www.cafetinnova.org

ISSN 0974-5904, Volume 10, No. 03

DOI:10.21276/ijee.2017.10.0327

International Journal of Earth Sciences and Engineering

June 2017, P.P. 673-676

Behavioral, Physical and Biochemical Responses Induced by Amoxicillin Exposure from *Cyprinus carpio*

REKHA RAO, BASAVARAJU MANU AND ARUN KUMAR THALLA

Department of Civil Engineering, National Institute of Technology Karnataka, Surathkal, Srinivasnagar Post-575025, Karnataka, INDIA

Email: *rekharao89@gmail.com, bmanu8888@gmail.com, thallpce@gmail.com*

Abstract: *Amoxicillin was tested onto the freshwater fish C. carpio (fingerlings and adult). The motivation was to understand and analyze the toxicity of amoxicillin (AMX) exposure on Cyprinus carpio, in terms of behavioral, physical, and biochemical abnormalities in the target fishes. The behavioral changes were analysed using parameters: Swimming and Breathing Patterns, and Appetite. The Changes in Physical Appearances: discoloration of scales accounted for the physical abnormalities. The Enzyme Assays: Acid phosphatase, Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Lactate dehydrogenase, Acetylcholine esterase, conducted on the liver, muscle and brain samples of the fish were the indicators of the biochemical changes in the fishes. The concentrations tested on the fishes were chosen in a way to identify the Lowest Observed Effect Concentration, LC-50 (Lethal Concentration) and 100% mortality for both the fingerlings and the adult Cyprinus carpio. It was found that the Lethal Dose (100% mortality) for fingerlings is less than Lowest Observed Effect Concentration of adult Cyprinus carpio. Also, Amoxicillin is toxic to the liver. Liver failure of the target fishes led them unto death.*

Keywords: *Amoxicillin, enzyme assay, behavioral responses, Cyprinus carpio*

1. Introduction

The pollution of freshwater sources is a primary concern. It substantially affects all life forms- aquatic as well as terrestrial (Wright, P. A. [1]; Li, W. *et al.* [2]). The fresh water sources with a dump of pharmaceutical wastewaters are even more concern. Due to the complexity of the compounds and their frequent use in both human and veterinary medicines, they become a severe threat to the quality of water and the inhabitants of the same (Corcoran, J. *et al.* [3]; Gao, L. *et al.* [4]).

1.1 Literature Review

The onset of toxicity is first noticed in behaviors. Change in normal behaviors is not natural in fishes. The *Cyprinus carpio* (*C. carpio*) are known for their considerable adaptability as well as relative vulnerability (Lu, X. *et al.* [5]). The observation of changes in behaviors and physical appearances complement the results obtained by Enzyme Assays (Tocher, D. R. [6]).

1.2 Objectives

The objective was to understand and analyze the toxicity of amoxicillin (AMX) exposure on *Cyprinus carpio*, in terms of behavioral, physical, and biochemical abnormalities in the target fishes.

2. Materials and Methodology

The fingerlings chosen were of size: 1-1.5 inch, weight: ~1.2mg and adults were 3.5-4 inch; weight: ~4.2mg for this study. They were bought from College of Fisheries, Mangalore, India and maintained in a glass tank. The amoxicillin (AMX) concentrations used on fingerlings were 2, 5, 8, 10, 11, 13, 15mg/L and 25, 40, 70, 80mg/L on adults. The concentrations were selected such as to observe Lowest Observed Effect Concentration (LOEC), LC-50 and 100% mortality. The objective of this study was to understand and analyze the toxic effects of AMX exposure on *C. carpio*, on the functioning of vital tissues like liver, muscle and brain; abnormalities in physical appearances and behaviors, along with identification of sublethal and lethal concentrations.
Behavioral and physical changes were recorded for parameters such as swimming patterns (swimming speed, distance and direction of swimming) (Kane, A.S. *et al.* [7]), appetite (regularity of food intake), breathing patterns, and scale color. The behavioral and physical changes were observed every hour and were recorded and tabulated manually. If fishes survived the 4-day (96hrs) run time, they were dissected to obtain the liver, brain and muscle tissues. The tissues were homogenized using 10% saline (Lomholt, J. P., & Johansen, K. [8]).

The water quality was inspected daily and the regular parameters like pH, Dissolved Oxygen levels and Turbidity levels were found to be considerably consistent and well within limits. The Enzyme Assays: Acid phosphatase (ACP) (EC 3.1.3.2), Alkaline phosphatase (ALP) (EC 3.1.3.1), Aspartate aminotransferase (AST/GOT) (EC 2.6.1.1), Alanine aminotransferase (ALT/GPT) (EC 2.6.1.2), Lactate dehydrogenase (LDH) (EC 1.1.1.27), Acetylcholine esterase (AchE) (EC 3.1.1.7) were carried out using

Received: January 02, 2017; Accepted: May 21, 2017; Published: June 30, 2017
International Journal of Earth Sciences and Engineering, 10(03), 673-676, 2017, DOI:10.21276/ijee.2017.10.0327

standard procedures (Hayes, A. W. [9]). Protein estimation was done using Lowry *et al*. method. The enzyme activity was calculated and graphs were plotted. The statistical analysis of the results was done by using Newman-Keul's test and Duncan's multiple-range test. Values are expressed as mean ± SD, with the significance at $P \leq 0.05$.

3. Results and Discussion

The liver samples undergo ACP, ALP, AST and ALT enzyme assay. The muscle samples undergo AchE and LDH enzyme assays and brain samples undergo only AchE enzyme assay.

3.1 Behavioral and physical abnormalities

At the concentrations ≥10mg/L of AMX, the fingerlings and adults alike would be very lethargic for about 5-6hrs. They also schooled themselves in a lower posterior corner of the tank. No free, normal movement within the tank was observed. Hyperventilation was also observed. Subsequently, lack of appetite followed by complete respite from food intake was recorded. They sometimes drifted to some other lower corner of the aquarium, but hardly came to the surfaces. They lay low and motionless. Other than this, there was no other motion-related response. So again, the quantification of swimming capacity goes void.

The breathing went from shallow to ragged followed by direct breathing at the water surface, from the atmosphere directly (suffocation symptom). It went from Normal to Hypoventilation and then Hyperventilation. The physical impacts were in the form of change of fish scale color from silvery to deep gray. Again, the implications of AMX exposure on *C. carpio* (fingerlings and adults, alike) can be put together as: discoloration of scales, hyper lethargy, suffocation, desperation, death. The discoloration was more obvious in adults than in fingerlings.

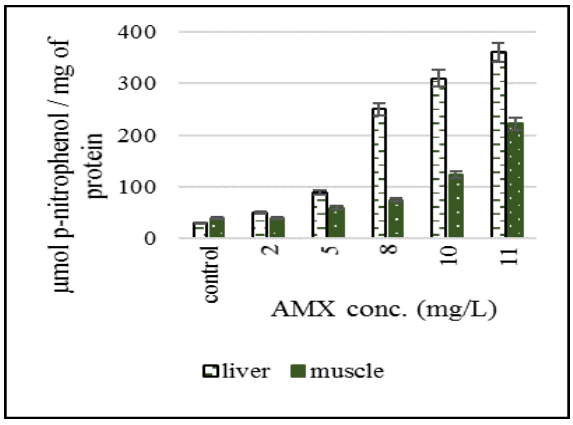

Figure 1. *ACP levels in Liver and Muscle in Fingerlings C. carpio due to AMX intake**

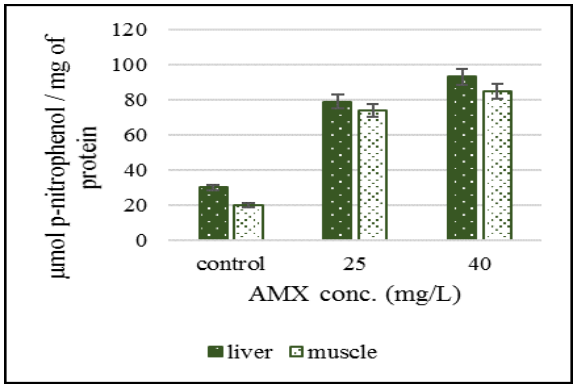

Figure 2. *ACP levels in Liver and Muscle in Adult C. carpio due to AMX intake**

3.2 Biochemical changes

The protein levels were estimated for both non-exposed and exposed fish. Undesirable conditions facilitate/block expression of particular proteins. Increased protein levels were observed in liver, muscle and brain samples for all the tested concentrations of AMX in both fingerlings and adults. The ACP (Figure 1 and 2) and ALP levels (Figure 3 and 4) were estimated in both liver and muscle samples. The impact is not distinctively noticeable for <5mg/L concentrations for fingerlings; <25mg/L for adult, and no mortality observed for those dosages.

Then it rose in dose-dependent manner and was enough high in the dosages where mortality was observed. This confirms the rise is apparently due to less extensive harm and significant rises are indicative for extensive tissue damage leading to death. The change in behavior was as described earlier. It also showed less appetite compared to fish in the control tank. The significant increase gradually had a toll on the fish behavior as well as its health.

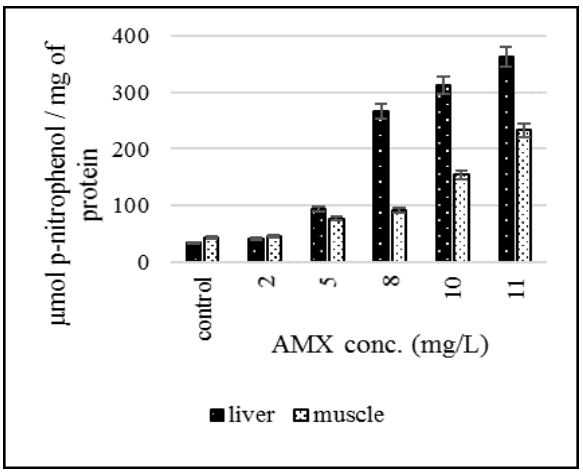

Figure 3. *ALP levels in Liver and Muscle in Fingerlings C. carpio due to AMX intake**

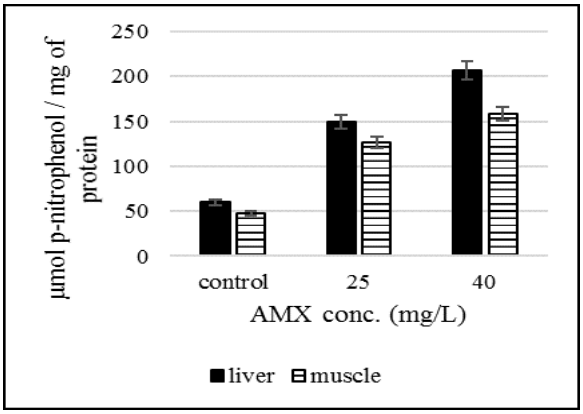

***Figure 4**. ALP levels in Liver and Muscle in Adult C. carpio due to AMX intake**

Like others, AST and ALT levels in liver (Figure 5 and 6) for fingerlings and adults rose significantly at all LOEC. The overall health of the fish had undergone significant changes on the negative side. There were only minor toxicity symptoms in the muscle and brain samples. The liver samples, however, show maximum deviation from a std. linear graph; which implies that the liver tissues suffered maximum damage when compared to muscle and brain tissues.

3.3 Toxicity in Liver

The AMX showed major impacts on the liver of the fishes. It is due to increased lysosomal activity in the liver and muscle (Gül, Ş. *et al.* [10]) It is apparent that AMX increased ACP activity in the liver by interacting with lysosomes. AMX, being a moderately lipophilic substance, could interact with the plasma membrane and cause alteration in its function. ALP is basically a membrane-bound enzyme, and any perturbation in the membrane property caused by interaction with AMX could lead to alteration in the ALP activity.

The rise in AST and ALT points at the fact that the AMX stimulates the glutamate transaminase activity, which is a mitochondrial enzyme (Anderson, P. M. [11]) Increased glutamate transaminase activity in the liver is due to toxic injury brought about by AMX (Zhang, J. *et al*. [12]).

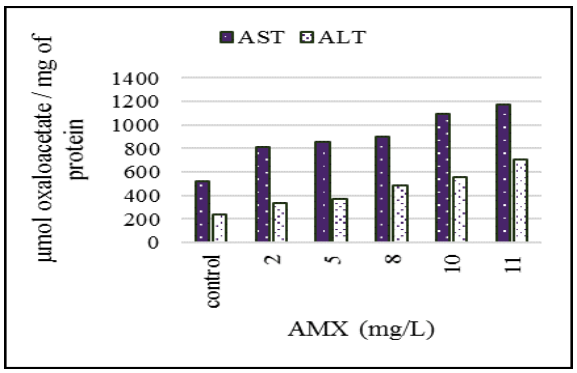

***Figure 5**. AST and ALT levels in Liver in C. carpio Fingerlings due to AMX intake**

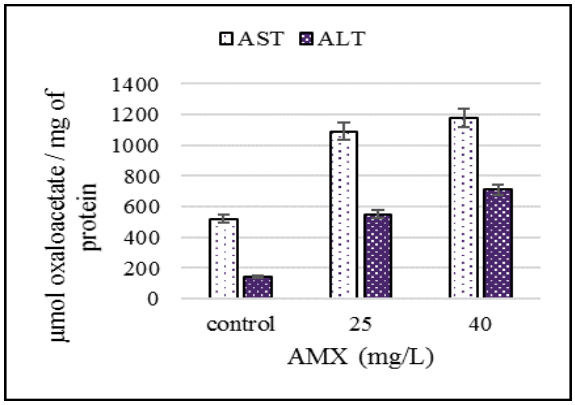

***Figure 6**. AST and ALT levels in Liver in Adult C. carpio due to AMX intake**
**significance given at mean ± SD where P ≤ 0.05*

Thus, fish exhibits decreased respiration rate as a response to the stimulus. The discolored scales were indicative of loss of selective-permeability and initiation of fin rot, henceforth allowing full access to AMX into the body. Since the muscles were susceptible by then, sluggish behavior and irregular swimming patterns were observed henceforth (Minguez, L. *et al.* [13]; Ramos, A., *et al.* [14]).

4. Conclusions

Amoxicillin has mild lipophilicity but due to the vulnerability of the fingerlings, the compound was able to disintegrate the scales enough to gain access to fish blood stream and thus accelerate the toxic effects. It also caused liver failure. It repeats similar effects on adults, thus conforming that, extensive liver damage is the cause of the mortality. It can also deter the physical and behavioral patterns of the fish. It can interfere with the body mechanisms and thus cause toxic effects onto the fish population.

The damage is inflicted at the tissue level in the form of disruption of membranes, dilution of blood and vital fluids, blocking of certain metabolic pathways and variation in enzyme activity levels. The fingerlings succumbed to death at lower concentrations than the adults.

Fingerlings: The 8mg/L is the LOEC. 50% mortality was observed at 11mg/L at 36hrs. 100% mortality was observed for 15mg/L in 45hrs.

Adults: 25mg/L as the LOEC after 96hrs. The 40mg/L and 80mg/L were the 50% and 100% mortality rates at 72 and 48hrs respectively.

References

[1] Wright, P. A., "Nitrogen excretion and enzyme pathways for ureagenesis in freshwater tilapia (oreochromis niloticus)", Physiological Zoology, PP. 881-901, 1993, DOI: 10.1086/physzool.66.6.30163745

[2] Li, W., Shi, Y., Gao, L., Liu, J., & Cai, Y., "Occurrence of antibiotics in water, sediments, aquatic plants, and animals from baiyangdian

lake in north china", Chemosphere, 89(11), PP. 1307-1315, 2012, DOI: 10.1016/j.chemosphere.2012.05.079

[3] Corcoran, J., Winter, M. J., & Tyler, C. R., "Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish", Critical Reviews in Toxicology, 40(4), PP. 287-304, 2010, DOI: 10.3109/10408440903373590

[4] Gao, L., Shi, Y., Li, W., Liu, J., & Cai, Y., "Occurrence, distribution and bioaccumulation of antibiotics in the haihe river in china", Journal of Environmental Monitoring, 14(4), PP. 1247-1254, 2012, DOI: 10.1039/C2EM10916F

[5] Lu, X., Dang, Z., & Yang, C., "Preliminary investigation of chloramphenicol in fish, water and sediment from freshwater aquaculture pond", International Journal of Environmental Science & Technology, 6(4), PP. 597-604, 2009, DOI: 10.1007/BF03326100

[6] Tocher, D. R., "Fatty acid requirements in ontogeny of marine and freshwater fish", Aquaculture Research, 41(5), PP. 717-732, 2010, DOI:10.1111/j.1365-2109.2008.02150.x

[7] Kane, A.S., Salierno, J.D., and Brewer, S.K. "Methods in Aquatic Toxicology: Fish models in behavioral toxicology, Automated techniques, updates and perspectives", Vol. 2, Ch. 32, Lewis Publishers, PP. 559-590, 2005, ISBN: 9780203501597

[8] Lomholt, J. P., & Johansen, K., "Gas exchange in the amphibious fish, amphipnous cuchia", Journal of Comparative Physiology, 107(2), PP. 141-157, 1976, DOI: 10.1007/BF00691221

[9] Hayes, A. W., "Principles and Methods of Toxicology" CRC Press, 2007, ISBN: 9781420005424

[10] Gül, Ş., Belge-Kurutaş, E., Yıldız, E., Şahan, A., & Doran, F., "Pollution correlated modifications of liver antioxidant systems and histopathology of fish (cyprinidae) living in seyhan dam lake, turkey." Environment International, 30(5), PP. 605-609, 2004, DOI: 10.1016/S0160-4120(03)00059-X

[11] Anderson, P. M., "3 urea cycle in fish: Molecular and mitochondrial studies", Fish Physiology, 14, PP. 57-83, 1995, DOI: 10.1016/S1546-5098(08)60242-3

[12] Zhang, J., Shen, H., Wang, X., Wu, J., & Xue, Y., "Effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of freshwater fish carassius auratus", Chemosphere, 55(2), PP. 167-174, 2004, DOI: 10.1016/j.chemosphere.2003.10.048

[13] Minguez, L., Pedelucq, J., Farcy, E., Ballandonne, C., Budzinski, H., & Halm-Lemeille, M., "Toxicities of 48 pharmaceuticals and their freshwater and marine environmental assessment in northwestern france", Environmental Science and Pollution Research, 23(6), PP. 4992-5001, 2016, DOI: 10.1007/s11356-014-3662-5

[14] Ramos, A., Correia, A., Antunes, S., Gonçalves, F., & Nunes, B., "Effect of acetaminophen exposure in oncorhynchus mykiss gills and liver: Detoxification mechanisms, oxidative defence system and peroxidative damage", Environmental Toxicology and Pharmacology, 37(3), PP. 1221-1228, 2016, DOI: 10.1016/j.etap.2014.04.005