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RESEARCH ARTICLE

Exposure to cobalt chloride alters hemato-biochemical indices and erythrocyte morphology in stinging catfish, *Heteropneustes fossilis*

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Abstract. Higher amounts of cobalt chloride ($CoCl_2$) released by industries are regarded as environmental pollutants. The goal of the current investigation was to assess the acute toxicity of $CoCl_2$ from its effects on erythrocyte morphology and hemato-biochemical indicators in stinging catfish, *Heteropneustes fossilis*. The fish were subjected to $CoCl_2$ at five different concentrations of 0, 50, 100, 200, and 300 mg L^{-1}, which were referred to as control, CC50, CC100, CC200, and CC300, respectively. Red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), platelet counts, white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes, granulocytes, serum protein, and blood glucose level were among the hemato-biochemical parameters of stinging catfish that were measured following a 96 h exposure period. Additionally, erythrocytic nuclear abnormalities (ENA) and erythrocytic cellular abnormalities (ECA) were evaluated in the experimental fish. As the quantity of $CoCl_2$ increased, there were notable reductions in RBC, Hb, Hct, WBC, platelet counts, MCH, MCV, MCHC, lymphocytes, granulocytes, serum protein, and blood glucose levels compared to the control. When the $CoCl_2$ concentration increased, the frequencies of ECA and ENA also increased significantly ($P < 0.05$). This work demonstrated the significant toxicity of high $CoCl_2$ concentrations caused alterations in hemato-biochemical indices and deformities in the erythrocytes of stinging catfish. Thus, it is imperative to take all necessary corrective action to guarantee that the amount of $CoCl_2$ in the aquatic environment stays below permissible limits.

Keywords: cobalt chloride, environmental pollution, erythrocytes, hemato-biochemical parameters, stinging catfish

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Introduction

Fishes are the organisms in aquatic ecosystems that most commonly exhibit biological consequences of environmental pollution. Researchers worldwide are interested in aquatic pollution by various heavy metals (Dutta and Dalal 2008). Naturally occurring metallic elements with an atomic number higher than 20 and a high atomic density are known as heavy metals (4 g cm^{-3} or five times that of H_2O), which can

be toxic to many living organisms at very low concentrations (Tchounwou et al. 2012). The effects of heavy metals on fish health have been the focus of many studies in recent times (Javed and Usmani 2019). Furthermore, heavy metal contamination has a catastrophic impact on environmental quality and substantially disrupts the biological equilibrium of ecosystems (Farombi et al. 2007).

Cobalt chloride ($CoCl_2$) is an inorganic compound of cobalt salt, a heavy metal, and chlorine. It is extremely concerning when heavy metals, particularly cobalt (Co), build up in waterways and agricultural areas as a result of human and natural processes. Although it is a vital component of vitamin B_{12}, increased exposure to it impacts fish substantially (Saravi et al. 2009). Co compounds that dissolve quickly in water are often more toxic than those that do not (ATSDR 2004). Unpolluted natural waters normally have no more than one microgram per liter of the metal (Marr et al. 1998). In contrast, areas impacted by agricultural or industrial activities can exhibit substantially higher (290 μg L^{-1} to 5,670 μg L^{-1}) concentrations of Co in natural waterbodies (ATSDR 2004). According to Nagpal et al. (2004), the concentration of 110 μg L^{-1} signifies potential acute pollution, where exceeding this concentration poses immediate toxic risks to aquatic life, including mortality and physiological stress. In contrast, 4 μg L^{-1} cobalt concentrations indicate that levels above this threshold over a 30-day period suggest chronic pollution, adversely affecting the health, growth, and reproductive success of aquatic organisms.

Hematological indices can be excellent indicators of $CoCl_2$ toxicity in fish because hematological parameters are used to describe the health status of fishes. Variations in fish blood parameters are caused by environmental factors (Asgah et al. 2015). It has been established that hematological indicators are useful instruments for evaluating the physiological condition of fishes. Hematological analysis, including measurements of red blood cells, white blood cells, hematocrit, and hemoglobin, is a valuable research tool for assessing fish health and monitoring stress responses (Parthipan and Muniyan 2013). The effects of some heavy metals on the hematological aspects of fishes are reported by Hedayati and Darabitabar (2017), Kumar et al. (2017), and Verma et al. (2019). Atamanalp et al. (2010) documented changes in hematological parameters of brown trout, *Salmo trutta*, exposed to different concentrations of $CoCl_2$. Changes in tissues (gill, liver, muscle) and key hematological parameters were reported in Mozambique tilapia, *Oreochromis mossambicus*, exposed to different doses of $CoCl_2$, with an LC_{50} estimated at 340 mg L^{-1} (Suganthi et al. 2015).

Stinging catfish is widely recognized on the Indian subcontinent for its nutritional and economic importance. The species is valued greatly for its nutritional and therapeutic qualities in addition to its great taste and high market value (Chakraborty and Nur 2012, Saha et al. 2022). This species can withstand low dissolved oxygen levels in water and can survive in adverse conditions because of its accessory air-breathing organ (Fatma and Ahmed 2020). Pathogen infection, reduction of natural habitats, and heavy metal contamination limit their productivity and muscle quality (Hossain et al. 2010). However, studies on the acute toxicity of $CoCl_2$ in stinging catfish based on hemato-biochemical indices and erythrocyte morphology are rare. Therefore, the present study was conducted to assess the acute effect of varying concentrations of $CoCl_2$ on hemato-biochemical parameters and erythrocyte morphology of stinging catfish.

Materials and Methods

Experimental fish and laboratory condition

The stinging catfish (average standard length: 18.58 ± 0.15 cm and average weight: 43.40 ± 1.16 g) were procured from a local fish market and then transferred to the Wet Laboratory of Gazipur Agricultural University (GAU) in Gazipur, Bangladesh with minimum handling. In the wet laboratory, the fish were acclimatized in two aquaria for one week. Fifty fish were kept in each aquarium (76 cm × 46 cm × 46 cm) containing 120 L tap water with sufficient aeration. The fish were fed

a commercial diet with 35% protein at the satiation level twice daily during the acclimatization period. Every day, half the water was exchanged and feces and other waste were cleaned from the tanks.

Experimental exposure

Analytical grade cobalt chloride ($CoCl_2$) was obtained from Sigma-Aldrich and used without further purification. Based on the previously determined 96 h LC50 value of $CoCl_2$ (390 mg L^{-1}), various sublethal concentrations below this value were selected to assess its toxicity. The fish were exposed to five different concentrations of $CoCl_2$ of 0, 50, 100, 200, and 300 mg L^{-1} that were referred to as control, CC50, CC100, CC200, and CC300, respectively. This experiment was carried out in three replications in aquaria with 40 L water capacity for 96 h with 20 fish stocked in each aquarium. During the experiment, sufficient aeration was provided. To keep the $CoCl_2$ concentration consistent, it was added to the water every 24 h with regular total water exchange. Water quality parameters such as temperature, dissolved oxygen (DO), pH, and dissolved ammonia were measured during the experimental period. Each aquarium was continuously aerated with an air pump (RESUN ACO-001). The dissolved oxygen content was measured with a digital DO meter (Lutron DO-5509) and the pH was determined with a digital pH meter (Hach Co., Colorado, USA). Ammonia levels (mg L^{-1}) were measured with an ammonia measuring kit (HANNA Instrument Test Kit). An external water heater (Sobo HF-300) maintained water temperature.

Hemato-biochemical analysis

Blood samples were drawn from the caudal artery of the fish after 96 h of exposure to $CoCl_2$. For hematological evaluation, eight fish (n = 8) from each aquarium were sacrificed, and blood samples were taken from each group using heparinized syringes in EDTA (Ethylene Diamine Tetra Acetic Acid) tubes. Hematological parameters of red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), platelet counts, white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes and granulocytes were determined using a completely automatic hematology analyzer (Model DH36, Dymind Biotechnology, China) at the Fish Nutrition Laboratory of the Department of Aquaculture, GAU. The hematology analyzer was calibrated for fish blood with a structured protocol provided by the manufacturer. Blood glucose level (mmol L^{-1}) was estimated using a portable glucometer (Sinocare safe AQ Smart Blood Glucose Meter).

To determine serum protein levels, fresh blood was drawn from the fish into non-heparinized tubes, allowed to clot, and then centrifuged (3000 rpm, 10 min, and 4.0°C) in a Sigma 3-18KS centrifuge (Germany). The supernatant serum was then carefully collected in another tube using a Pasteur pipette, and the serum protein levels were measured (g 100 mL^{-1}) with a portable hand refractometer (Erma, Japan).

Analysis of cellular and nuclear abnormalities of blood cells

To determine erythrocytic cellular abnormalities (ECA) and erythrocytic nuclear abnormalities (ENA), drops of blood were placed on sterile microscope slides and smeared immediately after collection. The smeared slide was air dried for fifteen minutes, fixed for ten minutes with methanol, and then stained for ten minutes with Giemsa solution. Finally, the stained slide was washed with running tap water and air-dried at room temperature for 4 to 5 h. Then the stained blood sample slide was viewed under an electronic microscope (ZEISS Primo Star, Germany) at a magnification of × 40. Three slides were prepared for each fish, and 2,000 cells were counted from each slide, and three fish per group were examined to investigate ECA and ENA.

Statistical analysis

Throughout the trial, all of the data were collected and recorded on a computer spreadsheet. Before statistical analysis, the Shapiro-Wilk test and Levene's

test were used to check all data for normality and homogeneity, respectively. SPSS Statistix 10.0 (IBM, USA) software was then used to perform one-way analysis of variance (ANOVA) on the data. Tukey's post-hoc test was performed to determine significant differences among treatments at $P < 0.05$.

Results and discussion

The water quality parameters showed little variation over the course of the trial, with ranges for dissolved oxygen of 6.00–6.39 mg L^{-1}, pH of 7.00–7.35, and ammonia of 0.20–0.29 mg L^{-1} (Table 1). These range of water quality parameters were suitable for the intensive culture of Asian stinging catfish, *Heteropneustes fossilis* (Das et al. 2021).

The results of the hematological analysis are presented in Table 2. The numbers of RBC ($\times 10^6$ μL^{-1}) at CC100, CC200, and CC300 after 96 h exposure were 1.40 ± 0.11, 1.32 ± 0.19, and 1.17 ± 0.06, respectively, which were significantly ($P < 0.05$) different from the control. These findings indicated that as $CoCl_2$ concentration increased, the quantity of RBCs dropped. Hb levels (g dL^{-1}) at CC50, CC100, CC200, and CC300 mg L^{-1} were 13.50 ± 0.22, 13.40 ± 0.44, 13.05 ± 0.02, and 12.60 ± 0.70, respectively, and indicated significantly ($P < 0.05$) decreased Hb levels compared to the control (16.25 ± 0.19).

Previous studies also reported that cobalt toxicity significantly reduced RBC counts and Hb levels in freshwater fish species, including *Labeo rohita*, *Cirrhinus mrigala*, and *Cyprinus carpio*, resulting in anemia and impaired oxygen transport from damage to erythropoietic tissues and cobalt accumulation in vital organs (Singh and Kumar 2013). The effects of a sublethal concentration of zinc (4.42 mg L^{-1}) on hematological changes in freshwater *Channa punctatus* were studied by Ganesan and Karuppasamy (2015). According to Hossain et al. (2015), who determined the effects of sumithion on hematological parameters in *C. carpio*, there was a comparable decrease in Hb

Table 1
Average body weight of stinging catfish and water quality parameters during exposure to various concentrations of $CoCl_2$ (Mean ± SD). *ABW – average body weight, DO – dissolved oxygen

	Concentration of $CoCl_2$ (mg L^{-1})				
Parameters	Control	CC50	CC100	CC200	CC300
ABW (g)	43.40 ± 1.16	43.90 ± 1.10	43.55 ± 1.90	43.69 ± 1.87	43.50 ± 1.21
0 hour of exposure					
DO (mg L^{-1})	6.00 ± 0.05	6.16 ± 0.03	6.23 ± 0.02	6.10 ± 0.09	6.20 ± 0.02
pH	7.11 ± 0.09	7.19 ± 0.09	7.00 ± 0.03	7.20 ± 0.02	7.12 ± 0.08
Ammonia (mg L^{-1})	0.21 ± 0.01	0.23 ± 0.02	0.20 ± 0.02	0.24 ± 0.03	0.23 ± 0.01
24 hours of exposure					
DO (mg L^{-1})	6.10 ± 0.04	6.20 ± 0.05	6.15 ± 0.03	6.22 ± 0.04	6.15 ± 0.02
pH	7.33 ± 0.02	7.29 ± 0.01	7.25 ± 0.02	7.17 ± 0.09	7.35 ± 0.04
Ammonia (mg L^{-1})	0.25 ± 0.03	0.27 ± 0.01	0.21 ± 0.02	0.23 ± 0.02	0.22 ± 0.04
72 hours of exposure					
DO (mg L^{-1})	6.30 ± 0.05	6.33 ± 0.04	6.39 ± 0.02	6.30 ± 0.06	6.28 ± 0.05
pH	7.13 ± 0.04	7.20 ± 0.01	7.16 ± 0.03	7.21 ± 0.04	7.20 ± 0.02
Ammonia (mg L^{-1})	0.27 ± 0.02	0.26 ± 0.01	0.25 ± 0.04	0.22 ± 0.03	0.24 ± 0.03
96 hours of exposure					
DO (mg L^{-1})	6.19 ± 0.03	6.15 ± 0.02	6.13 ± 0.05	6.22 ± 0.04	6.24 ± 0.03
pH	7.25 ± 0.05	7.22 ± 0.01	7.20 ± 0.02	7.28 ± 0.05	7.26 ± 0.04
Ammonia (mg L^{-1})	0.29 ± 0.03	0.28 ± 0.01	0.27 ± 0.03	0.25 ± 0.02	0.24 ± 0.02

Table 2
Hematological parameters of stinging catfish after 96 h exposure to various concentrations of $CoCl_2$ (mean ± SD). * Different letter superscripts in rows indicate significant ($P < 0.05$) differences among values. Hb – hemoglobin, RBC – red blood cell, WBC – white blood cell, Hct – hematocrit, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, MCV – mean corpuscular volume

	Concentration of $CoCl_2$ (mg L^{-1})				
Parameters	Control	CC50	CC100	CC200	CC300
RBC ($\times 10^6$ μL^{-1})	2.63 ± 0.17^a	2.55 ± 0.10^a	1.40 ± 0.11^b	1.32 ± 0.19^b	1.17 ± 0.06^b
Hb (g dL^{-1})	16.25 ± 0.19^a	13.50 ± 0.22^b	13.40 ± 0.44^b	13.05 ± 0.02^b	12.60 ± 0.70^b
Hct (%)	55.05 ± 0.63^a	54.58 ± 0.84^{ab}	53.28 ± 0.07^{bc}	53.31 ± 0.28^{bc}	52.33 ± 0.21^c
MCV (fl)	218.30 ± 3.32^a	214.75 ± 2.88^b	214.55 ± 0.21^b	214.10 ± 2.14^b	213.60 ± 4.36^b
MCH (pg)	63.35 ± 0.07^a	58.15 ± 0.63^b	48.76 ± 0.98^c	44.10 ± 0.28^d	44.20 ± 0.14^d
MCHC (g dL^{-1})	28.40 ± 0.70^a	26.60 ± 0.84^b	26.35 ± 0.07^b	25.20 ± 0.84^b	23.45 ± 0.49^c
Platelet ($\times 10^3$ μL^{-1})	15.50 ± 0.70^a	10.50 ± 0.50^b	10.60 ± 0.71^b	6.00 ± 0.00^c	5.50 ± 0.70^c
WBC ($\times 10^3$ μL^{-1})	39.21 ± 0.97^a	35.38 ± 1.27^b	33.51 ± 1.51^c	31.86 ± 1.13^d	26.66 ± 1.63^e
Lymphocyte (%)	96.55 ± 1.91^a	96.16 ± 1.20^a	94.53 ± 2.05^a	90.85 ± 0.74^b	90.43 ± 0.42^b
Granulocyte (%)	4.96 ± 0.17^a	4.85 ± 0.07^a	3.80 ± 0.14^b	3.50 ± 0.49^b	3.53 ± 0.14^b

levels. The present study showed a considerable drop in Hb production and RBC counts, which could have been attributed to structural heme changes and iron deficit. Additionally, the oxygen carrying capacity of the fish may have been compromised by $CoCl_2$.

The Hct (%) levels of 53.28 ± 0.07, 53.31 ± 0.28, and 52.33 ± 0.21 for CC100, CC200, and CC300, respectively, revealed significant ($P < 0.05$) variations compared to the control group. The platelet counts ($\times 10^3$ μL^{-1}) of 10.50 ± 0.50, 10.60 ± 0.71, 6.00 ± 0.00, and 5.50 ± 0.70 for CC50, CC100, CC200, and CC300, respectively, showed significantly ($P < 0.05$) lower values compared to the control group. The results of this experiment indicated that Hct levels and platelet counts decreased with exposure to increased concentrations of $CoCl_2$. In this study, the decrease in Hct levels after exposure to $CoCl_2$ in stinging catfish could be a sign of hemodilution. The Hct values observed in the fish might also be due to RBC lysis. Similar reductions in Hct levels were reported by Sayed and Shokr (2015), Atamanalp et al. (2010), and Mousavi and Yousefian (2012) in Nile tilapia, brown trout, and Caspian brown trout, respectively, that were exposed to zinc, cobalt chloride, and mercuric chloride, respectively, under laboratory conditions. Additionally, decreased platelet counts following $CoCl_2$ exposure also suggested a hemodilution mechanism, possibly due to osmoregulation impairment or gill damage. Previous studies have revealed comparable findings with substantial declines in platelet counts in mudfish, *Clarias gariepinus,* and the freshwater *C. carpio* exposed to malachite green (toxicant) and cadmium chloride, respectively (Musa and Omoregei 1999, Ali et al. 2018).

The results of this investigation showed that the number of WBC ($\times 10^3$ μL^{-1}) decreased with increased concentrations of $CoCl_2$, and the number of WBC (26.66 ± 1.63) at CC300 was significantly ($P < 0.05$) lower compared to the control group (39.21 ± 0.97). The decreased number of WBC, which is called leucopenia, might have been the result of the bio-concentration of $CoCl_2$ in the kidney and liver. Moreover, the decreased number of WBC might also have been related to an increased level of corticosteroid hormones, the secretion of which is a nonspecific response to any environmental stressor (Lemos et al. 2023). A similar decreased number of WBC was also reported in brown trout *Salmo salar* exposed to $CoCl_2$ (Atamanalp et al. 2010). This finding contradicted the knowledge that infections and intoxications stimulate WBC in fishes. In contrast,

Atamanalp et al. (2011) reported that WBC counts increased significantly in rainbow trout, *Oncorhynchus mykiss*, exposed to $CoCl_2$. The results indicated that MCV (fl) and MCH (pg) levels at CC300 were 213.60 ± 4.36 and 44.20 ± 0.14, respectively, which showed significant ($P < 0.05$) reduction in comparison with the control group. MCHC (g dL^{-1}) level at CC300 was 23.45 ± 0.49, which showed significant differences in comparison with the control group. The results of this experiment indicated that the MCV, MCH, and MCHC levels decreased with increased concentrations of $CoCl_2$. Decreased levels of MCV and MCHC with increasing concentrations of $CoCl_2$ could have been due to the release of proerythrocytes containing low Hb into circulation and hypochromic anemia. It is possible that a decrease in cellular blood iron caused the decrease of MCH. According to Milla et al. (2010), spleens in fish have been shown to contract when the animal is under stress, and MCV values would decrease if the spleen discharged fewer cells.

In this investigation, lymphocyte levels decreased with increasing $CoCl_2$ concentrations. Lymphocyte (%) levels at CC200 and CC300 were 90.85 ± 0.74, and 90.43 ± 0.42, respectively, which in contrast to the control group, displayed significant changes (96.55 ± 1.91). This concurred with the findings of Celik et al. (2013) from a study in which they exposed Mozambique tilapia, *Oreochromis mossambicus*, to several zinc concentrations. Further, it has been reported that zinc decreased lymphocytes in *C. carpio* (Witeska 2005), and this effect of this heavy metal may be explained by the suppressive effect of the cortisol hormone on lymphocyte production in the present study. In addition, the reduction of granulocytes (%) in this study was caused by tissue damage. Similar reductions were observed in *C. carpio* exposed to arsenic (Talas et al. 2012).

Blood glucose level is a common biological indicator of stress in fishes (Pacheco and Santos 2001). In the current investigation, blood glucose (mmol L^{-1}) levels decreased significantly in the CC100 (3.02 ± 0.12), CC200 (2.30 ± 0.14), and CC300 (2.23 ± 0.16) groups compared to the control group (3.66 ± 0.15) (Fig. 1). This result showed that blood glucose levels decreased with increasing concentrations of $CoCl_2$. At the beginning, this is usually associated with glycogen mobilization, where glycogen is broken down into glucose, and this process can lead to hyperglycemia (McLeay 1977). However, over time, exposure to $CoCl_2$ may deplete glycogen retention capacity, reducing blood glucose levels

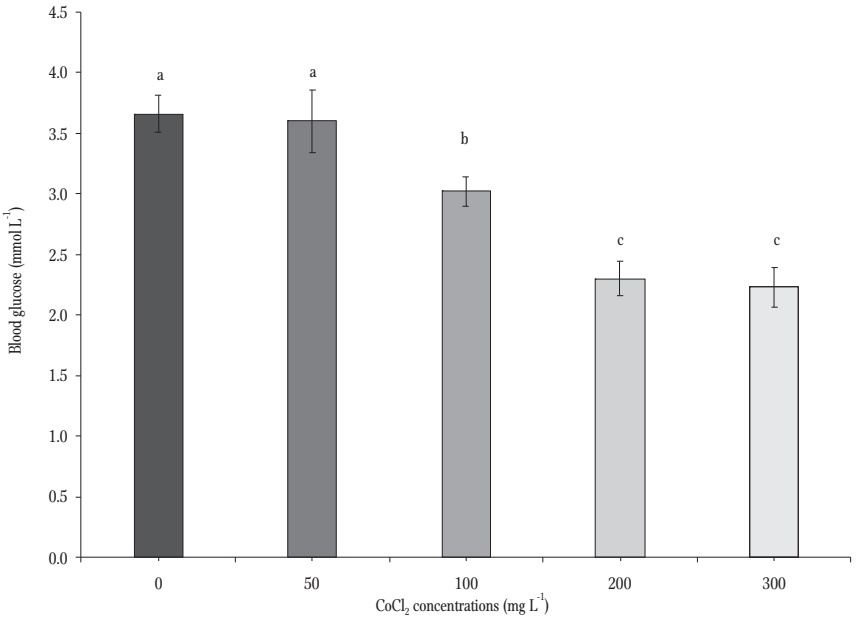

Figure 1. Variations in blood glucose levels following 96 h exposure to various $CoCl_2$ concentrations. Different letter superscripts in rows indicate significant ($P < 0.05$) differences among values. All values are expressed in mean ± SD.

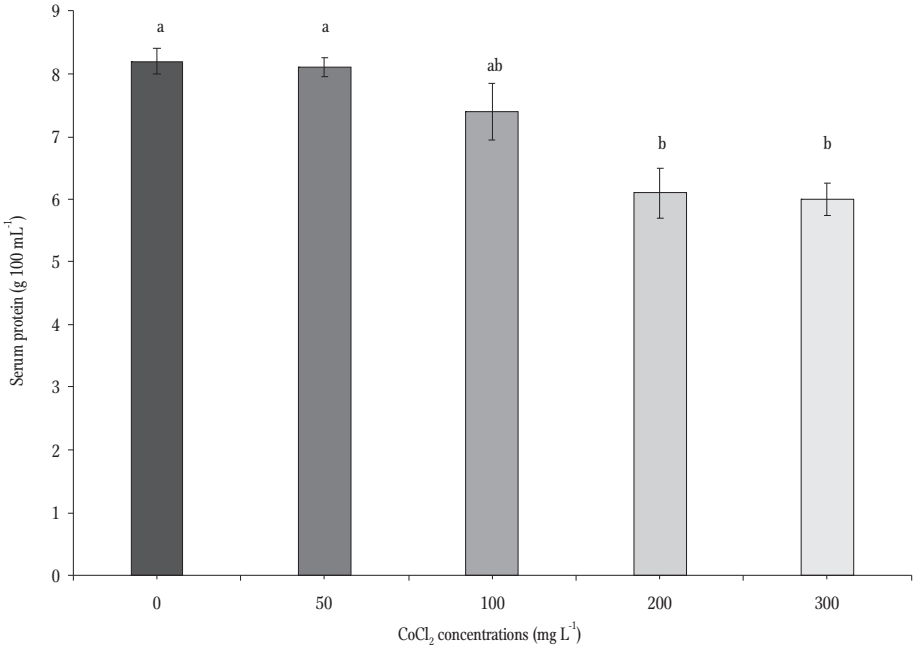

Figure 2. Alterations in blood protein levels following 96 h exposure to various $CoCl_2$ concentrations. Different letter superscripts in rows indicate significant ($P < 0.05$) differences among values. The mean ± SD is used to express all values.

(hypoglycemia). $CoCl_2$ exposure disrupts physiological and biochemical processes in fishes (*L. rohita*, *C. mrigala*, and *C. carpio*), causing liver glycogen depletion, potentially due to hepatic cell necrosis (Singh and Kumar 2013). Moreover, decreased glucose content in blood indicates that fish utilize stored carbohydrates excessively as a consequence of the metabolic toxic stress caused by the heavy metal during the treatment period (Lavanya et al. 2011). The findings of the present study concurred with those by Gashkina (2024), in which cobalt exposure in fish decreased blood glucose levels by inhibiting the liver's capacity to produce glucose (gluconeogenesis), disrupting the activity of enzymes that regulate glucose metabolism (phosphoenolpyruvate carboxykinase and glucose-6-phosphatase), increasing glucose uptake by tissues, inducing oxidative stress that affects metabolic functions, and altering the hormonal regulation of glucose levels.

In the current investigation, serum proteins (g 100 mL^{-1}) were shown to decline with rising $CoCl_2$ concentrations. In CC300, the serum protein level (6.00 ± 0.25) was significantly ($P < 0.05$) different compared to the control group (8.20 ± 0.21) (Fig. 2). This type of reduction (hypoproteinemia) was ascribed to renal excretion, reduced protein synthesis, or a liver disorder (Lavanya et al. 2011). In the current investigation, hypoproteinemia was caused by increased proteolysis (protein breakdown into amino acids, which may then be converted into nitrogen and other elementary molecules). Proteolysis is a physiological process that provides energy to cope with the stress of heavy metal toxicity (Srivastava and Prakash 2018). Sinha (2021) found that nickel exposure significantly reduced serum protein levels in spotted snakehead, *C. punctatus*. A similar reduction in serum protein was reported by Talas et al. (2012) in *C. carpio* exposed to arsenic.

Widely used as a key diagnostic tool, erythrocytes can also be used to assess the structural and functional health of fishes exposed to harmful chemicals. According to Sawhney and Johal (2000), erythrocytes react to environmental stresses and changes, which are the most common way they reflect toxins found in water bodies. After being exposed to various doses of $CoCl_2$, the stinging catfish blood smears showed various erythrocytic cellular abnormalities (ECA), including teardrop (Fig. 3a), elongated (Fig. 3b), echinocytic (Fig. 3c), hemolysis (Fig. 3d),

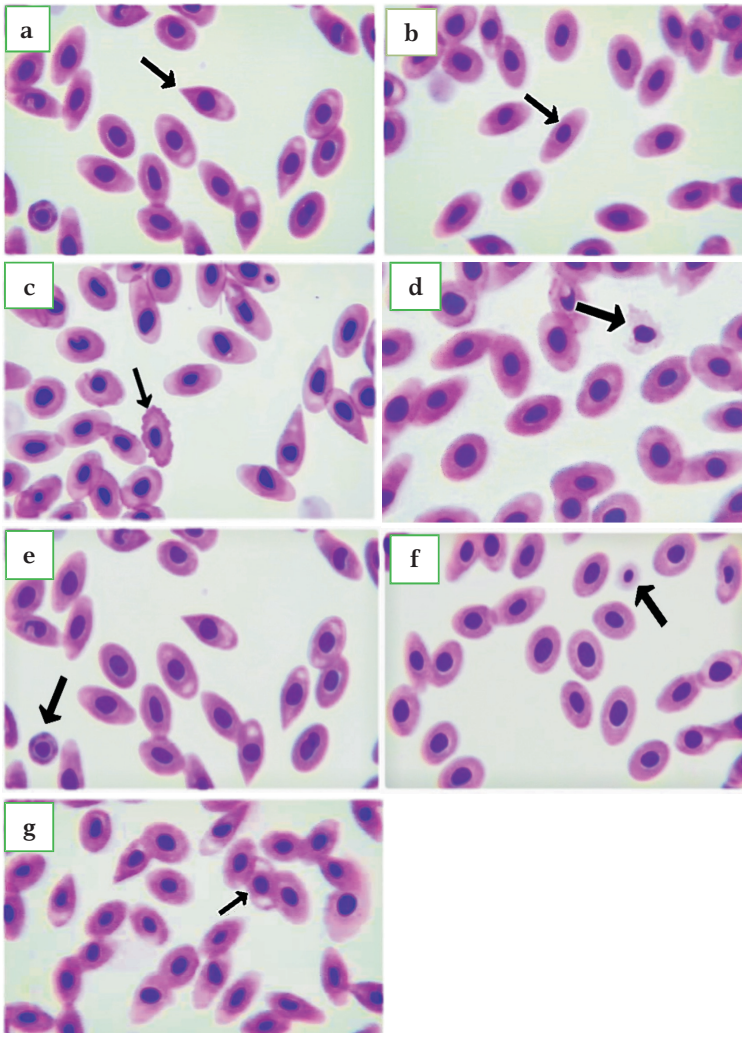

Figure 3. Various erythrocytic cellular abnormalities (ECA) in Giemsa-stained blood smears of stinging catfish treated with different concentrations of $CoCl_2$ (a) teardrop (b) elongated (c) echinocytic (d) hemolysis (e) erythroblast (f) microcyte (g) vacuolated cytoplasm.

erythroblast (Fig. 3e), microcyte (Fig. 3f), and vacuolated cytoplasm (3g). Among ECA, teardrop cells are twisted at the top to resemble a nipple that tapers to the erythrocyte terminals; elongated cells are long-shaped, slender structures; echinocytic cells are unevenly shaped and have serrated edges throughout the cell membrane; hemolysis refers to the rupture or destruction of erythrocytes, leading to the release of hemoglobin and other intracellular components into the surrounding fluid, such as plasma or serum; erythroblasts are immature, nucleated erythrocyte precursors found in the peripheral blood, typically indicating a disruption in normal erythropoiesis; microcytes are erythrocytes with reduced cell volume, appearing as small red blood cells in peripheral blood smears; erythrocytes with vacuolated cytoplasm refer to erythrocytes that contain visible, empty spaces or vacuoles within their cytoplasm.

The frequencies of ECA in stinging catfish exposed to various $CoCl_2$ concentrations are presented in Table 3. Frequencies of teardrop, elongated, erythroblast, and vacuolated cytoplasm increased significantly ($P < 0.05$) with higher $CoCl_2$ concentrations compared to the control. However, there was no significant difference in terms of hemolysis in the control, CC50, or CC100 treatments, while higher $CoCl_2$ concentrations (CC200, CC300) increased the frequency of hemolysis. Shah (2016) examined the impact of cobalt toxicity on the blood cells of *L. rohita* and found that exposure to cobalt resulted in significant anomalies in erythrocytic cells. Sadiqul et al. (2016) states that the cellular intake of hazardous substances, ion permeability, and the breakdown of cell membranes led to erythrocytic abnormalities such as echinocytic, elongated, and tear-drop-shaped cells. Additionally, Fatima et al. (2014) documented the effects of heavy metal exposure (chromium, nickel, and lead) on two fish species (*Channa striatus* and *Heteropneustes fossilis*) and observed various erythrocytic abnormalities, including vacuolated cytoplasm and microcytes, indicating severe cytotoxic and genotoxic effects. Increased levels of lipid peroxide molecules in the cell membrane can also cause erythrocyte shape to change (Bai et al. 2014). In the present study, erythrocyte structures were altered from the absorption of the toxic substance $CoCl_2$, which can cause cell membrane damage and increase the susceptibility of erythrocytes to rupture when passing through narrower capillaries.

Fig. 4 depicts various ENA in fish blood at different $CoCl_2$ concentrations, including cells that had

Table 3
Frequencies of erythrocytic cellular abnormalities (ECA) in stinging catfish exposed to various $CoCl_2$ concentrations for 96 h (mean ± SD). * Different letter superscripts in rows indicate significant ($P < 0.05$) differences among values. Three slides were prepared for each fish and 2,000 cells were counted per slide, and at least three fish from each group were analyzed

	Concentration of $CoCl_2$ (mg L^{-1})				
ECA	0	50	100	200	300
Tear-drop	0.20 ± 0.01^{d}	0.48 ± 0.04^{c}	0.68 ± 0.11^{bc}	0.75 ± 0.02^{b}	1.13 ± 0.04^{a}
Elongated shape	0.50 ± 0.00^{d}	1.15 ± 0.03^{c}	1.18 ± 0.14^{bc}	1.22 ± 0.03^{b}	1.27 ± 0.11^{a}
Echinocytic	0.18 ± 0.04^{c}	0.38 ± 0.03^{bc}	0.63 ± 0.18^{b}	1.23 ± 0.03^{a}	1.25 ± 0.00^{a}
Hemolysis	0.18 ± 0.04^{c}	0.30 ± 0.00^{c}	0.33 ± 0.04^{bc}	0.67 ± 0.11^{ab}	0.83 ± 0.11^{a}
Erythroblast	0.15 ± 0.02^{c}	0.28 ± 0.04^{b}	0.39 ± 0.01^{b}	0.68 ± 0.01^{a}	0.74 ± 0.04^{a}
Microcyte	0.12 ± 0.01^{c}	0.21 ± 0.01^{c}	0.42 ± 0.04^{b}	0.71 ± 0.08^{a}	0.81 ± 0.01^{a}
Vacuolated cytoplasm	0.11 ± 0.01^{d}	0.46 ± 0.03^{c}	0.68 ± 0.02^{b}	0.94 ± 0.08^{a}	0.98 ± 0.04^{a}

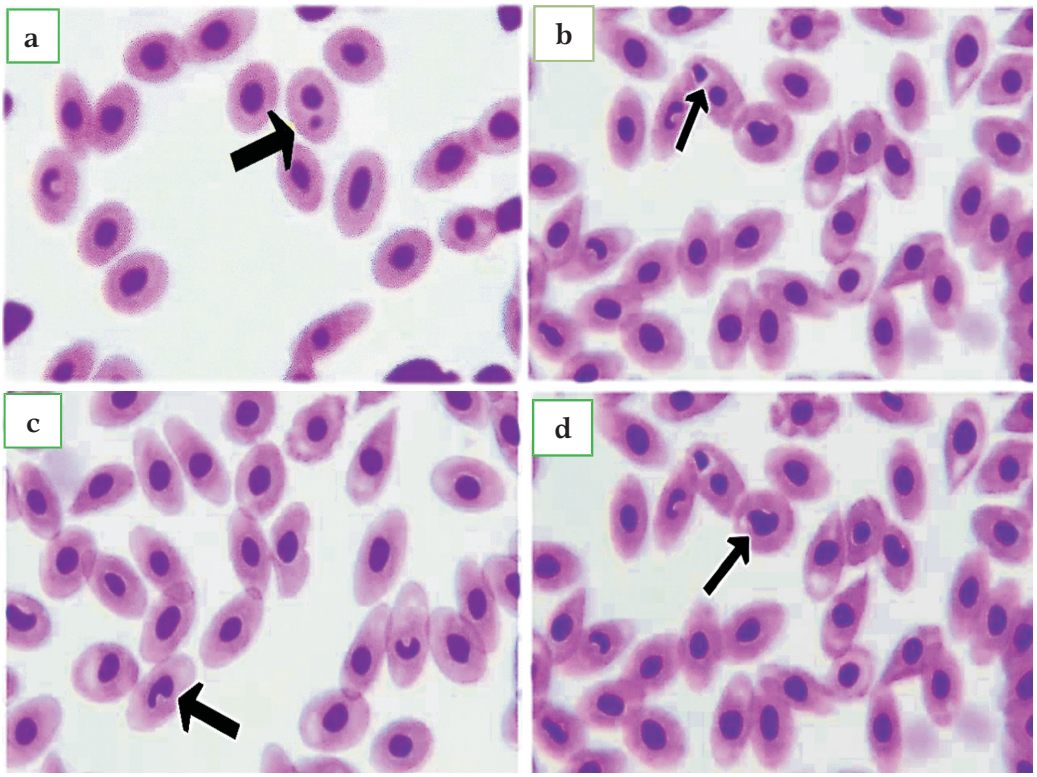

Figure 4. Giemsa-stained blood smears of stinging catfish treated with varying concentrations of $CoCl_2$ showed a variety of erythrocytic nuclear abnormalities (ENA): (a) micronucleus (b) binuclei (c) notched (d) blebbed.

micronuclei (Fig. 4a), binuclei (Fig. 4b), or notched nuclei (Fig. 4c), or were blebbed (Fig. 4d). ENA was classified by Carrasco et al. (1990) and Fenech et al. (2003) as binucleated, which refers to two nuclei that are entirely separated in a cell but almost the same size. Micronuclei are circular bodies formed from chromatin, with the same staining properties as the central nucleus. Osmosis in a hypertonic nucleus that lost all of its nuclear components as a result of shrinkage results in a nucleus with a notched shape. Blebbed is a condition where small protrusions of chromatin push against the nuclear membrane.

Table 4 displays the frequencies of ENA of stinging catfish treated with varying doses of $CoCl_2$.

Table 4

Frequencies of erythrocytic nuclear abnormalities (ENA) in stinging catfish exposed to various $CoCl_2$ concentrations for 96 h (mean ± SD). * Different letter superscripts in rows indicate significant ($P < 0.05$) differences among values. Three slides were prepared for each fish and 2,000 cells were counted per slide, and at least three fish from each group were analyzed

	Concentration of $CoCl_2$ (mg L^{-1})				
ENA	0	50	100	200	300
Micronucleus	0.28 ± 0.07^{c}	0.43 ± 0.02^{c}	0.73 ± 0.02^{b}	0.90 ± 0.06^{ab}	1.08 ± 0.05^{a}
Binuclei	0.20 ± 0.01^{e}	0.35 ± 0.05^{d}	0.63 ± 0.03^{c}	0.73 ± 0.04^{b}	0.85 ± 0.01^{a}
Notched	0.23 ± 0.02^{c}	0.33 ± 0.03^{c}	0.73 ± 0.01^{b}	0.83 ± 0.02^{b}	0.98 ± 0.04^{a}
Blebbed	0.28 ± 0.04^{c}	0.48 ± 0.02^{bc}	0.73 ± 0.06^{b}	1.08 ± 0.08^{a}	1.28 ± 0.03^{a}

Frequencies of micronuclei and notched and blebbed nuclei were significantly ($P < 0.05$) higher in stinging catfish blood exposed to the CC100, CC200, and CC300 treatments compared to CC50 and the control. The frequency of binuclei significantly increased with increasing concentrations of $CoCl_2$. Exposure to $CoCl_2$ at sub-lethal concentrations significantly induced micronuclei and other nuclear abnormalities in peripheral erythrocytes of *Cirrhina mrigala* (Minhas et al. 2022). Differential nuclear abnormalities of erythrocytes, such as binucleated, micronucleus, notched nuclei, etc., might have arisen as a result of tubulin polymerization failure under chromium stress as reported by Hussain et al. (2014) and Vardavas et al. (2016). Furthermore, binucleated cells and notched nuclei can arise as a result of mitotic fuses caused by aneugenic toxicant effects (Ventura et al. 2008). In the present study, $CoCl_2$ has the ability to induce nuclear abnormalities in fish erythrocytes and increasing concentrations led to increased frequencies of ENA in stinging catfish.

Conclusion

The present study revealed that $CoCl_2$ has profound toxic effects on the hemato-biochemical parameters and erythrocytic morphology of stinging catfish. Increasing concentrations of $CoCl_2$ led to significant reductions in RBC, Hb, Hct, WBC, platelet counts, MCH, MCV, MCHC, lymphocytes, granulocytes, serum protein, and blood glucose levels of stinging catfish. Additionally, higher $CoCl_2$ exposure resulted in a dose-dependent increase in erythrocytic cellular and nuclear abnormalities. The findings of this study provide critical insights into the sublethal impacts of $CoCl_2$ toxicity, offering foundational knowledge for ecological monitoring and the management of aquatic species exposed to pollutants.

Author contributions. F.S.B. – conducted the experiment, data acquisition, statistical analyses, data interpretation, drafting the paper; M.A.H. – conceptualization, study design, project administration, fund acquisition, supervision; M.D. – review of the paper, editing the paper; F.R.U. – editing the paper; M.R.B – review of the paper; M.J. – editing the paper; M.S.A.S. –supervision, revision of the paper.

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