

syringe into the muscle tissues at a right angle to the ventral surface of the fish until the blood was drawn into the syringe. Following that, the collected blood was transferred into a vial containing EDTA and placed in a freezer.

2.5.2 Preparation and estimation of haemoglobin

The haemoglobin fraction was isolated using the methodology outlined by Adisa et al. (2004) based on the principle of hypotonic lysis. To extract haemoglobin, the blood from all the fish in each treatment group were taken in a separate centrifuge tube and centrifuged at 3000 rpm for 10 minutes. After centrifugation, the RBC pellet was washed three times with a 0.14 M NaCl solution. Followed by washing, the RBC cell pellet was dissolved in phosphate buffer saline. Then one volume of RBC suspension was lysed by adding two volumes of 0.01M phosphate buffer (pH 7.4) and 0.5 volume of CCl₄. The haemolysate was further purified by centrifugation at 2300 rpm for 15 minutes at room temperature to remove any debris. The fraction with a high concentration of haemoglobin, specifically the upper layer was isolated and used to estimation of haemoglobin. The cyanmethemoglobin method, which is internationally recommended, was employed for determining the quantity of haemoglobin. 5 mL of cyanmethemoglobin reagent (Drabkin's solution) was transferred into sterilised test tubes. Subsequently, 20 µL of hemolysate from each of the experimental group fish were added separately to the tubes. Following that, the tubes were gently agitated and allowed to kept for 10 minutes at room temperature. After the incubation, the absorbance was measured in a spectrophotometer at 540 nm.

2.6 Determination of total RBC and WBC counts

The enumeration of RBC and WBC was performed using a Neubauer hemocytometer, following the methodology described by Martins et al. (2004).

$$\text{Total RBC count} = \frac{\text{Number of cells counted (N)} \times \text{Dilution factor}}{\text{Area} \times \text{Depth}}$$

$$\text{Total WBC count} = \frac{\text{Number of cells counted (N)} \times \text{Dilution factor}}{\text{Area} \times \text{Depth}}$$

2.7 Enumeration of differential leucocytes count

The differential blood count of the fish from each experimental group was conducted using the procedure outlined by Hudson and Hay (1991). To carry out this experiment, a minute quantity of blood was placed on a glass slide, and a thin smear was made by using oil and a spreader slide. Afterward, the blood smear was allowed to dry in the air, and then the slide was treated with Leishman's stain. The slide was incubated for 5 minutes and subsequently diluted with distilled water, followed by an additional incubation period of 10 minutes. After staining, the slides were rinsed with tap water and left to air dry. Finally, the slides were examined by placing them under the microscope.

2.8 Assessment of erythrocyte sedimentation rate (ESR)

The ESR of the fish from all the experimental groups was determined using the Westergren method. In order to perform ESR, the blood drawn from each sample was transferred into a Westergren-Katz tube up to the 200 mm mark immediately following blood collection. Subsequently, the tube was positioned vertically in a rack and left undisturbed for 1 hour at room temperature. During this period, measurements of the distance between the lowest point of the surface meniscus and the topmost layer of the red cell sediment were taken. The ESR is a measure of the distance that erythrocytes decrease in millimetres over the course of one hour.

2.9 Statistical analysis

Results were given as Mean $\pm$ Standard Deviation (SD) obtained from three independent experiments, and the data was assessed by one-way analysis of variance (ANOVA). The '*p*' values less than 0.05, 0.01 and 0.001 were considered as significant, highly significant and extremely significant difference between the groups.