Biospectra : Vol. 12(2), September, 2017, pp. 65-70
An International Biannual Refereed Journal of Life Sciences

18(AS).
Animal Science

ISSN : 0973-7057

Effect of atrazine (Herbicide) on blood parameters of common carp (*Cyprinus carpio)*

Swarnima Kumari* & Arun Kumar
University Department of Zoology, B.N. Mandal University, Madhepura, Bihar, India

Received : 15th July, 2017 ; Revised : 11th August, 2017

Abstract: In the present study an attempt was made to investigate the acute toxicity of atrazine (ATR) a herbicide on an economically important fish, *Cyprinus carpio*. In which, 24 h median lethal concentration (24 h LC_{50}) of herbicide, atrazine to *C. carpio* was 18.5 ppm. Due to fish exposure to 24 h LC_{50} of atrazine for 24 h, red blood cells (RBCs) count (-63.17%), hemoglobin (-27.35%), plasma glucose (-6.78%) and plasma protein (-18.73%) levels were decreased; whereas white blood cells (WBCs) count (+3.73%) was enhanced. The differences in haematological and biochemical values were statistically significant ($p < 0.05$). However, WBC count was not significantly changed. The alterations of the above parameters could be used as an important tool for the assessment of pathological conditions of fish.

Keywords : Atrazine, acute toxicity, hematology, biochemical, *Cyprinus carpio*

INTRODUCTION

The rapid industrialization, application of synthetic fertilizers and use of various insecticides and pesticides, the natural water resources are fast degrading in the water quality. Aquatic ecosystems that run through agricultural or industrial areas have high probability of being contaminated by runoff and ground water leaching by a variety of chemicals.[1] Agricultural pesticides are released into the atmosphere by the spray drift, post application, volatilization and wind erosion of soil.[2] Pesticides presents in aquatic environments can affect aquatic organisms in different ways.[3] In India, more than 70% of the chemical formulations are employed in agricultural practices and to find their way to freshwater bodies, ultimately affect non target organisms.[4]

*Corresponding author :
Phone : 9430561914
E-mail : dr.swarnima.2016@gmail.com

The use of herbicides to control aquatic weeds has applied in fish management where they are used in aquatic habitats especially rice fields and some fish farms.[5] ATR has been one of the most widely used herbicides to control broadleaf weeds in corn or crops, including green vegetables.[6] After spraying on crops, it can enter watercourses, because of its high mobility through soil.[7] It is also pointed out that ATR reaches aquatic environments due to proximities of the agricultural country sides to the water places, or directly due to the careless application in such environments.[8] After reaching the environment the atrazine or triazine based herbicides are not degraded by microbial or hydrolytic process.[9] However, reports pointed out that atrazine can be degraded in surface water by photolysis and microorganisms and the half- lives of 20-50 days at 20-25°C have been found under laboratory conditions and increasing at lower temperatures.[10] It has

been detected in natural and surface waters at concentrations exceeding 0.1 gl^{-1} in some areas and also accumulated in a variety of tissues.[11-14] Many authors have reported the impact of atrazine on the physiology and metabolism of aquatic organisms particularly on fishes.[15-18]

Fish are one of the most widely distributed organisms in an aquatic environment and being susceptible to environmental contamination may reflect the extent of the biological effects of environmental pollution in waters. Monitoring of blood parameters, both cellular and non-cellular may have considerable diagnostic value in assessing early warning signs of pesticide poisoning.[19] In India, ATR still is one of the most widely used herbicides controlling broad leave weeds and grasses. The possible effects of ATR on aquatic eco-system have stimulated studies to understand the mechanisms and measurements of the toxic effects of it to aquatic organisms. Hence, the present study is designed to study the acute effect of ATR as a herbicide on blood parameters of a freshwater teleost fish *C. carpio*.

MATERIALS AND METHODS

C. carpio was selected for the present investigation and the healthy specimens were collected from local market of Madhepura. Fish were acclimatized to laboratory conditions for about 15 days before the commencement of the experiment. During this period, fish were fed *ad libitum* with rice bran and oil cake in the form of dough daily. Water was replaced every 24 h after feeding in order to maintain a healthy environment with enough oxygen. The aquarium water was analyzed for physico-chemical characteristics according to APHA (1998)[20].

Five hundred fish were stocked in a large cement tank (4m×6 m×3m) after cleaning and disinfected with potassium permanganate. Fish with an average weight of 6 g and length about 7-8 cm were selected for the experiment. The LC_{50} value of the herbicide atrazine (ATR), (2-chloro-4-ethylamino-6-isopropylamino- 1,2,3-triazine) (18.5 ppm) to fish was calculated following the method of Finney (1978) with a confidence limit of 95%. The acute toxicity experiment was carried out in two circular glass tanks, filled with 40 l of water. A normal pH (6.3) and ATR concentration of 18.5 ppm (LC_{50} 24h) were maintained throughout the experiment. Twenty fish, which were already with held from feeding for 48h, were introduced into each tub. The control was maintained in two circular glass tanks with 20 fish per tub. After 24 h, 40 randomly sampled fish, 20 each for the control and ATR treated groups were used for the haemato-biochemical assay. Care was taken to minimize disturbances to the animals. After the stipulated time period (24 h) fish from control and ATR treated tanks were sacrificed and blood was collected by cardiac puncture using heparinised syringes and kept at low temperature (4°C). All analyses were performed on pooled blood samples. Whole blood was used for the estimation of red blood cells (RBCs), white blood cells (WBCs) and haemoglobin (Hb) content. RBCs and WBCs were counted by the method of.[21] Haemoglobin was estimated by cyanmethemoglobin method.[22] Then the pooled blood samples were centrifuged for 15 min at 10 000 rpm, the plasma was withdrawn and transfer- red into clean vials for plasma glucose and protein estimation. Glucose was estimated by *O*-Toluidine method of Cooper.[23] Protein was estimated according to the method.[24] For the experiment and control group (toxicant free water), three replicates were maintained.

RESULTS AND DISCUSSION

The calculated LC_{50} for 24 h with a confidence limit of 95% of atrazine (ATR) to the carp, *C. carpio* was 18.5 ppm indicating the moderate toxicity of ATR to the fish.

During above exposure period the fish shows various behavioral responses like increased opercular movement, mucous secretion, jerky movement, floating on the sides, hypersensitivity showing violent erratic and fast swimming etc. The abnormal behaviour of the fish indicates the toxic effect of ATR on central nerves system (CNS) and cardiovascular system as suggested by Antychowicz (1979)[25]. Documented effects of ATR in fish include a slowdown in reflexes, swimming activity and feeding. Puigdoller (1978)[26] reported that fish Atlantic salmon exposed to 100 gl^{-1} ATR had reduced food consumption after 10 and 15 days of exposure. These behavioral changes were the result of decreased acetyl cholinesterase activity.

The effects of environmental stressors on the peripheral blood of fishes are well documented in the literature. ATR is toxic; often bioaccumulative and persistent.[27] The blood alterations of carp *C. carpio* exposed to acute concentration of ATR is shown in Figure 1. During acute treatment RBC count (-63.17%), hemoglobin (-27.35%), plasma glucose (-6.78%) and plasma protein (-18.73%) levels were lowered when

compared to that of their control group whereas WBC count (+3.73%) was increased. The differences in haematological and biochemical values were statistically significant ($p < 0.05$). However, WBC count was not significantly changed. Hussein *et al*. (1996) reported decreased RBCs number, hemoglobin concen- tration and haematocrit percentage of *Oreochromis nitoticus* and *Chrysichthyes auratus* when exposed to 3 and 6 mg/l ATR. There also occurs significant increase in hematocrit in Atlantic salmon when exposed to ATR. found that damage of the gill lamellae causes decreased respiratory capacity in *Tilapia mosambica* exposed to 1.1 mgl^{-1} ATR.[28] Found a decline in the number of erythrocytes after exposure of *C. carpio* to 0.1 mg/l atrazine.[29] A high frequency of micronuclei and nuclear abnormalities in *O. niloticus* exposed to different concentrations of ATR is also shown by other authors.

Erythrocyte level was found to be depressed in fishes subjected to stressful conditions. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to gill damage and the nature of the changes shows a release of erythrocytes from the blood depots.[30] Inhibition of erythropoiesis and increase in the rate of erythrocyte destruction in hematopoietic organs is the cause of decrease in RBC count found significant decrease of RBCs, Hb and packed cell volume (PCV) in ATR exposed fish species and indicated the toxic effect of ATR on spleen, liver and anterior kidney.[31] In the present study, the significant decrease in RBCs and hemoglobin content might have resulted from the lowering of the oxygen content of the water due to the presence of atrazine in the test media. Further reduction in the total erythrocyte count (TEC) might have attributed to a decrease in the erythropoietic activity of the kidney or to the haemodilution resulting from impaired osmoregulation across the gill epithelium. Leucocytes are involved in the regulation of immunological function and their numbers increase as protective response in fish to stress. Such an increase in total leucocyte count (TLC) occurs by the increase in lymphoperisis and/or enhanced release of lymphocytes from lymphoid tissues.[32] Other scientists also reported that WBC increase could be due to an induced proliferation as a result of the chemical toxicity, of pluripotential hematopoietic cells that in turn may be a consequence of a depletion circulating differrentiated cells.[33] The increase in WBC count in the present study indicates the stress condition of the fish caused by ATR which might have produced hypoxia and gill damage. Changes in carbohydrate metabolism measured as plasma glucose can be used as general stress indicators in fish. Reduction in serum glucose levels after exposure to toxicants appears to be caused by hypoxic conditions leading to an excess utilization of stored carbohydrates. Significant decrease in serum glucose was also found. This decrease could be attributed to the toxic effect of ATR on the liver.[34] In the present study, the decreased level of plasma glucose during acute treatment might have resulted from hypoxic condition caused by the herbicide ATR. Further, it is seen that reduction in food intake of ATR treated fish could be also considered another possible reason for decrease in plasma glucose.

The concentration of protein in the serum of fish has been used as an indicator of their general state of health. Reported that the higher energy demand might have triggered an increase in protein catabolism, a process in which both blood and structural protein are converted to energy, thereby reducing serum protein.[35] They further reported that dilution of plasma volume after haemolysis and shrinkage of RBC could also cause a small reduction in protein percentage in serum. Failure of haemopoiesis is a characteristic indicator of kidney damage. Kidney damage causing increased renal excretion of blood protein may also have contributed to the depletion of serum protein in the fingerlings. However, various reports also indicates decrease of total protein in ATR treated fish *Oreochromis niloticus* and *Chrysichthyes auratus* was mainly due to globulin, explaining the toxic effects of ATR on the immune system of these fishes. In the present study, the reduction of plasma protein of fish from acute treatment indicates the toxic effect of ATR on spleen, liver and kidney. Various other researchers also found that carp exposed to 100 gl^{-1} ATR for 72 h had significantly lower plasma protein concentrations, which suggested a haemodilution effect operating in these fish.

From the present study, it is concluded that, atrazine (ATR) has a profound influence on the blood profiles of the treated fish. Hence, the use of the herbicide ATR should be minimized and these parameters could be effectively used as potential biomarkers of herbicide toxicity to *C. carpio*.

REFERENCES

1. **Todd NE, Leuwen MV. 2002.** Effect of sevin (carbaryl insecticide) on early life stages of zebra fish, *Danio rerio*. Ecotoxicol. Environ. *Saf*. **53:**267-272.
2. **Qiu X, Zhu T, Li J, Pan Li Q, Miao G, Gong J. 2004.** Organochlorine pesticides in the air around the Taihu Lake, China. Environ. *Sci. Technol*. **38:**1368-1374.
3. **Ventura BC, Angelis DF, Maria AM, Morales M. 2008.** Mutagenic and genotoxic effects of the Atrazine herbicide in *Oreochromis niloticus* (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay. Pestic. *Biochem. Physiol*. **90:** 42-51.
4. **Bhatnagar MC, Bana AK, Tyagi M. 1992.** Respiratory distress to *Clarias batrachus* (Linn.) exposed to endosulfan a histological approach. *J. Environ. Biol.* **13:** 227-231.
5. **Wu TL, Lambert L, Hastings D, Banning D. 1980.** Enrichment of the agricultural herbicide atrazine in the micro surface water of an estuary. *Bull. Environ. Contam. Toxicol*. **24**: 411-414.
6. **Cui H, Hwang HM, Zeng K, Glover H, Yu H, Liu Y. 2002.** Riboflavin- photosensitized degradation of atrazine in a freshwater environment. *Chemosphere*. **47:** 991-999.
7. **Waring CP, Moore A. 2004.** The effect of atrazine on Atlantic salmon (*Salmo salar*) smolts in fresh water and after sea water transfer. *Aquat. Toxicol*. **66:** 94-104.
8. **Hussein SY, El-Nasser MA, Ahmed SM. 1996**. Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, *Egypt. Bull. Environ. Contam.Toxicol*. **57:** 503-510.
9. **Gamble DS, Khan SU, Tee QS. 1983.** Atrazine hydrolysis: Part I. Proton catalysis at 25 8C. *Pestic. Sci* **14:**537-45.
10. **WHO. 1996.** Guidelines for drinking-water quality, 2nd ed. Vol.2. Health criteria and other supporting information. *World Health Organization, Geneva*, 1996.
11. **Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW, Williams WM. 1996.** Ecological risk assessment of atrazine in North American surface waters. *Environ. Toxicol. Chem*. **15:** 31-76.
12. **Power M, Attrill MJ, Thomas RM. 1999.** Trends in agricultural pesticide (atrazine, lindane, simazine) concentrations in the Thames Estuary. *Environ. Pollut*. **104:**31-39
13. **Environment Agency. 1997.** Pesticides in the Aquatic Environment. Update of the Report of the National Rivers Authority, Water Quality Series No. 26. National Centre for Toxic and Persistent Substances
14. **Du Preez HH, van Vuren JHJ. 1992.** Bioconcentration of atrazine in the banded tilapia, *Tilapia sparrmanii*. *Comp. Biochem. Physiol.* **101(C):** 651-655.
15. **Prasad TAV, Srinivas T, Rafi GM, Reddy DC. 1991.** *In vivo* effect of atrazine on haematology and O2 consumption in fish *Tilapia mossambica*. *Biochem. Int*. **23:**157-161.
16. **Grobler E, van Vuren JJJ, du Preez HH. 1989.** Routine oxygen consumption of *Tilapia sparrmanii* (Cichlidae) following acute exposure to atrazine. Comp. *Biochem. Physiol.* **93C:** 37-42.
17. **Srinivas T, Prasad TAV, Raffi GM, Reddy DC. 1991.** Effect of atrazine on some aspects of lipid metabolism in fresh water fish. *Biochem. Int*. **23:**603-609.
18. **Phyua YL, Warne MStJ, Lim RP. 2006.** Toxicity and bioavailability of atrazine and molinate to the freshwater fish (*Melanotenia fluviatilis*) under laboratory and simulated field conditions. *Sci. Total Environ* **356:**86-99.
19. **Pant J, Tewari H, Gill TS. 1987.** Effects of aldicarb on the blood and tissues of a freshwater fish. Bull. Environ. *Contam. Toxicol*. **38:**36- 41.
20. **APHA (American Public Health Association). 1998.** Standard methods for the examination of water and wastewater, 20th Ed. Washington, DC.

21. **Rusia V, Sood SK. 1992.** Routine hematological tests. In: Medical laboratory technology Kanai, (Ed., Mukerjee L), Vol. I. Fifth reprint. Tata McGraw Hill Publishing Company Limited, New Delhi. pp. 252-258.

22. **Drabkin DL. 1946.** Spectrometric studies, XIV-The crystallographic and optimal properties of the hemoglobin of man in comparison with those of other species. *J. Biol. Chem*. **164:** 703-723.

23. **McCormick SD. 2007.** Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. *Aquat. Toxicol.* **84:**27-37.

24. **Lowry OH, Rosebrough NJ, Farr AL, Randall RI. 1951.** Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193:** 265-275

25. **Antychowicz J, Szymbor E, Roszkowski J. 1979.** Investigations upon the effects of some pesticides on carp (*Cyprinus carpio*) Bull. Vet. Inst. *In Pulawy*. **23(3/4):** 124-130

26. **Puigdoller KN, Bjornsson BT, Rehwoldt RE. 1978.** Investigations into the acute toxicity and some chronic effects of selected herbicides on several freshwater fishes. *App. Eco*. J. **4:** 316-327

27. **Fernando MD, Alcaron V, Ferandez-Casalderrey A, Gamon M, Andreu- Moliner E. 1992.** Persistence of some pesticides in the aquatic environment. *Bull. Environ. Contam. Toxicol.* **48:**747-755.

28. **Prasad TAV, Srinivas T, Reddy JS, Reddy DC. 1995.** Atrazine toxicity on transport properties of hemocyanin in the crab (*Ozioteiphusa senex*). *Ecotoxicol. Environ. Saf.* **30:**124-6.

29. **Horn G, Hanke W. 1980.** Hämatologische Veränderungen bei karpfen unter dem Einf lu v o n Atrazin.

30. **Drastichova J, Svobodova Z, Luskova V, Machova J. 2004.** Effect of cadmium on hematological indices off common carp, *Cyprinus carpio* (L.). *Bull. Environ. Contam. Toxicol*. **72:**725-732.

31. **Joshi PK, Bose M, Harish D. 2002.** Changes in certain hematological parameters in a siluroid cat fish, *Clarias batrachus* (L.) exposed to cadmium chloride. *Pollut. Res*. **21(2):** 129-131.

32. **Johansson-Sjobeck ML, Larsson A. 1978.** The effect of cadmium on the hematology and on the activity of delta-amino leverlinic acid dehydratase (ALA-D) in blood and hematopoietic tissues of the flounder, *Platichthys flesus* (L.). *Environ. Res.* **17:** 191-204.

33. **Fink NE, Salibian A. 2005.** Toxicological studies in adult amphibians: effects of lead. *Appl. Herpetol*. **2(3):** 311-333

34. **Braunbeck T, Burkhardt-Holm P, Gorge G, Nagel R, Negele RD, Storch V. 1992.** Rainbow trout and zebra fish, two models for continuous toxicity tests: relative sensitivity, species and organ specificity in cytopathologic reaction of liver and intestines to atrazine. *Schriftenr- ver-wasser-Boden-Lufthyg*. **89:**109-145.

35. **Das PC, Ayyappan S, Jena JK, Das M. 2004.** Acute toxicity of ammonia and its sublethal effects on selected haematological and enzymatic parameter of mrigala, *Cirrhinus mrigala*. (Hamilton). *Aquat. Res*. **35:**134-143.

***