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Evaluation of some Hematological and Biochemical Parameters in the Grass Carp (*Ctenopharyngodon idellus*) Exposed to Sub-Lethal Concentrations of Benzene

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ABSTRACT

The current study aimed to investigate the impact of long-term exposure to sub-lethal concentrations of benzene on the biochemical and hematological parameters of grass carp *Ctenopharyngodon idellus*. Healthy 80 fish were randomly divided into four treatment groups: T1 (control group), T2, T3 and T4 exposed to different levels of benzene concentrations (0, 0.25, 0.5 and 0.75ppm) for a period of 96 h. The concentration of cellular components of blood including sugar and proteins was estimated; as well as the red and white blood cells counts, contents of blood lymphocytes, neutrophils, hemoglobin (Hb), and packed cell volume (PCV). The results revealed that there were significant decreases in the total number of erythrocytes and glucose in case of T2, T3, and T4 as compared to control group (T1) ($P \leq 0.05$). Similarly, there were significant decreased total numbers of leukocytes and lymphocytes between T3-T4 groups with those in T1 group at $p \leq 0.05$. Comparison with the control treatment (T1), none of PCV, TP and neutrophils showed any differences among treatments (T2,T3 and T4) at $P>0.05$. On Hb level on the other hand decrease significant was observed between groups T4 versus T1($p \geq 0.05$). Biochemical parameters like glucose and total protein levels had been changed when grass carp exposed to sub-lethal doses of benzene leading to alterations in physiological condition.

INTRODUCTION

Benzene's impact on the environment is that it can pollute water sources and accumulate in aquatic ecosystems. For example, it can enter streams via industrial effluent, crude oil leakage and careless disposal of containers with benzene **(Chatterjee et al., 2023)**. Many studies have shown that fishes are exposed to benzene cause changes in their biochemistry and hematological parameters. These change may have several significant implications for fish populations' general health as well as wellness, since perturbations of biochemical pathways and hematologic processes can impair certain crucial physiological functions including metabolism, organ function and immune response **(Duman & Şahan, 2018)**.

Therefore, long term researches on benzene effects on grass carp are important because they are a key species in fresh water ecology globally and also in aquaculture **(Wildhaber *et al.*, 2023)**. In fact it is straightforwardly visible as its food is easily found within Iraq's marshes **(Taher, 2018)**. It is important to understand the consequences of chronic exposure to sublethal levels of benzene in grass carp for the purpose of assessing the potential risks associated with this form of pollution and developing effective management plans aimed at protecting aquatic ecosystems as well as fish populations' health **(Solaiappan & Prakash, 2021)**.

Benzene is a poisonous compound which can motive adjustments in liver enzyme stages, kidney harm, lipid peroxidation, oxidative strain, and hematological values inclusive of packed mobile quantity (PCV) and hemoglobin (Hb) concentration **(D'Andrea & Reddy 2014; Sharique *et al.* 2021; Solaiappan & Prakash 2021)**.
Consequently, such research would assist in understanding the specific biochemical and hematological changes that occur due to benzene exposure in grass carp hence will provide relevant information in assessing potential risks associated with fish populations exposed to benzene pollution and subsequent development of effective management strategies. The study results may also contribute to the area of environmental toxicology by revealing sublethal effects of benzene on biochemical and hematological parameters among grass carps. By doing so it will be possible to develop guidelines and regulations aimed at reducing the negative impacts of benzene contamination on aquatic ecosystems and conservation of fish species.

The aim of current study was to evaluate the effect chronic exposure to low levels of benzene on the blood profile of a grass carp population as well as identify any haematogical alterations resulting from prolonged sub lethal exposure to varying concentrations of benzene, evaluate the potential risks and hazards of benzene pollution on the health of grass carp populations and determine the threshold level of benzene concentration that may cause significant alterations in biochemical and hematological parameters in grass carp.

MATERIALS AND METHODS

Collection and maintenance

Grass carp were obtained from Aquaculture unit, College of Agriculture, and kept for 3 days in a controlled laboratory environment for acclimatization prior to the experiment. About 80 fish (weight range 10.4- 19.8g and length range 10.3- 12.4cm) were selected for the study.

Fish were randomly divided into five groups: a control group (not exposed to benzene) and four experimental groups exposed to sub-lethal concentrations (0.25, 0.5, 0.75, and 1ppm) of benzene.

Water quality parameters, such as water temperature (thermometer), dissolved oxygen, salinity, pH were monitored daily to ensure optimal conditions for fish (Table 1).

During the acclimation period, the fish were fed on a diet that was prepared using feed materials containing fishmeal, soybean meal, wheat, wheat bran, starch, and a mixture of vitamins and minerals (Table 1). However, they were not fed during the acute tests.

Table 1. Components of the feed (%)

Ingredients	**%**
Fishmeal	25
Soybean meal	22
Wheat meal	29
Wheat bran	20
Starch	2
Vitamins and minerals premix	2
Total	100

Preparing of water-soluble fraction of benzene

The water soluble fraction (WSF) of benzene was prepared by diluting 394mg of benzene in one liter of distilled water in a glass container and slowly stirred for 20h at room temperature (20± 2°C). After mixing, it was allowed to be separated for 1 to 6h and the upper layer was siphoned off, leaving the residual solution composed of saturated benzene represented the stock solution. Subsequently, it was stored in a closed glass container at 10°C to prevent evaporation and contamination **(Anderson *et al*., 1974; NAS, 1975)**.

Calculating concentration the median-lethal of benzene

The acclimated fish were distributed into five 30L covered glass aquaria, each contained 5 animals, with three replicates (n=5 fish/treatment). Aqueous static renewal tests were conducted to estimate four days median lethal concentration (LC_{50}) values concentrations for benzene under standard laboratory conditions.

Grass carp in the experimental group were exposed to various concentrations of benzene (0.25, 0.5, 0.75, and 1ppm) according to the following treatments (T2, T3, T4 and T5), respectively, while the control group (T1) was kept in the same condition without exposure to benzene.

The chemical analysis ratios were performed using a rapid content analyzer. The proximate analysis gave the chemical composition of the experimental diets which included 25.22% protein, 3.7% lipids, 12.63% ash, 2.06% fiber, 45.26% nitrogen free extract (NFE), and 11.26% moisture.

Blood standard

Following the exposure period (96h), blood samples were collected from each fish heart using 2ml glass sterile syringe and transferred into a test tube with an anticoagulant for hematological analysis using a Mindray bc-30s equipment, manufactured in China.

The hematological analysis included measuring parameters, such as the number of leukocytes and erythrocytes, lymphocytes (%), neutrophils (%), Hb content, and PCV. The glucose and total protein levels were also determined in blood plasma. The kits from the Chinese company "Mindray" were used to analyze the blood samples for these parameters.

Statistical analysis

The hematological analysis data were statistically analyzed using a complete random design (CRD) in order to examine the impact of various treatments on the parameters being studied. To determine significant differences between the means, the analysis of variance (ANOVA) was employed, and the LSD test was used at a significance level of 0.05. The statistical analysis was performed using the SPSS program (Version 26).

RESULTS

In the acute toxicity test, the mortality rate increased with increasing benzene concentrations after 96h of exposure in grass carp. The mortality rate for grass carp exposed to benzene concentrations of 0, 0.25, 0.50, 0.75, and 1ppm were 0, 11.1, 38.87, 44.4, and 100%, respectively (Table 3). The results showed that LC_{50} of benzene was 0.619ppm for grass crap (Fig. 2).

In biochemical and hematological parameters *C. idellu*s were exposed to different concentrations (0, 0.25, 0.5 and 0.75 ppm) of sub-lethal concentrations of benzene according to the following treatments: T1 (control), T2, T3, and T4, respectively.

Table 2. Environmental factors measured in benzene test.

Environmental factor	Value measurement
Temperature (°C)	20± 0.32
Salinity (psu)	2.3± 0.21
pH	7.8± 0.62
Dissolved oxygen (mg/L)	7± 0.34

Table 3. Mortality percentages of *C. idellus* exposed to different concentrations after 96h of exposure to different concentrations of benzene.

Treatment (ppm)	Mortality rate (%)
Control	0
0.25	11.1
0.5	38.87
0.75	44.4
1	100

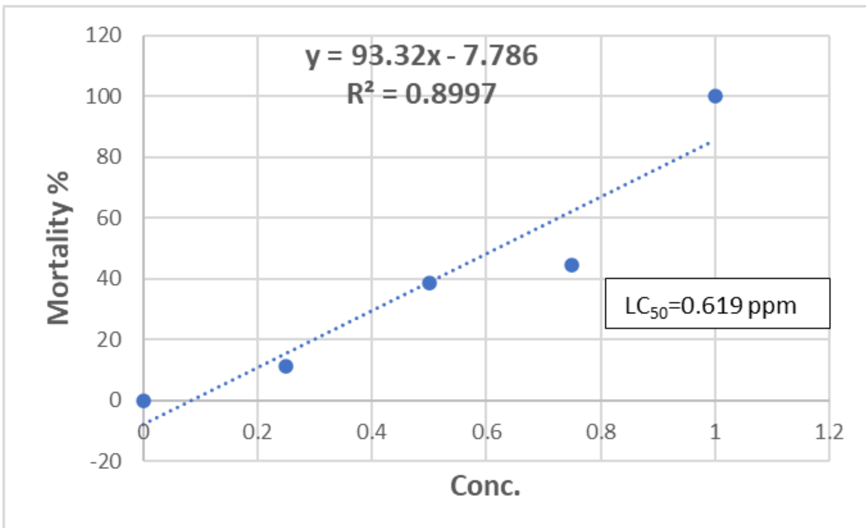

Fig. 1. LC_{50} of benzene on *C. idellus* after 96h.

It was shown that there were significant decreases ($P\leq 0.05$) in the total number of erythrocytes and glucose levels in the treatments (T2, T3, and T4) compared to the control group (T1; Figs. 2, 8). It was also noted that, there were significant decreases ($P\leq 0.05$) in the total number of leukocytes and lymphocytes (%) between the T3 & T4 groups with the T1 group (Figs. 3, 4). However, there was no significant differences ($P> 0.05$) observed between the T2 and T1 groups. No significant differences ($P> 0.05$) were observed in PCV, total protein level, and neutrophils (%) among the treatments (T2, T3, and T4) compared to the T1 group (Figs. 5, 6, 9). For blood Hb, a significant decrease difference ($P\leq 0.05$) was found between T4 and T1, and no significant differences ($P> 0.05$) were observed between the other treatments.

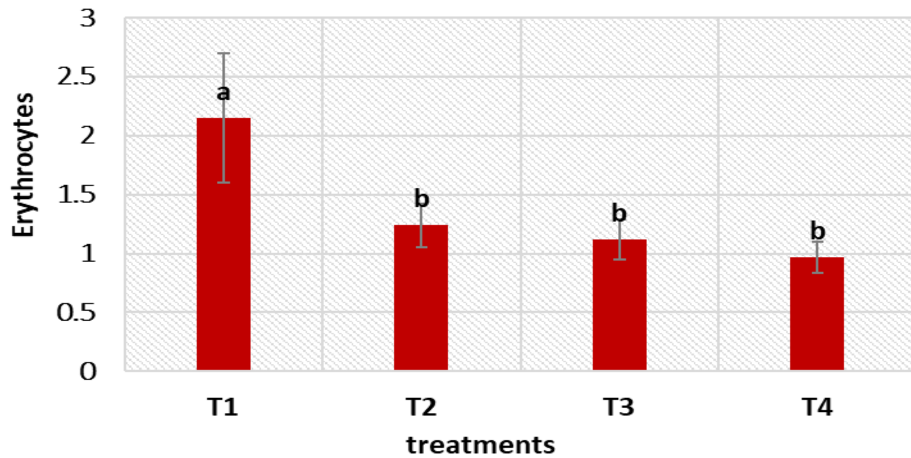

Fig. 2. Erythrocytes count (cell × 10^6/ mm^3) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

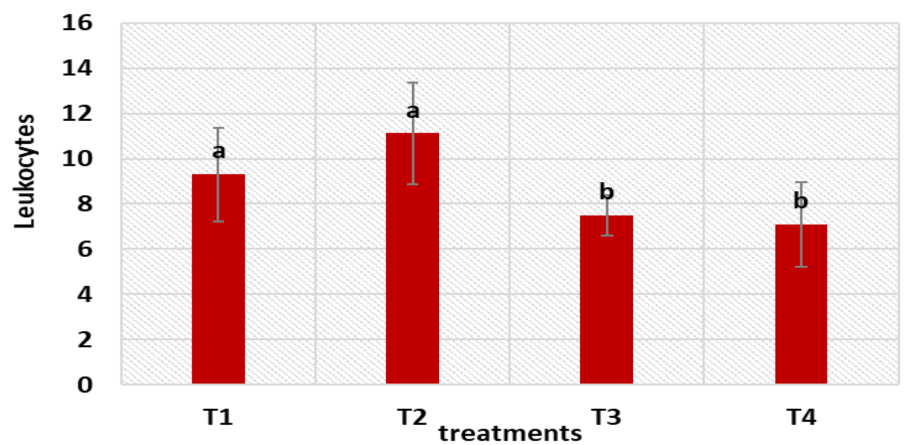

Fig. 3. Leukocytes count (cell × 10^3/ mm^3) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

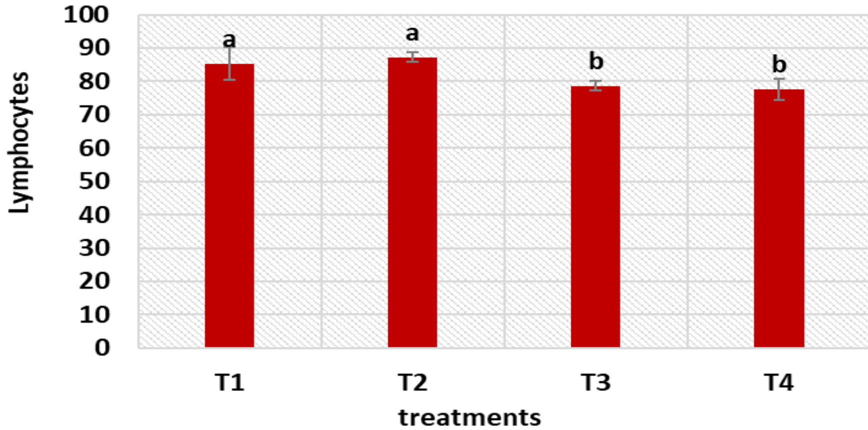

Fig. 4. Lymphocytes (%) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

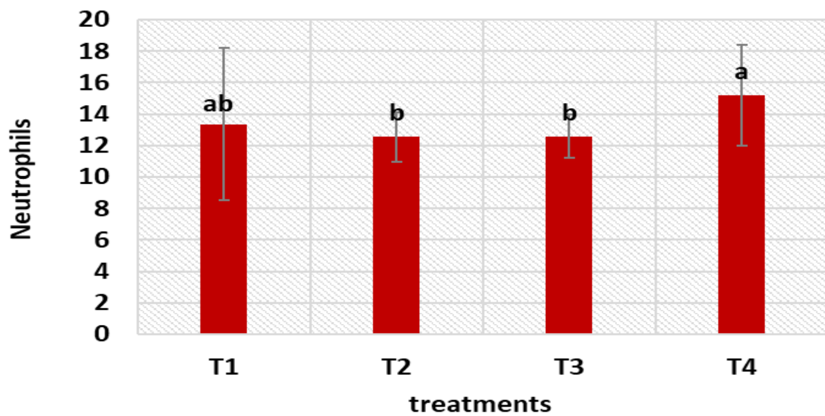

Fig. 5. Neutrophils (%) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

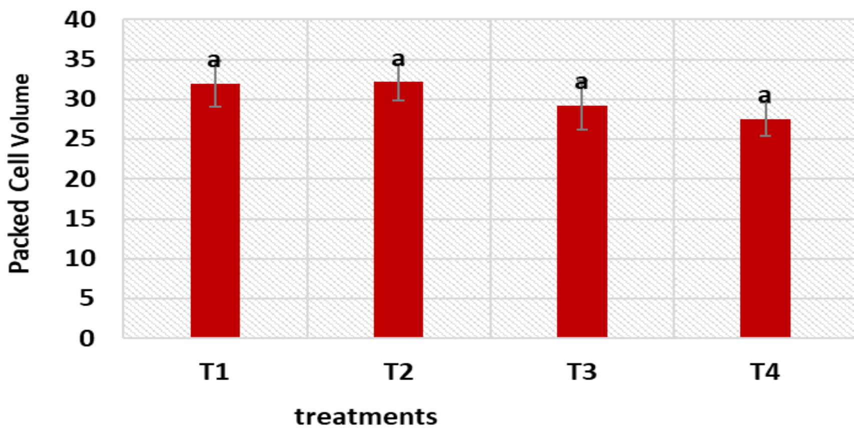

Fig. 6. Packed cell volume (PCV %) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

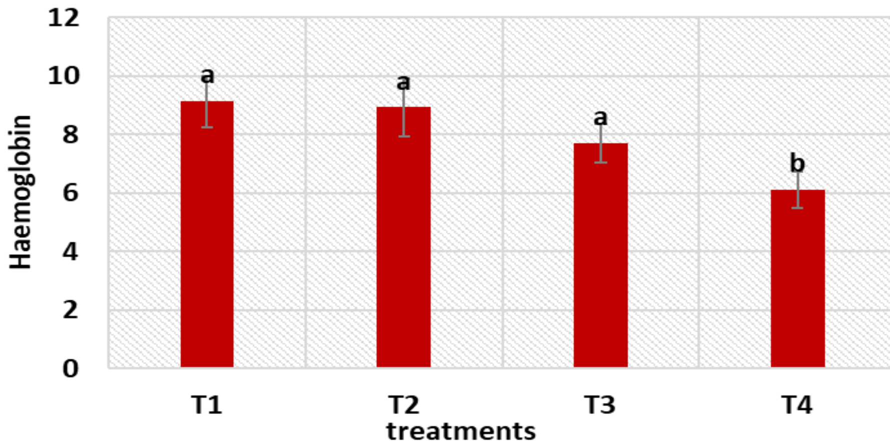

Fig. 7. Hemoglobin (Hb) (g/ 100ml blood) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

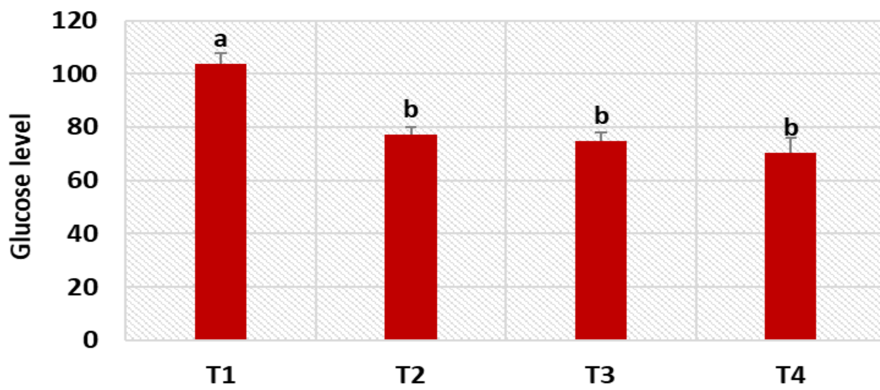

Fig. 8. Glucose level (mg/ 100ml blood) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

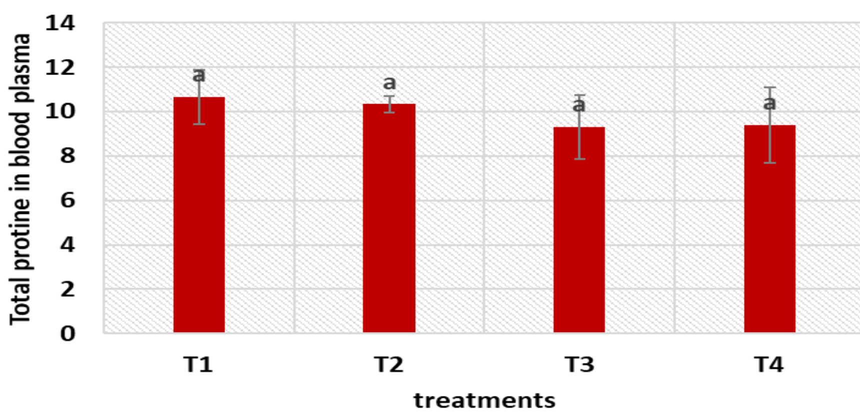

Fig. 9. Total protein in blood plasma (mg\ 100ml blood) of *C. idellus* exposed to sub-lethal concentrations of benzene for a period of 96h. Different letters in one row indicate significant differences ($P \leq 0.05$)

DISCUSSION

Water pollution issues represent significant challenges to the ecosystem community **(Lin *et al*., 2022; Pereira *et al*., 2011)**. Among several polluting compounds, there are three specific monocyclic aromatic hydrocarbons which play a prominent role, including benzene, toluene, and xylene. These substances are utilized in several household and industrial applications, either individually or in combination, as documented by the Environmental Protection Agency **(EPA, 1980)**. The current study revealed that the mortality ratio of grass carp exposed to different concentrations of benzene, with an LC_{50} of 0.619ppm, increased as the concentration of benzene increased (Fig. 1).

The study of blood in different species of fish draws attention to how the coadaptation of blood characteristics, phylogeny, locomotor activity, habitat and the potential to acclimate to different environmental conditions can be understood. **(Tavares-Dias and Moraes, 2004)**.

Changes in the hematological characteristics of fish can serve as a reflection of the alterations in the surroundings experienced by these organisms. As a result, these characteristics function as invaluable markers of the condition of aquatic creatures that are exposed to contaminants since they embody one of the primary observable stress reactions prompted by shifts in the environment **(Davison *et al*., 1993)**.

Moreover, hematology analysis plays a vital role in fish health assessment, and evaluating physiological biomarkers, as it revealed their potential to serve as biomarkers in such an assessment **(Maftuch *et al*., 2020)**.

In a previous study, fish exposed to alkyl benzene sulfonate at concentrations equivalent to 10 and 20% of the 96-hour LC_{50} showed a slight decrease, though notable, in the levels of erythrocytes, Hb, and PCV. The concentrations of alkyl benzene sulfonate used were 0.006 and 0.012mg/ l, respectively. This decrease in hematological parameters could be due to the harmful effects of the surfactant on hematopoietic tissues. Similarly, lower levels of erythrocytes and Hb can result from erythrocyte destruction in blood-forming tissues, hemolysis, abnormal heme-synthesis, increased generation of free radicals, and insufficient oxygen supply by gills **(Ghaffar, 2020)**.

It was observed in the present study that exposure to sub-lethal concentrations of benzene can cause significant or insignificant decrease in the chemical and biological parameters of grass carp. The decrease in PCV levels may be due to the lysis of erythrocytes or owing to the benzene-induced damage to the bone marrow, where red blood cells are produced. Therefore, the reduction in erythrocytes, Hb, and PCV levels is a clear indication of anemia **(Ololade & Oginni, 2010)**. Moreover, the decrease in erythrocytes, Hb, and PCV values may be attributed to altered erythropoiesis activity **(Ghaffar, 2020)**.

In another study, the exposure of fish to benzene for 96 hours resulted in significant decreases in the values of red blood cells, white blood cells, lymphocytes, and glucose levels in the blood plasma **(Maduenho & Martinez, 2008)**.

There was also a slight decrease in neutrophil cells, PCV, Hb, and total protein, although it was not statistically significant. Alteration of the permeability of erythrocytes to hinder oxygen transport is the key way in which hydrocarbons in petroleum can cause serious hemolysis **(Duarte et al., 2010)**.

The effects of alkyl benzene sulfonate toxicity on *Oreochromis mosambicus* were evaluated by the fish being exposed to 10% and 20% concentrations of their 96-hour LC50. In the exposed fish there was a rise in levels of erythrocytes, Hb, PCV, plasma protein and plasma glucose followed by a decline. A fall in hematological parameters indicates that alkyl benzene sulfonate had an adverse effect on the fish **(Ghosh *et al.*, 2022)**.

Stress responses are important for organisms' survival. This is because glucose availability provides energy substrates to tissues such as brain, muscles and gills thereby allowing animals to respond defensively to stressors. Polycyclic aromatic hydrocarbons (PAHs) exposure has been observed to increase plasma glucose levels in previous studies **(Vijayan *et al.*, 1997; Pacheco & Santos, 2001)**.

It is well known that organisms' immune systems are vulnerable to xenobiotic burden, and that hydrocarbons can cause diverse effects on fish's immune components and functions **(Reynaud & Deschaux, 2005)**. In sea bass, a prolonged exposure to Arabian crude oil led to a considerable drop in white blood cell counts specifically lymphocytes. The fall of lymphocytes shows no sign of escape of white blood cells out of the circulatory system on their way to probably damaged tissues. Rather, it suggests a disturbance in apoptosis or necrosis activity, which can be attributed to the cytotoxic effects of PAHs due to their water solubility and lipophilicity **(Schirmer *et al.*, 1998)**.

In the current study, an increase in neutrophil percentage and a decrease in lymphocyte percentage in T4 compared T1 were observed after exposure to the sub-lethal concentrations levels of benzene. Moreover, similar effects have been observed in mammals injected with substances derived from petroleum representing the activity of the first and second lines of defense against cellular damage **(d'Azevedo *et al.*, 1996)**. Studies in cultured fish *Oreochromis aureus* have also confirmed increases in monocytes and neutrophils, along with a decrease in lymphocytes, during different stressors. Neutrophils are believed to have phagocytic activity, which may explain their increased percentage during infectious situations **(Silveira-Coffignya *et al.*, 2004)**.

Blood levels of Hb in the fish are generally used to distinguish abnormalities and negative signs of fish health **(Casanovas et al., 2021)**. For example, exposure to lethal doses of butachlor caused severe hematologic changes in fishes. Higher values of erythrocyte count, Hb concentration, and PCV in exposed fish recommend stress-induced erythrocyte production and Hb **(Habiba, 2012)** Butachlor exposure also increased white blood cells numbers increased intensely (WBC) due to immune response. Additionally, the percentage of lymphocytes increased in fish after butachlor exposure, signifying an immune response **(Habiba, 2012)**.

CONCLUSION

Our results showed that short-term exposure to sub-lethal concentrations levels of benzene causes toxicity and have a deleterious impact on grass carp's physiological state, biochemical and hematological parameters, and overall health and leading to a reduction in immune capacity. It can also drastically lower the productivity of fish and other aquatic organisms in water bodies. Further investigations into the effects of benzene and hematological as well as other biochemical markers need to be examined in different types of animals.

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