free juveniles of fresh water fish *C. punctatus* were collected from Hanumantal fish market (Jabalpur) M.P. ranging from 6 to 10 cm in length and 35 to 48 gms in weight and brought to the laboratory. The fishes were dipped in tubs and were disinfected with 0.01% of $KMnO_4$ solution and washed thoroughly to prevent dermal infection and fishes were maintained in aquariums with dechlorinated water which was continuously aerated. These fishes were acclimatized in the laboratory for two weeks prior to the experimentation.

The fishes were fed once a day with cut pieces of earth worm. Twenty five percent of the tank water was renewed in alternate days of the experiment. The tanks were overseen for water quality Test by using a commercial analysis kit (standard Jal-TARA Water Testing Kit). Temperature, pH and ammonia concentration were measured every day. Hardness, Alkalinity and dissolved oxygen, chloride and nitrate concentration were measured once a week (Table 1) (Borges *et al*., 2004).

Table 1. Physical and chemical parameters of water quality.

Parameter	Value
Temperature (^oC)	23.4
Chloride (mg l^{-1})	31.5
NO_2–N (mg l^{-1})	0.2
NO_3–N (mg l^{-1})	3.4
NH_3 un-ionized (mg l^{-1})	0.008
pH	7.4
Dissolved oxygen (mg l^{-1})	7.0
Total alkalinity (mg l^{-1})	78.5
Total hardness (mg l^{-1})	82.6

The whole experiment was conducted in two steps viz. determination of LC_{50} concentration (acute toxicity test) and biochemical studies.

Determination of LC_{50} concentration or (Acute toxicity test)

The experiments consisted of a control group and 6 experimental groups. A total number of twelve *C. punctatus* juveniles were used in this study to determine the zero and 100 % of mortalities, as well as the 96 hours of LC_{50}. The juveniles were divided into 7 groups (each group contains 12 fishes) and kept in glass aquarium (size 60 cm×30 cm×40 cm) fishes per group were exposed to different concentration of Atrazine 25 mg/L, 30 mg/L, 35 mg/L, 40 mg/L, 45 mg/L and 50 mg/L, (except control group). During the 96h acute toxicity experiment, water in each aquarium was aerated and had the same conditions as the acclimation period. After every 24 hours the dead fishes were removed and the numbers of survivals were recorded, the lethal concentration values were obtained and analyzed by Finney's probit analysis method (Elia *et al.,* 2002). Regression line was drawn on the basis of two variables. Log dose and empirical probit and was used to determine the expected probit necessary for LC determination. In the present study, calculated LC_{50} value of Atrazine is 40 mg/L. Refer Table 2, 3 and Figure 1.

Biochemical studies

The various sublethal concentrations viz. 5mg/L, 10 mg/L and 15 mg/L ie 12.5%, 25 % and 37.5% of LC_{50} value (40 mg/L) of 96 hours for 15 and 30 days respectively were given. The experiment was designed in seven glass aquariums, each containing 6 specimens of fish (one control and the other six with sub lethal concentrations 5, 10, 15 mg/L) for 15 days and 30 days respectively.

Collection and processing of blood samples

The blood samples were taken immediately from cardinal vein using 2 ml syringe and transferred into a tube containing 0.2% EDTA as anticoagulant. The blood was immediately centrifuged at 1500 rpm for 10 min. Serum was then removed and stored at 40°C prior to immediate determination of biochemical parameters, Glucose, Protein, Cholesterol. Glucose was determined by Folin-Wu method, Protein was determined by Reinhold's method and Cholesterol was determined by Zak's method using commercial kits.

Statistical Calculations

The significance of sample means between control and Atrazine treated fish was tested by using Students't' Test (Table 4 and 5).Values are expressed mean ± SD of observations Values are significant at $P<0.05$, $P<0.01$, $P<0.001$.

RESULTS AND DISCUSSION

The fresh water fish *C. punctatus* showed varied degrees of mortality with different concentration of Atrazine. The percentage of mortality was significantly increased in concentration and duration dependent manner of Atrazine, are presented in Table 1 and table 2 and the fish also showed many biochemical changes viz glucose, protein and

cholesterol, when exposed to various sub lethal concentrations, are presented in Table 3 and table 4. The mean values have been taken, Glucose were increased significantly ($p<0.001$) when compared to control groups. On the other hand the levels of Protein and cholesterol levels decreased significantly ($p<0.001$) when compared to control group.

Analysis of serum biochemical constituents shows useful information in detection and diagnosis of metabolic disturbances and diseases in fishes (Jamalzadeh and Keyvan, 2009). The present results show that Atrazine is highly toxic to various freshwater fish species. Atrazine herbicides are used to control broadleaf weeds in maize, sorghum, vines, top-fruit, citrus, sugar cane, bananas, coffee and oil palms. It is used in combination with many other herbicides (Alvarez and Fuiman, 2005; Zhou *et al.*, 2008). Atrazine has high mobility so that after application it can move to untargeted areas, especially to aquatic bodies (Waring and Moore, 2004).

When *C. punctatus* were exposed to 25 mg/L Atrazine, only 8.33 % died out of total 12 fishes after 96 h, whereas at concentrations of 30 , 35 ,40 45 and 50 mg/L, the number of dying fishes were 2,4,6,9 and 12 i.e., 16.67%, 33.33%, 50%, 75%, and 100% mortalities respectively. However, all the fishes died after an exposure of 96 h at 50 mg/L concentration of Atrazine. 96h LC_{50} values of Atrazine herbicide against *C. punctatus* were calculated as 40 mg/L. Imbalance, vertical swimming, loss of appetite, bleeding at the base of the fins were important clinical symptoms observed in *C. punctatus* at high concentrations of Atrazine.

Different concentrations of Atrazine have shown different alterations in biochemical parameters of the fish. The Atrazine has its own target sites of action and predominantly acts as a metabolic depressor. It generally affects the activity of biological active molecules such as enzymes (SGOT, SGPT, amylase, lipase, etc.) and promotes the fluctuations in the concentration of glucose, proteins, cholesterol etc in the blood.

Plasma concentration of glucose is regulated by complex interactions of hormones such as glucagon, cortisol and Insulin. Determination of glucose concentration in blood serum is widely used as an indication of stress response. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma (Percin and Konyalioglu, 2008). In present study hyperglycaemic condition was noticed in the sub-lethal concentrations, 5 mg/L, 10 mg/L and 15 mg/L of Atrazine for 15 and 30 days respectively. This may be considered as a manifestation of stress induced by Atrazine herbicide. Similar observations were recorded in *C. punctatus* when exposed to Chloropyrifos (Ramesh and Sarvanan, 2008). Hyperglycemia was observed in common carp when exposed to a mixture of heavy metals (Parvathi *et al.,* 2011).

Hypergycemia is caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses (Wedemeyer and Mcleay, 1984). Stressors are found to increase the levels of circulating adrenal hormones such as glucocorticoids (Hontela and Daniel, 1996) and catecholamines (Nakano and Tomlinson, 1967). Both the categories of hormones are responsible for hyperglycemia.

Protein is also one of the important biochemical parameters used to understand the general state of fish health. Proteins are main building blocks of the cell. During stress conditions, fish needs significant energy to detoxify the harmful substances to overcome stress. The depletion in total protein content may be due to augmented proteolysis and possible utilization of their product for metabolic purposes (Ravinder and Suryanarayana, 1988).

In the present study, total protein concentration depleted as the concentration of Atrazine was increased. The most probable reason could be inhibition of metabolizing enzymes in the presence of toxicant. (Jamalzade and Keyvan, 2009). The quality of protein also gets affected due to impaired incorporation of amino acids in the proteins (Hussein and El-Nasser, 1996). Significant depletion of total protein has also been observed in *Oreochromis niloticus* and *Hrysichthytes auratus* after acute exposure to Atrazine (Wedemeyer and Mcleay, 1984). In the present study there was hypoproteinemia after 15 and 30 days exposure to different concentrations of Atrazine. This may be due to the liver damage where most plasma protein synthesis usually occurs (Singh and Sharma, 1998).

Cholesterol content in blood serum is linked to lipid metabolism and depends on the calorific value of the food. In the present investigation, cholesterol showed continuous decline with increase in sub-lethal concentrations of Atrazine. This is supposed to be due to active cholestrololysis (George *et al.,* 1981). Hypocholesterolemia has also been observed in the serum of *Channa punctatus* during prespawning phase when exposed to nickel-chrome electroplating effluent (Kaur and Kaur, 2006). Jyothi and Narayan (2001) investigated that decline in serum cholesterol is due to liver disfunction by carbaryl stress in *Clarias batrachus*. Whereas, Gill and Pant (1988) reported that decrease in serum cholesterol is due to the cadmium stress affecting absorption of dietary cholesterol in fish.

Table 2. Rate of mortality of Juvenile *C. punctatus* exposure to Atrazine for 96 hrs.

Concentration	Log dose	24	48	72	96	Total
Control	0	0	0	0	0	0
25	1.39794	0	0	0	1	1
30	1.477121	0	0	1	1	2
35	1.544068	0	1	1	2	4
40	1.60206	1	1	2	2	6
45	1.653213	1	2	3	3	9
50	1.69897	2	3	3	4	12

Table 3. Calculate log dose and probit values.

Concentration	Log dose	Mortality	Percentage	Probit
25	1.39794	1	8.33	3.59
30	1.47712	2	16.67	4.01
35	1.54406	4	33.33	4.56
40	1.60206	6	50	5
45	1.65321	9	75	5.67
50	1.69897	12	100	7.33

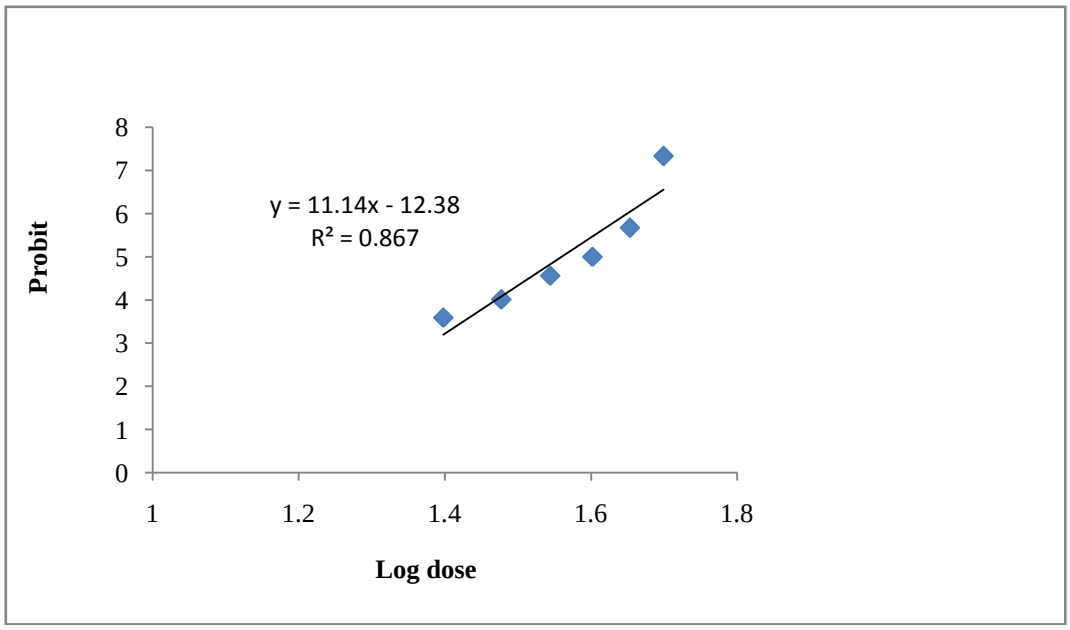

Figure 1. Linear relationship between probit response and log concentration of Atrazine on Juveniles of *C. punctatus.*

Table 4. Alteration in Serum biochemical parameters of *Channa punctatus* (Bloch.) in control and after exposed to 5mg/L, 10 mg/L and 15 mg/L, treatments of Atrazine for 15 days.

Exp. Set	control	5 mg/l	10 mg/l	15 mg/l
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Glucose	21.55 ± 3.22	26.35 ± 2.47*	28.32 ± 2.73**	29.41 ± 3.12 **
Protein	5.14 ±0.66	4.05 ± 0.76*	3.44 ± 0.68**	2.75 ± 0.52***
Cholesterol	228.36±23.18	205.34±23.78	194.04±22.07*	172.08±26.42**

*Significant (P<0.05); **Highly-significant (P<0.01); ***Very highly significant (P<0.001).

Table 5. Alteration in Serum biochemical parameters of *Channa punctatus* (Bloch.) in control and after exposed to 5 mg/L, 10 mg/L and 15 mg/L treatments of Atrazine for 30 days.

Exp. Set	control	5 mg/l	10 mg/l	15 mg/l
Glucose	21.555±3.22	28.88 ± 2.70**	30.23 ± 2.68**	31.51±2.79***
Protein	5.14±0.66	3.43±0.79**	2.61±0.84***	1.95±0.46***
Cholesterol	228.36±23.18	183.74±19.76**	165.22±8.79***	136.02±17.25***

*Significant (P<0.05); **Highly-significant (P<0.01); ***Very highly significant (p<0.001).

CONCLUSION

Protein and cholesterol decreased significantly while glucose increased in the blood serum of *C. punctatus* with the exposure to increasing concentration of Atrazine. Therefore, Atrazine herbicide has a profound effect on serum biochemical profiles of *C. punctatus*. Hence the current investigations are helpful for evaluating the physiological status and metabolic activity levels of Atrazine treated fish.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of zoology, Govt. M. H. College of Home Science and Science for women for the facilities provided to carry out this research work.

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