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Impact of Arsenic Exposure on Haematological and Biochemical Parameters of the Catfish, *Heteropneustes fossilis*

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Abstract

Arsenic contamination in groundwater is a significant global issue, adversely impacting both human health and aquatic ecosystems. Anthropogenic activities are major contributors to arsenic pollution in water. Previous studies have demonstrated that arsenic exposure alters behavioural, haematological, and biochemical parameters in fish by increasing the production of reactive oxygen species. Multiple organs, including the liver, kidney, gills, and cardiovascular system, are severely affected by arsenic toxicity. In the present study, *Heteropneustes fossilis* was exposed for 96h and 7 days to two sublethal concentrations, 4.88 mg/L and 14.64 mg/L, of dibasic sodium arsenate heptahydrate and effects on haematological and biochemical parameters were analyzed. The results indicated significantly decreased haemoglobin content and red blood cell count in fish exposed to arsenic under both exposure durations, while total white blood cell count increased. Superoxide dismutase (SOD) enzyme activity in the kidney increased approximately 25-fold and 2-fold in fish exposed to 4.88 mg/L and 14.64 mg/L dibasic sodium arsenate heptahydrate for 96h, respectively. In contrast, SOD activity was reduced to 0.46-fold and 0.36-fold in fish exposed for 7 days to 4.88 mg/L and 14.64 mg/L dibasic sodium arsenate heptahydrate, respectively, compared to control fish. Approximately 2-fold increased catalase activity was observed in fish exposed to both concentrations of dibasic sodium arsenate heptahydrate for 96h. Catalase activity was also enhanced by $\sim$ 1.5-fold and $\sim$ 1.75 -fold in the fish exposed to both concentrations for 7 days. These findings indicate that arsenic is a potent toxicant, and continuous monitoring of arsenic levels in aquatic environments is essential to ensure healthy and safe fish production.

Keywords and Phrases: Arsenate, Arsenite, *Heteropneustes fossilis*, Superoxide dismutase, Catalase, ROS.

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Introduction

Arsenic toxicity in the groundwater is a global concern as it imposes harmful effects on human beings and aquatic animals. Among several forms, the inorganic forms of arsenic, i.e., arsenite (III) and arsenate (V), are considered highly toxic to organisms. Major sources of arsenic are natural and geogenic (Shaji et al., 2021; Marghade et al., 2023). However, anthropogenic activities, including various industrial processes such as glass manufacturing, the paints and pigments industry, and the textile industry, as well as agricultural practices involving insecticides and pesticides, the pharmaceutical industry, mining, and smelting, significantly contribute to the contamination of water with arsenic Chung et al. (2014), Patel et al. (2023). Studies have reported that arsenic exposure affects the health and physiology of fish, including reduced growth and development and inhibition of sperm production Rachamalla et al. (2024). Almost every vital organ of fish, including gills, liver, kidneys, and intestines, is affected by arsenic, suggesting the gravity of its toxicity to fish and other organisms Kumari et al. (2017). Earlier studies have highlighted that arsenic exposure modulates the blood profile of the fish, including red blood cells, white blood cells, haemoglobin content, haematocrit level, and other parameters. It also affects the biochemical profile, such as total lipid content, protein content, and glucose level, and other parameters in the organisms exposed to arsenic. In addition, studies have shown that arsenic toxicity influences the synthesis of reactive oxygen species and several antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) Olmedo et al. (2013), Kumari et al. (2017), Patra et al. (2024). Consumption of fish and other aquatic organisms contaminated with arsenic may pose a potential threat to human health, including risks of cancer, as well as respiratory and dermatological conditions Castro-González et al. (2008). Assessment of these parameters acts as an early warning sign for the health conditions of the fish as well as aquatic environment, and thus is helpful in monitoring the aquaculture. A few studies suggest that arsenic possesses the potential to damage DNA by oxidative stress, alter the DNA methylation pattern, and impair the DNA repair machinery Hei and Filipic (2004), Faita et al. (2013),Tam et al. (2021). These arsenic-induced genotoxicity results in structural and numerical chromosomal aberrations, and increased risk of cancer Ozturk et al. (2022).

Arsenic contamination in the groundwaters of Indian subcontinent, especially in the Indo-Gangetic plain, is very high and has been reported to severely affect the human population residing in these plains Das et al. (1994), Mazumder et al. (1998), Harvey et al. (2005). Acute exposure to arsenic has been reported to cause gastrointestinal (abdominal pain, diarrhoea, vomiting) and cardiovascular complications (cardiac arrhythmia, tachycardia). Moreover, acute exposure also causes severe neurological distress, including muscle cramping, numbness or tingling in the extremities Rat-

naike (2003), Prakash and Verma (2021). In contrast, the chronic exposure to arsenic causes irreversible changes in the skin such as skin lesions, skin cancers, damage of vital organs, especially liver and kidney, and developmental issues Ratnaike (2003), Ozturk et al. (2022), Garkal et al. (2024).
In the present study, *Heteropneustes fossilis*, also known as catfish, has been used as a model organism to assess the effects of certain sublethal doses of arsenic. It is frequently utilized in ecotoxicological investigations due to its modest size, availability, adaptability, short life cycle, variety of habitats, sensitivity, direct interaction with toxins, and genetic and physiological resemblance to human beings Schartl (2014). The findings of the present work will shed more light on the toxicity of arsenic to fish.

Materials and Methods

Collection and acclimatization of fish in laboratory conditions

Healthy *H. fossilis* fish of nearly equal weight and length were obtained from the local market. Fish were disinfected by potassium permanganate solution ($KMnO_4$, 2mg/L) for $\sim$ 2 hrs and treated fish were transferred into glass containers having ground tap water for acclimatization in the laboratory conditions. Fish were fed twice a day with standard food. The water quality parameters, including the pH of the ground tap-water, dissolved oxygen, biochemical oxygen demand and dissolved carbon dioxide, were measured following APHA guidelines (2005). Dead fish were removed immediately from the tank to prevent severe water pollution and disease outbreaks. Nearly 1/3 of the water from the tank was renewed every 24 h to maintain water quality.

Determination of LC_{50} of dibasic sodium arsenate heptahydrate at 96h

The median lethal concentration (LC_{50}) value of dibasic sodium arsenate heptahydrate ($Na_2HAsO_4.7H_2O$, M.W.- 312.02g) was determined at 96h according to the Miller and Tainter method, 1944 Randhawa (2009). The determination of LC_{50} of dibasic sodium arsenate heptahydrate in *H. fossilis* was performed as a collaborative work in the laboratory (Singh et al., 2026, in press). Fish were distributed into a control group and six experimental groups, 10 fish per group. The fish of the control group were kept without exposure, while the fish of the experimental groups were exposed to varying concentrations of dibasic sodium arsenate heptahydrate, ranging from 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L, respectively. The water of each container was replenished every day, and treatment was contin-

ued for 96h with respective concentrations of dibasic sodium arsenate heptahydrate. The number of dead fish in each group was recorded. The percentage mortality was converted into probit value using a probit table, and a graph was plotted against the log concentration of the dibasic sodium arsenate heptahydrate used. Log concentration equivalent to the probit value 5.0 was selected and converted to antilog value to get the LC_{50} value of dibasic sodium arsenate heptahydrate. All the ethical considerations were followed, and experiments were performed according to the standard regulatory guidelines of the Organisation for Economic Co-operation and Development (OECD, 2019).

Experimental design and arsenic exposure

In the present study, two short-term (acute) exposure periods, i.e., 96h and 7 days, and two sub-lethal concentrations of dibasic sodium arsenate heptahydrate, viz. 10% (application factor = 0.1) and 30% (application factor = 0.3) of the LC_{50} value were used to assess the acute toxicity on the health of *H. fossilis*. For both 96h exposure, lab-acclimatized fish were divided into three groups, namely control, experimental group 1, and experimental group 2, five fish per group. Likewise, for the 7- day exposure period, fish were divided into three groups with 5 fish per group, i.e., control, experimental group 1, and experimental group 2. Fish of experimental group 1 and experimental group 2 were treated with 4.88 mg/L (10% of LC_{50}) and 14.64 mg/L (30% of LC_{50}) of dibasic sodium arsenate heptahydrate, respectively; while fish of the control groups were left untreated. Exposure to the afore-mentioned concentrations of arsenic solution was continued for 96h in both the experimental group 1 and experimental group 2, with renewal of water every day. For the 7- day exposure, both the experimental group 1 and experimental group 2 were exposed to their respective concentrations for 7 days, consecutively.

Collection of blood sample and assessment of haematological parameters

Haematological parameters such as red blood corpuscles (RBC) and white blood cells (WBC), haemoglobin (Hb) content, and cellular and nuclear deformities act as fast and sensitive indicators to assess the toxic effects of arsenic and other toxicants in fish Witeska et al. (2023). Therefore, the caudal peduncle of the fish from both control and experimental groups 1 and 2 was cut by sharp blades, and blood was collected in EDTA-vacutainers to assess the haematological parameters.
Measurement of Hb content was done using Sahli's method. In brief, 0.1 N HCl was taken in a graduated tube up to 20 marks and 20 μl of blood was added. Blood and HCl were mixed properly and kept for 10 minutes. Further, distilled water was added to the graduated tube till the colour of the blood-HCl mixture matched the

reference tube, and the reading was recorded. The process was repeated three times for each blood sample, and the mean Hb content was calculated.
For counting of RBC, the blood sample ($2mu$l) was taken and diluted with Hayem's solution (200 times) and mixed gently. The diluted blood (10 μl) was dispensed on the improved Neubauer's haemocytometer, covered with a coverslip and counted in 5 RBC counting chambers at 40X magnification, cautiously. The process was repeated three times for each blood sample, and the mean RBC number was calculated using the formula given below:
Total RBC count = no. of cells in 5 squares X 5 X dilution factor X 10
For total WBC counting, 2 μl of blood was taken and diluted with 38 μl WBC diluting solution to get a final dilution of 20 times. 10 μl of the diluted blood were dispensed on the improved Neubauer's haemocytometer, and a coverslip was applied. WBCs were counted in 4 WBC counting chambers based on the nuclear morphology, and the total number of WBC was calculated by the formula given below:

Total WBC count = Total WBCs counted X dilution factor X 10/4

Assessment of biochemical parameters

Toxicants enhance the synthesis and activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), to neutralize the reactive oxygen species (ROS) produced due to metal toxicity. Thus, biochemical assays offer the earliest signs of toxicity caused by pollutants. Therefore, the activity of SOD and catalase enzyme was measured in the kidney tissue of fish from control and experimental groups Sinha (1972), Das et al. (2000). Fish were ice-cold anesthetized, and kidney tissue was removed and washed in 1X phosphate-buffered saline (PBS) to remove blood traces. Kidney tissue was homogenized in 1X PBS (ice-cold), and 10% tissue homogenate was prepared. The tissue lysate was centrifuged (12000 rpm, 20 min, 4°C), and the supernatant was collected for SOD and catalase assay.
For the SOD assay, a reaction mixture was prepared from 1X PBS (50 mM), L-methionine (20 mM), Triton-X (1%), hydroxy-hydrochloride (10 mM), and EDTA (50mM). Reaction mixture was dispensed in glass tubes (1.4 ml/tube) and 100 µl of 10% kidney tissue homogenate was added, followed by incubation for 5 min at 37°C. Further, 10 mM riboflavin solution was added (80 μl/tube) and photo-activated for 10 minutes. After incubation, 2 mL of freshly prepared Griess reagent was mixed into each test tube. Now, 300 μl of pink/purple coloured product was dispensed in microplate, and the intensity of the colour was measured at 543 nm in a plate reader (Agilent Biotek Epoch-SN). Each reaction was performed in triplicate, and activity was calculated Das et al. (2000).
Catalase enzyme breaks H_2O_2 into water and oxygen and thus protects organisms by regulating its level in the body. For assessment of catalase activity, 500 μl of 0.8

M H_2O_2 was dispensed in glass tubes (dark condition), followed by the addition of 625 μl of 1X PBS to each test tube. Further, kidney tissue homogenate was added (125 μl/tube) and incubated for 1 min at room temperature. After incubation, 2 ml freshly prepared $K_2Cr_2O_7$ solution was mixed in each test tube, blocked with cotton and kept for 10 minutes at 95°C – 100°C to obtain a coloured product. Now, 300 μl of this product was distributed into a microplate in triplicate, and the intensity was measured at 570 nm (Agilent Biotek Epoch-SN). Catalase enzyme activity was calculated in the fish from the control and experimental groups and plotted as the mean fold change Sinha (1972).

Result and Discussion

Calculation of LC_{50} of dibasic sodium arsenate heptahydrate

The mean weight and length (mean ± SD) of fish used in the present work were 22.5±1.32 g and 18.5±1.32 cm, respectively. The LC_{50} value of $Na_2HAsO_4.7H_2O$ at 96h was determined by the Miller and Tainter method (1944). The details of concentrations, mortality, corrected percentage mortality, and probit values will be available on request. The calculated LC_{50} value of sodium meta- arsenate was found to be 48.80 mg/L.

Effects of arsenic on haematological parameters of the catfish, *H. fossilis*

Exposure to heavy metal toxicants often affects several haematological parameters in fish and other model organisms. Our data showed that Hb content was decreased significantly at 96h exposure in the fish of both experimental group 1 (exp.1, 12.27 g/dl) and experimental group 2 (11.80 g/dl), compared to control group (Fig. 2A). At 7-day exposure, Hb content was also found to decrease significantly in both experimental groups 1 (12.13 g/dl) and experimental group 2 (11.47 g/dl), compared to control group at (Fig. 2B).
Besides Hb content, total RBCs and WBCs were also counted. Our findings showed a significant decrease in the total RBC count in experimental group 1 and experimental group 2, compared to the fish of the control group, at 96h exposure to dibasic sodium arsenate heptahydrate. Similar results were observed when fish were exposed to arsenic for 7 days. In contrast, total WBC count was found to be significantly increased in the fish of both experimental groups 1 and 2, at both 96h and 7 days exposure periods. The details of the alterations observed in haematological parameters are given in Table 1.

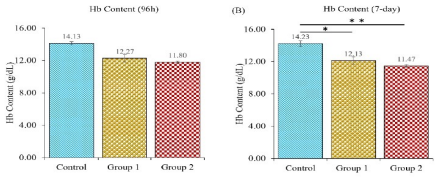

Fig. 2A Hb Content of fish of control and both the experimental group 1 and group 2, at 96h exposure period. Fig. 2B Hb Content of fish of control and both the experimental groups 1 and 2, at 7-day exposure period. p-value < 0.05 considered statistically significant.

Effects of arsenic exposure on Superoxide dismutase activity

Superoxide dismutase activity assay was performed to assess the effects of arsenic toxicity on the kidneys of fish. Our SOD assay data revealed a $\sim$ 25-fold and $\sim$ 2-fold increased SOD activity in the fish of experimental groups 1 and 2, respectively, compared to control at 96h exposure period (Fig. 3A). In contrast, $\sim$ 0.46 -fold and $\sim$ 0.36 -fold decreased SOD activity was observed in the fish of both experimental groups 1 and 2, respectively, compared to control group at 7-day exposure period (Fig. 3B).

Table 1: Changes in the haematological parameters of *H. fossilis* exposed to dibasic sodium arsenate heptahydrate

Haematological Parameters	96 h Exposure			7-day Exposure		
	Control	Treated Group 1	Treated Group 2	Control	Treated Group 1	Treated Group 2
Total RBC ($\times 10^6$/mm^3)	5.7 ± 0.02	$4.8 \pm 0.02^*$	$4.5 \pm 0.03^{**}$	5.6 ± 0.03	$4.8 \pm 0.03^*$	$4.6 \pm 0.03^*$
WBC ($\times 10^3$/mm^3)	16.1 ± 0.06	$18.63 \pm 0.04^{**}$	$19.5 \pm 0.03^{**}$	16.2 ± 0.02	$18.6 \pm 0.03^{**}$	$19.5 \pm 0.03^*$
Hb (g/dL)	14.1 ± 0.02	$12.3 \pm 0.5^*$	$11.8 \pm 0.2^{**}$	14.2 ± 0.3	$12.1 \pm 0.5^*$	$11.5 \pm 0.2^{**}$

Values represented are mean $\pm$ SE. For statistical analysis, one-way ANOVA and Student's t-test was applied and $p < 0.05$ was considered significant*.

Effects of arsenic exposure on catalase activity

Besides the SOD assay, effects of arsenic toxicity on the kidneys of fish were also investigated by catalase assay. Our findings of the catalase assay revealed significantly increased catalase activity ($\sim$ 2-fold) in the fish of both the experimental groups 1

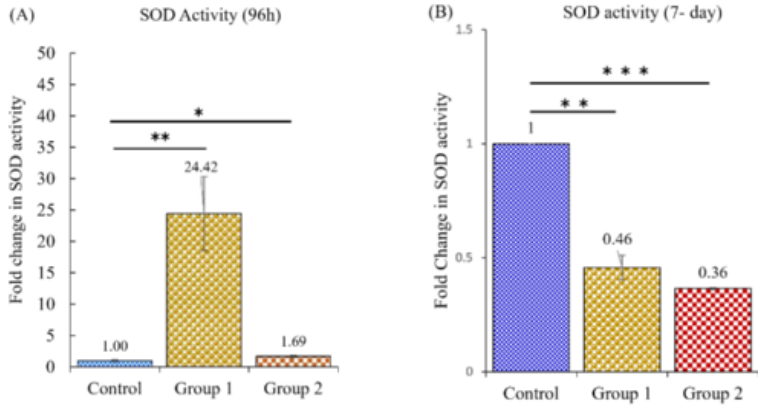

Fig. 3A Assessment of SOD activity in control and experimental groups 1 and 2 at 96h exposure to dibasic sodium arsenate heptahydrate, Fig. 3B SOD activity in control and experimental groups at 7-day exposure to dibasic sodium arsenate heptahydrate. p-value < 0.05 considered as significant.

and 2, compared to the control at 96h exposure period (Fig. 4A). Similar results were found in the fish exposed to arsenic for 7 days. Approximately 1.5-fold and 1.7-fold enhanced catalase enzyme activity was detected in the fish of experimental groups 1 and 2, respectively, compared to the fish of the control group (Fig. 4B).

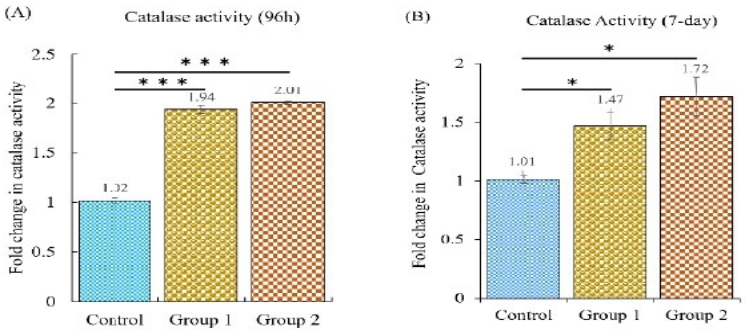

Fig. 4A Assessment of catalase activity in control and experimental group 1 and group 2 at 96h exposure to sodium arsenate. Fig. 4B Catalase Activity in control and experimental groups at 7-day exposure to sodium arsenate. p-value < 0.05 considered as significant.

Discussion

Fish are crucial aquatic animals that play a critical role in the maintenance of ecosystems, food web dynamics, nutrient cycling, and water quality of aquatic environments. They provide $\sim 20\%$ of the world's animal protein and are fundamental to human survival. Moreover, fish are used as bioindicators to assess the health conditions of aquatic environments. Additionally, they are used as model organisms in ecotoxicological research to study the toxicity of aquatic pollutants and their transfer through food webs (Boudou & Ribeyre, 2018). In the present work, *H. fossilis* has been used to assess the toxic effects of dibasic sodium arsenate heptahydrate on certain haematological and biochemical parameters.

Our data showed that the LC_{50} value of dibasic sodium arsenate heptahydrate at 96h was 48.80 g/L for *H. fossilis*, suggesting that it is a highly toxic inorganic compound to fish. Earlier toxicological studies on sodium arsenate have also reported its lethality on other fish species, including Indian major carp Catla catla (43.78 mg/L), Cnesterodon decemmaculatus (7.32 mg/L), Oryzias latipes (30.3 mg/L, 7 days), and Danio rerio (258.8 mg/L) Ohki et al. (2002), Liu et al.(2005), Kavitha et al. (2010), Gonzalez Nunez et al. (2022). The variation in the LC_{50} value of the dibasic sodium arsenate heptahydrate in different species could be due to differences in the species of fish, age, size, and life stage of the fish used in different experiments Capkin et al. (2006). Other factors, such as duration of exposure, route, and method of administration of the compound, may result in different LC_{50} values even for the same compound Connell et al. (2016). Additionally, water quality parameters, including temperature, pH, dissolved oxygen, and the hardness of water, may affect the chemical behaviour of the toxicant and metabolic rate of the organism and thus cause variation in the LC_{50} of the compound Hall & Anderson (1996) and Capkin et al. (2006).

In the present work, haematological parameters were measured to assess toxic effects, as variations in the blood parameters can be detected quite early. The decreased level of haemoglobin was observed in the fish exposed to the arsenic compound compared to the fish in the control group. The decreased level of Hb could be due to reduced production of RBCs or increased destruction. Earlier studies have reported that heavy metal toxicants may inhibit the synthesis of heme (the iron-containing part of Hb), cause oxidative damage to RBCs, and haematopoietic tissues of fish, such as kidney and spleen Kumar and Banerjee (2016), Han et al. (2019), Shahjahan et al. (2022). Like earlier studies, the decreased number of RBCs was seen in the fish exposed to varying concentrations of arsenic at both 96h and 7 days of exposure. The decreased RBC count in the treated fish could be due to reduced haematopoiesis because of damage to the kidney and spleen Onita et al. (2021), Zheng et al. (2025). Other factors responsible for the decreased number of RBCs could be haemolysis, inhibition of haemoglobin synthesis, and internal bleeding due

to exposure to arsenic Kavitha et al. (2010) and Jamil Emon et al. (2023).
In contrast, significantly increased levels of WBCs were observed in the fish treated with the arsenic compound compared to fish of the control group at both 96h and 7 days of exposure. The increase in the WBCs is possibly because they act as key components of the fish's immune system, and their number often changes in response to environmental stressors and toxicants. Studies have reported a marked increase in the WBC count when fish are exposed to arsenic and other toxic metals such as chromium, cadmium, and lead Kumar & Banerjee (2016), Huang et al. (2022). These increased WBCs are considered a physiological defense response of the fish to oxidative stress and tissue damage induced by arsenic to counteract the toxicity and inflammation resulting from reactive oxygen species (ROS) generated by arsenic exposure. Elevated leukocyte levels, especially phagocytes and lymphocytes, facilitate phagocytosis and detoxification of foreign metallic toxicants circulating in the bloodstream Kavitha et al. (2010) and Witeska et al. (2023).
Antioxidant enzymes are biochemical biomarkers used extensively in ecotoxicology to evaluate the effects of environmental stress and aquatic pollutants. The SOD enzyme and catalase work together as the first line of defense to counteract the oxidative damage caused by reactive oxygen species Dar et al. (2019) and Kumar et al. (2020). In our study, SOD enzyme activity was increased 25-fold in the fish of experimental group 1 compared to the fish of the control group at 96h exposure. The increased activity is possibly to neutralise the toxic effect caused by the arsenic as a compensatory defence against oxidative stress. The upregulated activity of SOD prevents the accumulation of free radicals, maintains redox balance, and protects the cells from damage Vutukuru et al. (2006). However, the activity of the SOD enzyme was increased only 2-fold in the fish of experimental group 2 at 96h exposure, suggesting that the high concentration of arsenic overwhelmed the SOD defence mechanism Kim et al. (2015). In contrast, the activity of the SOD enzyme was found to be decreased significantly in both experimental group 1 and experimental group 2 at 7 days' exposure, compared to the fish of the control group. The observed decrease in SOD activity in 7 days' exposure could be possible due to inhibition of enzyme activity by the toxicant, co-factor displacement, saturation of the defence mechanism, and overwhelming accumulation of the reactive species that exceeds the cell's compensatory capacity Kim et al. (2015), Sac & Yeltekin (2023). Unlike SOD, the activity of the catalase enzyme was found to be significantly increased in the fish of both the experimental groups at 96h as well as 7 days of exposure to arsenic, compared to controls. The increased enzymatic activity of catalase could be in response to oxidative stress caused by arsenic, as has been reported in earlier studies Atli et al. (2006), Vinodhini & Narayanan (2009). The variable effects of arsenic possibly could be due to differential binding of arsenic to the sulfhydryl (-SH) groups of the active sites of the SOD and catalase enzymes, causing conformational changes and misfolding of proteins Tamas et al. (2014),

Wang et al. (2024).

Conclusion

In the present study, the effects of arsenic on the haematological and biochemical parameters were investigated in the H. *fossilis*. Our data showed marked differences in the haematological parameters of fish treated with specific concentrations of arsenic for 96h and 7 days, suggesting that arsenic affects fish blood parameters. Changes in biochemical parameters, such as SOD and catalase activities, further support our finding that arsenic is a potent toxicant and may affect the overall health of fish. Further studies, including the expression of stress-induced biomarkers and proteins, will shed more light on the molecular mechanism of arsenic toxicity. Additionally, our study suggests that continuous monitoring of the aquatic environment is essential for keeping fish healthy and, thereby, the human population consuming such fish for their dietary intake.

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