

each filled with 10 litres of water for the experiment. A completely randomised design was adopted. From the stored extracts, 3000 mg/25 cL, 5000 mg/25 cL, and 7000 mg/25 cl of distilled water were used as a stock solution, from which 5 ml of each concentration was infused daily into the plastic tanks.

## 2.6 Behavioural studies

During the 23-day sub-acute toxicity test, *C. gariepinus* exposed to graded concentrations of *S. occidentalis* was closely observed for the subsequent behavioural changes: air gulping, stunned positioning, skin peeling, aggression, and erratic swimming (fast and spiral movement). Weak, moderate, and high rankings were given to the observed changes.

## 2.7 Haematological test

According to Erhunmwunse and Ainerua (2013), haematological parameters, including red and white blood cell counts, packed cell volume, haemoglobin, and white blood cell differential counts, were performed following standard procedures.

### 2.7.1 Determination of haemoglobin

Using the Randox kit, haemoglobin was measured spectrophotometrically. Potassium ferricyanide (0.61 mmol/L), potassium cyanide (0.77 mmol/L), potassium phosphate (1.03 mmol/L), and 0.1% v/v surfactant are all present in the reagent.

Procedure:

The test tubes were labelled as blank, standard, and tests. 20 µL of whole blood was added to each tube. Additionally, 5 mL of the reagent was added to each tube, which was then incubated at room temperature for 3 minutes. The absorbance of all tubes was read at 540 nm against the reagent blank.

Haemoglobin conc. (g/dL) = Abs of sample X 36.77 --- (1)

### 2.7.2 PCV (Packed Cell Volume)

Blood was poured into a simple capillary tube until it was about  $\frac{3}{4}$  full. Plasticine was used to seal the tube's open end. The sealed tube was centrifuged at exactly 12,000 revolutions per minute for five minutes in a Hawksley micro-hematocrit centrifuge. The packed cell volume value was determined and expressed as a percentage for each tube after placing it in a micro hematocrit reader.

### 2.7.3 RBC (Red Blood Cell count)

The hemocytometer was used to determine the red blood cell count. Blood was diluted 1:200 with red blood cell diluting fluid using a red blood cell pipette. After mixing the dilution and waiting two minutes, the hemocytometer's counting chamber was filled, and the red blood cells were counted using a 40x microscope objective. The total number of counted cells was expressed in cubic millimetres or litres and multiplied by 10,000.

### 2.7.4 Total WBC (White Blood Cell Count)

The haemocytometer's white blood cell pipette was used to dilute the blood 1:20 with WBC diluting fluid. The liquid was slowly combined with the blood. The dilution was added to the counting chamber, and the WBCs were counted using a microscope's x10 objective. The total cell count was expressed in millilitres or litres and then multiplied by 50.

### 2.7.5 WBC differential count

A drop of blood was evenly distributed across a clean, grease-free slide to create a thin blood film using a smooth-edged spreader. After staining the blood film with aqueous stains, it was fixed in acetone-free methyl alcohol for approximately three to five minutes to prevent hemolysis upon contact with water. It was then left to dry. After applying the field, A and B stains to the blood film, 100 white blood cells were separated under a microscope with oil immersion objectives.