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Research Article

Haematological Changes in Fish *Labeo rohita* Exposed to the Azo Dye Tartrazine

Athira N* and Jaya DS

Department of Environmental Sciences, University of Kerala, India

***Corresponding Author:** Athira N, Department of Environmental Sciences, University of Kerala, India. **Email:** n.athira20@gmail.com

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Abstract

For the present study, azo dye tartrazine (acid yellow 23) having wide range of application in textiles and synthetic foods, was used to assess the toxic effects and biomarker responses in fish *Labeo rohita* (Hamilton, 1822), commonly known as rohu. In toxicity studies haematological estimation is recognized as significant before many years. Acute (T1, T2 and T3 for 96 hours) and chronic (CT1 and CT2 for 75 days) bioassays were conducted. Major physico-chemical characteristics of aquaria water were analysed and recorded in regular intervals of experiment. After the experimental period, blood was collected and major haematological parameters; Hb, TC, RBC, PCV, MCV, MCH and MCHC were analysed. The results show that parameters Hb, RBC, PCV, MCV, MCH were significantly reduced and TC was significantly increased in highest concentration of acute test (T3) and in chronic tests (CT1 and CT2). The changes in haematological parameters can reflect the physiological status of animal and indicates the environmental conditions where they being. The experimental study establishes that haematological indices such as Hb, TC, RBC, PCV, MCV, and MCH are good biomarkers of the tartrazine exposure in fishes..

Keywords: Azo Dyes; Acid Yellow 23; Fish Biomarker; Haematological Indices; Tratrazine

Abbreviations

COD: Chemical Oxygen Demand; K^+EDTA: Potassium Ethylene Diamine Tetra Acetic Acid; Hb- Haemoglobin; TC: Total Differential Count; RBC: Red Blood Corpuscles; PCV- Packed Cell Volume; MCV: Mean Cell Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Introduction

Dyes are the colorants become an essential component in textiles, cosmetics, junk foods and pharmaceutical products. Due to the wide range of applications, dyes can be present in wastewaters released from several industries and the effluent contamination of aquatic resources forms a global concern. In the case of synthetic dyes such as azo dyes, conventional treatment technologies are not successful because of the complex nature, high stability and lack of information regarding its toxic effects.

Dyes and pigments are important industrial chemicals widely used in textiles, cosmetics, food stuffs, beverages and pharmaceutical products. Among the synthetic dyes, the usage of azo dyes is significant because of their properties like good fastness, high stability, wide range of gamut, simple mode of application and low cost [1]. Some of the azo dyes are banned in several countries due to toxicity, non-biodegradability, carcinogenicity and mutagenicity [1]. Unfortunately developing countries are still depending on these synthetic colorants. So, dyes and their degradation products are detectable in several industrial effluents (textile, paper, cosmetics and food industry) and municipal sewage. The aquatic ecosystem receives both treated and raw effluents leading undesirable changes to water body [1,3]. The azo dyes are highly water soluble, bright coloured and resistant to conventional treatment methods, pose high ecological risk. Industrial effluents can make undesirable changes in water quality which leads to massive destruction of aquatic flora as well as fauna.

Fish forms a non-target organism for several contaminants and can be used as a bio-indicator for assessing ecosystem health. Chemical poisoning induced changes can be easily monitored in body fluids of higher order organisms like fishes. So, the haematological parameters can be successfully employed as the physiological and pathological indicator in environmental monitoring. The azo dye tartrazine or acid yellow 23 is widely using in textiles, cosmetics and food stuffs and there are no other scientific documentations on its toxicity in lower vertebrates. Therefore, tartrazine was selected for bio-marker studies in Fresh water carp- *Labeo rohita* (Hamilton, 1822) [4]. Present experimental study aimed to evaluate the alterations in haematological indices of fishes exposed to acid yellow 23 in both 96 hours acute study and 75 days chronic study.

Materials and Methods

The azo dye, acid yellow 23 or Trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(*E*)-(4-sulfonatophenyl) diazenyl]-1*H*-pyrazole-3-carboxylate (95%) was purchased from the TCI chemicals (Tokyo Chemical Industry Co., Ltd., India). Experimental animal in the present study is Indian major carp- *Labeo rohita* (Hamilton, 1822) commonly known as 'Rohu' was brought from the National fish seed farm at Neyyar Dam, Department of Fisheries, Kerala State Govt., Thiruvananthapuram, Kerala. After disinfection the fishes were acclimatized to the laboratory conditions for 21 days with *adlibitum* diet. For both acclimatization and experiments chlorine free water was used and major water quality parameters were analysed according to the standard procedures in (5). 42 healthy fishes of 15 ± 2 cm length and 28 ± 2 gram body weight were selected for the experimental study and are divided into test and control groups. For the acute studies, 10 ppm (T1), 30 ppm (T2) and 60 ppm (T3) of acid yellow 23 were used and the fishes were exposed for 96 hours in the aquarium (25L). For the chronic studies 5 ppm (CT1) and 10 ppm (CT2) concentrations of the dye were used and exposed the fishes for 75 days under controlled conditions in aquarium (25L). The control group fishes were also maintained with both acute and chronic tests and the tests were done triplicates. During both static-acute and static renewal-chronic assays, the important water quality parameters were determined and fish behaviour, swimming patterns etc. were also noticed and recorded. After the experimental period, blood samples were collected from the caudal peduncle of fishes, in EDTA (K^+ EDTA) tubes and major haematological indices – Haemoglobin (Hb), Total Count (TC), Red Blood Cell count (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) were analysed following the standard procedures [6].

The analytical data obtained were expressed as mean ± SD (standard deviation), and the comparison between the control group (C and CC) and test groups (T1, T2, T3, CT1 and CT2) were made by Analysis of Variance (ANOVA) in SPSS 15 for windows. The level of significance was considered at $P < 0.001$.

Results and Discussion

Water quality parameters

The results of the important physico-chemical parameters of water in the acute and chronic assay are given in table 1 and figure 1a to 1e. The mean values of T1, T2 and T3 for acute and CT1 and CT2 for chronic experiments are referred as test value.

In acute assay the water quality parameters were analysed at regular intervals of 12, 24, 48, 96 hours. The parameters colour, pH, dissolved oxygen, dissolved carbon dioxide, chemical oxygen demand and ammonia show variations in the subsequent intervals of exposure periods. In chronic assay, renewals of aquaria water were done around 15 times and the major physico-chemical characteristics of water were analysed each 1st and 5th days of renewal up to 75 days. The test values are the mean of both CT1 and CT2.

Very small amount of dye can cause high visibility in water because of the bright colour. The colour decreases after 48 hours may be due to the absorption in fish body and photodegradation. The rise in pH is associated with the ammonia production in water as result of fish excretion. As the aquaria water is stagnant and highly coloured there was a chance of oxygen deficiency and raise in dissolved carbon dioxide. The dye tartrazine itself causes change in COD of water just after the addition because of the presence aromatic amines formed by the dissociation of complex dye in water. Experimental fishes show avoiding behaviour in feeding and hyperactivity as abnormal swimming and jumping. As a result of fish excretion concentration of ammonia and dissolved carbon dioxide increased in water leads to rise in pH.

The major changes in water quality parameters are more visible in the acute experiment than in chronic experiment because of the nature of assay. In acute experiment, it was due to the non-renewal of test solutions and comparatively high concentrations of azo dye, acid yellow 23 (tartrazine) used in the experiment. The water quality parameters like pH, temperature and colour can influence the other physico-chemical characteristics of water and toxicity of contaminant present in it. And also, the poor quality of water affects the health and immunity of organisms like fishes in it. This may lead in the easier toxicological response in the fishes due to the presence of a xenobiotic. Toxicity induced abnormal metabolism and decayed cells of test fishes also leads in the water pollution and vice versa. The water quality parameters were changed in accordance with the duration of experiment. Therefore, the present study, points out that tartrazine has high potential to pollute the aquatic systems indicated by changes in water quality.

Water quality parameters- Acute experiments Figure 1a to 1e.

Parameters	Group	1st day	5th day
Temp (⁰C)	Control	28	28
	Test (CT1 and CT2)	28	28
Colour (HU)	Control	0	5
	Test (CT1 and CT2)	550	500
pH	Control	6.68	7.65
	Test (CT1 and CT2)	6.79	7.98
DO (mg/L)	Control	7	6.01
	Test (CT1 and CT2)	6.4	5.2
DCO2 (mg/L)	Control	8.9	10.1
	Test (CT1 and CT2)	10.4	13.4
COD (mg/L)	Control	0	0.8
	Test (CT1 and CT2)	24	59
NH3 (mg/L)	Control	0.21	0.59
	Test (CT1 and CT2)	0.57	1.52

Table 1: Physico-chemical characteristics of aquarium water (Chronic assay).

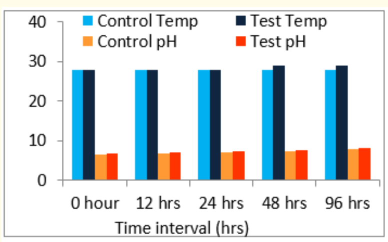
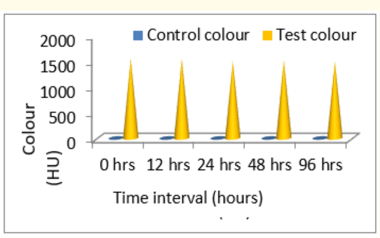


Figure 1a: Variation in temperature and pH of aquaria water -control and test groups,



1b: Variation in colour of aquaria water - control and test groups.

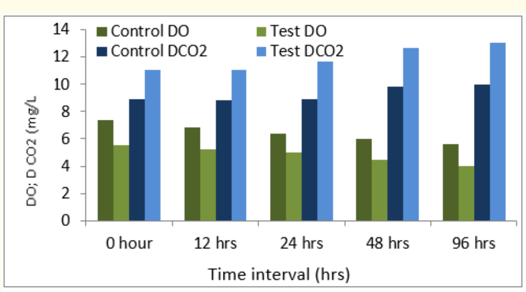
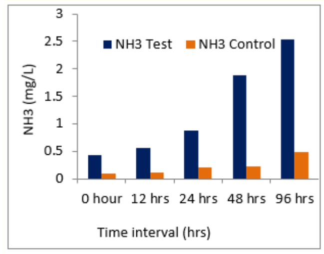


Figure 1c: Variation in DO and DCO2 in aquaria water of control and test groups.



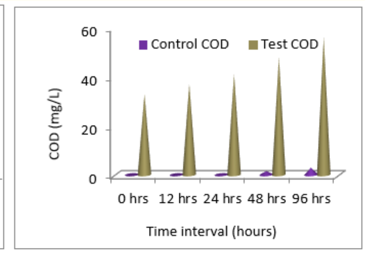


Figure 1d: Variation in ammonia in aquaria water -control and test groups.

Figure 1e: Variation in COD in aquaria water- control and test groups.

Haematological changes in fishes

The results of heamatological studies in fish *Labeo rohita* were given in table 2 and 3. Analysis shows a remarkable decrease in Hb, RBC, PCV, MCV, MCH in highest concentration of acute experiment (T2 and T3) and chronic experiments (CT1 and CT2).

Parameter	Group			
	C	T1	T2	T3
RBC (10^6/mm^3)	3.13 ± 0.10	2.87 ± 0.08	2.79 ± 0.08	2.61 ± 0.09
WBC (10^3/mm^3)	57.58 ± 0.81	58.78 ± 1.13	60.43 ± 0.30	62.17 ± 0.42
Hb (gm/dL)	9.42 ± 0.75	8.87 ± 0.10	8.75 ± 0.15	7.50 ± 0.89
PCV (%)	28.80 ± 0.9	28.08 ± 0.08	27.53 ± 0.22	23.82 ± 0.08
MCV (fL)	92.82 ± 0.08	96.45 ± 0.10	94.38 ± 0.08	85.72 ± 0.12
MCH (Pg)	30.33 ± 0.10	30.63 ± 0.10	31.18 ± 0.08	27.28 ± 0.08
MCHC (%)	32.58 ± 0.17	31.75 ± 0.11	33.15 ± 0.10	31.36 ± 0.12

Table 2: Haematological parameters of Fishes - Acute Experiment.

[All values are mean ± SD; n = 6].

Parameter	Group		
	CC	CT1	CT2
RBC (10^6/mm^3)	3.35 ± 0.11	2.74 ± 0.13	2.32 ± 0.12
WBC (10^3/mm^3)	57.11 ± 0.61	59.32 ± 0.72	64.18 ± 0.94
Hb (gm/dL)	9.40 ± 0.89	7.75 ± 0.11	6.40 ± 0.63
PCV (%)	28.65 ± 0.11	27.38 ± 0.12	21.35 ± 0.16
MCV (fL)	92.68 ± 0.85	92.60 ± 0.14	82.25 ± 0.11
MCH (Pg)	30.82 ± 0.08	30.55 ± 0.19	25.43 ± 0.10
MCHC (%)	32.38 ± 0.12	31.77 ± 0.45	30.87 ± 0.48

Table 3: Haematological parameters of Fishes - Chronic Experiment.
[All values are mean ± SD; n = 6].

In acute experiment, high RBC count was observed in control (C) group fishes as 3.13 ± 0.10 × 10^6 mm^3 and lowest was in T3 group fishes as 2.61 ± 0.09 × 10^6 mm^3. Test fishes (T1, T2 and T3) shows highly significant (P < 0.001) reduction of RBC count with respect to control (C). T3 group fishes also show highly significant (P < 0.001) decrease of RBC count with respect to T1. There is no significant change in RBC count among T1 and T2 and as well as among T2 and T3.

In chronic experiment, highest RBC count was observed in control (CC) group fish as 3.35 ± 0.11 × 10^6 mm^3 and lowest was in CT2 group fishes as 2.32 ± 0.12 × 10^6 mm^3. Test fishes (CT1 and CT2) shows highly significant (P < 0.001) reduction of RBC count with respect to control (CC) group fishes. CT2 group fishes also show highly significant (P < 0.001) decrease of RBC count with respect to CT1.

In the present study, the reduction of RBC count in the tartrazine exposed fishes may be due to the oxygen deficiency induced physiological stress. The water quality analysis also showed low dissolved oxygen content (Table 1 and Figure 1) in aquarium water during the experiment. The significant reduction in the RBC count indicates hypoxic condition, anaemia, erythrocytopenia and haematopoietic tissue damage and this may be due to tartrazine exposure. In chronic experiments, anaemia may be resulted from avoidance behaviour of food and due to the tartrazine induced blood loss by internal bleeding. Haematological study of fish on exposure to toxicants reveals that erythrocytes are the major and reliable indicators of various sources of stress [7].

The haemotoxic substances can cause structural damage in RBC membranes resulting in haemolysis. RBC count may also get altered due to stress related inhibition in release of RBCs from the spleen. Similar decreasing trend in RBC count was reported in fish *Cirrhinus mrigala* treated with leather dyes; bismark brown and acid leather brown [8]. The change in RBC count of test fishes in the present study agreed with the studies [9] in fingerlings of *Labeo rohita* exposed to textile azo dye, C.I. Acid orange 7. Studies in fish *Tilapia mossambica* also reported drastic reduction of RBC count exposed to untreated textile effluent [10].

In acute experiments, total count (TC) of white blood cells was high (62.17 ± 0.42 × 10^3mm^3) in T3 group fishes and in low in control (C) group fishes as 57.58 ± 0.81 × $10^3/mm^3$. T3 group fishes show highly significant (P < 0.001) increase in TC with respect to control group and T1 group fishes, and significant (P < 0.01) increase with respect to T2 group fishes. T2 group fishes also showed significant (P < 0.01) increase in TC with respect to T1 group fishes.

In chronic experiments, highest value of TC (64.18 ± 0.94 × 10^3mm^3) was observed in CT2 group fishes and lowest was in control (CC) group fishes as 57.11 ± 0.61 × 10^3mm^3. CT2 group fishes show highly significant (P < 0.001) increase of TC with respect to CC and CT1 group fishes. CT1 group fishes also show highly significant (P < 0.001) increase of TC with respect to control (CC) group fishes.

The total count (TC) of WBC was generally employed for detecting the lethal and sub lethal effects of toxic effluents in fishes [11]. The major types of WBC in fishes are lymphocytes, monocytes, neutrophils, eosinophils and basophils [12]. Each type WBC has specific features and definite functions in organism body. In the present study both acute and chronic test fishes show increase of TC with respect to control fishes and may be due to the tartrazine induced toxicity. Increase in WBC count in tartrazine exposed fishes was due to induced luecocytosis as a result of immune response against intoxicated dye or due to leukemic condition. An immediate activation of the fish immune system is proved by increase in leucocytes [13]. Total number of leucocytes (as included as TC) is a diagnostic feature of many diseases. The stimulation of the immune system also causes an increase in lymphocytes due to injury or tissue damage and may be responsible for rise in WBC. Similar results were also reported in the studies on *Poecilia reticulate* exposed to methyl red [14]. The leucocytosis was also detected in *Cirrhinus mrigala* exposed to leather dyes [8].

The rise in total number of leucocytes (WBC) indicates the physiological stress to fishes due to the exposure to tartrazine and dye induced changes in water quality parameters of aquaria water (Table 1 and figure 1). Lymphocytes are the most numerous cells which function in the production of antibodies and chemical substances serving as defense against infection. The primary consequence of observed changes in TC in stressed fish is suppression of the immune system and increased susceptibility to disease as suggested by [15]. The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of fish [16]. In the present study, increase in WBC count indicates hypersensitivity of immune system to tartrazine and these changes could be immunological reactions to produce antibodies to cope up with stress induced by the toxicant. During exposure period of chronic experiment TC or WBC got enhanced, indicating that the fish can develop a defensive mechanism to overcome the toxic stress of pollutant [17]. The results of this study agree with the observations in the studies in fish *Heteropneustes fossilis* in which there was significant increase in the total WBC count after exposure to malachite green [18].

In acute experiment, lowest Hb content was observed in T3 group fishes as 7.50 ± 0.89 gm/dL and highest value was observed in control (C) group fishes as 9.42 ± 0.75 gm/dL. Analysis shows significant (P < 0.001) decrease of Hb in test fishes (T1, T2 and T3) with respect to control (C) group fishes. T3 group fishes also show highly significant (P < 0.001) decrease of Hb with respect to T1. There is no significant change of Hb content between T1 and T2 and as well as betweenT2 and T3.

In chronic experiment, lowest Hb content was observed in CT2 group fishes as 6.40 ± 0. 63 gm/dL and highest was observed in control (C) group fishes as 9.40 ± 0.89 gm/dL. Statistical analysis shows significant (P < 0.001) decrease of Hb content in test fishes (CT1 and CT2) with respect to control (CC) group fishes. CT2 group fishes also show highly significant (P < 0.001) decrease of Hb with respect to CT1.

In the present study, the reduction of haemoglobin content in test fishes of both acute and chronic experiments indicates the physiological stress induced by tartrazine. The destruction of red blood cells also causes the reduction or inhibition of Hb synthesis. So, any changes in the Hb content can be employed as an indication of erythrocyte indemnities. The azo dye may inhibit the synthesis of Hb through lowering the nutrient or iron uptake during exposure period. Deficiency of dissolved oxygen also causes the decline of Hb in fishes. Low dissolved oxygen was found in test aquaria water (Table 1 and figure 1) due to tartrazine, which may also lead in the reduction of Hb level in test fishes. Low level can be indicating haemolysis, is related with the destruction of RBCs, and the formation of methaemoglobin (indicates a change to the ferric state). Both haemolysis and methaemoglobin formation diminish the oxygen-carrying capacity of blood [19]. Low Hb in test fishes of present study agrees with the studies in *Labeo rohita* exposed to petrochemical effluents [20]. In chronic experiment, the nutrient imbalances cause the inhibition of RBC and haemoglobin synthesis. Similar RBC and Hb variations were also reported in fish Nile tilapia exposed to dye malachite green [21].

In acute experiment, highest packed cell volume (PCV) was observed in control (C) group fishes as 28.80 ± 0.9% and lowest was in T3 group as 23.82 ± 0.08%. T3 group fishes show highly significant (P < 0.001) reduction of PCV with respect to control (C), T1 and T2. T2 group also shows highly significant (P < 0.001) reduction of PCV with respect to control (C).

In chronic experiment, highest packed cell volume (PCV) was observed in control (CC) group fishes as 28.65 ± 0.11% and lowest was in CT2 group fishes as 21.35 ± 0.08%. CT2 group fishes show highly significant (P < 0.001) reduction of PCV with respect to CC and CT1. CT1 group also shows highly significant (P < 0.001) reduction of PCV with respect to control (CC).

In the present study PCV of test fishes show a clear decreasing trend in experimental fishes especially in T3, CT1 and CT2. Similar observations were reported in fish *Clarias batrachus* exposed to sago factory effluent [22]. The studies on azo dyes in human beings reported high potential health risk is caused by adsorption of azo dyes and their breakdown products (toxic amines) through the gastrointestinal tract, skin, lungs, and also formation of hemoglobin adducts and disturbance of blood formation [23]. So, inhibition of blood formation in dye treated fishes was confirmed. Fingerlings of fish *Catla catla* exposed to different concentrations of the dye reactive red (RR 120) showed marked changes both in their blood cells' morphology and in nuclear material as formation of micronuclei (MN), nuclear buds (NB), fragmented apoptotic cells (FA) and bi-nucleated cells (BN) respectively [24].

In acute experiment, high MCV was observed in T2 group fishes as 96.45 ± 0.10 fL and lowest MCV was in T3 group fishes as 85.72 ± 0.12 fL. T3 group fishes show highly significant (P < 0.001) reduction of MCV with respect to control (C), T1 and T2 group fishes. T2 and T1 group fishes show highly significant (P < 0.001) increase of MCV with respect to control (C) group fishes.

In chronic experiment, high MCV was observed in control (CC) group fishes as 92.68 ± 0.85 fL and lowest MCV was in CT2 group fishes as 82.25 ± 0.11 fL. CT2 group fishes show highly significant (P < 0.001) reduction of MCV with respect to CC and CT1 group fishes. CT1 group fishes show highly significant (P < 0.001) increase of MCV with respect to control (CC) group fishes.

In the present study, MCV increased in T2 group fishes of acute experiment and decreased in other groups (T1 and T3) of acute and all test fishes (CT1 and CT2) of chronic experiment. Increased MCV can be considered as manifestation of normochromic macrocytic anaemia in treated fishes and this result is in accordance with the findings in swiss albino mice fed with tartrazine [25]. As MCV represents the cell volume of erythrocyte, any swellings and shrinkage due to toxicity may reflects in MCV. The variation in MCV of test fishes may differ with respect to the individual fishes and quality of inhabiting water.

The decrease in MCV and aemoglobin content in tartrazine treated fishes indicates that red blood cells shrink, either due to hypoxia or a microcytic anaemia. Reduced haemoglobin weakens oxygen supply to various tissues in fishes leading to slow metabolic rate and hypoxia that promote erythropoiesis. This would impose oxygen deficit in fish, promoting anaerobic respiration as a result of high carbon dioxide level in the blood. Under the existing situation, the fish would start to produce immature erythrocytes as a compensatory and adaptive reaction, in an attempt to transport more oxygen to the tissues. The toxicity of tartrazine in the fish may cause changes in the spleen contraction that reduce the ousting of erythrocytes to the circulatory system. Similar changes of low MCV were also reported in fish *Clarias gariepinus* due to lead (Pb) poisoning [26]. Haemoglobin concentrations reflect the oxygen supply to an organism and the organism itself tries to maintain them as much stable as possible.

In acute experiment, high MCH was observed in T2 group fishes as 31.18 ± 0.08 Pg and lowest was in T3 group fishes as 27.28 ± 0.08 Pg. T3 group fishes show highly significant (P < 0.001) decrease in MCH with respect to control (C), T1 and T2. T2 shows highly significant (P < 0.001) increase in MCH with respect to control (C) and T1.

In chronic experiment, high MCH was observed in control (CC) group fishes as 30.82 ± 0.08 Pg and lowest was in CT2 group fishes as 25.43 ± 0.10 Pg. CT2 group fishes show highly significant (P < 0.001) decrease in MCH with respect to CC and CT1. CT1 shows significant (P < 0.01) decrease in MCH with respect to control (CC).

In the present study, MCH increased in T2 group fishes of acute experiment as a response to tartrazine toxicity. However, MCH was decreased in test fishes of chronic experiment due to the long-term tartrazine exposure and induced inhibition of blood cell synthesis. There may be variation in MCH of test fishes depends upon the individual fishes. The variation of MCH indicates the anaemia due to chemical stress. Tartrazine can affect the blood cell formation and may cause the abnormal sized erythrocytes. Studies in swiss albino mice reported similar changes in MCH treated with tartrazine [27]. In chronic experiments, MCH was decreased in test fishes in accordance with the increasing concentration of exposed tartrazine. Decreased MCH in fish blood might be due to the high level of immature RBC in circulation. The reasons behind the formation of immature RBC include haematotoxicity of tartrazine, oxygen deficiency and inadequate nutrition. Decline in MCH in test fishes of present study agrees with the studies in fish *Labeo rohita* exposed to petrochemical effluents [20].

In acute experiment, high mean corpuscular haemoglobin concentration (MCHC) was observed in T2 group fishes as 33.15 ± 0.10% and lowest was observed in T3 group fishes as 31.36 ± 0.12%. T2 group fishes show (P < 0.01) significant increase in MCHC with respect to control (C) and T1 group fishes. T3 group fishes show highly significant decrease in MCHC with respect to control (C), T1 and T2 group fishes.

In chronic experiment, high mean corpuscular haemoglobin concentration (MCHC) was observed in control (CC) group fishes as 32.38 ± 0.12% and lowest was observed in CT2 group fishes as 30.87 ± 0.48%. CT2 group fishes show highly significant (P < 0.001) decrease in MCHC with respect to control (CC) group fishes. CT2 group fishes show significant (P < 0.01) decrease in MCHC with respect to CT1 group fishes. There is no significant change in MCHC among CT1 and (CC) group fishes.

The mean corpuscular haemoglobin concentration (MCHC) is a good indicator of red blood cell swelling or impairment in Hb synthesis [28]. The results of MCHC in this study also show a dynamic change in acute experiments indicating abnormal sized erythrocytes as a result of shrinkage and swelling of RBCs due to tartrazine exposure. In the fishes of T2 group the MCHC was increased in comparison with control and it may be due to the irregular swelling of erythrocytes as owed to sudden changes in the body. MCHC shows fluctuating results in acute experiments unlike other parameters. The variation of MCHC in test fishes were not in accordance with the concentration of exposed tartrazine. Similar variations were also reported in fish *Channa punctatus* exposed to tannery mill effluents [29].

In chronic experiments, MCHC were decreased in test group fishes (CT1 and CT2) due to chronic tartrazine exposure and low nutrient availability. The variation in of MCHC indicates the destruction of RBCs which leads anaemia, erythropenia and leucopoiesis. The variation in Hb content due to decreased synthesis also reflects in MCHC. Similar observations were reported in fish *Labeo rohita* exposed to plywood industry effluents [30].

The fluctuation in red cell indices such as MCV, MCH and MCHC were observed in studies on fish *Labeo rohita* exposed to paint, dye and petroleum industry effluents [31]. The changes in these indices indicate the impairment in blood formation and anaemic conditions. So, the present experimental study on Labeo rohita exposed to azo dye tartrazine confirmed the earlier findings. The study agrees that haematological analysis in fish culture is becoming more popular and is imperative for toxicological research; environmental monitoring and fish health conditions [32]. The water quality also has great influence on the haematological indices of fishes especially in cultural conditions [33].

Conclusion

As dyes, are important industrial chemicals used in food industry and textile industries, it can be detected in varying quantities in wastewaters discharged to the nearby water bodies. Acid yellow 23 (tartrazine) is one of the dyes used in textiles, food stuffs and medicines. Because of the stability and resistance to biodegradation, dyes and pigments can create lot of ecological and health problems.

In conclusion, the present study reveals that even short-term exposure of fish (96 hours) to high concentration of tartrazine causes considerable changes in their haematological parameters like RBCs, TC, Hb, PCV, MCV, MCH and MCHC due to the dye absorption in the circulatory system. The parameters like RBC, Hb, PCV, MCV, MCH and MCHC decreased and total count of WBC or TC increased in test fishes with respect to control fishes. Increase in WBC might be due to the adaptive mechanism against toxicity of tartrazine. The changes in haematological parameters due to the chronic exposure of fish *Labeo rohita* to sublethal concentrations of tartrazine are more severe than that of acute exposure to high concentrations of azo dye. Therefore, this experimental study proves the physiological stress in the organism on tartrazine exposure, and such disturbances endorsed that the organism is more susceptible to water pollution due to azo dyes.

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Conflicts of Interest

The authors declare no conflict of interest.

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