Journal of Life and Social Sciences
eISSN: 3006-2675
www.bbasrjlifess.com
*J. Life Soc. Sci, 2025; Volume, **4**: 34*

Original Research Article ***Open Access***

EVALUATION OF TOXIC EFFECTS OF XYLENE IN *LABEO ROHITA L.*

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(Received, 7th September 2023, Revised 20th December 2024, Published 1st January 2025)

Abstract *The aquaculture farming of aquatic animals is an important source of food in Asia and worldwide. Aquaculture includes a stream, a lake the ocean, or an estuary. Present research work aimed to estimate the toxic effect of xylene on Labeo rohita. For the experimental study, Fish were collected from Manawa Hatchery, Lahore, Pakistan. Forty selected fish were divided into different four groups. To compare the toxic effects of xylene in different concentrations were applied ranging from 5ml, 10ml, and 15ml xylene up to 30 days. The blood of fish was collected individually from every group using standard methods and blood samples of different fish samples were also tested through different standard protocols. It was found from results that the complete blood count of fish was negatively correlated with xylene, as white blood cell (WBC), red blood cell (RBCs), and complete blood count (CBC) reduce with an increase in the concentration of xylene. The results suggested that waste and or use of xylene should be controlled to avoid losses through diseases and toxicity in fish as well as other aquatic animals.*

*[Citation: Kiran, N., Waleed, R., Hayyat, Q., Sherazi, S.H.U.H., Ishaq, S. (2025). Evaluation of toxic effects of xylene in labeo rohita L. J. Life Soc. Sci, **4**: 34]*
Keywords: *Labeo rohita; xylene; complete blood count (CBC); toxic; Aquaculture*

Introduction

The aquatic organisms may include plants, and various categories of fishes; crabs, shrimp, clams, mussels, oysters etc. (Mizuta et al., 2023). Aquaculture can serve a variety of purposes like securing endangered species, harvesting any commercially important crop, or raising the fish for stocking public water (i.e, commercial fishing or sport fishing purposes) (Mizuta et al., 2023). Aquaculture is also a source of employment for over 41 million people (Action, 2020). Sustainable aquaculture is very important for providing nutritious food and is earning a livelihood for millions of mankind (Rasak et al., 2023). Fish is also good source of essential fatty acid that is necessary for development of body and brain (Khalid et al., 2022). Aquaculture production has been reported to increase by 7-11 folds per year in the last three decades (Bartley, 2022). 2012 survey reported the utilization of approximately 44 million metric tons (mmt) of finfish,15mmt of mollusks,6mmt crustaceans, and 23.8 mmt of aquatic plants (Eskola et al., 2020). Also, global demand for fish meal and fish oil increased by three folds in the aquaculture feed industry. While aqua culturing, natural aquatic resources are consumed and are transformed into commodities that may have a direct or indirect impact on biodiversity (Naylor et al., 2021). The magnitude of aquaculture impact depends upon the type of resources used, species type to be cultured, intensity of farming system and methods and management (Ferreira et al., 2021). Cages, tanks and pond are some alternates to farm finfish. By providing the supplementary feeds, tropical and subtropical fresh water herbivores or omnivores species, especially majority of the carps are grown in ponds (Jana). While on the contrary, temperate and tropic species of marine water and most diadromous are provided with fish oil-based diet or whole fishmeal nutritious diet and are reared in floating net cages (Delgado and Ruzzante, 2020). This intensive or semi-intensive farming costal pond arrangement result to disturb the biodiversity, decreased DO in water, also detrimental metabolites flushed out due to diluted sea water pollution m (Tran et al., 2022). Although aquaculture belief to redirect the flow of energy of an ecosystem but it would always have consequence to the environment and biodiversity to

some extent (Prakash and Verma, 2022). Aquaculture systems can be used as nature's subsidies for the sustainable production of aqua species on local and global scale (Gephart et al., 2020). Aquaculture needs to expand in order to improve production of aquatic food in tuned with the ecosystem (Boyd et al., 2020). Fishes are cold blooded creatures who have streamline body. They have cartilaginous or bony structures and latera1 line sensory organ (Webb, 2023). It is also enriched with omega 3 polyunsaturated fatty acid. Rohu (*Labeo rohita*) was the most important of the three Indian major carp species used in carp polyculture systems (DEEPTI, 2021). It has effectively established itself in almost every riverine system in India, including the freshwater of Andaman(Zhang et al., 2023). This carp's traditional culture extends back hundreds of years in the little pons of Eastern Europe (Kang et al., 2023). This fish can be found in Pakistan, India, Nepal, Bangladesh, Burma, Thailand, China, Cambodia, and Sri Lanka (Perera et al., 2022). The *Labeo rohita* is a large silver-coloured fish with a cyprinid body shape (Clavel and Morlon, 2020). Each scale usually has a red mark on it beneath (Munsell, 2022). The average size of *Labeo rohita* that can be harvested is 1-1.5 kg, and it takes 8 to 12 months to attain this size (Okmalasari and Prakasa, 2023). The snout was depressed and extends beyond the mouth, the eye dorsolateral are hidden views from the outside of the head the mouth is small, and the inferior lip is absent (Baker et al., 2022). A single dose of 0.4-0.5 ml body weight was given to female fish and 0.2-0.3 ml to male's fish was utilized when synthetic formulations were use (DHARA et al., 2023).

A spaying breeding tank, an incubation hatching tank, and a water storage and delivery system are the three primary components of this type of hatchery (Rajts et al., 2022). The density of brood stock varies from (5 kg to3kg). The breeding tank can have a depth of up to 1.5m of water. Several chemical industries use the petroleum source to manufacture xylene (Shi et al., 2021). Generally, wellbore soaking using diesel and xylene is done on the petroleum production system to remove the formed organic plugging (Walters et al., 2020). However, these chemicals exert passionate effects and constant threats to field personnel and environment through storage and its flow backs into the waste pit and to the aquatic system (Morrison, 2022). A concern that contamination could harm the health and genetic makeup of fish and shellfish stocks has grown recently (Fox et al., 2020). Xylene was generated in the United States using catalytic reforming of petroleum (approximately 95%). In the United State, there were 20 mixed xylenes, producing facilities (Dantas et al., 2022). Collectively, these three isomers of xylene are known a ttal xylene (Ye, 2022). Xylenes are extracted from the catalytic reforming as part of the BTX aromatics benzene, toluene, and xylenes. It's a solvent used in the printing, rubber, and leather industries, cleaning agents, paint thinners and varnishes (Müller and Schackmann, 2023). Wax is not soluble in water; hence xylene is used instead (Theyab, 2020). Xylene still has no antidote (Razak et al., 2020). The meta and para isomers, which have comparable boiling temperatures, are separated from the less volatile ortho isomer via fractional distillation (Hermann et al., 2023). It also affects one's hearing, it irritates the skin when it comes into contact with it (Yliluoma and Palonen, 2020). This chemical is also required for the manufacture of xylene chlorides and xylene bromides (Dutta and Bhat, 2023).

Material and Methods

This study was carried out at the University of Lahore's Institute of Molecular Biology and Biotechnology (IMBB) Zoology Lab. 40 fresh water *Labeo rohita* samples were collected from a Manawa Hatchery, Lahore, Pakistan. Average weight of these samples was 40g and length ranged 3-4 inch. These samples were taken to the fish tank house of the Institute of Cellular Biology and Biotechnology (IMBB), University of Lahore, in polythene bags that were filled with oxygen and water. Since only healthy fish were employed in the experiment and all samples were acclimated to laboratory settings for two weeks, the obtained fish fingerlings were treated with 0.01% $KMnO_4$ solution for 15 minutes and monitored for any injuries or diseases during that time. Once a day, fish were fed a full and typical amount of food. Third of it was replaced in alternate days with tap water to eliminate any form of contamination that might have occurred. The water physiochemical parameters were determined daily in the study. The fish were put in four equal groups each containing / consisting of 10 fish. Each group was placed into different tanks with a tank capacity of 60L depending on their respective labels of group A, B, C and D. The first group was the control group and was fed with normal water that was not treated in anyway. Others three groups were experimental groups. Xylene doses of different concentrations (5mL,10mL and 15mL concentration) were prepared by disolving required amountin solvcnt (Sathishkumar et al., 2024). The solutions were renewed once in a 48h exposure period where the 30 L volume of water was removed and substituted by the fresh water to maintain the drug dose at the petty constant concentrations. After the completion of trial fish was removed from aquarium and blood samples were collected by cardio-puncture of fish using 1 CC plastic disposable syringe. After collection blood was transferred immediately into EDTA tubes in order to prevent blood from clotting. Haematology analysis of CBC count was done by " sysmex XP-100"', which followed the following principle for measuring RBCS, WBCS, platelets count etc. Other factors such as MCV, MCH, MCHC were demonstrated using standard formulas (Ozawa and Sakaue, 2020).

Results

Complete blood count of *Labeo rohita* was analyzed after 30 days exposure with 5mL,10mL and 15mL

dose concentration of Xylene and results showed gradually decrease in RBCs, WBCs count as concentration of dose increased as compared to control group. However, WBCs count increased on 5mL exposure and then decreased as the dose of Xylene increased. A significant difference in WBCs was recorded in dose dependent manner to that of the control group. Also, mean corpuscular volume (MCV), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) also decreased in dose dependent manner when compared to control group. For determining the kind of anaemia and evaluating the size and shape of erythrocytes, haematological indices including MCH, MCV, and MCHC are essential. Whether or not cell division during erythropoiesis is normal is indicated by the values of MCV and MCHC. In contrast to the control group, the experimental groups' MCV, MCHC, and MCH levels progressively decreased as the concentration rose in this investigation. Haematological indices of *Labeo rohita* with respect to xylene concentrations is described in the given analysis (Figure 1).

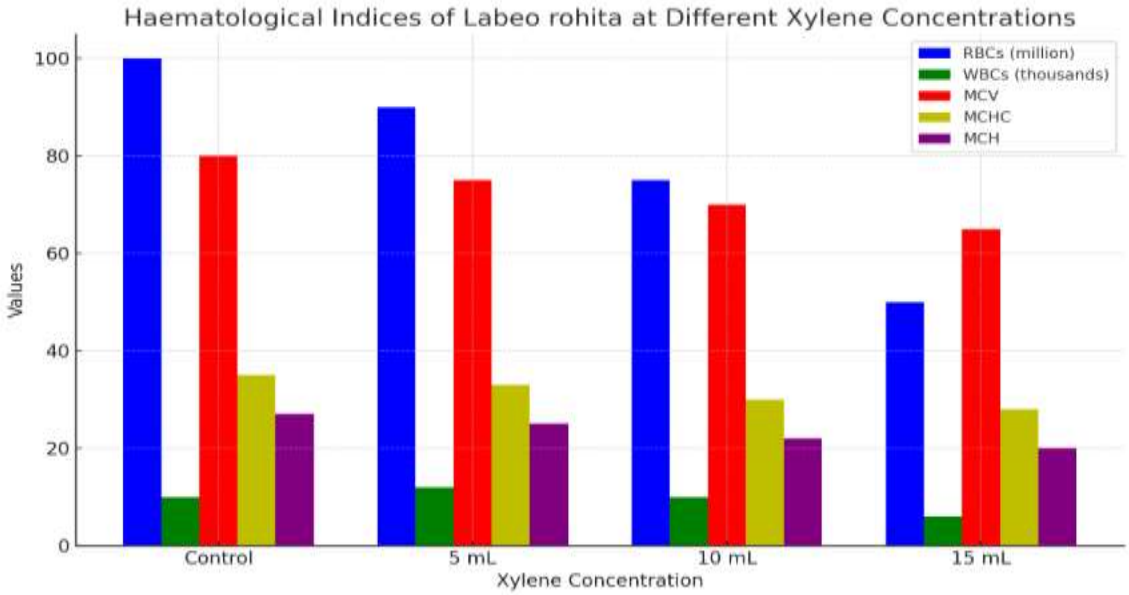

Figure 1. Haematological indiced of Labeo rohita at different xylene concentrations

Low mortality was recorded any concentration of Xylene exposure to *Labeo rohita* over the trial duration. There were able to detect some behavioural abnormalities which includes; they were more secreting mucus, they exhibited less swimming, feeding behaviour was also considered.

Discussion

Constituting a large part of the demand for aromatic hydrocarbons, xylene being one of the thirty most manufactured chemicals in the United States based on volume. Xylene is produced by chemical companies from petroleum (Condle et al., 1988). When dissolved, they release fish membranes into the blood stream and transport them into the tissue cells to be further split into other toxic components which act on the macromolecules of the exposed fish (Lewis et al., 2007). Histopathological analysis proved useful in identifying the influence of chemicals on an organism, which was used as a biomarker that represents the overall health of ecosystem residents (DHARA et al., 2023).

Other related similar findings negative effect on haemoglobin caused by Xylene exposure also highlighted by many other researchers (Chakraborty et al., 2024). This inhibited haemoglobin content in Xylene exposed fish may attributed to damage or destroy haemopoietic tissue to exposed fish (Mandal et al., 2024). Low MCH, MCV and PCV indicate the possible reduced size of RBCs due to the present study discovered anaemia in *Labeo rohita*'s gills and muscle tissue, which may be related to erythropoiesis caused by Xylene poisoning. WBCs are the white blood cells in living body, which fights against every outside invader or disease and infection causing matter. This remains the case when an infection causing agent enters the body, the quantity of WBCs rises. That is if there is an increase of WBCs then there is presence of a toxic agent studies (Boyd et al., 2020). Certain biomarkers such as WBCs increased in some studies our study however, showed contrary results with a decrease in WBCs. However, WBCs were high in the 5mL of low dose exposure, but afterwards, the sample reduced the levels of WBCs in the high dose exposure. This reduced in WBCs were parallel to the study conducted, this result may imply that our fishes might had a low immune competence or some environmental stressors such as low water temperature which may reduce WBC countages. Xylene is also known to delay the lymphocites mitogenesis (Condle et al., 1988) and for the production of MCP-1(Monocyte chemoattractant protcin-1). The low doses of xylene interfere with the

immunity aid cytokine level. In the same way as mentioned above the decrease in Hot levels is an indication of anemia in fish given that it is an expression of the physical response of the body of fish due to stress after exposure to Xylene (Suaidi et al., 2022). This could be due to reduction of Hb levels leading to a low MCHC (Niaz et al., 2015). There is tension with MCH and MCV values in which in this research it has slightly declined (Cakmak et al., 2020).

Conclusion

In this present study, Xylene caused damages were observed in *Labeo rohita* blood cells with different doses of concentration.

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Declarations

Author Contribution statement

NK performed experiment. RW, SHUHS, QH, and SS performed data analysis and helped in interpretation of results. All authors read final version of manuscript and approved for publication.

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Funding

Not applicable

Conflict of Interest

Regarding conflicts of interest, the authors state that their review was carried out independently without any affiliations or financial ties that could raise concerns about biases.