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Responses of *Clarias gariepinus* Juveniles to Varied Concentrations of *Paullinia pinnata* Aqueous Leaf Extract

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Abstract

The study assessed Clarias gariepinus juveniles' response to Paullinia pinnata leaf aqueous extract. Four treatments (1.50, 3.00, 4.50, and 6.00 g/ mL) were made from the stock solution (50 g/ L) for a 96-h main experiment, following the range-finding test (0.00, 0.25, 0.375, and 0.50 g/ mL) whose highest treatment posed no toxic effect. The phytochemical screening indicated alkaloids, flavonoids, glycosides, tannins, and saponins. The in-situ water parameters significantly ($p < 0.05$) differed: temperature < 27 ˚C, E.C.: 809-1460 µS/ cm, and TDS: 460-580 mg/ L, except pH: 8.73-9.00. The fish exhibited neither hyperactivity nor mortality every 24 h of the main experiment. The WBC, RBC, PCV, MCH, and MCHC differed significantly ($p < 0.05$). An increase in the WBC, RBC, and PCV led to a decrease in the MCH and MCHC by ≈75.86 %. The skin indicated moderate hyperplasia to club cell degeneration, the gill indicated lamellae fusion to pillar capillaries' congestion, and the liver indicated moderate venule congestion. The aqueous extracts influenced damage to the fish's health, suggesting cautious usage and disposal of the plant in the water body.

1.0 Introduction

There is an abundance of medicinal plants whose scientific importance has not been fully explored. Plants have widely served as the richest source of raw materials for traditional medicine and modern medicine, particularly in Africa and Asia [1]. Despite the availability of conventional drugs, medicinal plants treat infections and provide opportunities across developing countries for accessibility and affordability [2]. Phytochemicals in plants serves a huge biological function and is responsible for the medicinal usefulness of plants as drugs. The treatment of rickets, leprosy, fever disability, post-partum pain, localised pain, infectious diseases, cough, whooping cough, eye ailments and complex treatments for jaundice and yellow fever has been traced to medicinal plants [3].

Paullinia pinnata isa sub- and woody climber of the Sapindaceae family, originates from tropical America, and now common in tropical Africa and Madagascar savanna zones. "Bread and cheese plant" and "sweet gum" are its common names. The local names include Kakansenla or Ogbe-okuje in Yoruba, Aza, Igala; Egwubi, Omekpa in Edo, Enu Kakanchela in Nupe, Goorondoorinaa in Hausa, basa in Liberia, Gbe-se, Togo (AnyiAnifo); Tolundi, Sierra Leone (Kono); Kamakagu and Ghana (AdangmeKrobo); Akplokinakpa [4]. Aqueous decoctions and powdered roots from the plant are used as traditional medicine [5,6]. The plant properties are variously used; the leaf, stem, and root aqueous decoction exhibited activity against *Bacillus subtilis* and *Staphylococcus aureus* [7]. The antibacterial activity in plant extracts has been attributed to some secondary metabolites that make the plant useful in drug research and development [8]. The methanolic leaf extract of the plant indicated anxiolytic properties, which were clinically applied to treat and manage anxiety disorders in rats [9]. Its root elemental contents were bio-markers that indicated aphrodisiac usage and were adopted to manage erectile disorders in Ghana [10]. The ethanolic leaf extract of *paullina pinnata* was also used in treating *Shigella flexneri* induced diarrhoea in wistar rats, this study also reviewed its toxicity effect on the rat, showing the appropriate dosage required in treating diarrhoea in wistar rats [11]. The use of plants in animal disease treatment is traditionally practised, albeit with non-standardised doses, as a significant shortfall; the fundamental belief further compounds that the plant extracts are non-toxic [5]. Its aqueous and methanolic extract improved its analgesic and anti-inflammatory effects on mono-arthritis and supported its medicinal application [5]

However, the plant was introduced to Africa as a fish poison, or ichthyotoxic plant, and is used to stun or kill fish [12]. In small-scale fish production, most farmers use a combination of conventional and ethnopharmacological formulations to treat diseases and parasite infections. The obnoxious fishing method in Nigeria involves using ichthyotic plants to catch fish, causing mass fish mortality in ponds, contaminating the freshwater bodies, and affecting non-target organisms [13]. The physicochemical changes in the culture environment often cause

physiological changes in fish; thus assessing the water quality is crucial because the water parameters determines productivity and survival of the fish. Changes in the behaviour of the fish are also monitored, as behavioural changes are good indicators of damage to the fish due to exposure to toxic agents [14]. Family Clariidae is one of the world's most commonly cultured fish species [15]. C. gariepinus juveniles were used for the toxicity test because they were more sensitive than adults (18). The current study examined the toxicological effect of varied concentrations of P. pinnata leaf aqueous extract on Clarias gariepinus haematological and histopathological characteristics.

2.0 Materials and Methods

2.1 Collection and preparation of plant material

Fresh leaves of the *Paullinia pinnata* were collected (handpicked) at the back of the unity building along the road to the College of Management Science in the Federal University of Agriculture Abeokuta (FUNAAB) in April 2023. The collected leaves were identified at the Forestry and Wildlife Management of FUNAAB, washed under running tap water, and rinsed with distilled water. The leaves were then air-dried with a dehydrator at room temperature for 2 hours, powdered and lastly kept in an airtight container at room temperature for further laboratory analysis. Among plants' medicinally significant natural products are alkaloids and saponins, known for their unique medicinal properties (17). The *P. pinnata* leaf was screened for alkaloids, tannins, flavonoids, saponins, and glycosides.

2.2 Stock solution preparation, range-finding test, and the main experiment

Fifty grams (50 g) of the powdered *P. pinnata* leaves were weighed, soaked in 1 L distilled water for 24 h, and intermittently shook every 3 h. The broth was sieved into a bowl, and the collected filtrate was stored in the refrigerator as a stock solution. The stock solution of the *P. pinnata leaf* aqueous extract was used to make three serial treatments (0.250, 0.375 and 0.500 g/ mL) for the range-finding test (RFT). The 180 *Clarias gariepinus* juveniles for the experiment were procured from Mustard Fingerling Agribusiness Ventures, Kotopo Farm Extension, Abeokuta, Ogun State, Nigeria. The fish were transported in a cut-out keg containing the pond water and acclimatised in a circular fibre tank for seven days in the new environment (18) in the Fish Hatchery Centre of FUNAAB. The fish were distributed in ten across 15 experimental tanks of 30 L capacity for five treatments in replicate. The experimental triplicate tanks were thoroughly washed and rinsed with running tap water before putting the fish. The tanks were half-filled from the FUNAAB borehole, and the cultured water was tested for in-situ parameters throughout the experiment. The RFT was determined using the method described by Silva *et al* (19). The experiment went through a completely randomised design with three plastic tanks for each treatment. The RFT as a preliminary test lasted 96 hours (5 days). The treatments were dropped with a 5 mL syringe in each experimental tank every 24 h, when the four in-situ water parameters (pH, temperature, total dissolved solids, and conductivity) were monitored with Hannah handheld kit and the fish were checked for mortality.

Haematology and histopathology (skin, liver, and gill) analyses were carried out for each replicated treatment at the end of 96 h. With

no mortality, even at the highest (0.500 g/ mL) treatment during the RFT, the concentration was increased for the main experiment, having its treatment tanks labelled 1.50, 3.00, 4.50, and 6.00 g/ mL with the control tank labelled 0.0 mg/ L. At the terminating stage of the experiment, the blood sample was taken from the caudal peduncle of the test fish to collect 1 to 1.5 mL of the blood samples according to the procedure of Argungu *et al.* (20). The blood sample was emptied into a 10 mL sample bottle containing anticoagulant EDTA (Ethylenediaminetetraacetic acid) vial. Blood samples were estimated for haematological (PCV, erythrocyte count, haemoglobin, leucocyte count, etc.) and histopathological (skin, liver, and gill) parameters at the Veterinary Clinic, FUNAAB to know the likely changes that might have occurred in the fish.

2.3 Data analysis

The obtained data were entered in the Excel Spreadsheet. They transferred to Statistical Package Social Sciences version 23 for descriptive (mean and standard deviation), inferential (ANOVA and Duncan Multiple Range Test for means separation), and Pearson Correlation (for likely associations among the analysed parameters) analyses.

3.0 Results

3.1 Water Quality Parameters

The water pH dropped slightly compared with the control tank pH value due to the impact of varied treatments on cultured water quality. The E.C. of the control tank was lower than 4.50 and 6.00 mg L tanks while higher than 1.50 and 3.00 mg/ L tanks (Table 1). The assessed in-situ water parameters, except the pH, had significant ($p < 0.05$) differences across the experimental tanks (Table 2).

Table 1: Water parameters of the tank containing the experimental fish

Water parameter	Treatments	N	Mean	Std. Deviation	Minimum	Maximum
Temp. (˚C)	1.50 g/ mL	12	26.47[b]	0.69	25.60	27.70
	3.00 g/ mL	12	26.39[b]	0.75	25.50	27.80
	4.50 g/ mL	12	26.18[b]	0.41	25.70	26.70
	6.00 g/ mL	12	25.68[a]	0.04	25.60	25.70
	Control	12	26.25[b]	0.54	25.60	27.10
	Total	60	26.19	0.60	25.50	27.80
pH	1.50 g/ mL	12	8.73[a]	0.57	7.99	9.99

	3.00 g/ mL	12	8.79[a]	0.33	8.31	9.41
	4.50 g/ mL	12	8.88[a]	0.31	8.46	9.46
	6.00 g/ mL	12	8.95[a]	0.26	8.64	9.44
	Control	12	9.00[a]	0.30	8.65	9.58
	Total	60	8.87	0.37	7.99	9.99
EC (µS/ cm)	1.50 g/ mL	12	1145.67[b]	82.89	1035.00	1304.00
	3.00 g/ mL	12	1460.42[c]	739.22	1017.00	2685.00
	4.50 g/ mL	12	809.92[a]	117.97	616.00	910.00
	6.00 g/ mL	12	899.00[ab]	46.42	819.00	972.00
	Control	12	976.08[ab]	111.91	831.00	1123.00
	Total	60	1058.22	402.51	616.00	2685.00
TDS (mg/ L)	1.50 g/ mL	12	580.00[b]	40	520	650
	3.00 g/ mL	12	460.00[a]	80	340	570
	4.50 g/ mL	12	460.00[a]	40	420	520
	6.00 g/ mL	12	480.00[a]	60	430	600
	Control	12	500.00[a]	60	420	560
	Total	60	490.00	70	340	650

A water parameter whose mean values had the same alphabet was not significantly different ($p > 0.05$) across the treatments. N: The number of times the water parameters were assessed for the mean values to be taken.

Table 2: Analysis of variance of the water parameters

		Sum of Squares	Df	Mean Square	F	Sig.
Temp	Between Groups	4.532	4	1.133	3.780	0.009

	Within Groups	16.485	55	0.300		
	Total	21.017	59			
pH	Between Groups	0.590	4	0.148	1.074	0.378
	Within Groups	7.556	55	0.137		
	Total	8.146	59			
E.C.	Between Groups	3157932.767	4	789483.192	6.783	0.000
	Within Groups	6401085.417	55	116383.371		
	Total	9559018.183	59			
TDS	Between Groups	.117	4	0.029	8.441	0.000
	Within Groups	0.190	55	0.003		
	Total	0.307	59			

Pearson's correlation indicated an indirect and weak significant (at 0.05) association between temperature and pH, while a direct and moderate association (at 0.01) between temperature and TDS. The association implied that as the temperature of the cultured water increased, the pH reduced ($r = 0.271$, $R^2 = 0.0734$) by 7.34 %, while TDS increased ($r = 0.460$, $R^2 = 0.2116$) by 21.16 % (Table 3). For the impact of varied treatments on water quality over the main experiment period (Fig. 1): the temperature was lowest at 48 h (3rd day) and highest at 96 h (5th day); however, the cultured water maintained a room temperature. The water pH increased from 24 to 96 h but decreased at 96 h; maintained alkaline media, which indicated an excellent experimental culture. The water E.C. was highest at 24 h but reduced and maintained from 48 to 96 h, while TDS increased over the 96 h period.

Table 3: Correlations between the water parameters

	Temp	pH	EC	TDS
Temp	1			
pH	-0.271*	1		

EC	0.174	-0.162	1	
TDS	0.460**	0.090	-0.226	1

A blood parameter with * and ** was correlated at 0.05 and 0.01 significance levels with each other across the treatments, respectively. – value indicated negative correlation, + value indicated positive correlation.

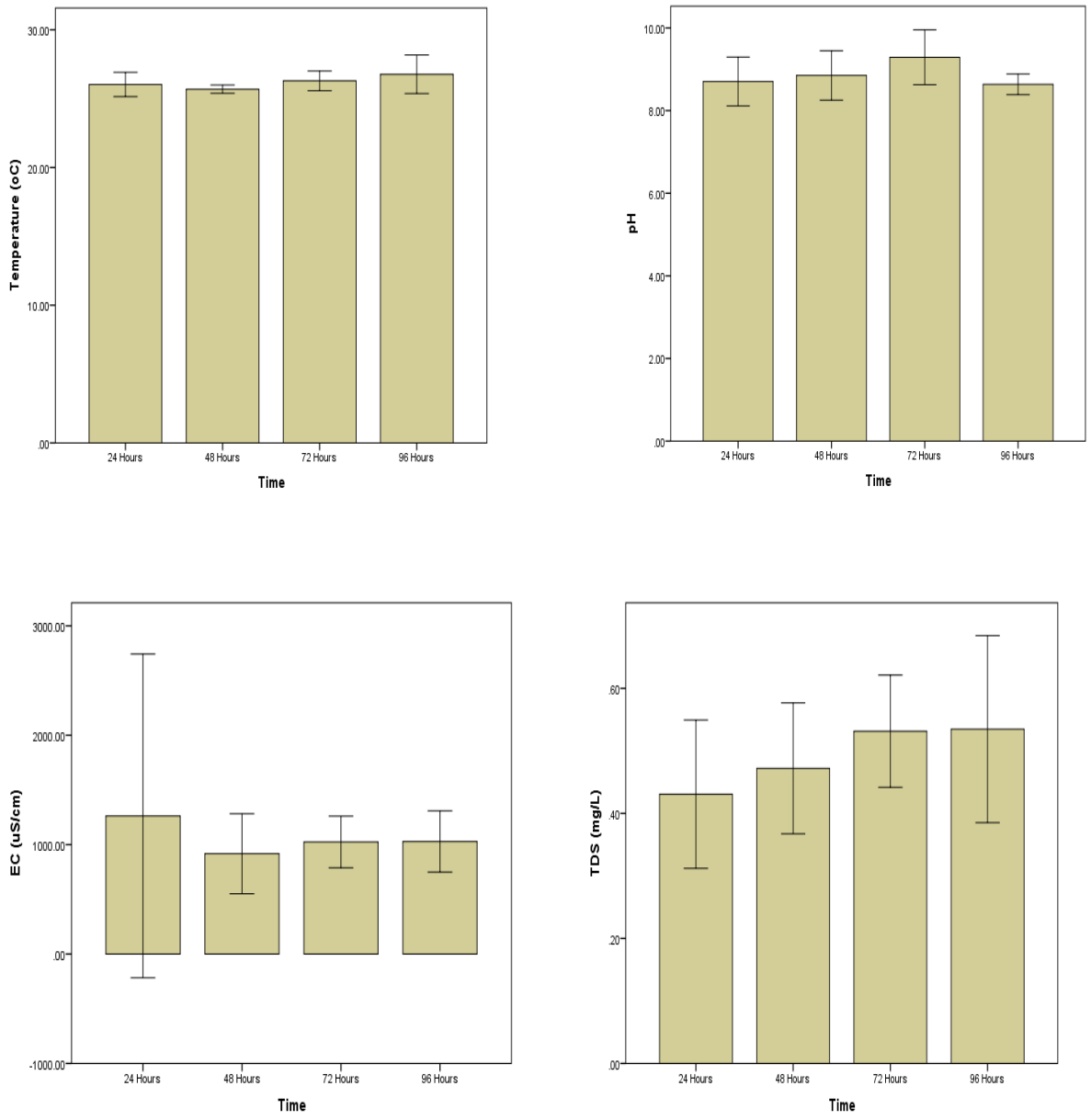


Figure 1: Effects of *P. pinnata* aqueous extracts on water in-situ parameters over the 96-h period.

3.2 MORTALITY ESTIMATION

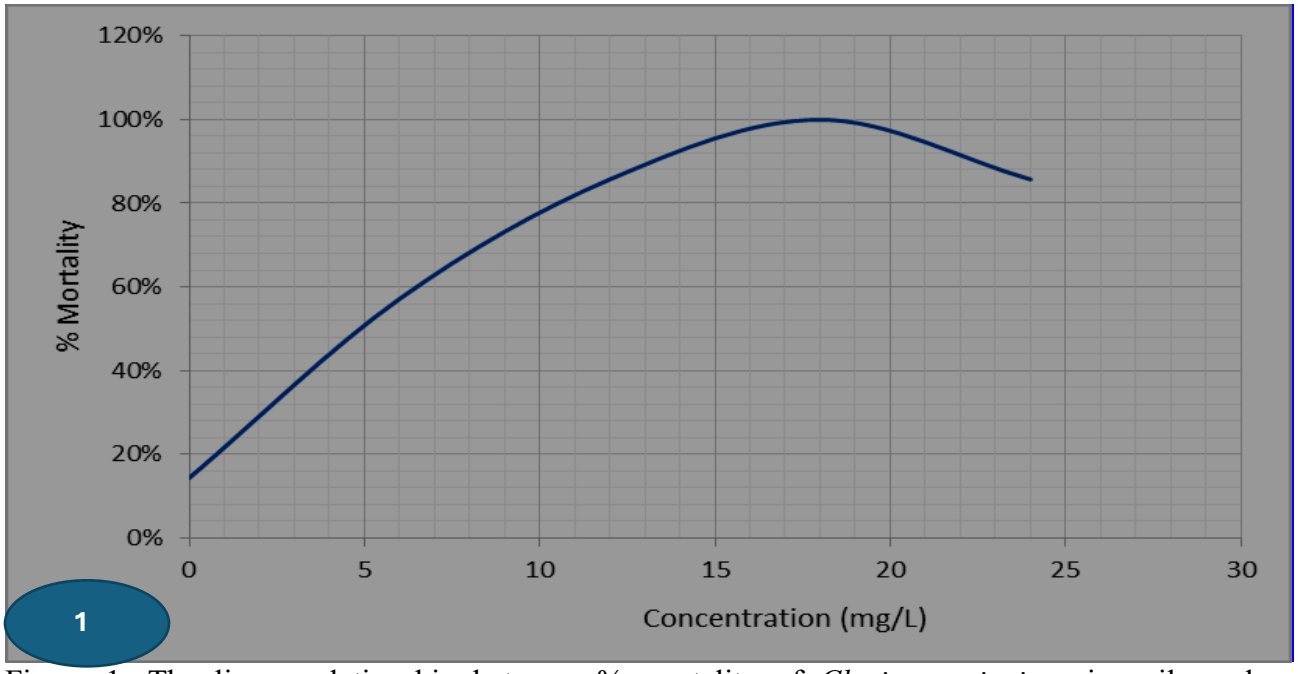


Figure 1: The linear relationship between % mortality of *Clarias gariepinus* juvenile and concentrations of aqueous *P. pinnata* leaf extract. LD_{50} of the extract = 4.8 mg/ L.

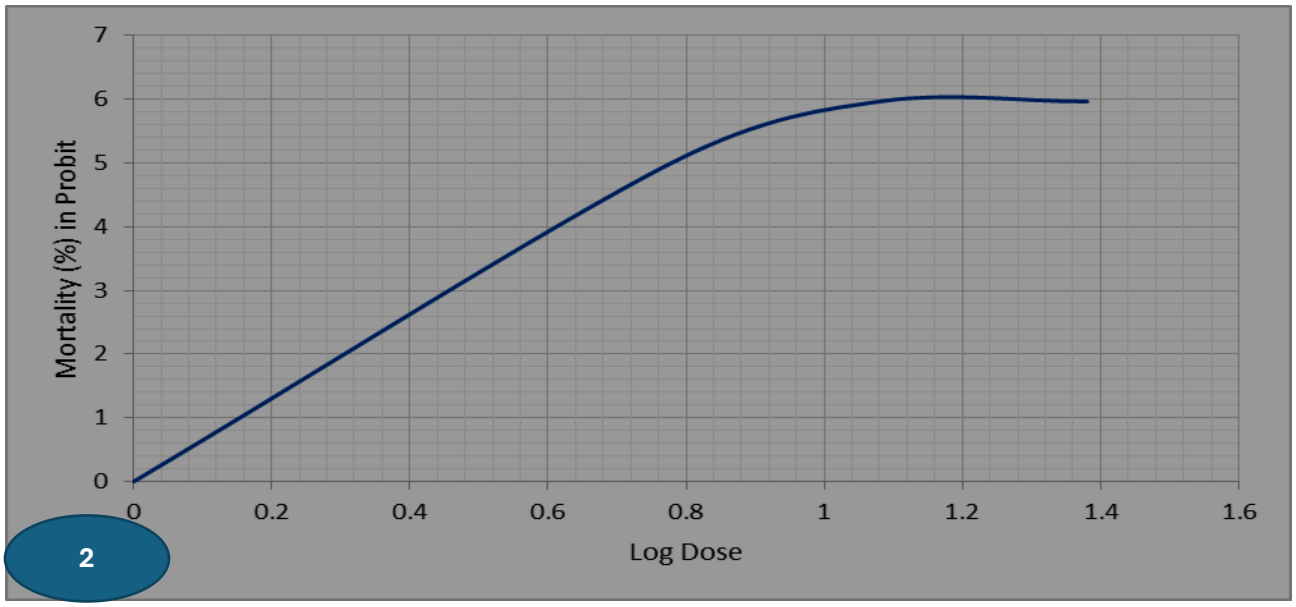


Figure 2: Percentage (%) mortality in probit after 14 days and log dose of aqueous *P. pinnata* leaf extract.

3.3 HAEMATOLOGICAL ANALYSIS

Table 4: Descriptive and inferential statistics of blood parameters

Blood parameter	Treatment	N	Mean	Std. Deviation	Min.	Max.
HB	1.50 g/ mL	3	48.25[a]	54.80	9.50	87.00
	3.00 g/ mL	3	9.40[a]	1.27	8.50	10.30
	4.50 g/ mL	3	13.05[a]	0.21	12.90	13.20
	6.00 g/ mL	3	11.90[a]	0.57	11.50	12.30
	Control	3	32.60[a]	31.68	10.20	55.00
RBC	1.50 g/ mL	3	1.05[a]	0.21	1.70	2.00
	3.00 g/ mL	3	2.15[ab]	0.49	1.80	2.50
	4.50 g/ mL	3	4.20[c]	0.42	3.90	4.50
	6.00 g/ mL	3	3.15[b]	0.35	2.90	3.40
	Control	3	2.40[ab]	0.42	2.10	2.70
WBC	1.50 g/ mL	3	8.10[a]	0.85	7.50	8.70
	3.00 g/ mL	3	8.90[a]	1.41	7.90	9.90
	4.50 g/ mL	3	15.10[b]	0.57	14.70	15.50
	6.00 g/ mL	3	13.30[b]	0.85	12.70	13.90
	Control	3	9.00[a]	0.14	8.90	9.10
HET	1.50 g/ mL	3	31.00[a]	1.41	30.00	32.00
	3.00 g/ mL	3	29.50[a]	2.12	28.00	31.00
	4.50 g/ mL	3	28.50[a]	0.71	28.00	29.00
	6.00 g/ mL	3	28.50[a]	2.12	27.00	30.00
	Control	3	28.00[a]	1.41	27.00	29.00

LYM	1.50 g/ mL	3	67.50[a]	3.54	65.00	70.00
	3.00 g/ mL	3	69.50[a]	2.12	68.00	71.00
	4.50 g/ mL	3	70.50[a]	2.12	69.00	72.00
	6.00 g/ mL	3	70.50[a]	2.12	69.00	72.00
	Control	3	70.50[a]	2.12	69.00	72.00
EOS	1.50 g/ mL	3	0.50[a]	0.71	0.00	1.00
	3.00 g/ mL	3	0.00[a]	0.00	0.00	0.00
	4.50 g/ mL	3	0.00[a]	0.00	0.00	0.00
	6.00 g/ mL	3	0.00[a]	0.00	0.00	0.00
	Control	3	0.00[a]	0.00	0.00	0.00
BAS	1.50 g/ mL	3	0.50[a]	0.71	0.00	1.00
	3.00 g/ mL	3	0.00[a]	0.00	0.00	0.00
	4.50 g/ mL	3	0.50[a]	0.71	0.00	1.00
	6.00 g/ mL	3	0.50[a]	0.71	0.00	1.00
	Control	3	0.50[a]	0.71	0.00	1.00
MONO	1.50 g/ mL	3	0.50[a]	0.71	0.00	1.00
	3.00 g/ mL	3	0.50[a]	0.71	0.00	1.00
	4.50 g/ mL	3	0.50[a]	0.71	0.00	1.00
	6.00 g/ mL	3	0.50[a]	0.71	0.00	1.00
	Control	3	1.00[a]	0.00	1.00	1.00
MCV	1.50 g/ mL	3	116.33[a]	1.87	115.00	117.65
	3.00 g/ mL	3	110.34[a]	8.96	104.00	116.67
	4.50 g/ mL	3	114.88[a]	11.60	106.67	123.08

	6.00 g/ mL	3	135.40[a]	8.46	129.41	141.38
	Control	3	122.22[a]	15.71	111.11	133.33
MCH	1.50 g/ mL	3	49.34[c]	2.60	47.50	51.18
	3.00 g/ mL	3	44.21[bc]	4.26	41.20	47.22
	4.50 g/ mL	3	31.26[a]	3.66	28.67	33.85
	6.00 g/ mL	3	37.92[ab]	2.46	36.18	39.66
	Control	3	41.51[bc]	5.28	37.78	45.24
MCHC	1.50 g/ mL	3	42.40[d]	1.56	41.30	43.50
	3.00 g/ mL	3	40.05[c]	0.61	39.62	40.48
	4.50 g/ mL	3	27.19[a]	0.44	26.88	27.50
	6.00 g/ mL	3	28.00[a]	0.07	27.95	28.05
	Control	3	33.97[b]	0.05	33.93	34.00
PCV	1.50 g/ mL	3	21.50[a]	2.12	20.00	23.00
	3.00 g/ mL	3	23.50[ab]	3.54	21.00	26.00
	4.50 g/ mL	3	48.00[c]	0.00	48.00	48.00
	6.00 g/ mL	3	42.50[c]	2.12	41.00	44.00
	Control	3	29.00[b]	1.41	28.00	30.00

A blood parameter whose mean values had the same alphabet was not significantly different ($p>0.05$) across the treatments. H.B.: haemoglobin, RBC: red blood cell, WBC: white blood cell, HET: heterocyte, LYM: lymphocyte, EOS: eosinophil, BAS: basophil, Mono: monocyte, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MHCH: mean corpuscular haemoglobin concentration, PCV: packed-cell volume or haematocrit

Table 5: Analysis of variance of blood parameters

		Sum of Squares	Df	Mean Square	F	Sig.
H.B.	Between Groups	2273.774	4	568.444	0.709	0.619

	Within Groups	4008.630	10	801.726		
	Total	6282.404	14			
RBC	Between Groups	7.110	4	1.778	11.468	0.010
	Within Groups	.775	10	0.155		
	Total	7.885	14			
WBC	Between Groups	77.696	4	19.424	25.693	0.002
	Within Groups	3.780	10	0.756		
	Total	81.476	14			
HET	Between Groups	11.400	4	2.850	1.056	0.464
	Within Groups	13.500	10	2.700		
	Total	24.900	14			
LYM	Between Groups	13.600	4	3.400	.557	0.704
	Within Groups	30.500	10	6.100		
	Total	44.100	14			
EOS	Between Groups	0.400	4	0.100	1.000	0.486
	Within Groups	0.500	10	0.100		
	Total	0.900	14			
BAS	Between Groups	0.400	4	0.100	.250	0.898
	Within Groups	2.000	10	0.400		
	Total	2.400	14			
MONO	Between Groups	0.400	4	0.100	.250	0.898
	Within Groups	2.000	10	0.400		
	Total	2.400	14			
MCV	Between Groups	749.947	4	187.487	1.746	0.276
	Within Groups	536.924	10	107.385		
	Total	1286.871	14			
MCH	Between Groups	368.717	4	92.179	6.385	0.034

	Within Groups	72.189	10	14.438		
	Total	440.905	14			
MCHC	Between Groups	378.049	4	94.512	158.076	0.000
	Within Groups	2.989	10	0.598		
	Total	381.039	14			
PCV	Between Groups	1107.400	4	276.850	58.904	0.000
	Within Groups	23.500	10	4.700		
	Total	1130.900	14			

The value below 0.05 indicated a significant ($p<0.05$) difference across the treatments. H.B.: haemoglobin, RBC: red blood cell, WBC: white blood cell, HET: heterocyte, LYM: lymphocyte, EOS: eosinophil, BAS: basophil, Mono: monocyte, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MHCH: mean corpuscular haemoglobin concentration, PCV: packed-cell volume or haematocrit.

Table 6: Correlation coefficients between the blood parameters

	HB	RBC	WBC	HET	LYM	EOS	BAS	MONO	MCV	MCH	MCHC	PCV
HB	1											
RBC	-0.440	1										
WBC	-0.433	0.918**	1									
HET	0.312	-0.318	-0.279	1								
LYM	-0.478	0.282	0.329	-0.926**	1							
EOS	0.851**	-0.394	-0.395	0.613	-0.746*	1						
BAS	0.246	0.299	0.034	0.078	-0.369	0.408	1					

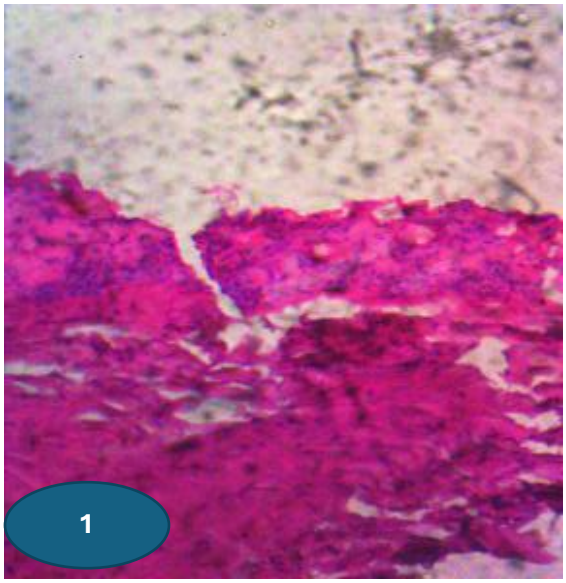
MONO	0.382	-0.184	-0.235	0.052	-0.311	0.272	0.250	1				
MCV	0.164	-0.053	0.244	-0.378	0.388	-0.064	-0.261	0.141	1			
MCH	0.547	-0.971**	-0.860**	0.366	-0.316	0.519	-0.295	0.143	0.097	1		
MCHC	0.395	-0.871**	-0.902**	0.544	-0.490	0.496	-0.164	0.030	-0.400	0.871**	1	
PCV	-0.399	0.948**	0.970**	-0.417	0.397	-0.404	0.200	-0.180	0.260	-0.908**	-0.962**	1

A blood parameter with * and ** was correlated at 0.05 and 0.01 significance levels with the other across the treatments, respectively. – value indicated negative correlation, + value indicated positive correlation.

H.B.: haemoglobin, RBC: red blood cell, WBC: white blood cell, HET: heterocyte, LYM: lymphocyte, EOS: eosinophil, BAS: basophil, Mono: monocyte, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MHCH: mean corpuscular haemoglobin concentration, PCV: packed-cell volume or haematocrit

($r = 0.871$, $R^2 = 0.7586$) 75.86 % of MCHC. Associations of each (MCH and MCHC) with PCV ($r > -0.908$, $R^2 = 0.8245$): WBC ($r > - 0.86$, $R^2 = 0.7396$) and RBC ($r > -0.90$, $R^2 = 0.81$) were indirectly strong to influence 82.45, 73.96 and 81.00 % reduction in PCV, WBC, and RBC, respectively.

3.4 HISTOPATHOLOGY



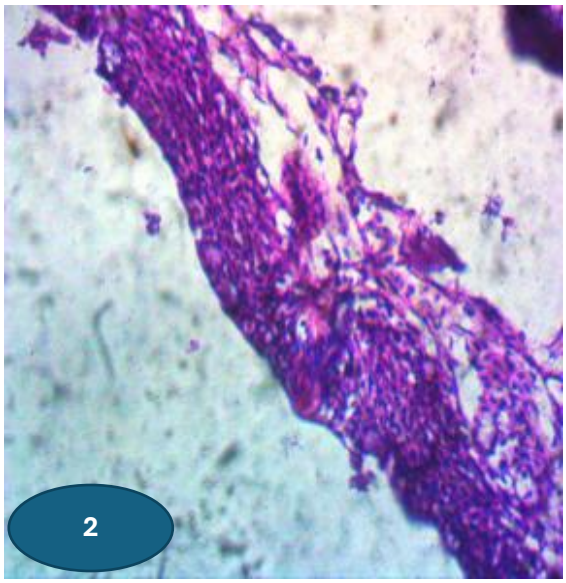
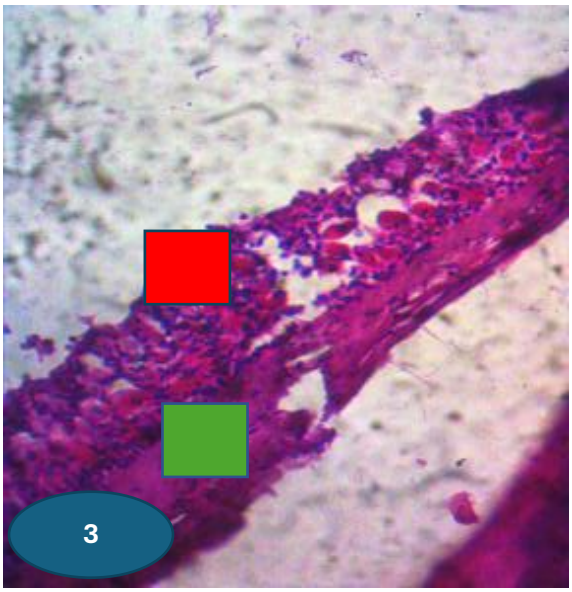


Plate 1: Skin of Cg in 0.0 g/ mL Pp leaf extract at 96 h Plate 2: Skin of Cg in 1.50 g/ mL Pp leaf extract at 96 h
Plate 1: No observable lesion. HE x400
Plate 2: Moderate hyperplasia of keratinocytes. HE x400
Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*



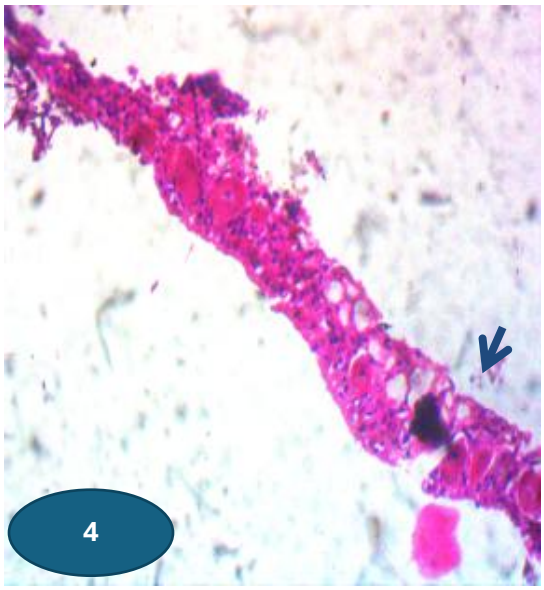
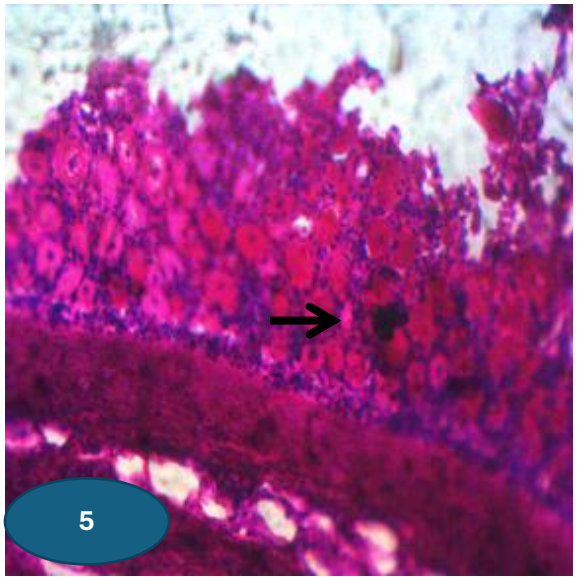


Plate 3: Skin of Cg in 3.00 g/ mL Pp leaf extract at 96h Plate 4: Skin of Cg in 4.50 g/ mL Pp leaf extract at 96h
Plate 3: Moderate hyperplasia of keratinocytes (red) and club cells (lemon). HE x400
Plate 4: Atrophy of keratinocytes and degeneration of club cells (black arrow). HE x400
Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*



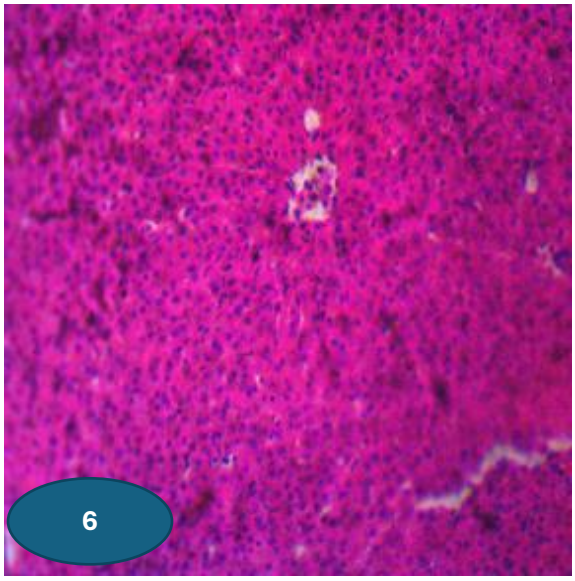
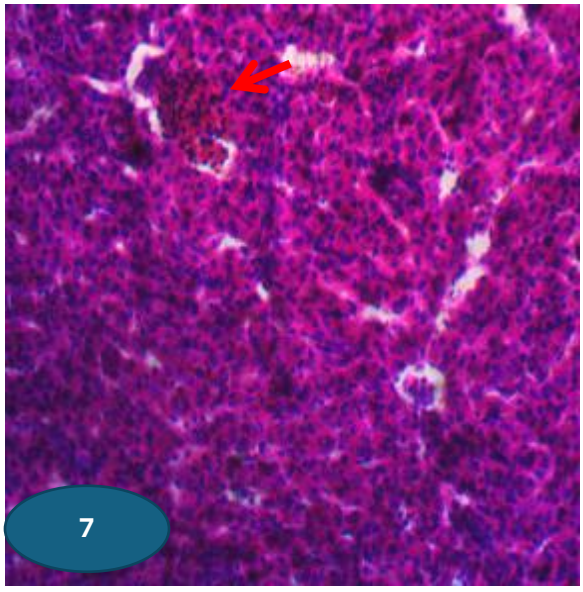


Plate 5: Skin of Cg in 6.00 g/ mL Pp leaf extract at 96h Plate 6: Liver of Cg in 0.0 g/ mL Pp leaf extract at 96h

Plate 5: Hyperplasia of club cells and keratinocytes (black stain). HE x400

Plate 6: No observable lesion. HE x400

Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*



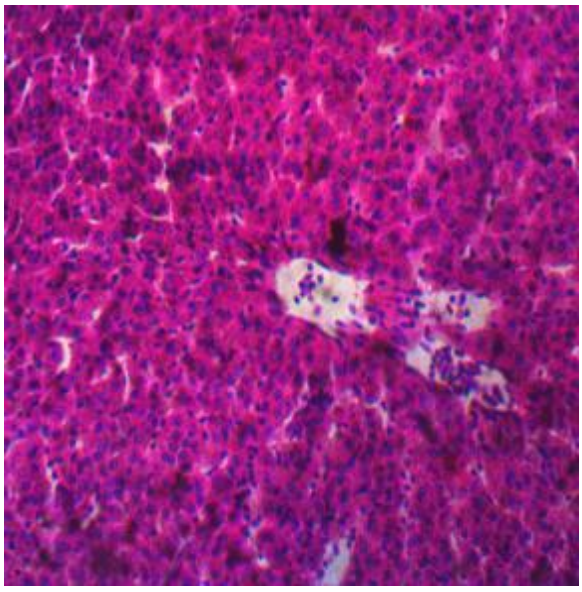
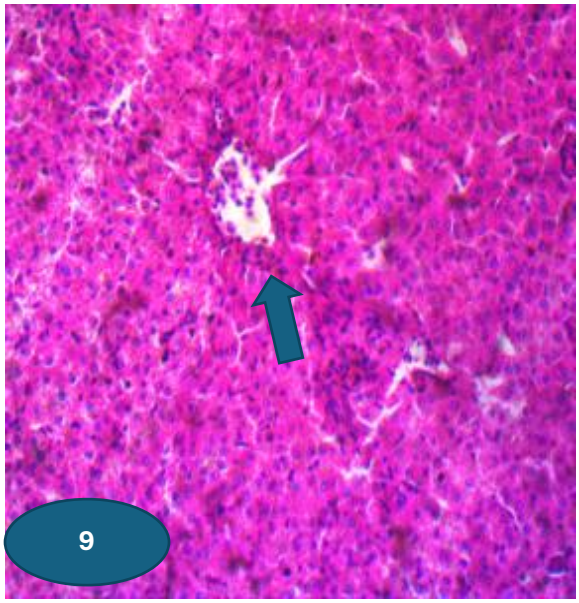


Plate 7: Liver of Cg in 1.50 g/ mL Pp leaf extract at 96 h Plate 8: Liver of Cg in 3.00 g/ mL Pp leaf extract at 96 h

Plate 7: Moderate congestion of the central venules (red arrow). HE x400

Plate 8: Moderate congestion of the central venules (black arrow). HE x400

Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*



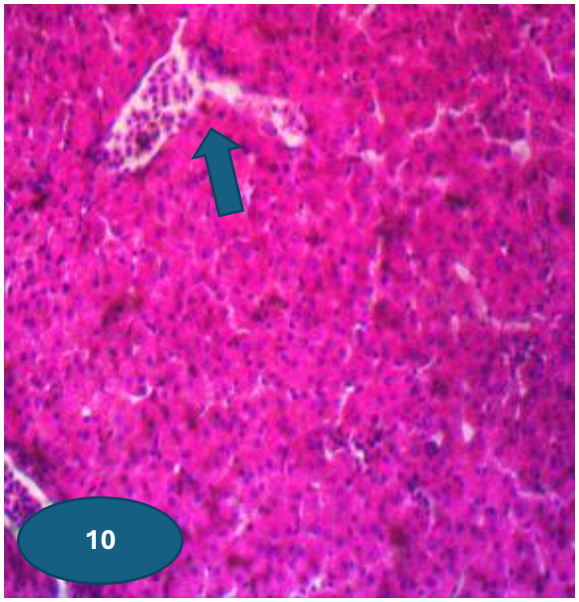
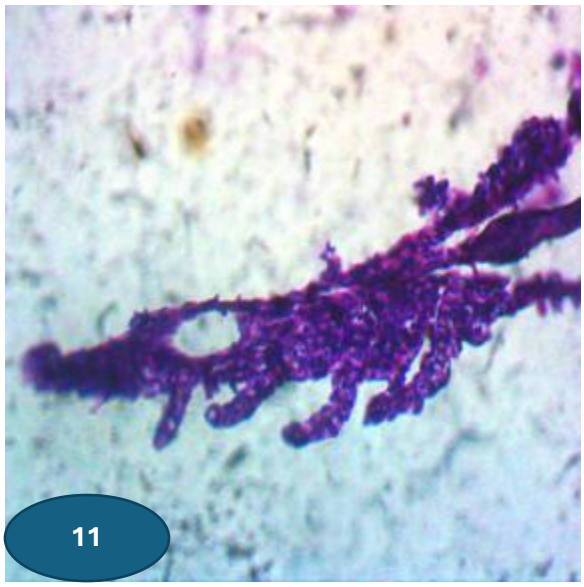


Plate 9: Liver of Cg in 4.50 g/ mL Pp leaf extract at 96 h Plate 10: Liver of Cg in 6.00 g/ mL Pp leaf extract at 96 h
Plate 9: Moderate congestion of venules (arrow). HE x400
Plate 10: Moderate congestion of venules (arrow). HE x400
Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*



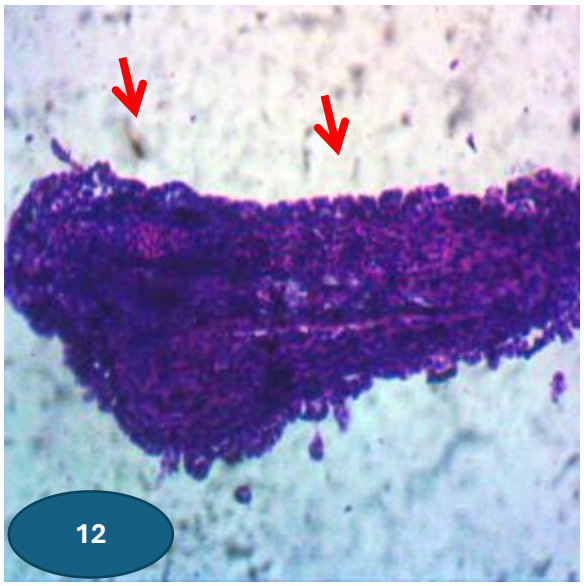
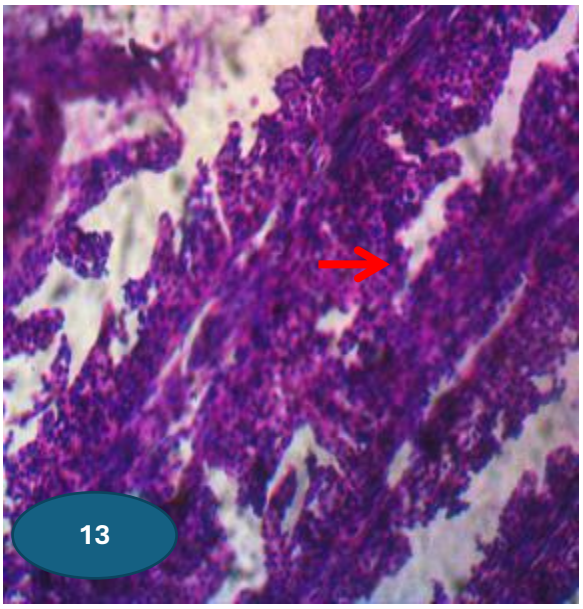


Plate 11: Gill of Cg in 0.0 g/ mL Pp leaf extract at 96 h Plate 12: Gill of Cg in 1.50 g/ mL Pp leaf extract at 96 h
Plate 11: No observable lesion. HE x400
Plate 12: Moderate hyperplasia and fusion of lamellae (arrows). HE x400
Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*



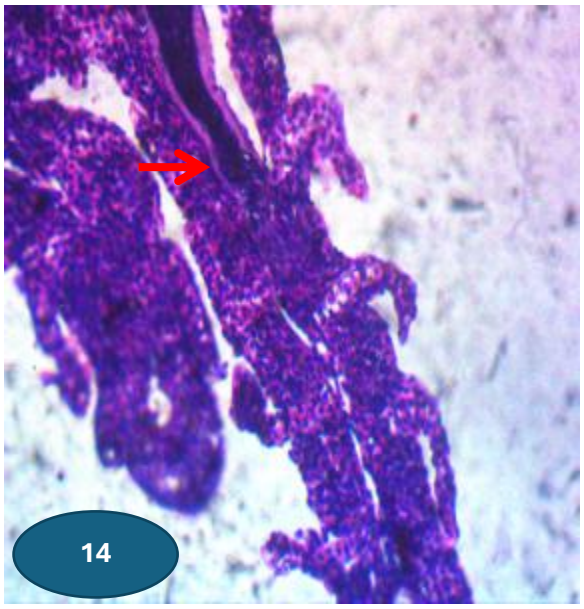


Plate 13: Gill of Cg in 3.00 g/ mL Pp leaf extract at 96 h Plate 14: Gill of Cg in 4.50 g/ mL Pp leaf extract at 96 h

Plate 13: The secondary lamellae have moderate hyperplasia (red arrow). HE x400

Plate 14: There is congestion pillar capillaries (arrow). HE x400

Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*

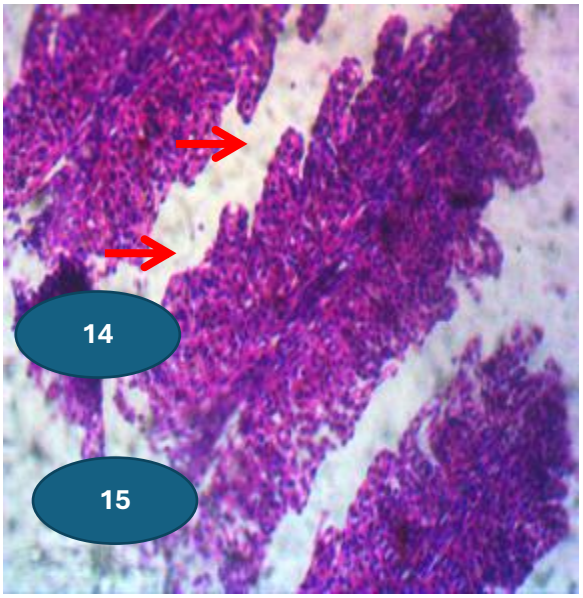


Plate 15: Gill of Cg in 6.00 g/ mL Pp leaf extract at 96 h

Plate 15: Hyperplasia of secondary lamellae (arrows). HE x400

Cg: *Clarias gariepinus*, Pp: *Paullinia pinnata*

4. Discussion

The aqueous leaf extract of *Paullinia pinnata* indicated alkaloid, glycoside, flavonoid, tannin, and saponin from the phytochemical screening; this is in agreement with an earlier study (18), which conducted a phytochemical analysis of *Kigelia Africana* and *Raphia vinifera*. (21) reported similar observations while studying the phytochemical and antibacterial properties of the leaf, stem and root of *P. pinnata,* [12] identified the secondary metabolites of *P. pinnata* as alkaloids, flavonoids, and tannins. Alkaloids are a group of nitrogen-containing compounds in plants, fungi, bacteria and animals [22]. Nitrogen atoms in the alkaloid structure give the compound drug properties. Alkaloids are reported to be the most therapeutically efficient phytochemicals [23]. Alkaloids are primarily found in higher plants, especially root, fruit, and stem. Alkaloid-rich plants exhibit a wide range of pharmacological properties such as anti-cancer [24], antibacterial, analgesic [25] and antidepressant [26]. Tannin is known for its antioxidant and antimicrobial properties and soothing diuresis. Cardiac glycoside moderates the heart muscles and renal flow (diuresis). Tannins are sequestered in vacuoles within the plant cell as secondary metabolites which protect other cell components. They usually occur in any plant's bark, roots, wood, leaves and fruits [27]. Flavonoids are widely spread in plants for their aromatic qualities and range of functions and explored in conventional herbal medicine thereby being understudied in antibacterial, antimicrobial, anti-cancer, and anti-diarrhoea activities [28]. Saponins are a class of non-synthetic compounds abundantly found in more than 100 families of plants [29]. More specifically, they are naturally occurring glycosides described by the soap-like foaming. Consequently, they produce foam when shaken in aqueous solutions. Saponins are important in medicine because they are used in antioxidants, anti-cancer, anti-inflammatory and body loss herbal preparations. Saponins are piscicides [30,31] ; known to exhibit antiviral: antiprotozoal and antibacterial and fungicidal activities [29]. The use indicates that saponin is bioactive and functional as a bio-pesticide ingredient. Plant glycosides are secondary metabolites comprising a sugar portion linked to a non-sugar moiety. Given their proven bioactivities and traditional use, they are widely present in plants, a large group of structurally diverse secondary metabolites. The glycoside is crucial in pharmacognosy.

The tendency to introduce the plant's toxicants into the water body necessitates an assessment of cultured water quality, fish behavioural responses, and physiological changes using haematology and histopathology [32].

The *Clarias gariepinus* juveniles exposed to the varied *P. pinnata* leaf aqueous extract exhibited no hyperactivity. So, the experimental fish did not jump outside of the water, had no loss of balance, no erratic swimming, no respiratory distress, no rapid opercula movement, no incessant gulping of air, no spiral movement, no discolouration of the whole body and no excessive mucus secretion throughout the 96 h. However, the fish clustered on one side of the tank, and bubbles were noticed on the water's surface anytime the dose was introduced. The behaviour responses contradicted the observations of [33,34 and 35], who subjected their experimental fish to varied concentrations of plant extracts and expressed that erratic behaviour associated with the impact of phytochemicals (which might be toxic) on fish. Exposure of the *P. pinnata* leaf aqueous extract on *C. gariepinus,* even at 6.00 g/ mL, did not cause mortality of the experimental fish throughout the 96 h. The similarity was observed in the previous work [18], which investigated the

influence of *Kigelia Africana* aqueous extract on *Oreochromis niloticus* (Nile Tilapia). [38] observed that *P pinnata* leaf ethanol extract was pathologically efficient against infectious diarrhoea without residual side effects after the 5th day (96 h). Still, modification in the liver and kidney at the higher dose indicated sub-acute toxicity. The current study determined the LD_{50} of *P. pinnata* aqueous extract at 4.8 mg/ L (Figure 1).

The test fish indicated changes in the haematology (blood parameters) and histopathology (gill, liver, and skin). The changes were initiated by the introduced irritant from the *P. pinnata* leaf aqueous extract, which contacted the skin and accumulated in the gill and liver. Haemoglobin (H.B.) of the test fish was higher in treatment at 1.5 g/ mL but three-fold lower than the control (Table 4). Alterations of the H.B. content and damages in erythrocytes showed the treatment impacts on the fish and its habitat (such as the *P. pinnata* in this study) and the purposes of ecological biomonitoring.

Fish H.B. dictates the blood red colour and exchanges gases (oxygen and carbon dioxide) within the tissues; it is susceptible to pollution in the aquatic surroundings, and morphology is a bioindicator of toxicants[37]. The values of RBC (red blood cell), WBC (white blood cell) and PCV (packed-cell volume) were lower in 1.50 and 3.00 g/ mL but higher in the 4.50 and 6.00 g/ mL treatments than the control. In contrast, the MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) were higher in 1.50 and 3.00 g/ mL and lower in the 4.50 and 6.00 g/ mL treatments than the control (Table 4). High or low content of red blood cells for PCV can indicate the impact of certain diseases, treatments, or foreign substances. The implication was that the fish developed a defence mechanism (WBC) and associated RBC and PCV more in the higher concentrations of the *P. pinnata* aqueous extracts (Table 4).

There were significant ($p < 0.05$) differences in five out of 12 haematological parameters: RBC, WBC, MCH, MCHC and PCV (Table 5). Significant responses to the five parameters corroborated the views of [38] that the fish haematological parameters respond differently to the pollutant's action in the wild. [39] (2021) referred to the RBC, H.B. and PCV as the main haematological parameters. The value of MVC (mean corpuscular volume) was higher in the 6.00 g/ mL (highest treatment) than in control; this implied that more oxygen might have been needed at the highest concentration of the *P. pinnata* leaf aqueous extract to corroborate the view of [37] and conclusion of [38] that the most uncomplicated response of test organism to oxygen deficiency is increasing in the MCV.

The RBC had a very strong (at 0.01) and direct association with WBC and PCV in a way that the production of RBC ($r > 0.90$, $R^2 > 0.81$) from the fish bone marrow influenced the production of more than 81 % of WBC and PCV from the Pearson's correlation (Table 6). Both MCH and MCHC were so strongly associated (at 0.01) that the presence of MCH influenced

For responses of the *C. gariepinus* to the *P. pinnata* leaf aqueous extracts, there was a progressive alteration in the fish skin (Plates 1 to 5): from moderate hyperplasia of keratinocytes and degeneration of the club cells to hyperplasia of keratinocytes and club cells as the treatments increased. The additional observations were accompanied by increased mucus production, peeling of the fish skin, and possible reduction in gaseous and ionic exchange, which occurred after 96 h and seven days (sub-acute) of aqueous extracts introduction.

The results of liver histology indicated moderate congestion of the central venules in

the lowest (1.50 g/ mL) and highest (6.00 g/ mL) concentrations of the *P. pinnata* leaf aqueous extract (Plates 6 to 10). Similar changes were observed in the Euphorbia hirta leaf aqueous extract on the liver and gill of *C. gariepinus* [40]. The liver alterations indicated the fish's response to detoxify the treatments from their metabolism and excretion [41]. The mechanism of the liver impairment of the venules' congestion, regardless of the extent, was said by [42] to indicate the association between the introduced extract and hepatic structures and functions, the extract's harmful impacts on the liver histology, and exposure period and the extract's phytochemicals.

The gill histology indicated gradually increased impairments as the concentrations of the P. pinnata leaf aqueous extracts increased (Plates 11 to 15): evidence of the *C. gariepinus* gill responses and defence of the fish against the increasing concentrations of the *P. pinnata* leaf aqueous extract, whose phytochemicals diffused into the bloodstream [43]. The phytochemicals tend to deplete the oxygen supply to the gill filament, causing gill cellular degeneration [44], intercepting functions of vital operations (respiratory chain): resulting in dysfunctions of osmotic balance and enzymes' active centres [45,46]. The moderate impact of varied *P. pinnata* leaf aqueous extract in this study was due to the used solvent (aqueous, water); this can be deduced from the previous studies [47,48,49]. Unlike water alone, the gathered observations combined with organic solvents efficiently and mostly wriggle, exhume, adsorb and infuse antioxidant products and phytochemicals out of the trial plants. In addition, the potential of solvent hazards follows the order ethanol > water > others (organic solvents like acetone, acetonitrile, isopropanol and methanol with water) for human consumption. In the above order, the solvents influence the efficiency of antioxidant extraction of most photoproducts and chemicals [50].

5.0 Conclusion

The study observed that the varied concentrations of *P. pinnata* leaf aqueous extract had effects on the fish's haematology and histopathology (skin, gill, and liver) compared with the main experiment control fish over 96 h. Mortality did not occur during the 96 h (acute toxicity test) until the seventh day (sub-lethal toxicity test). The probit analysis determined LD_{50} of *P. pinnata* leaf aqueous extract after 96 h at 4.80 g/ mL. The research indicated that the introduction of *Paullina pinnata* leaves into ponds or water bodies might threaten the fish and their habitat over time with damaging effects. Thus, caution must be taken when using and disposing of the plant in the ponds and water bodies as chronic (long-term) effects are possible on the fish.

Declaration

Ethical Approval

The study obtained its certificate of ethical approval from the College of Veterinary Medicine Research Ethics Committee with reference number FUNAAB/COLVET/CREC/2023/04/06.

Consent for publication

Not applicable

Availability of data

All data generated during this study are included in this manuscript

Conflict of Interest

The authors declare that they have no conflict of interest

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No funding was received for this research

Authors' contribution
AA and AT developed the original idea and experimental design; AT and AE drafted and analyzed the data and wrote the manuscript. All the authors provided manuscripts correction, edited and revised critically the manuscript for important intellectual and final approval of the version to be published. All authors approved the final draft of the manuscript

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