



ORIGINAL ARTICLE

TOXICOLOGICAL ASSESSMENT OF *LAGENARIA BREVIFLORA* WHOLE FRUIT EXTRACT IN JUVENILE AFRICAN CATFISH (*CLARIAS GARIEPINUS*)**Olayinka Remilekun Anifowose^{1*}, Solomon Ayomide Ogunyemi², Tolulope Ademola Olakojo², Bisi Olajumoke Adeoye², Gbolahanmi Akinola Oladosu¹, Olayinka Ayotunde Oridupa²**¹Department of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria; ²Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Oyo State, Nigeria OPEN ACCESS*Correspondence: dranifowose@gmail.com

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Plant extracts have been used in farmed fish to control fish bacterial infection, fish fry predators, to replace chemical pesticides and piscicides. This study was carried out to assess the toxicological effect of methanol fruit extract of *L. breviflora* in juvenile African catfish (*Clarias gariepinus*). Forty-five (45) juvenile catfish were randomly and equally divided into 9 groups. The first five groups were administered varying concentrations of *L. breviflora* fruit extracts (25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 125 mg/L) and LC₅₀ was calculated to be 63.9 mg/L. The remaining four groups were labelled A to D. The fish were exposed daily to graded concentrations of *L. breviflora*: Group A (Control), Group B (6.25 mg/L), Group C (12.5 mg/L) and Group D (25 mg/L) for 14 days. Blood samples of exposed fish showed significant decreases in the red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, and mean corpuscular haemoglobin. The histology results show erosion of the epidermis and hyperplastic alarm cells in the skin, areas of diffuse vacuolation, periportal degeneration and necrosis in the liver, mild degeneration of the lamella core, congestion of the submucosa and expanded lamella core in the skin. The plant extract can therefore be recommended for use at concentrations < 10 mg/L.

Keywords: aquaculture; *Clarias gariepinus*; *Lagenaria breviflora*; toxicology

INTRODUCTION

Aquatic food in the form of fish is increasingly appreciated for nutritional value addition through fish consump-

tion, which is driven by growing demand for wholesome and healthy fish [1]. Production of fish species like African catfish is important for the high demand of fish in Nigeria, and the reason for the species is due to its characteristics,

such as fast-growing, productive, efficient, and adaptive nature to the environment. In addition, aquaculture attributes of *C. gariepinus* include rapid growth, good feed conversion, excellent flesh quality, and high plasticity in its feeding habits, as well as good market potential [2].

The increased demand for African catfish is high, and many low-income earners depend on catfish as a major protein source [3]. Nigeria is making a substantial and prominent contribution to aquaculture production in Africa [4]. Nigeria is the largest aquaculture producer in Sub-Saharan Africa and ranks second in Africa. Nigeria is a distinguished second top producer of catfish, and this accounts for 64% of total fish production annually [4]. Despite being the top producer of catfish, Nigeria faces significant obstacles in bridging the demand-supply gap in its fishery market. Meanwhile, the growth of aquaculture is harshly endangered by the challenges and difficulties, such as extreme temperature fluctuation and diseases [5].

Moreover, diseases outbreaks due to bacterial infection pose serious economic devastation to the rapid growth of aquaculture in Nigeria [6]. The morbidity and mortality caused by bacterial infection are usually high due to environmental and biological factors. The causes of bacterial infection in farms include lack of biosecurity measures, stocking density, poor water quality, contaminated feed, and lack of prompt diagnosis and treatment of infected fish [7].

Over the last several decades, plant extracts have been used in farmed fish to control fish bacterial infection and fish fry predators, to replace chemical pesticides and piscicides due to extensive and indiscriminate use of these non-biodegradable synthetic chemicals, which have had harmful effects on aquatic environments and pose a high risk to non-targeted organisms [8, 9]. Plant extracts are considered promising agents because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability, and reduced toxicity [10]. The roles of plant extracts include disease management, water quality improvement, antibacterial effects (as an alternative to antibiotics), growth promotion, immunity improvement, and anti-stress effects [11].

Recently, the acceptance of traditional medicine as an alternative form of healthcare and the development of antimicrobial resistance to the available antibiotics have led to the increased isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations. One such plant that

has been used is *Lagenaria breviflora* (*L. breviflora*). *L. breviflora* was used in herbal treatment of inflammatory diseases such as colitis, skin diseases, jaundice, ulcers, and congestive heart failure [12]. Previous reports indicated that *L. breviflora* possessed antibacterial, antioxidant, anti-nociceptive, and anti-inflammatory attributes [12, 13]. The effects of dietary *Lagenaria breviflora* leaf extract (LBLE) on the growth performance, feed utilisation, and haematological parameters of juvenile African catfish were assessed by Paray et al. [14]. The usage of *L. Breviflora* in livestock production cannot be exaggerated, and rural rearers extensively utilized the fruit as curative and preventive options. *L. breviflora* was reported to contain phytochemicals, bioactive molecules, and vitamins such as flavonoids, carotenoids, alkaloids, phenols, 1,2-benzenedicarboxylic acid, mono[2-ethylhexyl], and vitamins C, B, and E, which can act as anti-inflammatories, antioxidants, and antibacterial agents, meanwhile, observed toxic effect of the extract may be due to the presence of alkaloids and other constituents [15]. This study was carried out to assess the toxicological effect of the methanol fruit extract of *L. breviflora* in juvenile African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

Preparation of plant extract

Fresh fruits of *L. breviflora* were purchased from a commercial seller in the market. The fruits were air-dried for 6 weeks at room temperature. The dried blended *L. breviflora* whole fruit (2.3 kg) was extracted in a glass container using 96% methanol (7 L). The mixture was constantly stirred and allowed to extract for 72 hrs. The filtrate was filtered using Whatman filter paper (1 mm). A repeat extraction was done and filtered. The combined filtrate was then concentrated with a rotary evaporator (Heidolph Laborota 400 efficient, made in Germany, model 517-01002-002) set at 40°C, after which the concentrate was further concentrated using a vacuum oven set at 40°C with a pressure of 700 mmHg. The weight of the crude extract was 99 g and the percentage yield was 4.3%.

Experimental animals and acclimatization

Forty-five juveniles of *Clarias gariepinus* (n = 45) with an average weight of 25.28 ± 0.81 g, length of 15.30 ± 0.6

cm were procured from a commercial fish farm in Ibadan. The fish were stored in 100 L plastic tanks and acclimatized for 2 weeks. The fish were earlier disinfected in 250 ppm formalin for 1 hour and then randomly examined and observed to be free from ectoparasites, lesions, or clinical signs of any disease. Following acclimatization, the fish were randomly and equally divided into 9 groups in 2 L of water, and each group was labelled.

Acute toxicity

An acute toxicity test of *L. breviflora* fruit crude extracts (methanol) was carried out to determine the lethal concentrations (LC_{50}) to *C. gariepinus*. The *C. gariepinus* juveniles were subjected to *L. breviflora* fruit extract (methanol) toxicity test for 96 hours to determine the toxic concentration range and the related timing. The test was carried out using 2L plastic tanks, and the fish were distributed equally into 5 different groups; 20 fish were divided into five groups ($n = 4$). The groups were A, B, C, D, and E. Varying concentrations of *L. breviflora* fruits extracts (methanol) were added to the water at 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, and 125 mg/L respectively. Every 12 hours, mortality rates were monitored and recorded. The LC_{50} was calculated from the graph plotted.

Sub-chronic toxicity

Sub-chronic toxicity test of *L. breviflora* fruit methanol extracts was carried out to determine the toxic effect of the plant. The *C. gariepinus* juveniles were subjected to *L. breviflora* fruit crude extract (methanol) toxicity test for 14 days, followed by collection of a blood sample and harvesting of organs on day 15. The test was carried out using 2L plastic tanks, and the fish were distributed equally into 4 different groups. Twenty-four fish were divided into four groups ($n = 6$). The groups were A, B, C, and D. The fish were exposed daily to graded concentrations of *L. breviflora*: Group A (Control, 0 mg/L), Group B (6.25 mg/L),

Group C (12.5 mg/L) and Group D (25 mg/L). The fish were exposed to fresh extract-treated water daily.

Haematology

Blood was collected into lithium heparinized tubes from the *C. gariepinus* juvenile on day 15 post extract exposure using the caudal vein for determination of haematological parameters. Heparinised capillary tubes were filled with blood to about two-thirds of the tube and sealed with plasticine to determine the packed cell volume. The tubes were placed in the haematocrit centrifuge (Perkin Elmer, USA) for 5–6 min at 3000 rpm and the packed cell volume (PCV) was read in the haematocrit reader. Haematology was carried out according to the method described by Adeshina et al. [16]. The following parameters were measured: packed cell volume (PCV); haemoglobin (Hb); red blood cell (RBC); white blood cell (WBC); counts, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and mean cell volume (MCV).

Histology

Four fishes per group were sacrificed by stunning, and tissue samples of the gills, and other lymphoid organs (liver, spleen and kidney) were harvested and fixed in 10% formalin. Tissue specimens were obtained from skin, liver, kidney, and gills for histological examination. Four fish each ($n = 4$) were sampled from each exposed and control group. Tissue specimens from skin, liver, kidney, and gill were fixed with 10% neutral buffered formalin, dehydrated, infiltrated, embedded in paraffin, and stained with haematoxylin and eosin according to Roberts [17].

Statistical analysis

Statistical software SPSS version 23 was employed for statistical analysis of data. Haematology parameters were subjected to one-way ANOVA. Differences were considered significant at $p \leq 0.05$ for all the datasets.

Table 1. Number of dead and mortality rate of *Clarias gariepinus* juveniles following acute exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract

Concentration	24 Hrs	48 Hrs	72 Hrs	96 Hrs
25 mg/L	0 (0%)	1 (25%)	0 (0%)	0 (0%)
50 mg/L	0 (0%)	0 (0%)	1 (25%)	0 (0%)
75 mg/L	1 (25%)	1 (25%)	0 (0%)	0 (0%)
100 mg/L	4 (100%)	0 (0%)	0 (0%)	0 (0%)
125 mg/L	4 (100%)	0 (0%)	0 (0%)	0 (0%)

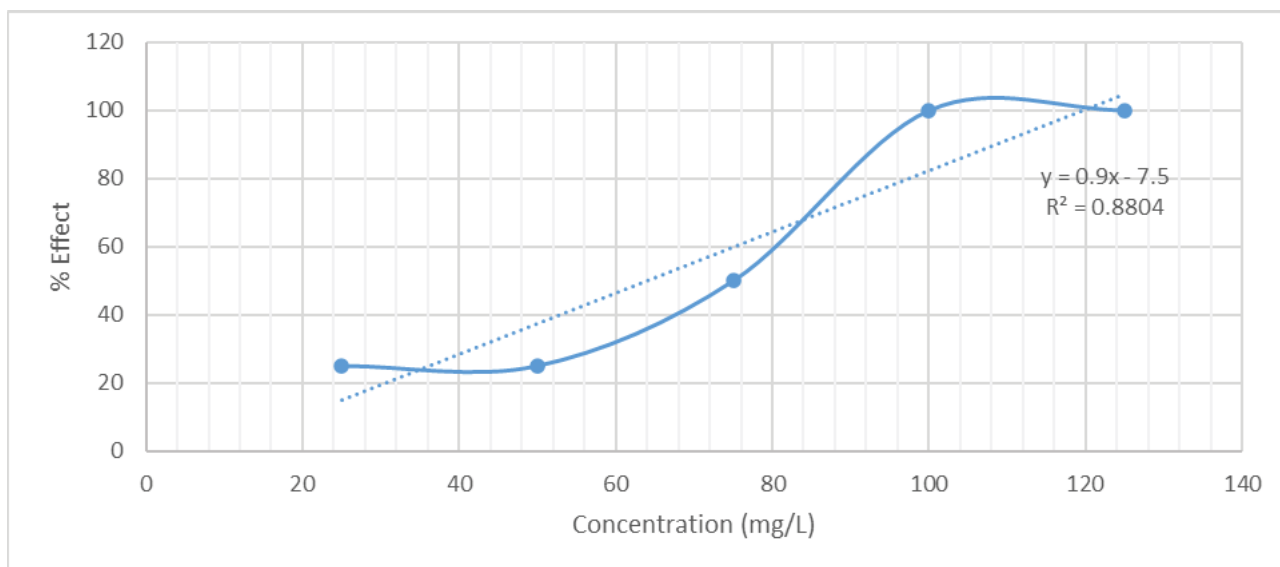


Fig. 1. LC₅₀ concentration of *Clarias gariepinus* juveniles following acute exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract; LC₅₀ = 63.9 mg/L

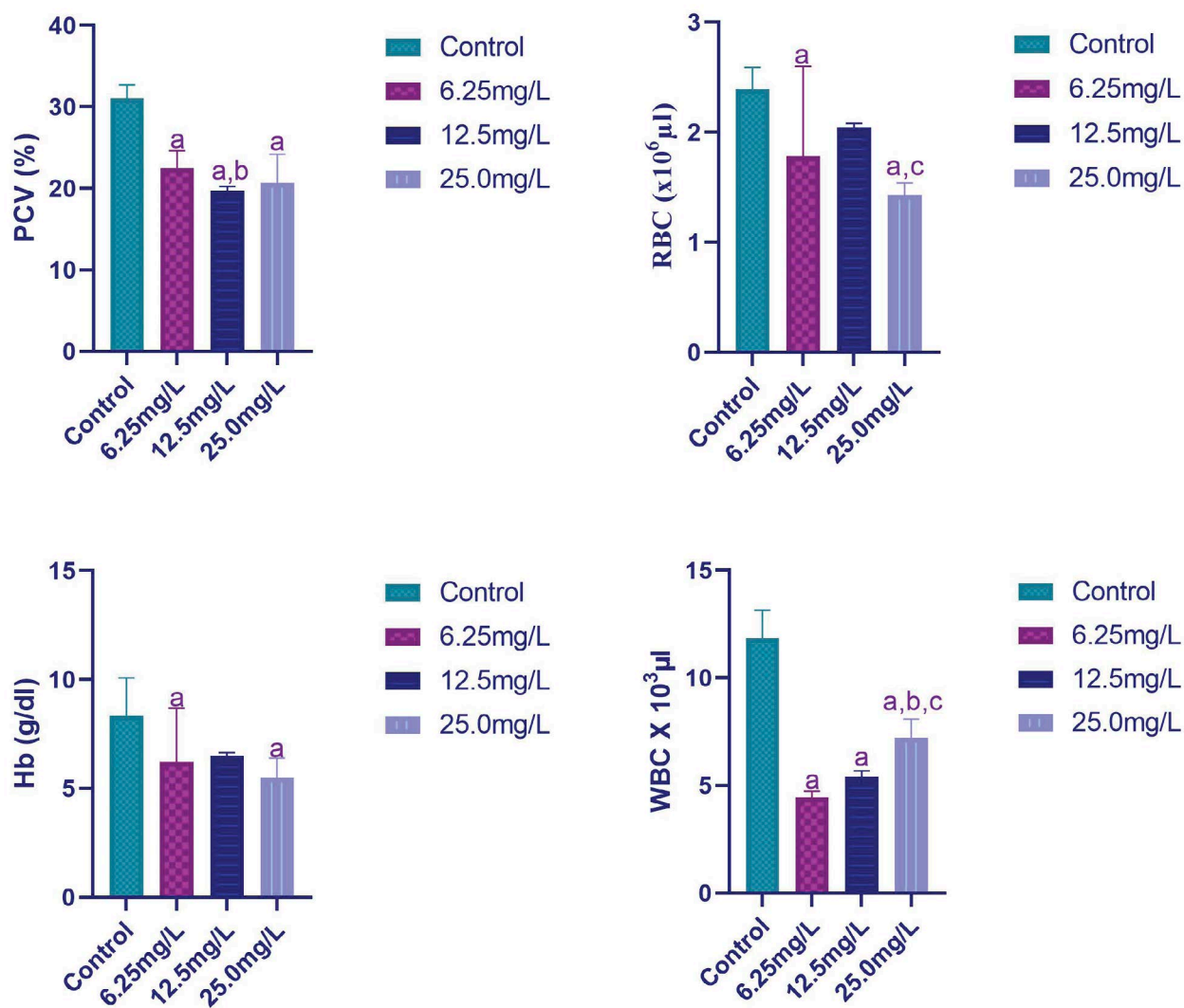


Fig. 2. Haematology of *Clarias gariepinus* juveniles following sub-chronic exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract (values with different superscripts indicate statistical significance at $p < 0.05$)

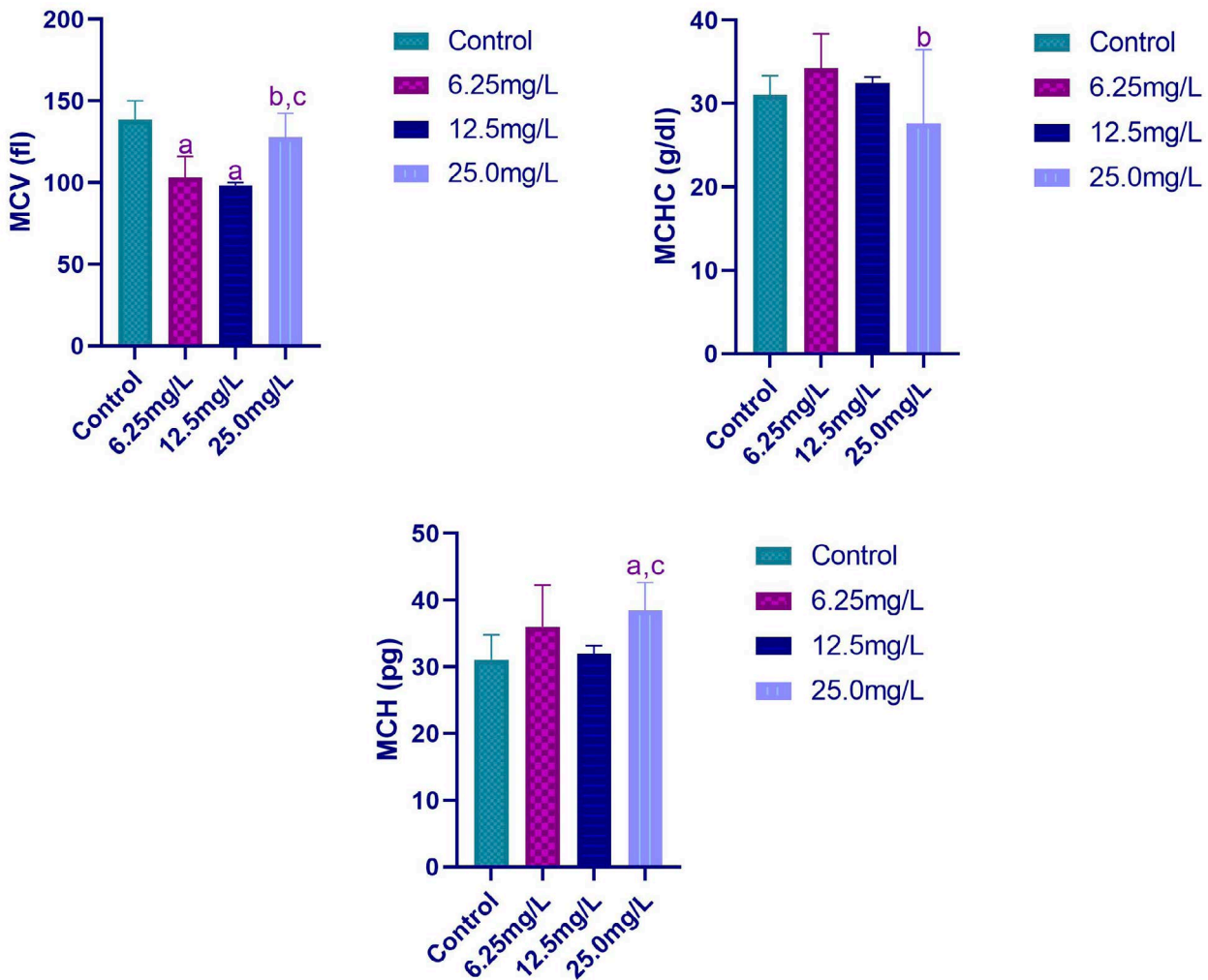


Fig. 3. Red cell indices of *Clarias gariepinus* juveniles following sub-chronic exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract (values with different superscripts indicate statistical significance at $p < 0.05$)

RESULTS

The fish in all the groups showed no obvious clinical signs on observation for 24 hours post-exposure to graded concentrations of *L. breviflora* methanol extract during acute toxicity. Mortality rate is shown in Table 1. LC_{50} was calculated to be 63.9 mg/L as shown in Figure 1. There was a significant decrease in the PCV ($p < 0.05$) of fish exposed to 6.25 mg/L, 12.5 mg/L and 25 mg/L compared with the control. A significant decrease ($p < 0.05$) was also observed in fish exposed to 12.5 mg/L of the extract compared to 6.25 mg/L. A significant decrease was also seen in the RBC ($p < 0.05$) of fish exposed to 6.25 mg/L and 25 mg/L compared with the control. In fish exposed to 25 mg/L of the extract compared to 12.5 mg/L, a significant decrease $p < 0.05$ was also observed. There is a significant

decrease in Hb ($p < 0.05$) of fish exposed to 6.25 mg/L and 25 mg/L of the extract compared to the control (Fig. 2). A significant decrease was detected in the MCV ($p < 0.05$) of fish exposed to 6.25 mg/L and 12.5 mg/L compared with the control. There was also a significant increase ($p < 0.05$) observed in fish exposed to 25 mg/L of the extract compared to 6.25 mg/L and 12.5 mg/L. There was a significant increase ($p < 0.05$) in the MCH of fish exposed to 25 mg/L compared with the control and 12.5 mg/L of the extract.

A significant decrease in the MCHC ($p < 0.05$) of fish exposed to 25 mg/L compared to 6.25 mg/L of the extract (Fig. 3). There was a significant decrease in the WBC ($p < 0.05$) of fish exposed to 6.25 mg/L, 12.5 mg/L, and 25 mg/L compared with the control. A significant increase ($p < 0.05$) was also observed in fish exposed to 25 mg/L of the extract compared to 6.25 mg/L and 12.5 mg/L (Fig. 2).

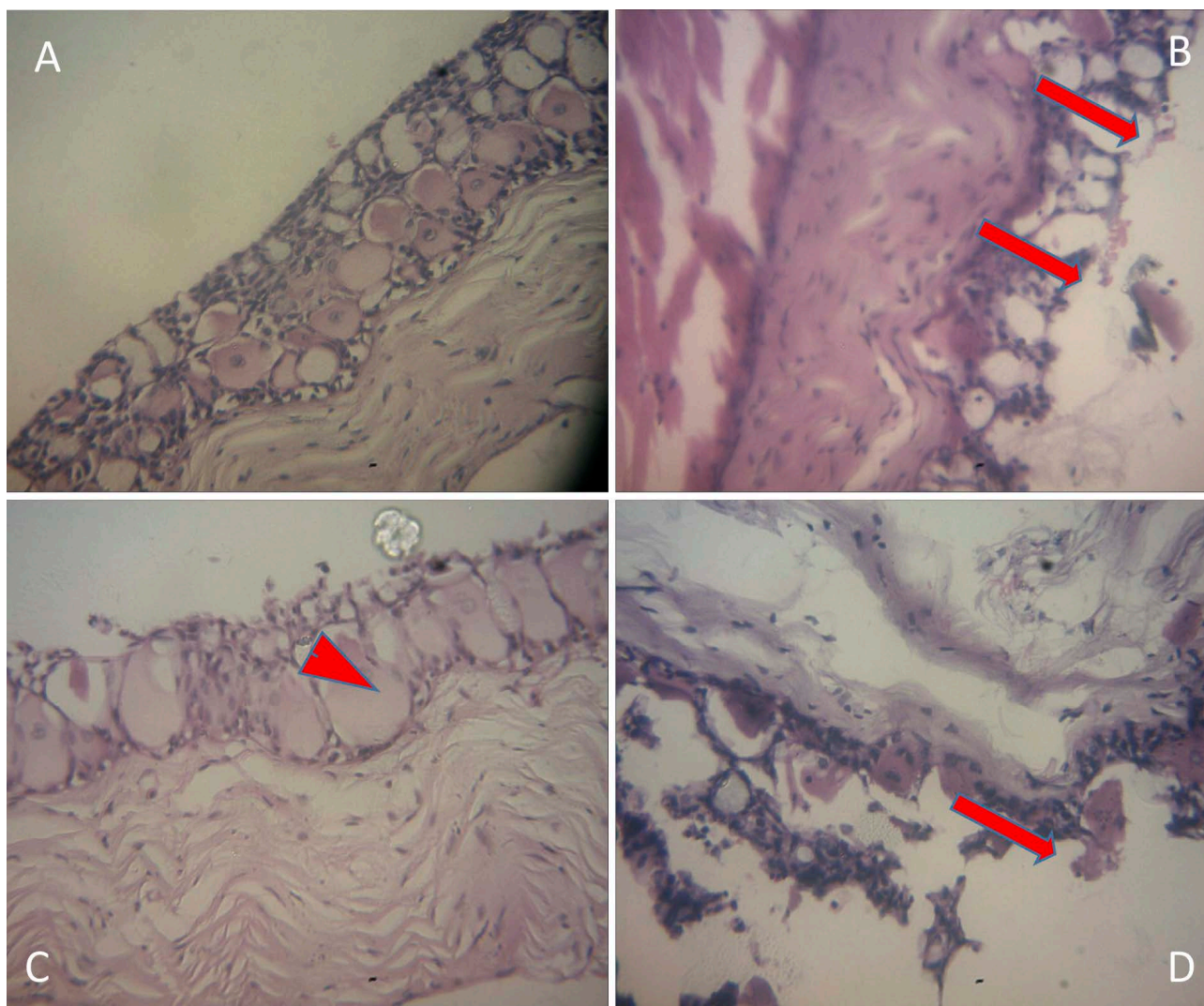


Fig. 4. Histology of the skin of *Clarias gariepinus* juveniles following sub-chronic exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract; H&Ex400; A (Control) – Normal, B (6.25 mg/L) – Erosion of the epidermis (arrow), C (12.5 mg/L) – Hyperplastic alarm cells (red arrow head), D (25 mg/L) – Erosion of the epidermis (red arrow)

The histology showed erosion of the epidermis in the skin of fish exposed to 6.25 mg/L, hyperplastic alarm cell in 12.5 mg/L, and erosion of the epidermis in 25 mg/L (Fig. 4). The histology of the liver showed periportal degeneration and necrosis in the fish exposed to 6.25 mg/L, periportal degeneration and necrosis with vacuolation in 12.5 mg/L, and periportal degeneration and necrosis in 25 mg/L (Fig. 5). The histology of the kidney showed moderate congestion of the interstitium in the fish exposed to 25 mg/L, meanwhile, there was abnormality observed in fish exposed to 6.25 mg/L and 12.5 mg/L, respectively (Fig. 6). The histology of the gill showed mild degeneration of the lamella core and oedema in the fish exposed to 6.25 mg/L, moderate congestion of the submucosa and expanded lamella connective tissue in 12.5 mg/L, and moderate congestion of the submucosa in 25 mg/L (Fig. 7).

DISCUSSION

In this study, there was a significant reduction in the PCV, RBC, HB, and WBC at the different doses of the extract when compared with the control. Increases in mean MCHC at dose 6.25 mg/L and 12.5 mg/L were not significant when compared with the control but a significant decrease was noticed at a concentration of 25.0 mg/L. The MCH results show a significant increase when the extract at dose 25.0 mg/L is compared with the control and 12.5 mg/L. There is a significant decrease in MCV when the extract at doses of 6.25 mg/L and 12.5 mg/L is compared with the control.

The low PCV, MCV, and a significant decrease in the highest concentration of MCHC are indicative of microcytic hypochromic anaemia [18]. This type of anaemia

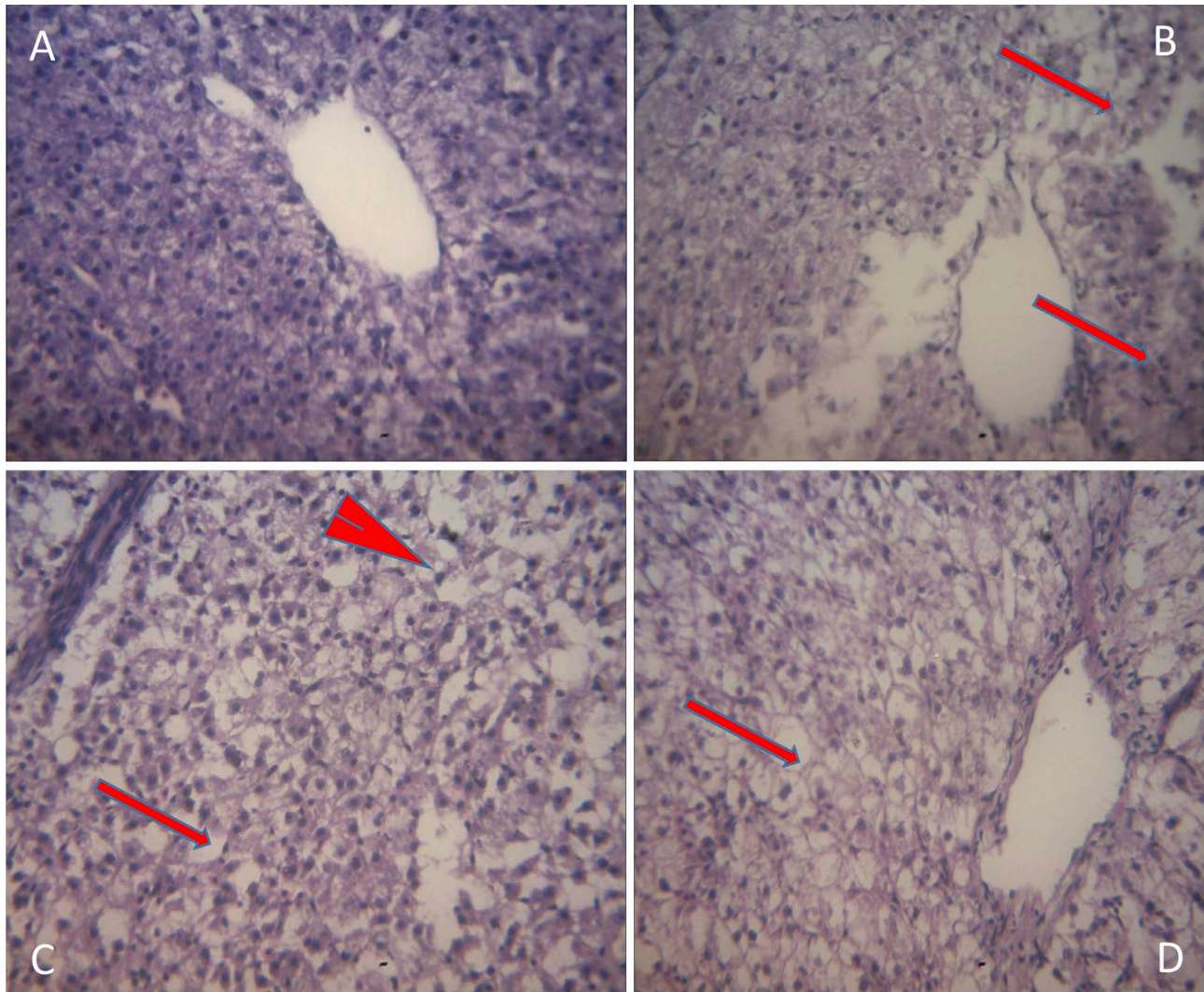


Fig. 5. Histopathology of the liver of *Clarias gariepinus* juveniles following sub-chronic exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract; H&Ex40; A (Control) – Normal, B (6.25 mg/L) – Periportal degeneration and necrosis (arrow), C (12.5 mg/L) – Periportal degeneration and necrosis (arrow) with vacuolation (red arrow head), D (25 mg/L) – Periportal degeneration and necrosis (red arrow)

has also been reported in healthy fish subjected to non-ylphenol and octylphenol [19]. The low WBC count may be due to prolonged stress in this situation, induced by the sub-chronic exposure to the extract [20]. Short-term stress sometimes results in an increase in WBC, but chronic and/or strong stress usually causes leukopenia [21].

The gills, skin, and liver of fish exposed to all concentrations of the extract showed signs of toxicity compared to the unexposed fish. However, the kidneys of fish exposed to the extract at concentrations of 6.25 mg/L and 12.5 mg/L were normal compared to the control, while 25 mg/L showed signs of toxicity following exposure to the extract. A significant pathology in the kidney was mainly the moderate congestion of the interstitium at 25 mg/L. This study indicates that the primary organs exposed to

the extract (skin and gills) may be involved with detoxification of the extract, as evident by the direct changes in the organs.

Periportal degeneration and necrosis, vacuolation, an increase in sinusoidal gaps, and poor hepatic cord structure were observed following sub-chronic exposure to *L. breviflora*. Vacuolation is due to the metabolic changes that occurred in the cytoplasm of the hepatocytes. According to Ezhilarasan et al. [22], oxidative stress is the main cause of liver necrosis. The changes in the liver may also explain the reduction of the haematological parameters in the fish. The primary organs responsible for erythropoiesis in fish are the kidneys. The main role of RBCs is to carry oxygen in the blood by the hemoglobin molecule. Therefore, erythropoiesis needs to be tightly regulated to maintain

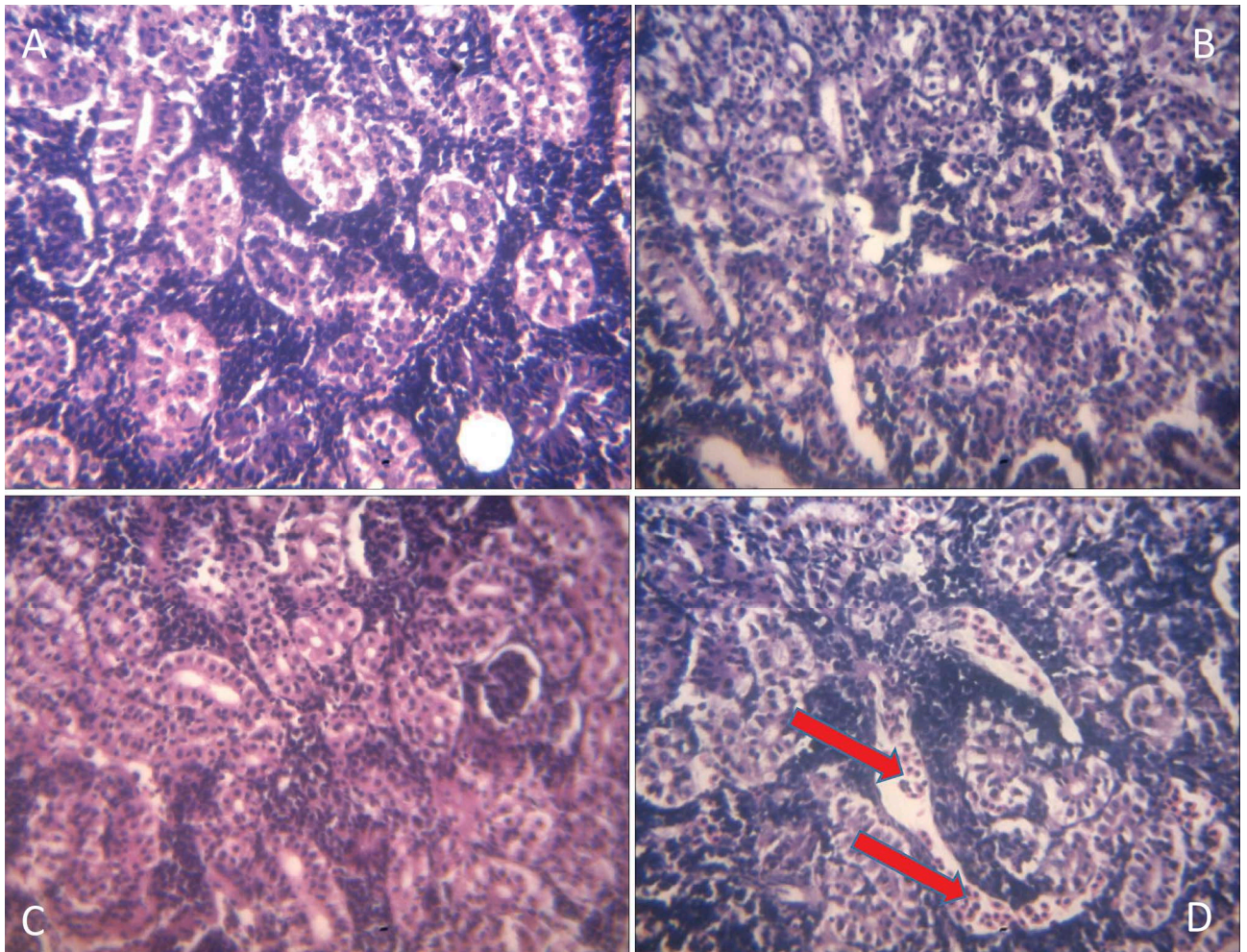


Fig. 6. Histopathology of the kidney of *Clarias gariepinus* juveniles following sub-chronic exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract; H&Ex40; A (Control) – Normal, B (6.25 mg/L) - Normal, C (12.5 mg/L) - Normal, D (25 mg/L) – Moderate congestion of the interstitium (red arrow)

homeostasis and to meet changes in oxygen supply and demand.

The study's findings of epithelial detachment and oedematous changes in the gill lamellae of the exposed fish suggest that the observed changes may have been caused by increased capillary permeability of the blood vessels of the afflicted gills. Similar oedematous gills in *C. gariepinus* treated with sub-lethal concentrations of methanol extract of *Raphia hookeri* were described by Adeogun et al. [23]. Despite the fact that mucous secretion helps to stop toxicants from reaching the gill epithelium, these effects may disrupt the gas exchange process and decrease respiration [24].

There was erosion of the epidermis, which may be a sequel to the loss of the protective functions carried out by the mucous cells. Prolonged exposure to the plant extract may have predisposed the skin to extensive loss and rapid exhaustion of the mucous cells. According to Chandel et

al. [25], *C. batrachus* skin exposed to the air experienced similar tear and wear that led to sloughing off of skin surfaces and haemorrhage.

CONCLUSION

The detected changes in the blood and histological abnormalities in the gills, liver, and skin of exposed fish, as well as the reported mortality in some of the exposed fish at acute toxicity, show that the ethanol extract of *L. breviflora* is toxic at the doses of exposure, particularly at concentrations >12.5 mg/L. The plant extract can therefore be recommended for use at concentrations <10 mg/L.

In conclusion, this work reported the safety evaluation of *L. breviflora* extract administered to *C. gariepinus*. It also contributed to basic research into alternative medicinal therapies in aquaculture.

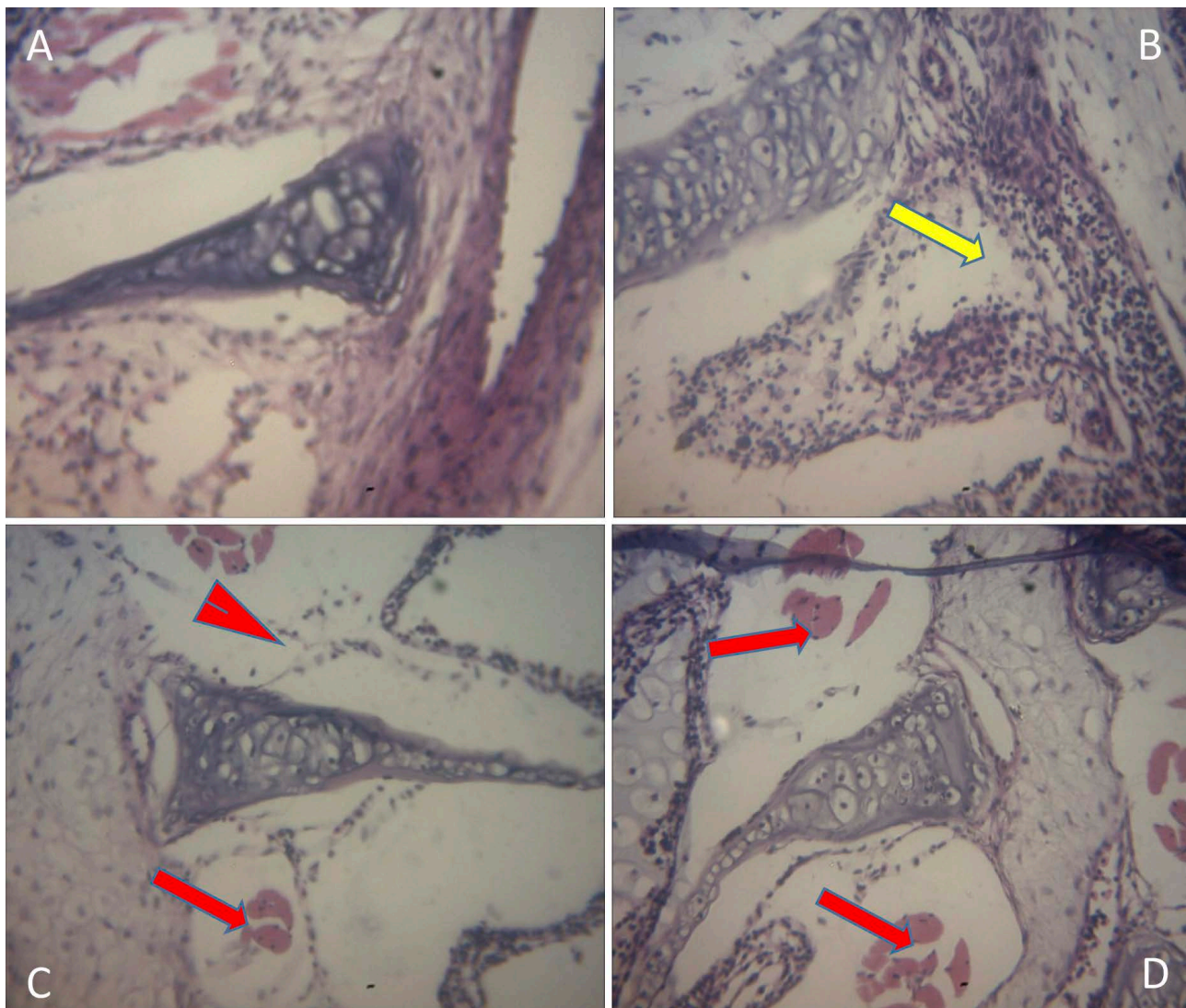


Fig. 7. Histopathology of the gills of *Clarias gariepinus* juveniles following sub-chronic exposure to graded concentrations of the *Lagenaria breviflora* whole fruit methanol extract; H&Ex400; A (Control) – (Normal), B (6.25mg/L) – Mild degeneration of the lamella core and oedema (yellow arrow), C (12.5mg/L) – Moderate congestion of the submucosa (red arrow) and expanded lamella connective tissue (arrow head), D (25mg/L) – Moderate congestion of the submucosa (red arrow)

Ethical Approval

The study adhered to the ethical guidelines for animal research and was approved by the University of Ibadan’s Animal Care, Use, and Research Ethics Committee (Approval No.: NHREC/ UIACUREC/08/12/2024).

Conflict of Interest

There is no competing interest to declare concerning this research work.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

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Generative AI Statement

This manuscript does not involve any generative AI.

Authors' Contributions

Motivation / Concept: OA
Design: OA, OR
Control/Supervision: OA, OR, GA
Data Collection and /or Processing: SA
Analysis and /or Interpretation: OA, OR, SA, TA, BO
Literature Review: SA, TA, BO, GA
Writing the Article: SA, TA, BO, GA
Critical Review: OR, OA

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