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EFFECT OF ACUTE AND SUBLETHAL CONCENTRATION OF *THEVETIA NERIIFOLIA* LEAVES ON THE GROWTH, SURVIVAL AND BLOOD PROFILES OF *CLARIAS GARIEPINUS* JUVENILES

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Key words: *Clarias gariepinus, Thevetia neriifolia*, haematology, histology, growth rate.

Abstract

The presence of predatory and unwanted fishes in cultured ponds is a serious problem for culturing edible freshwater fishes. The effects of acute and sublethal concentrations of *Thevetia neriifolia* leaves extract (TnLE) on the growth, survival and some haematological indices of *Clarias gariepinus* juveniles were investigated. The acute lethal toxicity (LC_{50}) of *T. neriifolia* leaves extract for 72-hour exposure of *C. gariepinus* was determined at 0.6 ml/L. *Clarias gariepinus* with mean weight of 15.65 ±0.02 g were exposed to TnLE at 0.00, 0.04, 0.08, 0.15, 0.30 and 0.60 ml/L concentrations for 4 weeks. The control and exposed groups were stocked with 20 fish per replicate and replicated three times. Data were analyzed using descriptive statistics and ANOVA at $p = 0.05$. This study revealed an increase in the weight of the fish exposed to TnLE, but the values decreased as the concentration of *T. neriifolia* increased. Significant ($p < 0.05$) increases were observed in the packed cell volume, haemoglobin, neutrophils and albumin of *C. gariepinus* juveniles exposed to *T. neriifolia* leaves extract compared to the control. The histology of the intestine revealed that no visible structural changes among the exposed groups while the skin, gills, and liver shows slightly abnormal structural changes in the exposed groups. This study can be concluded that the *T. neriifolia* leaves extract could serve as a biopiscicide in fish farming without compromising growth.

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Introduction

Aquaculture is a fast-growing sector in Nigeria, which contributes less than 5% of the total fish supply but has a growth rate of about 2% per year (MOSES 2006). Catfish farming and indeed aquaculture, offer strong potential for growth to meet the natural fish demand, thereby, reducing importation, providing employment, alleviating poverty and helping to meet the millennium development goals (WILLIAMS et al. 2007). Among the culturable fishes in Nigeria includes the African catfish (*Clarias gariepinus*), which is the most popularly cultured fish in Nigeria and indeed in Africa, and third in the world (GARIBALDI 1996) because of its hardiness, omnivores, ability to withstand adverse condition, and high economic value (HECHT et al. 1996).

Botanical piscicides are used by fishermen and fish farmers for catching or stupefying fish. They are applied during fishing or eradication of unwanted, predatory, and exotic fish species (CAGAUAN et al. 2004). The control of aquatic ecosystems with synthetic organic compounds reportedly caused health hazard (DALELA et al. 1978). Botanical ichthyotoxins are less expensive, biodegradable, readily available, easy to handle and safe for human and the environment (SINGH et al. 1996). Plant poisons are extracted from flowers, pulp, seeds, bark, fruits, root, leaves and even the entire plant (TYLER 1986). Some chemicals found in these plants will stun fish when passing through their gills or when ingested. The fish then float to the surface of the water for easy capture. The active ingredients in these plants are released by mashing and grinding the appropriate plants or plant parts, which are then introduced to the aquatic environment. Exposure of fish to these biocides may cause stress in fish without necessarily leading to death. Phytochemical analyses of piscicidal plants showed that they contain diverse toxic substances such as rotenones, saponins, flavonoids, alkaloids, glycosides, tannins, oxalic acids, solanine, selenium, nicotine, pyrethrum, and resin (AL ASHAAL et al. 2010). These substances are toxic to fish and other aquatic organisms at high concentrations and fade out within a short time (ADEWUNMI 1990).

Depending on the species of fish targeted, different plant species used as piscicides have different effects on fish (VAN ANDEL 2002). Piscicidal plants like *Blighia sapida, Kigelia africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa* and *Tephtosia vogelli* are commonly used by fisher folks to catch fishes because they are highly potent against fishes (FAFIOYE et al. 2004). The active principles in the plant part used (leaves, seeds, kernel and bark) have varying potencies and modes of action depending on forms of extracts, aqueous or alcohol used (SAMBA-

SIVAM et al. 2003). Lethal and sublethal concentrations of plant poisons are known to have effects on fish behaviour, growth, reproduction, feeding, respiration and generally other physiological processes of exposed organisms, which are visible also in haematological and histological studies (OLUSOLA et al. 2021). Several factors influence the response of organisms to toxicity tests which include age, disease, spawning time, condition, and water quality (OMONIYI et al. 2013).

Thevetia neriifolia (Juss) is an evergreen tropical, perennial shrub which belongs to the family Apocynaceae and is commonly known as "yellow oleander", but also called "milk bush", "trumpet flower" or "lucky nut". Utilization of this plant as a natural piscicide has not been well elucidated. In the present study, the piscicidal activity of aqueous extracts of the leaves of *T. neriifolia* plant was evaluated on *Clarias gariepinus* juveniles under controlled conditions.

Materials and Methods

Plant collection and identification

Mature leaves of *T. neriifolia* were obtained from the school premises of Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. The plant was identified at the Department of Biological Sciences (Botany Programme) of Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria.

Preparation of aqueous leaves extract

Two hundred and fifty grams (250 g) of fresh *T. neriifolia* leaves were washed with distilled water, macerated and squeezed in 30 litres of distilled water to obtain the aqueous leaves extract, and concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ml/L of *T. neriifolia* ($TnLE_{a–e,}$ respectively) were used for 72-hour range test.

Experimental fish

Four hundred and eighty *Clarias gariepinus* juveniles of average weight 15.64 ±0.02 g were obtained from a fish farm in Okitipupa, Nigeria. They were transported to the Fisheries and Aquaculture Technology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria in an adapted plastic jerry can containing 35 litres water from the farm. The fish were acclimatized for 7 days and fed twice a day at 3% body weight with 2 mm feed (Blue Crown) containing 40% crude protein.

Range test (72-hour LC_{50}) and sublethal toxicity test

After acclimation, the extract of the *T. neriifolia* leaves ($TnLE_{a–e}$ – 0.2, 0.4, 0.6, 0.8 and 1.0 ml/L, respectively) was introduced into 6 experimental bowls (40 cm × 20 cm × 20 cm) containing clean, dechlorinated and well aerated water. Fish were randomly selected and stocked at 8 juveniles per experimental bowl. Twenty-four hours prior to the test commencement, feeding of the fish was stopped. Each of the test solutions was introduced directly into the experimental tank in a single dose and replicate twice per treatment. The behaviour and mortality of the test fishes in each tank was monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours for the rest of the 72 hours' period. The 72 hours LC_{50} value was recorded and tested by probit analysis as described by FINNEY (1971).

The value 0.6 ml/L of LC 50 (concentration where mortality reached 50%) was obtained after 72 hours range test. For sublethal toxicity test, twenty fish per treatment were exposed to sublethal concentrations for a period of 28 days. Five groups of fish were exposed to different concentrations of *Thevetia neriifolia* leaves extract including 0.04, 0.08, 0.15, 0.30 and 0.60 ml/L ($TnLE_{1–6,}$ respectively), while one group, which served as the control group for *Clarias gariepinus* did not receive the extract. Each treatment group was replicated three times and water in each tank was replaced every three days throughout the period of the experiment using static water renewal methods to maintain relatively uniform physiochemical parameters and also to prevent fouling that may result from food residues. Fish were fed twice daily at 3% body weight (08:00 and 18:00 hrs) and they were weighed at the beginning of the study and weekly. Measurement of the weight change was performed weekly using sensitive weigh balance (SF – 400, 1000 g x 0.2 g, China) and the food amount adjusted weekly according to the new body weight

Histopathological examination

The skin, gills, intestine and liver of fish from the control and exposed groups were studied and compared for histopathological changes. Fishes (n = 2) were obtained from each treatment, dissected and immediately fixed in 10% formalin solution for 24 hours (KHOSHNOOD et al. 2014). At the end of 24 hours, tissue samples were washed in running water to remove traces of formalin. Specimens were dehydrated by passing through graded series of alcohol (30%, 50%, 70%, 95% and absolute ethanol) for two hours each. Specimens were later passed through xylene (clearing agent) to remove the alcohol, and molten paraffin wax was used to impregnate

the organs and tissues in a vacuum oven at 56°C and allowed to solidify following the procedures of LUNA (1968). Organs and tissues blocks were cut by trimming and attaching them to wood blocks in preparation for sectioning. Sectioning and staining of organs and tissues were done according to OMITOYIN et al. (1999). A light photomicroscope attached to a 35 mm camera was used to examine the organs sections.

Haematological and bio-chemical assessment

Prior to the collection of blood samples, the fish were anaesthetized with 2 – phenoxy ethanol and the blood samples (2 ml) were collected by the caudal ablation method from both the control and exposed fishes before the experiment, at the end of the experiment (28 days) and 14 days after post exposure (to assess recovery of fish). The blood samples were dispensed into tubes containing ethylene diamine tetraacetate (EDTA) anticoagulant and transported in ice-packed bags to the Microbiological Laboratory unit of Ondo State Specialist Hospital, Okitipupa for haematological analysis. Red blood cells (RBC) and white blood cells (WBC) were counted under a light microscope by improved Neubauer hemocytometer using Hayem's and Turk's solution as diluting fluids, respectively. Haemoglobin (Hb) was estimated by Cyanomethemoglobin method as described by BLAXHALL and DAISLEY (1993). Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated respectively using standard formula described by BLAXHALL and DAISLEY (1993).

Serum biochemistry

Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain serum biochemical parameters. Serum from the centrifuge blood was carefully siphoned out and the concentration of glucose, total proteins, albumin, globulin and Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined by the method described by OLUSOLA and NWOKIKE (2018). Glucose was measured in the laboratory using an electronic blood-glucose meter. A relatively small drop of blood from each sample was placed on a disposable test strip which interfaces with a digital meter. Within several seconds, the level of blood glucose was shown on the digital display.

Biological evaluation

Mean weight gain [g] = final body weight – initial body weight.

$$\text{Relative weight gain [\%]} = \frac{(\text{final body weight} - \text{initial body weight})}{\text{initial body weight}} \cdot 100 .$$

$$\text{Specific growth rate (SGR) [\%]} = \frac{(\log_e \text{final body weight} - \log_e \text{initial body weight})}{\text{experimental days}} \cdot 100 .$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{dry weight of the feed}}{\text{fish weight gained}}.$$

$$\text{Production performance index} = \frac{\text{final number of fish stocked} \cdot \text{weight gained}}{\text{experimental days}}.$$

$$\text{Survival rate (SR) [\%]} = \frac{\text{initial number of fish stocked} - \text{final number of dead fish}}{\text{initial number of fish stocked}} \cdot 100.$$

$$\text{Nitrogen metabolism [g]} = \frac{(0.549)\,(a + b)\,h}{2}.$$

where:
0.549 = constant value
a – initial mean weight of fish
b – final mean weight of fish
h – experimental periods in days (Bello et al. 2012).

Statistical Analysis

Growth performance and nutrient utilization, haematology and biochemical analysis resulting from the experiment was subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 20.0). Duncan multiple range test was used to compare differences among individual means.

Results

Behavioural responses of C. gariepinus juveniles exposed to different concentrations of *T. neriifolia* leaves extract

The results obtained in this study show that the behavioural changes of *C. gariepinus* juveniles exposed to the aqueous leaves extract of *T. neriifolia* increased with the concentration and the time of exposure. The fishes exhibited erratic swimming, air gulping, loss of reflexes, restlessness, and vertical positioning (Table 1).

Table 1

Behavioural changes and biological responses in *Clarias gariepinus* exposed to different concentrations of *T. neriifolia*; n = 8 specimens per treatment

Exp/Beh	24 hours						48 hours						72 hours					
CONC [ml/L]	0.00	0.20	0.40	0.60	0.80	1.00	0.00	0.20	0.40	0.60	0.80	1.00	0.00	0.20	0.40	0.60	0.80	1.00
A.G	–	–	–	–	+	+	–	–	–	+	+	N/A	–	–	+	+	N/A	N/A
E.S	–	–	+	+	+	+	–	–	+	+	+	N/A	–	+	+	+	N/A	N/A
L.R	–	–	–	–	+	+	–	–	–	–	+	N/A	–	–	–	+	N/A	N/A
LTG	–	–	–	–	+	+	–	–	–	+	+	N/A	–	–	–	+	N/A	N/A
M.S	–	–	+	+	+	+	–	+	+	+	+	N/A	–	+	+	+	N/A	N/A
V.P	–	–	–	–	+	+	–	–	–	+	+	N/A	–	–	+	+	N/A	N/A
L.B	–	–	–	–	+	+	–	–	–	–	+	N/A	–	–	–	+	N/A	N/A

Explanations: – = no change in behaviour found; + = change in behaviour found; A.G = air gulping; E.S = erratic swimming; L.R = loss of reflex; LTG = lethargy; M.S = mucus secretion; V.P = vertical positioning; L.B = loss of balance; N/A = not applicable (dead)

Growth performance and nutrient utilization of *Clarias gariepinus* exposed to *T. neriifolia* leaves extract

The growth performance and nutrient utilization of *C. gariepinus* exposed to aqueous leaves extract of *T. neriifolia* for 28 days revealed the highest body weight gain in $TnLE_2$ (0.04 ml/L) and lowest in $TnLE_6$ (0.60 ml/L) as 22.06 ±0.08 g and 15.93 ±1.58 g respectively. Generally, $TnLE_2$ showed better performance in all the parameters except survival rate, which was optimum in control (0.00 ml/L), $TnLE_3$ (0.08 ml/L), $TnLE_5$ (0.30 ml/L), and $TnLE_6$ (0.60 ml/L) each having 100.00 ±0.00%, and production performance index, which is optimum in $TnLE_3$ (0.08 ml/L) of 75.63 ±0.27. However, there were no significant differences ($P > 0.05$) in the mean final body weight, body weight gain, relative body weight gain, specific growth rate, feed conversion ratio, nitrogen metabolism and production performance index among the control and exposed groups (Figures 1–8).

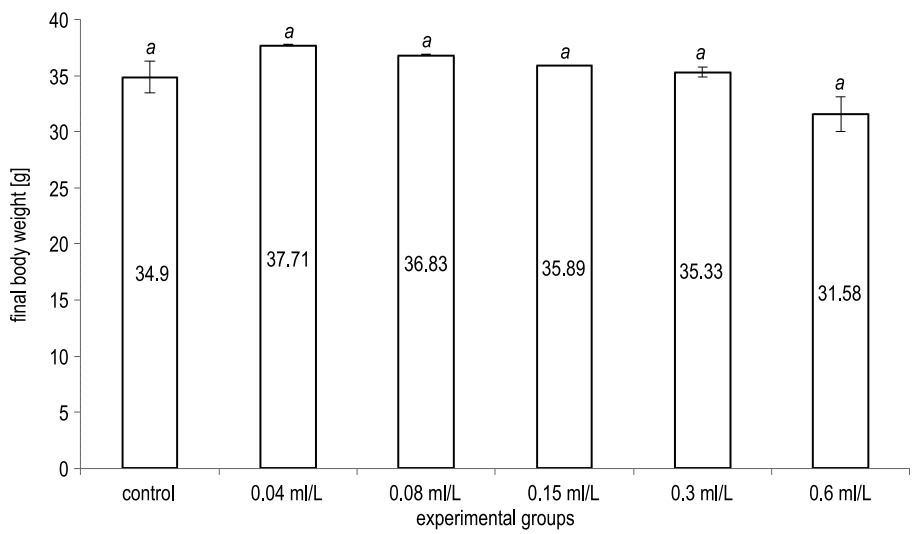

Fig. 1. Final body weight [g] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

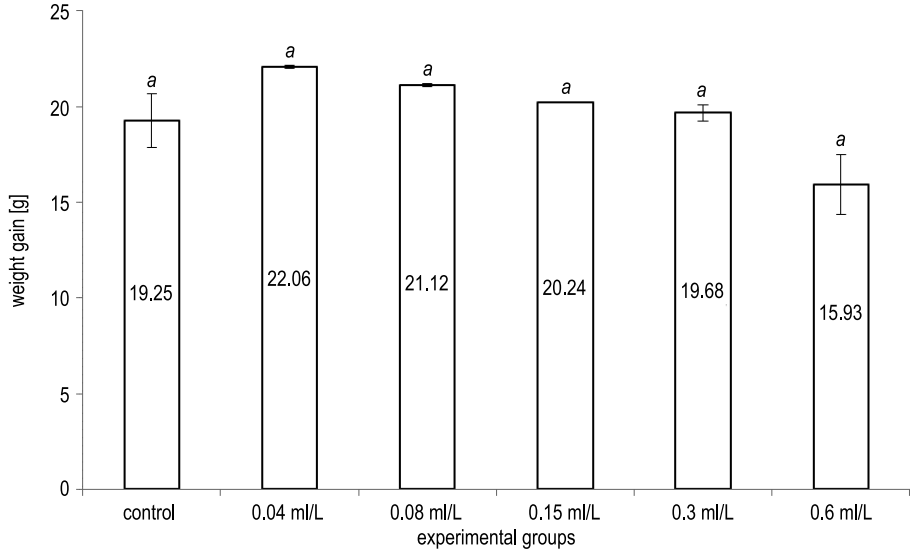

Fig. 2. Weight gain [g] of *C. gariepinus* juveniles 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

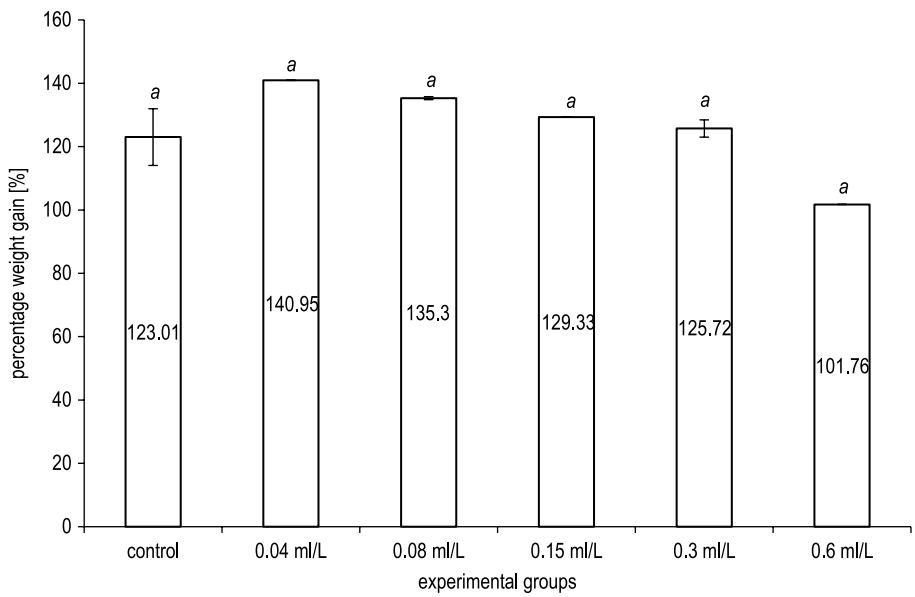

Fig. 3. Weight gain [%] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

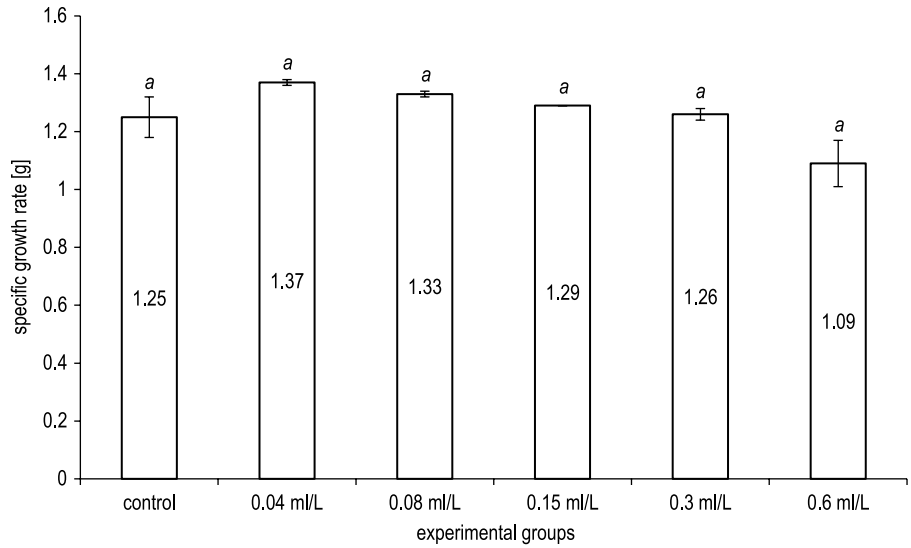

Fig. 4. Specific growth rate [%] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract

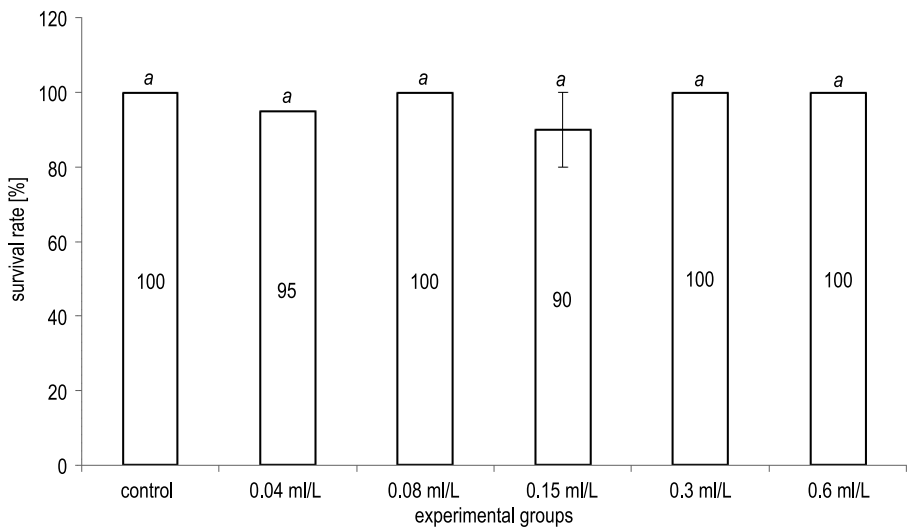

Fig. 5. Survival rate [%] of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract
n = 20 specimens per treatment; a – means with the same letter are not significantly different from each other ($p > 0.05$)

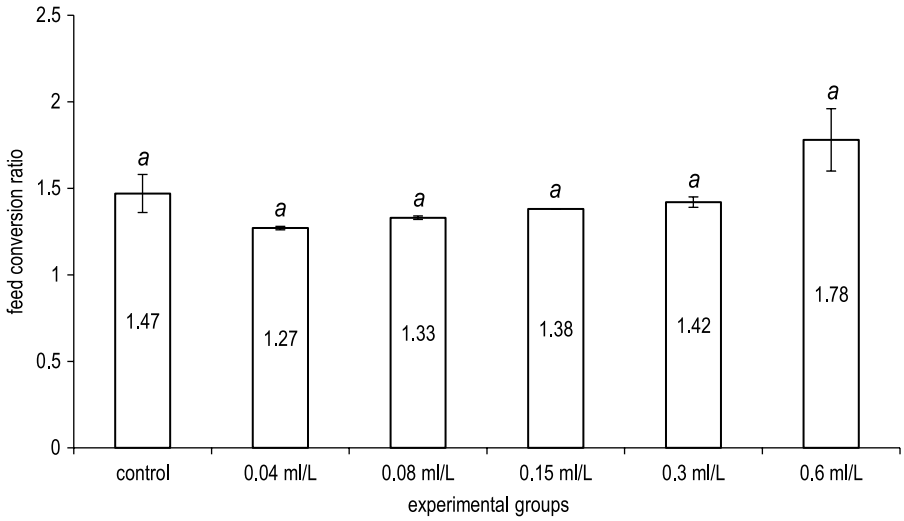

Fig. 6. Feed conversion ratio (FCR) of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; a – means with the same letter are not significantly different from each other ($p > 0.05$)

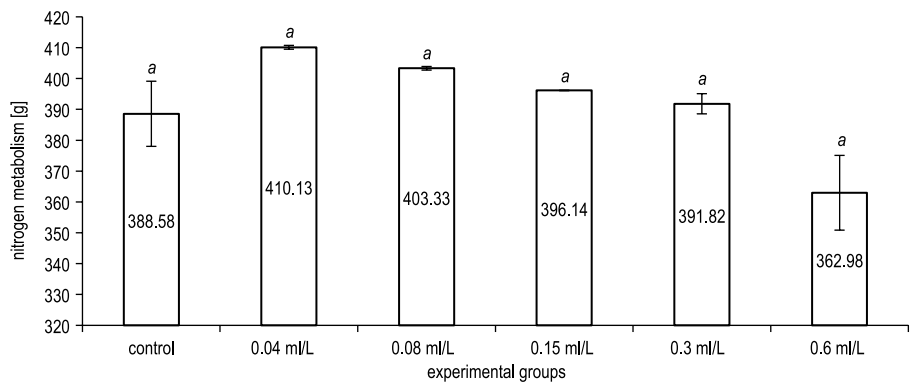

Fig. 7. Nitrogen metabolism of *C. gariepinus* [g] after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; a – means with the same letter are not significantly different from each other ($p > 0.05$)

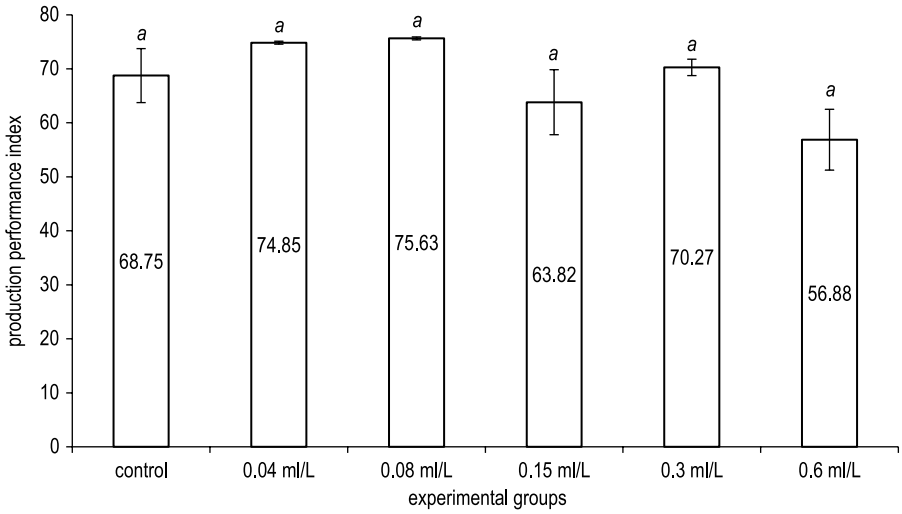

Fig. 8. Production performance index of *C. gariepinus* after 28 days' exposure to *T. neriifolia* leaves extract; $n = 20$ specimens per treatment; *a* – means with the same letter are not significantly different from each other ($p > 0.05$)

Mean haematological parameters of *Clarias gariepinus* exposed to sublethal concentrations of *T. neriifolia* leaves extract

The highest values of packed cell volume (PCV), haemoglobin (Hb) and white blood cells (WBC) were recorded in $TnLE_3$ (0.08 ml/L) as 46.00 ±2.00%, 15.30 ±0.20 g/dl and 12.20 ±0.20 · 10a/l, respectively. There were no significant differences ($p > 0.05$) in red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC) and monocytes. The values of PCV, Hb and platelets obtained after 14 days' post exposure were recorded to be decreasing compared to those obtained on exposure to TnLE (Table 2 and Table 3).

Mean plasma biochemistry and blood serum parameters of *C. gariepinus* exposed to sublethal concentrations of *T. neriifolia* leaves extract

The results of plasma biochemistry obtained in this study show that the values of albumin generally increased in the exposed groups when compared to the values obtained in the control. It was also revealed that the values obtained after 14 days' post exposure decreased when compared to those obtained before the experiment and during exposure to *T. neriifolia*. The result of the 14 days' post exposure shows a general decrease in ALT and AST when compared to those obtained before the experiment and during exposure to *T. neriifolia* (Table 4–5).

Table 2

Mean haematological parameters of *Clarias gariepinus* juveniles before and after exposure to different concentrations of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 [ml/L]	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
PCV%	39.00 ±2.00^a	41.00 ±2.00^{ab}	39.00 ±2.00^a	46.00 ±2.00^b	41.00 ±2.00^{ab}	43.00 ±2.00^{ab}	43.00 ±2.00^{ab}
HB [g/dl]	13.00 ±0.20^a	13.70 ±0.20^{ab}	13.00 ±0.20^a	15.30 ±0.20^c	13.70 ±0.20^{ab}	14.30 ±0.20^b	14.30 ±0.20^b
RBC · $10^{12/l}$	4.50 ±0.20^a	5.10 ±0.20^a	4.90 ±0.20^a	4.90 ±0.20^a	4.60 ±0.20^a	5.00 ±0.20^a	4.50 ±0.20^a
WBC · 10 a/l	12.70 ±0.20^c	10.80 ±0.20^a	10.70 ±0.20^a	12.20 ±0.20^{bc}	10.90 ±0.20^a	10.60 ±0.20^a	11.80 ±0.20^b
Platelets [m/µl]	20.50 ±0.20^a	28.70 ±0.20^b	29.90 ±0.20^c	31.70 ±0.20^e	29.80 ±0.20^c	30.60 ±0.20^d	33.00 ±0.20^f
MCV [F1]	86.70 ±0.20^a	80.40 ±0.20^c	79.60 ±0.20^b	93.90 ±0.20^f	89.10 ±0.20^e	86.00 ±0.20^d	95.60 ±0.20^g
MCH [pg/cal]	28.90 ±0.20^b	26.90 ±0.20^a	26.50 ±0.20^a	31.20 ±0.20^d	29.80 ±0.20^c	28.60 ±0.20^b	31.80 ±0.20^d
MCHC [g/dl]	33.30 ±0.20^a	33.40 ±0.20^a	33.30 ±0.20^a	33.30 ±0.20^a	33.40 ±0.20^a	33.30 ±0.20^a	33.30 ±0.20^a
NEU [%]	64.00 ±2.00^{ab}	64.00 ±2.00^{ab}	65.00 ±2.00^{ab}	69.00 ±2.00^b	60.00 ±2.00^a	70.00 ±2.00^b	70.00 ±2.00^b
LYMP [%]	31.00 ±2.00^a	33.00 ±2.00^a	33.00 ±2.00^a	29.00 ±2.00^a	40.00 ±2.00^b	30.00 ±2.00^a	29.00 ±2.00^a
MONO [%]	4.00 ±2.00^a	2.00 ±2.00^a	2.00 ±2.00^a	1.00 ±0.20^a	0.00 ±0.00^a	0.00 ±0.00^a	1.00 ±0.20^a
EOS [%]	1.00 ±0.20^b	1.00 ±0.20^b	0.00 ±0.00^a	1.00 ±0.20^b	0.00 ±0.00^a	0.00 ±0.00^a	0.00 ±0.00^a

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; PCV = Packed Cell Volume; HB = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; NEU = Neutrophils; LYMP = Lymphocytes; MONO = Monocytes; EOS = Eosinophil. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 3

Mean haematological parameters of *Clarias gariepinus* juveniles after 14 days' post exposure to different concentrations of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
PCV [%]	39.00 ± 2.00^{a}	44.00 ± 2.00^{a}	42.00 ± 2.00^{a}	40.00 ± 2.00^{a}	38.00 ± 2.00^{a}	41.00 ± 2.00^{a}	39.00 ± 2.00^{a}
HB [g/dl]	13.00 ± 0.20^{a}	14.70 ± 0.20^{d}	14.00 ± 0.20^{c}	13.30 ± 0.20^{abc}	12.70 ± 0.20^{a}	13.70 ± 0.20^{bc}	13.00 ± 0.20^{ab}
RBC · $10^{12/l}$	4.50 ± 0.20^{a}	4.70 ± 0.20^{a}	4.70 ± 0.20^{a}	5.00 ± 0.20^{a}	4.30 ± 0.20^{a}	4.60 ± 0.20^{a}	4.80 ± 0.20^{a}
WBC · 10 a/l	12.70 ± 0.20^{c}	11.40 ± 0.20^{bc}	11.70 ± 0.20^{c}	11.20 ± 0.20^{bc}	8.90 ± 0.20^{a}	10.80 ± 0.20^{b}	11.00 ± 0.20^{bc}
Platelets [m/µl]	20.50 ± 0.20^{a}	28.80 ± 0.20^{b}	30.10 ± 0.20^{c}	28.50 ± 0.20^{ab}	28.20 ± 0.20^{ab}	27.90 ± 0.20^{a}	28.40 ± 0.20^{ab}
MCV [F1]	86.70 ± 0.20^{a}	93.60 ± 0.20^{e}	89.40 ± 0.20^{d}	80.00 ± 0.20^{a}	82.20 ± 0.20^{b}	89.10 ± 0.20^{d}	86.70 ± 0.20^{c}
MCH [pg/cal]	28.90 ± 0.20^{b}	31.30 ± 0.20^{e}	29.80 ± 0.20^{d}	26.60 ± 0.20^{a}	27.30 ± 0.20^{b}	29.80 ± 0.20^{d}	28.90 ± 0.20^{c}
MCHC [g/dl]	33.30 ± 0.20^{a}	33.40 ± 0.20^{a}	33.30 ± 0.20^{a}	33.30 ± 0.20^{a}	33.20 ± 0.20^{a}	33.40 ± 0.20^{a}	33.30 ± 0.20^{a}
NEU [%]	64.00 ± 2.00^{ab}	70.00 ± 2.00^{a}	70.00 ± 2.00^{a}	69.00 ± 2.00^{a}	70.00 ± 2.00^{a}	65.00 ± 2.00^{a}	68.00 ± 2.00^{a}
LYMP [%]	31.00 ± 2.00^{a}	28.00 ± 2.00^{a}	30.00 ± 2.00^{a}	29.00 ± 2.00^{a}	28.00 ± 2.00^{a}	30.00 ± 2.00^{a}	29.00 ± 2.00^{a}
MONO [%]	4.00 ± 2.00^{a}	2.00 ± 2.00^{a}	0.00 ± 0.00^{a}	2.00 ± 2.00^{a}	1.00 ± 0.20^{a}	3.00 ± 2.00^{a}	2.00 ± 2.00^{a}
EOS [%]	1.00 ± 0.20^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.00 ± 0.20^{b}	2.00 ± 0.20^{c}	1.00 ± 0.20^{b}

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; PCV = Packed Cell Volume; HB = Haemoglobin; RBC = Red Blood Cell; WBC = White Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; NEU = Neutrophils; LYMP = Lymphocytes; MONO = Monocytes; EOS = Eosinophil. Mean of duplicate data; mean value in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 4

Mean Plasma Biochemistry parameters of *C. gariepinus* juveniles before and after exposure to different concentration of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
TP (g/dl)	76.00 ± 2.00^{a}	72.00 ± 2.00^{a}	72.00 ± 2.00^{a}	70.00 ± 2.00^{a}	76.00 ± 2.00^{a}	76.00 ± 2.00^{a}	72.00 ± 2.00^{a}
ALB (g/dl)	43.00 ± 2.00^{b}	34.00 ± 2.00^{a}	38.00 ± 2.00^{ab}	40.00 ± 2.00^{ab}	39.00 ± 2.00^{ab}	35.00 ± 2.00^{a}	37.00 ± 2.00^{ab}
GLO (g/dl)	33.00 ± 2.00^{ab}	38.00 ± 2.00^{bc}	34.00 ± 2.00^{abc}	30.00 ± 2.00^{a}	37.00 ± 2.00^{abc}	41.00 ± 2.00^{c}	35.00 ± 2.00^{abc}
ALB/GLO (ratio)	1.30 ± 0.20^{a}	0.90 ± 0.20^{a}	1.10 ± 0.20^{a}	1.30 ± 0.20^{a}	1.10 ± 0.20^{a}	0.90 ± 0.20^{a}	1.10 ± 0.20^{a}
ALT (U/I)	13.00 ± 2.00^{a}	12.00 ± 2.00^{a}	13.00 ± 2.00^{a}	11.00 ± 2.00^{a}	9.00 ± 2.00^{a}	12.00 ± 2.00^{a}	11.00 ± 2.00^{a}
AST (U/I)	12.00 ± 2.00^{a}	9.00 ± 2.00^{a}	10.00 ± 2.00^{a}	13.00 ± 2.00^{a}	8.00 ± 2.00^{a}	10.00 ± 2.00^{a}	10.00 ± 2.00^{ab}
GLUCO (mol/l)	$7.8.00 \pm 0.20^{e}$	5.70 ± 0.20^{d}	6.00 ± 0.20^{d}	4.30 ± 0.20^{ab}	5.00 ± 0.20^{c}	$4.90 \pm 0.20\text{b}^{c}$	4.10 ± 0.20^{a}

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; TP = Total Protein; ALB = Albumin; GLO = Globulin; ALB/GLO = Albumin-Globulin ratio. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different ($p > 0.05$)

Table 5

Mean plasma biochemistry parameters of *C. gariepinus* juveniles before exposure and after 14 days' post exposure of *T. neriifolia*; $n = 2$ specimens tested per treatment

Parameters	Before	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
TP [g/dl]	76.00 ±2.00^a	76.00 ±2.00^a	74.00 ±2.00^a	72.00 ±2.00^a	69.00 ±2.00^a	70.00 ±2.00^a	75.00 ±2.00^a
ALB [g/dl]	43.00 ±2.00^b	40.00 ±2.00^a	38.00 ±2.00^a	37.00 ±2.00^a	40.00 ±2.00^a	40.00 ±2.00^a	38.00 ±2.00^a
GLB [g/dl]	33.00 ±2.00^{ab}	36.00 ±2.00^{ab}	36.00 ±2.00^{ab}	35.00 ±2.00^{ab}	29.00 ±2.00^a	30.00 ±2.00^{ab}	37.00 ±2.00^b
ALB/GLO (ratio)	1.30 ±0.20^a	1.10 ±0.20^a	1.10 ±0.20^a	1.10 ±0.20^a	1.40 ±0.20^a	1.30 ±0.20^a	1.10 ±0.20^a
ALT [U/I]	13.00 ±2.00^a	12.00 ±2.00^a	10.00 ±2.00^a	9.00 ±2.00^a	13.00 ±2.00^a	12.00 ±2.00^a	10.00 ±2.00^a
AST [U/I]	12.00 ±2.00^a	11.00 ±2.00^a	9.00 ±2.00^a	9.00 ±2.00^a	9.00 ±2.00^a	11.00 ±2.00^a	8.00 ±2.00^a
GLUCO [mol/l]	7.8.00 ±0.20^e	4.70 ±0.20^a	6.00 ±0.20^{bc}	5.20 ±0.20^a	5.40 ±0.20^{ab}	7.30 ±0.20b^d	6.20 ±0.20^c

Explanations: TnLE = *Thevetia neriifolia* Leaves Extract; TP = Total Protein; ALB = Albumin; GLO = Globulin; ALB/GLO = Albumin-Globulin ratio. Mean of duplicate data, mean value in each row with similar superscripts are not significantly different ($P > 0.05$), fish ($n = 2$) per treatment

Histological examination of *C. gariepinus* juveniles exposed to sublethal concentrations of *T. neriifolia* leaves extract

There were slight observable changes in the gills and liver among all the exposed groups except the control. The control group recorded no visible abnormal structural changes also in the skin and intestine (Table 6).

Table 6

Histological changes observed different organs of *C. gariepinus* juveniles exposed to sub-lethal concentrations of *T. neriifolia* leaves extract ($TnLE_{1–6}$)

Organ	Histological changes	Control (0.00 ml/L)	$TnLE_2$ (0.04 ml/L)	$TnLE_3$ (0.08 ml/L)	$TnLE_4$ (0.15 ml/L)	$TnLE_5$ (0.30 ml/L)	$TnLE_6$ (0.6 ml/L)
Skin	moderate hyperplasia of keratinocytes	–	–	–	–	½	–
	atrophy of the epidermis and loss of keratinocytes	–	–	–	–	–	½
Gills	atrophy of secondary lamellae	–	–	½	–	–	–
	hyperplasia of secondary lamellae	–	–	–	½	½	½
Liver	moderate diffuse vacuolation of hepatocytes	–	½	–	½	½	–
	hepatocellular degeneration	–	–	½	–	–	–
	moderate diffuse atrophy of hepatocytes	–	–	–	–	–	½
Intestine	no visible structural changes	–	–	–	–	–	–

Explanations: ½ = present but mild; "–" = no lesion or morphological changes in tissue

Discussion

Aquatic species demonstrate alterations in their behavior in response to chemical stress, which can occur either as acute toxicity or sublethal toxicity (GRILLITSCH et al. 1999). The behavioural alterations exhibited by the fish subjected to *Thevetia neriifolia* were detected across varying levels of concentration. During the exposure period, the control group exhibited typical swimming behavior and maintained their natural coloration. However, as the concentration of the toxicant increased, the fish displayed an escalating frequency of anomalous behaviours. The observed behavioural alterations encompass a decline in equilibrium, ingestion of air and irreg-

ular swimming patterns. The fishes in the exposed groups exhibited heightened mucus secretion, potentially as an adaptation mechanism to mitigate the irritating effects of *T. neriifolia* on their body surface and mucus membrane.

The findings of this experiment indicated a notable overall increase in the weight of both the unexposed and exposed groups. The biggest increase in weight was observed in the $TnLE_2$ treatment, and weight growth reduced as the concentration of the extract increased. The $TnLE_2$ group exhibited superior performance across all biological indicators compared to the other exposed groups. However, statistical analysis revealed no significant differences ($p > 0.05$) among the exposed groups (Figure 2) There were notable disparities in the packed cell volume (PCV) between the control and exposed groups of fish, with statistical significance observed ($p < 0.05$). The recorded values exhibited an increase in comparison to the control treatment, apart from $TnLE_2$, where the result demonstrated a reduction (Table 2). The observed rise in red blood cell count (Table 2) may be attributed to several factors, such as an elevated number of red blood cells resulting from acute stress and spleen discharge, swelling of erythrocytes due to reduced blood pH and respiratory acidosis, or a decrease in circulating blood volume caused by the acidification of muscle tissue following exposure to stress or intense physical activity (GOMUŁKA et al. 2014). According to ETIM et al. (1999), haemoglobin is a significant indicator of fish survivability due to its direct correlation with the blood's capacity to bind and transport oxygen. This investigation revealed statistically significant variations ($p < 0.05$) in the levels of haemoglobin (Hb). The observed values exhibited an increase in comparison to the control treatment, except for $TnLE_2$ (Table 2).

The results indicate a marginal decline in the levels of red blood cells in the exposed groups as compared to the control group. However, statistical analysis revealed no significant differences ($p > 0.05$) between the control and exposed groups. A decrease in the white blood cell (WBC) values was observed in both the control and exposed groups, relative to the starting values recorded prior to the trial. Nevertheless, the experimental groups had greater values compared to the control group, except for $TnLE_2$ and $TnLE_5$, which showed somewhat lower values (Table 3). The observed elevation in the number of white blood cells in certain subjected fish can be related to modifications in their defensive mechanisms against the toxic effects of the extract (ZAGHLOUL 2001, ZAGHLOUL et al. 2005).

An elevation in mean cell volume (MCV) was observed in the groups that were exposed, apart from $TnLE_2$, where a reduction was noted, in comparison to the control group (Table 2). The elevation of mean corpuscu-

lar volume (MCV) is ascribed to the enlargement of erythrocytes due to hypoxia (insufficient oxygen levels) or disrupted water equilibrium (osmotic stress), leading to the development of macrocytic anemia in fish exposed to toxic substances (LARSSON et al. 1985). A decrease in lymphocyte count was observed in all treatment groups, apart from $TnLE_4$, which exhibited an increase (Table 2). The decrease in variable may be attributed to an immunological response, wherein the body produces antibodies to manage the stress caused by the hazardous substance. An observed elevation in the overall neutrophil count was documented (Table 2), either indicating the presence of a bacterial infection or arising from the influence of stress. The mean corpuscular hemoglobin concentration (MCHC) values exhibited a modest elevation compared to the control group, demonstrating continuous uniformity across the samples. However, it is worth noting that $TnLE_4$ displayed an identical numerical value to that of the control group (Table 2). Nevertheless, the study did not find any statistically significant differences ($p > 0.05$) between the control group and the groups who were exposed to the experimental conditions. This finding suggests that the exposure to the aqueous leaves extract of *T. neriifolia* did not have an impact on MCHC. The mean corpuscular hemoglobin (MCH) exhibited a statistically significant increase ($p < 0.05$) in numerical values when compared to the control group, except for $TnLE_2$ (Table 2).

Upon completion of the post-exposure period, the haematological analysis revealed a notable decline in the levels of PCV, Hb, MCV, and MCH within the exposed groups in comparison to the control group. Furthermore, there were no statistically significant differences observed in the values of PCV, RBC, MCHC, neutrophils, lymphocytes, and monocytes (Table 3). The findings additionally indicated a rise in the levels of white blood cells (WBC) in comparison to the values seen during exposure to the extract of *T. neriifolia* leaves. Nevertheless, the observed reversible hematological parameters after a 14-day post-exposure period (Table 3) provide evidence of the non-bioaccumulative action of the *T. neriifolia* extracts. The exposed groups exhibited minor variations in total protein (TP) and albumin/globulin (A/G) levels in comparison to the control group (Table 3). The globulin values of the exposed groups exhibited a substantial drop ($p < 0.05$) in comparison to the control group, except for $TnLE_5$. However, the exposed groups exhibited a modest yet statistically significant rise ($p < 0.05$) in albumin levels (Table 3).

Upon the conclusion of the post exposure period, it was observed that the levels of albumin (ALB) and the albumin-globulin ratio (A/B) in the exposed groups were comparable to those of their respective control groups. The observed data pertaining to the levels of total protein (TP) within the

groups that were exposed indicate a reduction in comparison to the control group. Nevertheless, the control and exposed groups did not exhibit any statistically significant differences ($p > 0.05$) in terms of TP, ALB, and A/B (Table 4). Substantial variations in the globulin (GLB) levels were observed within the exposed groups during post-exposure, in contrast to both the control group and the values recorded during exposure to *T. neriifolia* leaf extract. A notable decline in the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was observed in comparison to the measurements recorded prior to and after the administration of *T. neriifolia* leaf extract. No statistically significant differences ($p > 0.05$) were seen in the values of ALT between the control and exposed groups. However, a notable reduction was detected in the glucose levels, except for $TnLE_2$ (Table 4). At the conclusion of the post-exposure period, the study observed a statistically significant rise ($p < 0.05$) in glucose levels compared to those observed during exposure to *T. neriifolia*. The values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) exhibited an increase in comparison to the initial values acquired before to the commencement of the trial. No statistically significant differences ($p > 0.05$) were seen in the levels of ALT and AST between the control group and the exposed group (Table 5).

All fish organs in the study's control group were oriented similarly to those of other healthy finfish, with no tissue deformation or modification visible. However, as the concentration of the extract grew, there was clear evidence of changes in the structural formations of the gill tissues of the fish among the groups that were exposed to the extract. According to REDDY and WASKALE (2013), fish's gills are crucial in maintaining biological homeostasis in aquatic environments. Due to their proximity to the external environment and the large surface area of respiratory epithelium, the gills are susceptible to the negative impacts of contaminants and pollutants in the water. The gills of the fish subjected to a dose of 0.08 ml/L of *T. neriifolia* extract exhibited atrophy of the secondary lamellae. Additionally, hyperplasia of the secondary lamellae was detected at concentrations of 0.15 ml/L, 0.30 ml/L, and 0.60 ml/L. Similarly, the livers of the groups exposed to different amounts of the *T. neriifolia* extract exhibited varying degrees of structural deformations. The liver exhibited significant diffuse vacuolation of hepatocytes at a concentration of 0.04 ml/L. At a concentration of 0.08 ml/L, random hepatocellular degeneration was observed. Random swelling (vacuolation) of hepatocytes was observed at 0.15 ml/L and 0.3 ml/L concentrations. Likewise, moderate diffuse atrophy of hepatocytes was identified at a dose of 0.6 ml/L. The fish's skin exposed to an extract concentration of 0.3ml/L showed a modest increase in keratino-

cytes, indicating hyperplasia. Conversely, the skin exposed to a concentration of 0.6 ml/L had a reduction in the thickness of the outermost layer of skin (epidermis) and a decrease in the number of keratinocytes, indicating atrophy. No discernible alterations in the intestinal structure were identified between the control and exposed groups (Table 6).

Conclusion

The extract of *T. neriifolia* exhibited sublethal toxicity on the fish specimens, resulting in significant changes in the blood profiles of *C. gariepinus*. The observed variations in the fundamental characteristics of fish resulting from their exposure to the extract provide evidence of its mechanism of action as a piscicide. Therefore, it is plausible that this substance generated from plants could serve as a possible piscicide for the purpose of capturing fish or eliminating undesired fish populations in nursery and rearing ponds prior to stocking. This work has the potential to offer valuable insights for the potential future utilization of *T. neriifolia* in aquaculture applications. Further investigation is warranted to explore the effects of *T. neriifolia* leaf extracts on additional fish species, with the aim of comprehensively understanding their physiological and behavioral responses to these plant extracts.

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