IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed ( Group -I) Journal Volume 12, Iss 01, 2023

# CHRONIC TOXISITY EFFECTS OF HERBICIDE, ATRAZINE ON BEHAVIOUR RESPONSE & BLOOD METABOLITE OF Clarias batrachus (Linn.)

**[1]Ranjan Kumar Paswan, [2]Dr. Arti Kumari,**

Department of Zoology, C.M.Sc. College, L.N.M.U., Darbhanga
rranjankumar13@gmail.com

## ABSTRACT

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an herbicide widely used to weeds control in agriculture practice. The present study includes the blood metabolites alterations in airbreathing fish, Clarias batrachus (Linn.) induced to sublethal concentration (4 mg/L) of inorganic herbicide, atrazine. The treated fish group was showed significant blood biochemical alteration in the form of hyperglycemia, hypoproteinemia and hyperchlosterolemia diseases. Therefore the study reflect herbicide, atrazine at conc. 4 mg/l caused severe metabolic dysfunction in the fish Clarias batrachus. So, for optimum growth performance and survival rate of fish culture, water condition may be suitable at concentration of < 4 mg/L of atrazine contamination.

**Key words :** Herbicide, atrazine, metabolite, Clarias batrachus, hyperglycemia, hyperproteinemia, hyperchlosterolemia.

## INTRODUCTION :

The use of the herbicides appreciated after green revolution to improve agricultural production and yield but in other hand its harmful impact on non-target aquatic organisms, especially fish came in light. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an herbicide widely used to weeds control in agriculture practice. The electron transport in photosystem 11 is blocked by atrazine leading to chlorophyll destruction and ultimately photosynthesis system blocked. Atrazine showed infiltration into ground water due to its unique property as hydrolysis half-life of 30 days and relatively high water solubility (32 mg·L-1) (Orme & Kegley, 2004). Perry, 1990; Solomon, 1996; Selim, 2003 have reported the atrazine contamination in aquatic bodies, its conc. of 20 µg ·L-1 detected in surface water runoff, while concentrations as high as 700 µg ·L-1, repeated applications of atrazine for the control of weeds in agricultural fields resulted large quantities of the herbicide runoff into aquatic system.

Fish have been found as bio-indicators to detect the aquatic ecosystem pollution. Fish are used as biomarker for ecotoxicological studies due to their role in trophic web, accumulate toxic substances and respond to low concentration of mutagens, in first instance seen the effects of pollution in aquatic ecosystem (Van Der Oost, et al., 2003). Fish can be used to detect and assessing the potential of toxicants contamination in aquatic environment. Fishes are prime

 

concern of exposed to chemicals such as fertilizers, pesticides released from agricultural production wastes through surface run-off. Ultimately these toxicants introduce into food chain of ecosystem (Lakra & Nagpure, 2009).

Clarias batrachus (Linn.) is an air breathing cat fish, locally known as "Mangur", commonly found on this subcontinent is an integral part of paddy field culture. Such species directly concern to attack of severe herbicide toxicity from atrazine during the intensive application of it to eradicate weeds from the crop fields. C. batrachus has significant properties like wide distribution in the freshwater environment, locally availability throughout the year, easy acclimatization to laboratory conditions and commercial importance due to such it is recognized as an excellent test species for toxicity and biochemical studies.

Hence, in the present study effort has been made to illustrate the blood biochemical alterations and behavioral disorders in freshwater fish, Clarias batrachus induced to herbicide, atrazine.

**MATERIALS AND METHODS :**

Clarias batrachus was (average length (15±2.0 cm) and weight (30±2.2 g) procured live from the local fish market, Darbhanga. Fish were brought to lab in aerated open container and then washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish were acclimated for 15 days to laboratory conditions. Fish fed on commercial feed (containing 28% crude protein) at ration rate 3% of fish weight in the morning (10.00 AM).

For atrazine $LC_{50}$ values were determined through a static acute bioassays the mortality was recorded after 24, 48, 72 and 96 hr, and for these periods were 64.1, 50.1, 43.5 and 40.5 mg· L-1 concentration of atrazine respectively were calculated by the Finney method (1978). The 1/10th value of the $LC_{50}$ value for 96 hr was taken as the sublethal concentration according to method Sprague, (1971). 20 fish were exposed to a sub-lethal concentration (4 mg) of atrazine for 30 days. Side by side 20 fish were maintained as the control group. During exposure period behavioural abnormalities were assessed following a modified behavioural protocol checklist of Klesius et al. (2000). The individual fish in the experiment was assigned to get score as per following scoring system: 0, no observed changes in behaviour; 1, swimming abnormally, lethargic or unresponsive, changes in skin coloration; 2, hyperactive or jerk moement, rapid operculation; 3, death. Mean behaviour scores were calculated per replicate treatment.

On 30$^{th}$ day the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes then blood samples was extracted from the caudal dorsal of the test fish. Blood samples were processed for quantitative estimation of blood glucose (Sinha, 1990), serum protein ('Biuret method' of Varley et. al.,1980) and serum cholesterol (Kabara's method,1966).

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed ( Group -I) Journal Volume 12, Iss 01, 2023

**RESULT :**

**BEHAVIOURAL RESPONSE :** The control fish shows a normal movement at the bottom of the aquarium. In atrazine treatment aquarium fishes showed increased swimming, surfacing and hyperactivity. After 24 hrs observed restlessness, rapid surfacing, peeling of skin and colour fading were prominent. After 48 hr exposure the fishes showed slightly reduced activity and gradual increase in colour fading. At this stage exposed fish have a thin film of mucous with gill adhesion was noticed on gills, operculum and general body surface. After 72 hr exposed fish showed increased surfacing and gulping of air. At this stage loss of balance and jerky movements during swimming showed by exposed fish only. After 96 hrs exposed fish have ulceration on trunk, base of caudal and pectoral fins were prominent in 95% fish. Almost all exposed fish have covered with thick film of mucous on whole body and gills. Treated fishes were observed lost their natural colouration. Exposed fish showed loss of equilibrium before death settle down at bottom.

**BLOOD BIOCHEMISTRY STUDIES:** Carbohydrates, proteins and lipids are the principal constituents of the cell and also known as metabolites. Fishes exposed to stress conditions metabolites play a major role as energy precursors. For the proper and normal physiologic function of a cell required the optimum concentrations of these biomolecules. Exposure of atrazine disturb the normal physiology of the cell resulted alteration in the metabolites organization or concentration.

The fish, Clarias batrachus exposed to a sub-lethal concentration (4 mg/l) of atrazine reveals following changes in the blood biochemical parameters (table-1).

**BLOOD GLUCOSE:** The blood glucose level in the control fish group is assessed to be 65.66±0.73 mg/100ml of blood. The exposed fish showed an increase of 35.72% become hyperglyceric as evident by a highly significant ($p < 0.001$) elevated level of glucose in the blood (89.12± 1.30 mg/100ml) (Table-1). The values of the present observations are expressed as meant S.E. of 5 fish in each group.

**SERUM PROTEIN:** The atrazine exposed fish group show depletion in the level of serum protein 3.55 ± 0.10 g/100 ml as against the control group 6.05 ± 0.30 g/100 ml. The present value serum protein has been found to decrease by 41.32% became hypoproteinemia as evident by a highly significant ($P< 0.001$) (Table-1).

**SERUM CHOLESTROL:** The serum cholesterol level of control group has estimated 202±2.07 mg/100 ml. The treated fish group showed an increase 221.5±1.95 mg/100 ml. The present value of cholesterol has been found to increase by 9.15% became hypercholestrolemic as evident by a significant ($p< 0.05$) (Table-1).

 

**Tables:-1**

**Showing changes in the blood metabolite levels in Clarias batrachus exposed to atrazine (4 mg/l) for 30 days. Values are mean ± SE of 5 observations.**

| Parameters | Control | Atrazine exposed |
|---|---|---|
| Blood glucose (mg/100 ml) | 65.66±0.73 | 89.12± 1.25(+35.72%)*** |
| Serum protein (g/100ml) | 6.05 ± 0.30 | 3.55 ± 0.15 (- 41.32%) *** |
| Serum cholesterol (mg/100 ml) | 202±2.07 | 221.5±1.95 (+9.15%) * |

Valu

es indicate percent increase (+)

Or decrease (-) over control values significant at

* P<0.05, *** p<0.001

**DISCUSSION :**

In the present study, certain deformities and unusual swimming patterns, jerk movement were found in fish exposed to 4 mg/L of atrazine and above concentrations. The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions (Palanivelu et al., 2005; Ufodike and Onusiriuka, 2008). Behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1973). Acute toxic effect mercuric chloride was observed on H. fossilis by Pathak & Anand, (2020). The toxic effects of surfactant, dodecyl dimethyl benzyl chloride (1227) on larval locomotors of zebrafish was observed by Yanan, et al. (2015). It is, therefore, conclude that the toxicity of the herbicide, atrazine depend upon a number of physical, chemical and biological factors. Each of which may be used as a tool for herbicide toxicity to fish culture monitoring.

**BLOOD GLUCOSE:** Blood glucose level in serum of C. batrachus after treated with different concentration of atrazine showed highly significant increases (P<0.001) (89.12± 1.25 mg/100ml), in all groups compared to the control fish group (65.66±0.83 mg/100ml). Atrazine an effect is known to increase the levels of activating glycogenolysis and glyconeogenesis with a net result of increasing plasma glucose levels. Blood glucose increased due to stimulation of glucocorticoids in stressed catfish. Recently Jha (2009) and Poonam et al. (2010), Clair, et al.(2012), Koley (2013), Le Mer, et al. ( 2013), Pathak, (2020) and Mohan, (2020) have observed

similar result under the exposure of Nuvan, fenvalerate, endosulfan, glyphosate, Eklaux and atrazine in various fresh water fishes. Changes in blood glucose concentration is the most widely recognized and consistent response to stressor.

**PLASMA PROTEIN:** In the present investigation depleted levels of plasma proteins was observed. Serum proteins appear to be very sensitive to atrazine exposure as is evident by the apparent fall (-41.32%) in its content. The decrease in serum protein as observed during the present study suggests that the detoxification/ degradation of the toxicant either took place partially in the blood itself or involved the serum protein. This depletion further suggests a progressive protein degradation or biochemical transformation of the protein Nitrogen into other Nitrogenous products as suggested by Jha (2009), Clair, et al.(2012), Koley (2013), Le Mer, et al. ( 2013), Ajima et al.,(2015), Ahsan (2016), and Pathak, (2013) has observed similar result under the exposure of various toxicants Nuvan, fenvalerate, Atrazine, Eklaux heavy metals in various fresh water fishes.

Significant decrease in total plasma protein was recorded and it was found that the percent decrease was higher in 120 days exposure period (68.2%) then 96 hour (46.4%) in fish Clarias batrachus exposed to 11.12 ppm sublethal concentration of cadmium (Sastry & Shukla 1990). In present study agreement with Clair, et al. (2012) decrease in plasma protein within 30 days of exposure of atrazine at sublethal concentration to C. batrachus was recorded.

**SERUM CHOLESTEROL:** The present findings the toxicity of atrazine caused hypercholesterolemia in blood (Table-1) has conformity of earlier works as Kalaivani et al.,(2008) and Jha & Jha, (2010) ; Abedi et al., (2013); Le Mer, et al. ( 2013), Rani, et al.(2015), and Dilip, and Vidya (2016) has observed similar result under the exposure of Nuvan, fenvalerate, Eklaux, Trivalent chromium, Atrazine and heavy metals in various fresh water fishes.

Holmberg et al. (1972) have suggested that the elevated blood cholesterol level may be due to the hypermetabolic state of the fish or due to the impaired liver function. In response to toxicant found an increase in cholesterol may be due to its increased synthesis in the liver as the precursors for interval hormones. Madhavan & Elumalai (2016) have suggested that this increased cholesterol store might have found its way into the blood.

The present finding revealed that the behavioural responses as well as biochemical responses are dependent on stressor quantity and exposure time. Tchounwou, (2000) suggested that the herbicide may lead to the occurrence of transformation products in water with a potential or actual similar or higher toxicity than their parent. In the C. batrachus exposed to atrazine potentially could have affected the biochemical measurements in form of metabolites/breakdown products. To determine these biochemical changes and its transformation products in the fish exposed to atrazine, further study required.

 

## CONCLUSION:

The water contaminated with herbicide, atrazine concentration of > 4 mg/l is not suitable for the culture of Clarias batrachus with average weight 30.0± 2 g. The toxicity of atrazine on fish may be detected as alteration in blood biochemical properties in the form of hyperglycemia, hypoproteinemia and hyperchlosterolemia.

## ACKNOWLEDGEMENT:

The authors are thankful to the Department of Zoology, C.M. Sc. College, LN Mithila University, Darbhanga, for the provision of laboratory facilities used in this study.

## REFERENCES :

Abedi, Z., Khalesi, M.K. Eskandari S.K. 2013. Biochemical and Hematological Profiles of Common carp (C. carpio) under sublethal Effects of Trivalent chromium. Iran J. Toxicol**.7**:782-792.

Ahsan K., Nazish S., M., S.ayed K., Munawar S. A., Farooq, M. A., Sahibzada M. J., Hayat U, Ali M. Y., 2016. Quantitative Determination of Lethal Concentration $LC_{50}$ of Atrazine on Biochemical Parameters; Total Protein and Serum Albumin of Freshwater Fish Grass Carp (Ctenopharyngodon idella) Pol. J. Environ. Stud. Vol**. 25**, No. 4, 1555-1561.

Ajima, M.N.O., Ogo, O.A., Akpa, L.E. and Ajaero, I., 2015. Biochemical and haematological responses in African catfish Clarias gariepinus following chronic exposure to NPK (15:15:15) fertilizer. Afr. J. Aquat. Sci., **40:** 73-79.

Clair, A.; Mesnage, R.; Travert, C.; Seralini, G. E., 2012. A glyphosate based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicol. Vitro, **26 (2)**, 269−279.

Dilip, M. and Vidya B. 2016. Chromium induced changes in biochemical composition and gonado-somatic index of a teleost, Oreochromis mossambicus (peters) The Jour. Of Zool. St. **3(5):**28-34.

EIFAC (European Inland Fisheries Advisory Commission).1973. Water quality criteria for European freshwaterfish report on ammonia and inland fisheries. Water Research, **7:** 1011-1022.

Finney D.J. 1978. Statistical methods in biological assay. 3rd ed. London UK: Griffin Press; p. 508.

Holmberg, B., Jensen, S., S., Larsson, A., Lewander, K. and Olsson, M. 1972. Metabolic effects of technical pentachlorophenol (PCP) on the eel Anguilla Anguilla. Comp. Biochem. Physiolo. B **43**:171-183.

Jha, K.N. 2009. Biochemical reflections of cadmium chloride on the fish Channa punchtatus (Bloch) Ph.D. thesis of B. N. Mondal Uniersity, Laloonagar, Madhepura.

Jha, S.K. & Jha, M.M. 2010. Bio-chemical changes of mercury chloride on blood metabolite levels of C. batrachus (Linn). Indian J. of Environ & Ecoplann. Vol.**17**, No.(1).

Kabara, J.J. 1966. Determination and microscopic localization of cholesterol In:method of biochemical analysis (ed. D. Glick) New York: Interscience publicers Inc. 263-318.

Kalaivani, M., Kennadi, P. and Kannan, K. 2008. Heavy metal mercururi chloride induced Bio-chemical changes in the fresh water catfish Mystus vittatus, Indian J. Environ and Ecoplan **15**(1-2):210-212.

Klesius, P. H., C. A. Shoemaker, and J. J. Evans. 2000. Efficacy of a single and combined Streptococcus iniae isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (Oreochromis niloticus). Aquaculture **188**:237–246.

Koley 2013. Effect of Eklaux to Channa punctatus (Bloch), Ph. D. Thesis, L.N.M.U. Darbhanga.

Lakra, W.S.; Nagpure, N.S. 2009. Genotoxicological studies in fishes: A review. Indian J. Anim. Sci. 79, 93- 98.

Le Mer, C.; Roy, R. L.; Pellerin, J.; Couillard, C. M.; Maltais, D., 2013. Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (Gasterosteus aculeatus). Ecotoxicol. Environ. Saf., **89,** 174−181.

Madhavan P., Elumalai K. 2016. Effects of chromium ( VI ) on the lipid peroxidation and antioxidant parameters in the gill and kidney tissues of catfish , Clarias batrachus ( Linnaeus , 1758 ) ( Actinopterygii : Siluriformes). Int J Adv Res BiolSci**. 3**:249–255. 12.

Mohan K., 2020. Toxicity of Endosulfan on Total Lipid and Protein of Liver, Kidney and Gonads of Fish Channa punctatus (Bloch.). JETIR; Vol. **(7),** 10.

Orme, S.; Kegley, S. 2004. PAN Pesticide Database; Pesticide Action Network: San Francisco, CA, USA.

Palanivelu, V., Vijayavel, K., Ezhilarasi Balasubramanian, S. and Balasubramanian, M.P. 2005. Impact of fertilizer (urea) on oxygen consumption and feeding the freshwater fish Oreochromis mossambicus. Environmental Toxicology and Pharmacology, 19: 351–355.

Pathak P., Kumar A., 2020. Biochemical changes of mercuric chloride on blood metabolite levels of freshwater Fish Heteropneutes fossilis (Bloch.). JETIR, Vol. **07**, Issue-11.p-304.

Perry, C. 1990. Source, extent and degradation of herbicides in a shallow water aquifer near Hesston, Kansas. In Water-Resources Investigation Report 91–4019; US Geological Survey: Lawrence, KA, USA.

Poonam K., Amit K. J. & M. M. Jha 2010. Fish heath under tenvalerate stress. Indian Journal of Environment & Ecoplanning **17(3),** 517-520.

Rani, S., Gupta, R.K. and Manju Rani, 2015. Heavy Metal Induced Toxicity in Fish with Special Reference to Zinc and Cadmium. International Journal of Fisheries and Aquatic Studies. **3(2):** 118-123.

Sastry, K.V. and Shukla, V., 1990. Toxic effects of cadmium on some biochemical and physiolochemical parameters in the teleost fresh Channa punctatus. J. Biol. Vol. 2, No**. 2,** pp. 325-332.

Selim, H. 2003. Retention and runoff losses of atrazine and metribuzin in soil. J. Environ. Qual. 32, 1058–1071.

Sinha, K.P., 1990. Manual of practical biochemistry, Scientific book company, Patna.

Solomon, K.R.; Baker, D.B.; Richards, R.P.; Dixon, K.R.; Klaine, S. J.; La Point, T.W.; Kendall, R.J.; Weisskopf, C.P.; Giddings, J.M.; Giesy, J.P.; Hall, L.W.; Williams, W.M.1996. Ecotoxicological risk assessment of atrazine in North American surface waters. Environ. Toxicol. Chem. 15, 31–76.

Sprague, J.B., 1971. Measurement of pollution toxicity to fish. III. Sub – lethal effects and 'safe' concentration, Water Res.**5**: 245- 266.

Tchounwou, P.B.; Wilson, B.; Ishaque, A.; Ransome, R.; Huang, M.J.; Leszczynski, J. 2000. Toxicity assessment of atrazine and related triazine compounds in the microtox assay and computational modeling for their structure-activity relationship. Int. J. Mol. Sci. 1, 63–74.

Ufodike, E.B.C. and Onusiriuka, B.C. 2008. Acute toxicity of inorganic fertilizers to African catfish, Clarias gariepinus (Teugals). Aquaculture Research, **21**: 181186.

Van Der Oost, R.; Beyer, J.; Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharm. 13, 57–149.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed ( Group -I) Journal Volume 12, Iss 01, 2023

Varley, H.; Gowenlock, A.H. and Bell, M. 1980. Practical Clinical biochemistry, vol. 1.General topics and commoner tests. William Heinemann Medical Books Ltd. London.

Yanan, W,.Yuan Z., Sun M., and Zhu W. 2015. Exploring the effects of different types of surfactants on zebrafish embryos and larvae. Springer Nature. Sc. Rep. article No. 10107.

***