ABSTRACT

The present study aimed to prepare and examine blood smears of Labeo rohita for haematological evaluation to assess health status, detect diseases, and monitor environmental impacts. Blood samples were collected through caudal vessel puncture and cardiac puncture, followed by smear preparation, methanol fixation, and staining with Leishman and Giemsa stains. Microscopic observation at 40x magnification (wide field of view 0.45 mm; area 0.16 mm^2) revealed oval, nucleated erythrocytes with centrally placed nuclei, along with different nucleated leukocytes such as lymphocytes, monocytes, and granulocytes. Quantitative analysis showed reduced red blood cell counts (30- 35 cells) and elevated white blood cell counts (>500 cells), indicating possible infections or immune responses. These findings emphasize the importance of standardized haematological techniques as a non-lethal diagnostic tool in aquaculture, facilitating early disease detection, effective health monitoring, and sustainable fisheries management.

1. INTRODUCTION

- "Haematology is the science of studying the anatomical, physiological and pathological aspects of blood
- Haematology is a branch of medicine concerning the study of blood, blood-forming organs such as bone marrow, and blood-related disorders and diseases The word “heme” comes from the Greek word for blood. Haematological tests are used to detect and diagnose diseases

2. OBJECTIVE

- Assess fish health and detect diseases.
- Monitor effects of environment and water quality.
- Study physiological and immune responses in fish.

3. MATERIAL & METHOD

- Live fish (specimen for study)
- Syringe (5 mL) with fine needle (22–26 G)
- Anticoagulant: EDTA (for preventing clotting)
- Collection vial or purple top EDTA tube(1.5–2 mL, labelled)
- Clean, grease-free glass slides (minimum 2–3 per sample)
- Micropipette or fine dropper (for placing blood drop)
- Methanol (absolute, for fixation)
- Stains: Giemsa, Leishman, or Wright’s stain
- Distilled water or phosphate buffer (for stain dilution)
- Compound microscope with 40× and 100× objectives
- Immersion oil (for 100× viewing)

Step 1: Sample Collection

There are many different techniques that can be used to collect blood sample from fish ,blood may be taken by -

- Serving the caudal peduncle
- Puncturing the caudal vessel
- Cardiac Puncture
- Dorsal Aorta Puncture

Puncture of Caudal Vessel

This method can be used for repeated blood sampling from larger fish (>10 cm) with minimal mortality. In fish of ~200 g, 0.5–1 ml of blood may be withdrawn weekly. The procedure is as follows:

- Anesthetize the fish.
- Attach needle to syringe and flush with heparin solution.
- Insert needle on the mid-ventral line, posterior to the anal fin, advancing until the spinal column is contacted.

Fig no. 1 collecting blood from caudal vessel

- Apply gentle suction while slowly withdrawing until blood enters the syringe, avoiding air bubbles.
- Withdraw needle, detach, and transfer blood to a chilled, angled collection tube.
- Mix gently by inversion.

Cardiac Puncture

This is the method which can be used to take repeatedly Blood samples from larger fish directly from heart (usually >10 cm long).

- Render the fish unconscious in an anaesthetic Solution.
- Flush out the syringe with Anticoagulant like Heparin Solution.
- Hold the fish with ventral side on top Insert the needle vertically, midway between the Anterior base of pectoral fins. Apply negative pressure on plunger until blood enter the syringe , slowly withdraw the needle until blood enters the syringe.
- Slowly remove the needle from the fish completely
- Remove the needle and empty the contents of the Syringe into a tube held on ice

Fig no. 2 collecting blood by cardiac puncture

Step 2 : Blood smear preparation

- Place a single drop of blood about 2 cm from one end of a clean, grease-free glass slide.
- Use a second slide held at a 45° angle to the first slide. Touch its edge to the drop, then swiftly push it forward to let the blood spread into a thin, feathered smear.

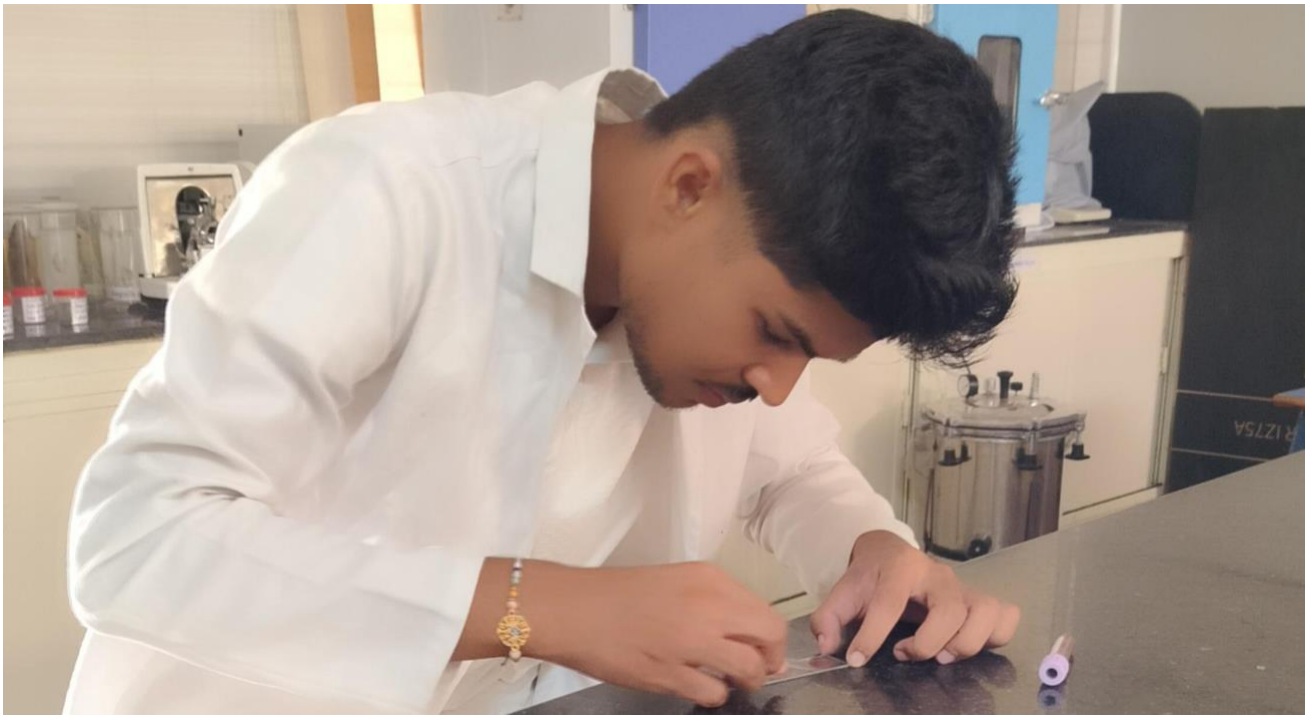
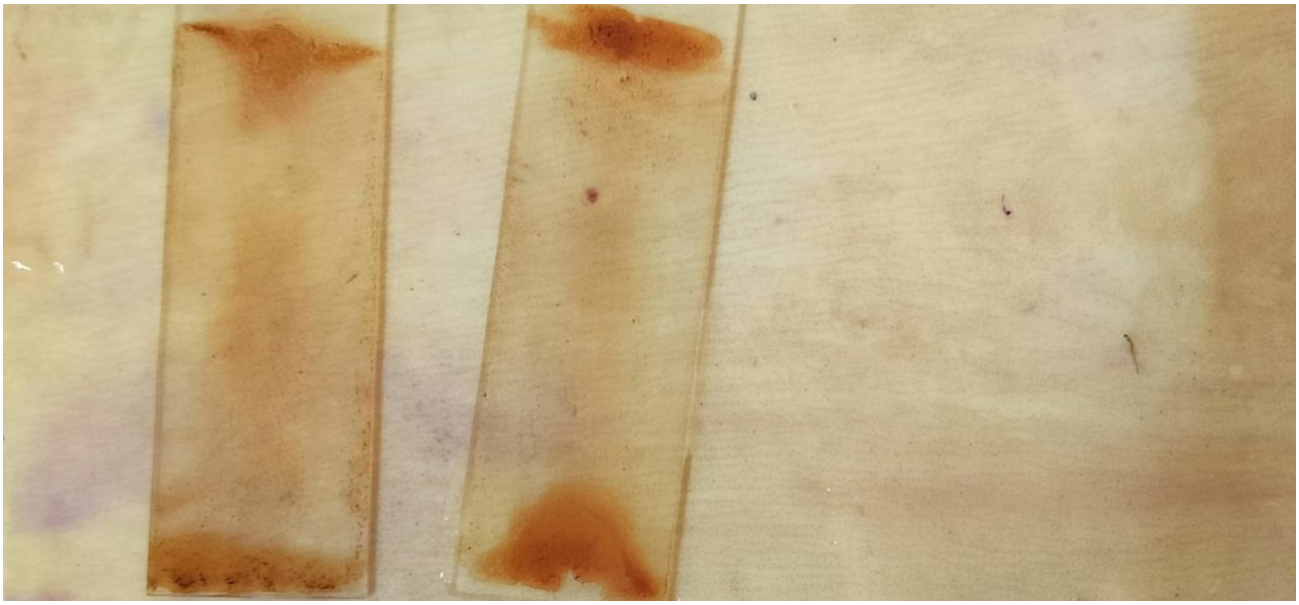


Fig no. 3 blood smear preparation

- Allow the smear to air-dry naturally—don't blow or heat



Step 3 : Fixation

- Fill the jar with absolute methanol until the level is high enough to cover the smear area on the slides.
- Place the air-dried slides vertically into the coupling jar.
- Keep slides in methanol for 2–5 minutes.
- Take the slides out carefully and let them air-dry upright on absorbent paper.

Fig no. 4 fix slide in methanol

Step 3 :Staining techniques

Fig no. 5 staining with leishman & giemsa stain

- Overlay each slide with 1 ml of Leishman stain
- After 1 to 2 minutes, overlay each slide with the Leishman stain still present, an equal volume of Giemsa working stain. Take care to evenly distribute the Giemsa solution throughout the length of the slide.
- After a few seconds, a metallic sheen should appear on the surface of the stain mixture. This indicates a proper staining mixture

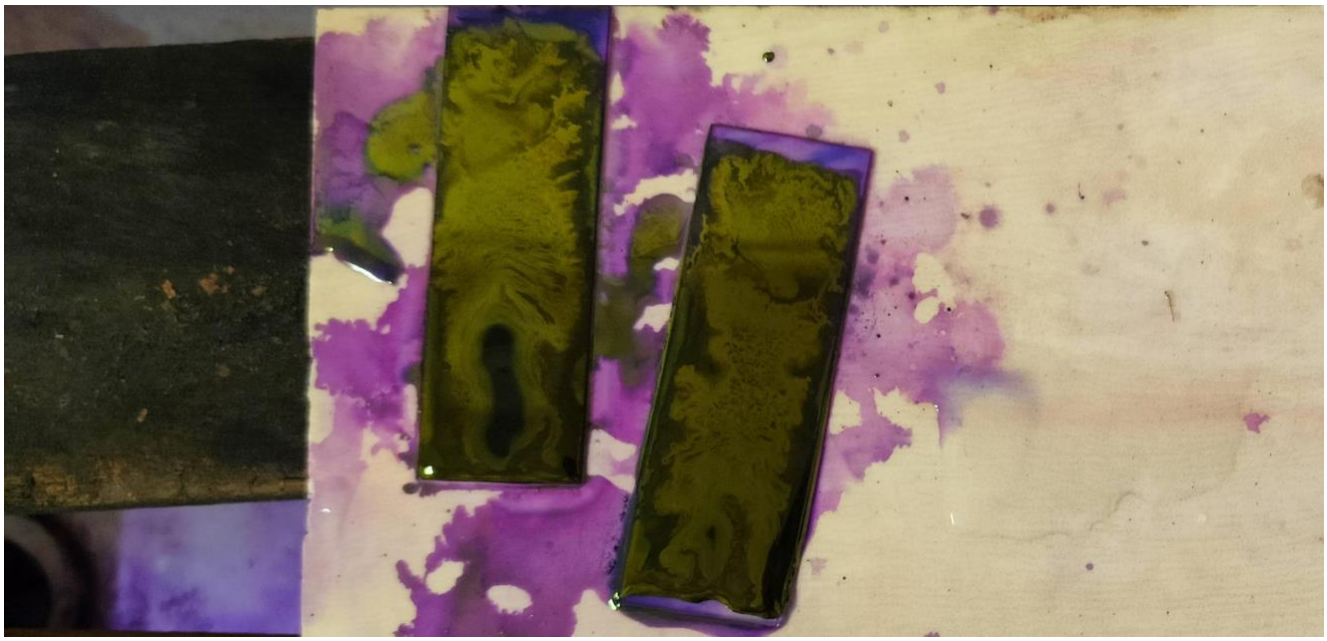


Fig no. 6 Metallic sheen observe in slide

- The staining time is variable (usually 14 to 20 minutes) at the end of which the stain is flushed from the slide with distilled water from a plastic squeeze bottle.
- The underside of the slide is wiped with an absorbent towel and the slide placed on edge to drain dry

4. RESULT

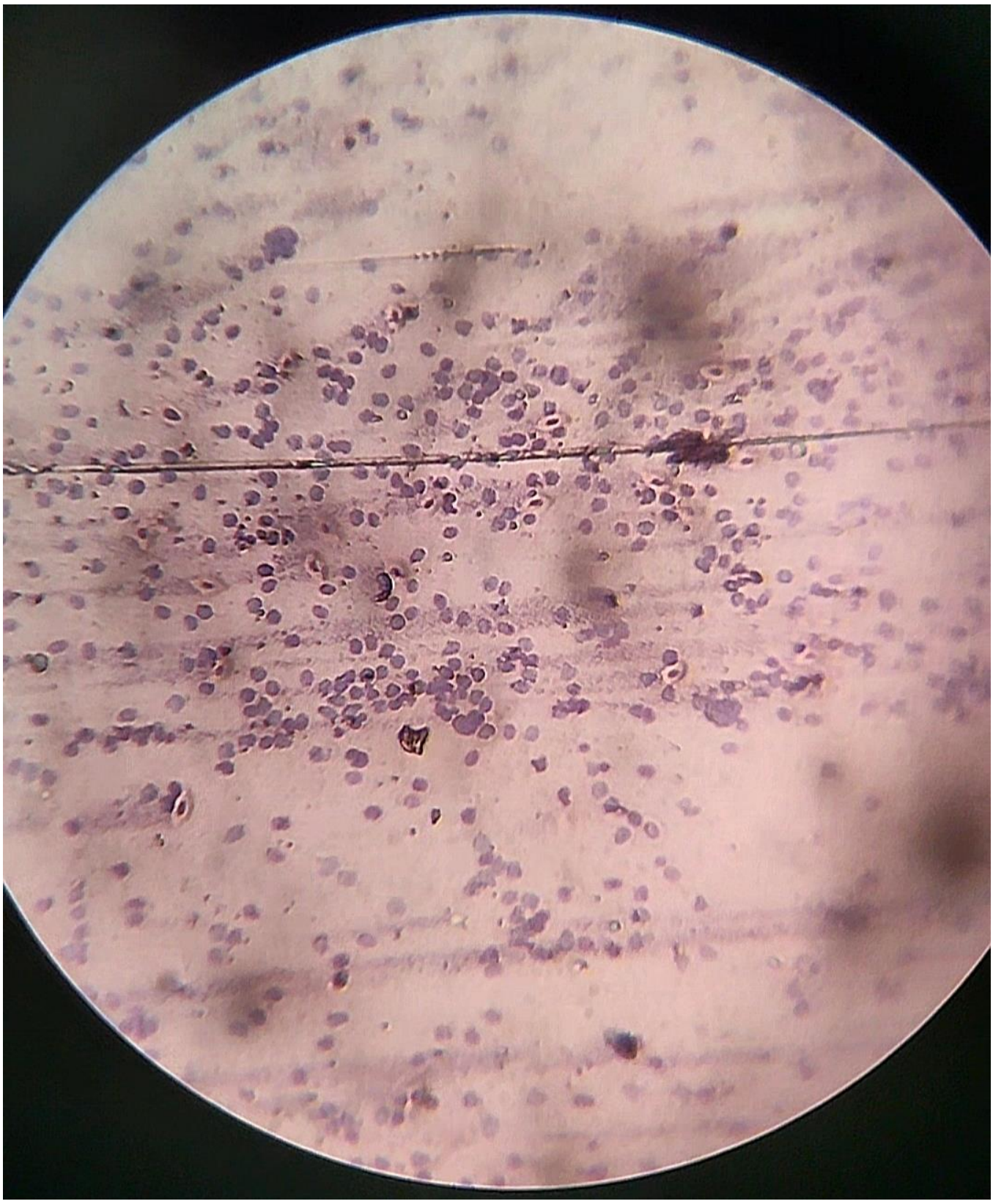
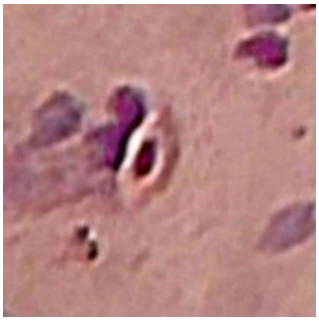
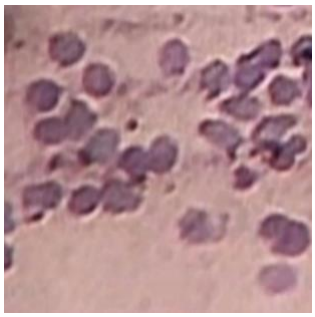


Fig. No. 7 Microscopic view of blood smear

Structural identification of blood cell

Microscopic Observation	Type	Shape and structure
	Erythrocytes (RBC)	Oval, nucleated red blood cells with centrally placed nucleus and haemoglobin-rich cytoplasm
	Leukocytes (WBC)	Nucleated white blood cells of various types (lymphocytes, monocytes, granulocytes) with diverse shapes, sizes, and granule patterns; function in immune defence.
	Monocyte	A fish monocyte is a large, round or oval white blood cell with a kidney-shaped nucleus and gray-blue cytoplasm

Result under microscope (40× Objective; FOV Ø 0.45 mm, Area ≈ 0.16 mm²)

- Specimen: Peripheral blood smear (wide field of view)
- Magnification: 40× objective (standard eyepiece)
- Field size: Diameter ≈ 0.45 mm; Area ≈ 0.16 mm²

Observations per field:

S.No	Blood cell type	Amount or No.
1.	Erythrocytes (RBC)	30- 35 cell
2.	Leukocytes (WBC)	>500 cell
3.	Monocyte	2 cell

- If a fish has fewer RBCs 20–35 (in wide field of view 0.45 mm; area 0.16 mm^2) and more than 500 WBCs,
- it usually suggests:
- Severe infection or inflammation — often due to bacterial, parasitic, or fungal disease, or possibly a chronic stress or immune response

5. CONCLUSION:

Fish haematology serves as a vital tool for assessing the physiological status, health, and stress levels of fish. By analysing parameters such as red and white blood cell counts, haemoglobin concentration, and haematocrit values, researchers and aquaculture professionals can detect diseases, monitor environmental impacts, and evaluate the effects of nutrition and management practices. As a non-lethal and reliable diagnostic method, haematological analysis contributes significantly to sustainable fisheries, improved aquaculture production, and the conservation of aquatic biodiversity.

6. BIBLIOGRAPHY

- https://www.researchgate.net/publication/343059764_Finfish_and_Shellfish_Physiology_Practical_Manual

- Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. Aquaculture, 500, 237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030
- Hematological methods in fish – Not only for beginners – ScienceDirect https://www.sciencedirect.com/science/article/pii/S0044848621011613
- https://pmc.ncbi.nlm.nih.gov/articles/PMC6716277/
- Madhuri sharma &priti Mishra practical manual for BFSc2nd year physiology of finfish and shellfish
- Satheeshkumar, P., Ananthan, G., Senthilkumar, D., Khan, A.B., &Jeevanantham, K. (2011). Comparative haematology and biochemical studies on wild marine teleost fishes from Vellar estuary, southeast coast of India. Comparative Clinical Pathology, 20(2), 127–134. https://doi.org/10.1007/s00580-009-0960-7