Toxicity Of Monotrim to Clarias Gariepinus Juveniles (Burchell, 1822): A Named Antibiotic

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ABSTRACT

The study is aimed at assessing the toxic effects of trimethoprim on juvenile sharptooth catfish (*Clarias gariepinus*). One hundred and eighty (180) juvenile catfish with length of 9 to 10cm were exposed to trimethoprim concentrations grouped into to six treatments category of 0.00 mg-L (control), 50 mgl, 100 mgl, 150 mgl, 200 mgl, and 250 mgl having two replicates each for a short acute toxicity test of 96 hour period. Ten *Clarias gariepinus* were placed in a rectangular aquaria for 21 days acclimatization period, trimethoprim was introduced into the fish holding tank prior to start of experimental observation, the juveniles measuring about the size of the human finger were however fed with 30% crude protein diet. Test specimens subjected to concentrations of 150 mgl, 200 mgl, and 250 mgl of trimethoprim revealed a predictable unperceptive, aggressive and inconsistent behavioral movements. At concentration of 0.00mgl referred to as the control, 50, and 100mgl no mortality was recorded, while mortality rate was at 25, 50 and 75% for samples exposed to trimethoprim concentration of ≥150mgl of trimethoprim. Fish mortality was dependent on antibiotic concentration as well the effect of exposure-time. The lethal concentration (LC_{50}) of this short acute toxicity test at 95% confidence limit was 165 ± 0.046mgl, indicating low toxicity. A dose-dependent relationship was reflected in the examined gills tissue. This paper revealed that antibiotics are harmful to aquatic resources particularly fish which form daily meal for man, hence, pose health challenges.

KEYWORDS: Exposure, Trimethoprim, Antibiotic, Lethal, Pharmaceutical, Catfish, Effect.

INTRODUCTION

Resistance to antibiotic is a universal issue of public health likened to antimicrobial application in food animals (Adelowo and Okunlola, 2019)). Little is reported about antibiotic application in farm fish in less developed nations that is associated to antibiotic resistance (Adelowo and Okunlola, 2019)). Antibiotics is regularly given as dose feed which serves as growth advocates, treatment of diseases, and as preventive standards in avoiding opportunistic microbe during the farming or transfer of fish (Au-Yeung *et al*, 2022). Antibiotics is regarded as the main approach for limiting huge losses caused brought about by bacterial infection (Cabello, *et al*, 2013). The relatively towering antibiotic intake by man and animals (Boyles *et al.*, 2017) produced a resultant let out of incomplete antibiotic metabolite that find their way into the pond, streams, lakes and rivers via sewage (Fick *et al*., 2015). Several researchers have indicated that consumed antimicrobic substances are faultily sucked up by fish then in the course of time excreted with their metabolites in excrement into the aquatic surrounding having still the antimicrobial activity undamaged (Iwu, *et*

al, 2020). After administration, antibiotics is capable of being conveyed through various route (Nguyen, *et al*, 2015). Sorption has been considered as the major process governing the mobility and transport of antibiotics in environment (Thiele, 2003). The decadence of antibiotic half-life may differ greatly, conditional on the chemical substance, biodegradation route and surrounding media. Trimethoprim is known to be most persistent in water systems thereby causing long term chronic effects to humans and fish (Nguyen, *et al*, 2015).

A large number of data on antibiotic decadence have it origin from oxygen rich surroundings or from laboratory findings that may well not or at most partly be interpreted to real field situations of fish farm culture approach (Nepejchalova *et al*, 2008) having differing water and air temperature, dilution by rainfall, appearance of other microorganisms, and limited oxygen supply (Nguyen, *et al*, 2015). Antibiotics and antibacterial, such as trimethoprim's, are constituent of the veterinary apparatus utilize for the determent and treatment of diseases in fish, though, the buildup of veterinary medicament remnant in fish could raise concerns in food safety (Songhee *et al*, 2022), Man and agricultural application of antibiotics have been mentioned as a relevant donor for the advancement and expansion of antibiotic resistance in deleterious and symbiotic strains of bacteria (Adelowo and Okunlola, 2019).

A large uncounted number of antibiotics is presently being applied in aquaculture and animal food production to boost their improvement, growth and avert pathogens than it being used among human population (Adelowo and Okunlola, 2019). Among the veterinary group which have antibiotics heavily utilized, specific consciousness is to be given to fish practices in ponds, lakes, rivers etc due to the direct influence they have on the aquatic habitat (Cabello *et al*, 2013). Findings from previous toxicity study have revealed great and high concentrations of pharmaceutical substances that end up in the water environment particularly from developing countries as a result of unmonitored released with incident repercussion for the for human health as end consumers of the sea food.

Nigeria has a weakly enforced law guiding the regulation and application of on-the-counter chemical substances by man, for veterinary application or to boost agricultural yields, hence, this applied and used chemical substance end up in the marine environment causing a build-up in biologic tissues Adelowo and Okunlola, 2019).. Most toxicity test experiment focuses on the chemical effect of sulfamethoxazole, flouroquinolones, Ormetoprim, dapsone in fish, however, this present paper aim to evaluate the toxic potentials and it effect on the human finger size catfish exposed to an antifolate antibiotic (trimethoprim).

MATERIALS AND METHODS

Experimental substance

Trimethoprim was purchased on-the-counter from a pharmacy in Calabar, Cross River State, Nigeria.

Test subject

Juvenile African sharptooth catfish (180) was obtained from a private fish farm situated in the city of Calabar, Cross River

State, Nigeria with the aid of a net for scooping the fish in the prompt hours of the morning to prevent unfavourable temperature and stress incidence.

Acclimation condition

The test subjects were placed in a laboratory settings preceding the start of the experimental procedure. The test specimens were spaced in to groups of ten unsystematically chosen individuals, preserved in transparent fish holding tank of diameter 30 x 30 x 50cm filled with water to a measured depth of about 9cm and left for a period of two weeks (14 days). Six experimental groups of treatment categories was design, with two replicates to a group. Dissolved oxygen was provided to the experimental design by way of aerators, and the young catfish were fed with 30% crude diet of protein twice daily for optimal feeding gauge, left overs of food pellets were taken out to avoid polluting the fish tank.

Preparation of stock solution

Trimethoprim is slightly soluble in water at 0.4mg/ml, in preparing the solution, tablets of trimethoprim was grinded and dissolved in 180ml of ethanol (100%), 180mls ethanol was then added to 270mls water bottle and trimethoprim was added to the 100% ethanol. A magnetic stirrer was placed into the solution and the water bottle inserted on the stirrer which was programmed at ~250-500rpm until the trimethoprim powder dissolved.

Range finding test

Range test was conducted by randomly taking ten test subject after habituation phase, placing them into the fish holding tanks (12) containing experimental solvent at different trimethoprim concentrations of 0, 150, 300, 600, 1200, 1500mg/l. Juvenile *Clarias gariepinus* were left in the test media without food for a 4 days (96H) period for careful observation of behavioral changes and mortality (Reish and Oshida, 1987). Range test showed concentrations >150mg/l to caused mortality to fish, while subject in the control category recorded no mortality, suggesting that lethal concentration (LC_{50}) is expected to be < 150mg/l to be considered safe for fish. Twenty samples were randomly chosen and placed in twelve rectangular aquaria having two replicate per treatment group to assess the 96H lethal concentration (LC_{50}) test following the end of the range finding test, test subject were deprived feed for 24hours before and during exposure (Reish and Oshida, 1987). The solvent concentrations of the treatments were 0mg/L, 100mg/L, 120mg/L, 140mg/L, 160mg/L and 180mg/L of Trimethoprim and subsequently used to determine the 96 hours LC_{50}. Observed behavioural responses such as aggressive, uncoordinated, sluggish movement, foaming on the surface of the water and fish mortality was evident and recorded at each concentration 50, 100, 150, 200 and 250mgl for the 96H test, however, as a means of preventing contamination to the experiment fishes which were observed and assessed to be death were removed.

Histological process

Organs of gills were extracted from samples drawn from each treatment group and maintained in 10% formal saline, 50% and 90% graded alcohol used for tissue dehydration for fifteen minutes, afterwards, cleared by submersion in three exchanges of xylene (xylene is preferred due to its short-term efficacy in clearing small tissue blocks) (Tayeb *et al.,* 2010), this was

followed immediately by impregnation in xylene paraffin wax placed at a temperature of 55°C for ~60 minutes. Tissue blocks were sectioned at 5µm thickness using a microtome. The tissue sections were remoisten in deionised water and the tissues stained with Hematoxylin-Eosin (H-E) (Tayeb *et al., (*2010), afterwards, examined under a light microscope.

Determination of physico-chemical parameters of test media

Recording of the physico-chemical parameters for Dissolved Oxygen, Temperature, pH, Conductivity and Turbidity of the experimental medium was established applying standard methods, recording was carried out in the morning and evening after proper calibration of the instrument for the 96H period American Public Health Association (APHA) (2005).

Length-weight measurement

The length-weight measurement of the *Clarias gariepinus* were measured using a backlit LCD display 110lb/50kg fishing scale and meter ruler to nearest grams and centimeters respectively.

Statistical analysis

Mortality data based on the response of juvenile *Clarias gariepinus* to different trimethoprim concentration was subjected to probit regression analysis to obtain the mean LC_{50}. The software Statistical Programme for Social Science version 15 was used to test differences between treatment categories. The microscopic investigation was subjective by viewing the gill and liver histological micrograph (Suhendrayatna *et al*, 2019).

RESULTS

Observed behavioral modification

Behavioral shifts from normal conditions noticed in the experiment is presented in table 1.

At elevated trimethoprim concentrations of 150mg/l to 250mg/l, test subject began displaying a characteristic slow, uncoordinated and aggressive movement liken to be caused by influence of the substance, however, spumes were created on the surface of the water at increased concentrations, and the percentage rate of mortality in each concentration gradient is presented in table 2. Samples in the control categories remain unaffected by any chemical actions, thereby, recorded no death (table 1).

Probit transformation

The percentage mortality – concentration relationship for *Clarias gariepinus* juveniles is shown in table 4 and illustrated in Figure 1. Mortality data of African sharp-tooth catfish (*Clarias gariepinus*) subjected to trimethoprim concentrations is presented in table 4. Probit regression result conducted on juvenile *Clarias gariepinus* showed mortality data of trimethoprim concentrations with positive and significant ($p<0.05$) coefficient of determination (r^2) = 0.74 among fish mortality and trimethoprim concentration (Table 3). The lethal concentration (LC_{50}) of trimethoprim on juvenile *Clarias gariepinus* at the 95% confidence interval was 170 mg/l ± 0.048 (Figure 1). The log transformation of trimethoprim concentration and mortality data obtained from probit transformation using the formula:

$$y = ax + b$$

Where; y = Expected percentage of *Clarias gariepinus* mortality, a = slope, x =

Trimethoprim concentration in mg/l, b = Intercept

Table 1: Noticeable Behaviour of juvenile's catfish subjected to different trimethoprim concentrations

Concentrations (mg/L)	Exposure duration (Hour)	Behaviour
0 (control)	24	Normal fish movement
	48	Normal fish movement
	72	Normal fish movement
	96	Normal fish movement
50	24	Normal fish movement
	48	Normal fish movement
	72	Normal fish movement
	96	Normal fish movement
100	24	Aggressive fish movement
	48	Aggressive fish movement
	72	Aggressive fish movement
	96	Sluggish fish movement
150	24	Uncoordinated fish movement
	48	Uncoordinated fish movement/surface water foaming
	72	Uncoordinated fish movement/surface water foaming
	96	Sluggish movement occasion by increased surface water foam
200	24	Uncoordinated fish movement of fish
	48	Uncoordinated fish movement/surface water spume
	72	Uncoordinated fish movement/surface water spume
	96	Uncoordinated fish movement/surface water spume
250	24	Uncoordinated fish movement
	48	Sluggish fish movement
	72	Sluggish movement of fish/increase surface water spume
	96	Fish death

Table 2: Percentage *Clarias gariepinus* survivability and mortality subjected to trimethoprim concentration level

Concentration (mg/L)	Mortality	% Mortality	Survival	% Survival
0	0	0	20	100
50	0	0	20	100
100	0	0	20	100
150	3	30	17	70
200	10	50	10	50
250	18	90	2	10

Table 3: Log Concentration–probit relationship of juvenile *Clarias gariepinus* subjected to trimethoprim concentration level

Conc. (Log Unit)	Response rate, p	Equation	determination Co-efficient, r^2	Significant level, α ($p<0.005$)
0.00	0.00			
2.000	0.00			
2.079	0.00	y = 0.42x - 20.80	0.75	0.05
2.146	0.30			
2.204	0.50			
2.255	0.90			

Table 4: Probit Transformation of *Clarias gariepinus* mortality data subjected to trimethoprim concentrations levels

Conc (mg/l)	Log Conc (x)	N	r (r/n)	P	M_R	Y	R_P	*P*
0	0.000	20	0	0.00	0	0.00	0.00	0.00
50	2.000	20	0	0.00	0	0.003	-0.003	0.00
100	2.080	20	0	0.00	0	0.214	-0.214	0.020
150	2.148	20	3	0.30	30	2.086	0.916	0.207
200	2.206	20	10	0.50	50	5.946	-0.946	0.593
250	2.258	20	18	0.90	90	8.783	0.219	0.877

N= Number of exposed fish, r = Number of responding fish, p = Rate of Response (r/n), MR = Rate of Mortality, Y = Expected probit regression result, RP = Residual probit, *P* = Probability

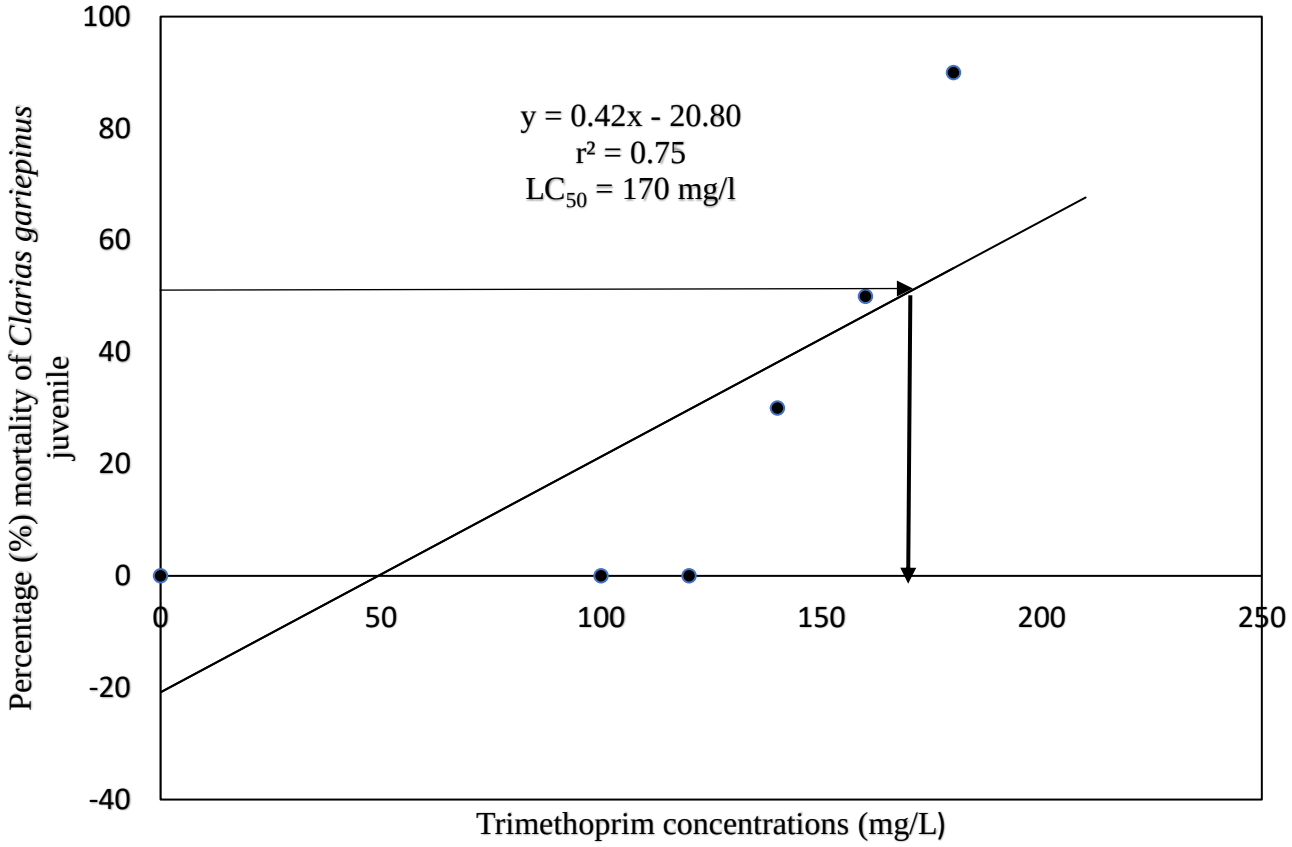


Fig 1: Probit graph of juvenile *Clarias gariepinus* subjected to trimethoprim concentration

Table 5: LC_{50} with 95% confidence limits of juvenile *Clarias gariepinus* juvenile subjected to trimethoprim concentrations

LC_{50} ± 95%CL	**Confidence limits**	
	Lower	Upper
0.074g ± 0.051	8.545	27.691

Physico-chemical parameters

The mean physico-chemical properties of the investigational design is presented in table 6. Concentration value of 250 mg/l showed significant ($p<0.05$) with peak value of 8.10 for pH in comparison with the control after exposure period of 72 H. The 200 mg/l concentration category of trimethoprim for pH revealed no significant difference value of 6.95 ($p>0.05$) in the 48 H exposure phase, the pH values at all concentration of trimethoprim were not significant ($P>0.05$). Table 6 showed the level of significance for all the other study metric at the various concentration levels in comparison with the world health organization standards (WHO).

Table 6: Mean physico-chemical parameters of experimental design at 96-hour exposure of *Clarias gariepinus* to trimethoprim

Physico-chemical parameters		Trimethoprim concentration						
	Exposure Time(H)	**Control**	**50 mg/l**	**100 mg/l**	**150 mg/l**	**200 mg/l**	**250 mg/l**	**WHO standard**
pH	24	6.59 ± 0.45^{a}	5.59 ± 0.05^{a}	6.14 ± 0.20^{b}	5.87 ± 0.12^{a}	6.24 ± 0.20^{b}	6.22 ± 0.19^{b}	6.5 – 8.5
	48	6.53 ± 0.53^{a}	5.92 ± 0.07^{b}	6.50 ± 0.13^{a}	6.55 ± 0.10^{a}	6.95 ± 0.06^{a}	6.40 ± 0.08^{a}	
	72	7.05 ± 0.03^{a}	6.59 ± 0.54^{b}	6.32 ± 0.31^{b}	6.32 ± 0.34^{b}	6.35 ± 0.05^{a}	8.10 ± 0.03^{b}	
	96	7.31 ± 0.07^{a}	6.24 ± 0.02^{b}	7.23 ± 0.24^{a}	6.75 ± 0.12^{b}	7.46 ± 0.13^{b}	5.88 ± 0.08^{b}	
DO (mg/l)	24	5.05 ± 0.43^{a}	4.89 ± 0.36^{a}	3.89 ± 0.59^{b}	4.63 ± 0.18^{a}	3.32 ± 0.07^{b}	3.70 ± 0.52^{b}	>5
	48	5.54 ± 0.19^{a}	3.70 ± 0.14^{a}	3.49 ± 0.21^{a}	3.57 ± 0.010^{a}	3.29 ± 0.30^{a}	3.49 ± 0.20^{a}	
	72	5.67 ± 0.10^{a}	3.51 ± 0.12^{b}	3.39 ± 0.11^{b}	3.40 ± 0.07^{b}	3.45 ± 0.15^{b}	3.53 ± 0.03^{b}	
	96	5.03 ± 0.47^{a}	3.48 ± 0.08^{b}	3.49 ± 0.09^{b}	3.38 ± 0.19^{b}	3.49 ± 0.19^{b}	3.35 ± 0.14^{b}	
Conductivity	24	195.00 ± 13.75^{a}	227.40 ± 9.29^{a}	287.01 ± 15.45^{b}	288.20 ± 20.00^{b}	307.65 ± 29.03^{b}	415.75 ± 55.30^{b}	250
	48	263.00 ± 5.04^{a}	283.35 ± 8.38^{a}	337.12 ± 5.34^{b}	405.01 ± 21.50^{b}	442.78 ± 31.5^{b}	655.70 ± 31.66^{b}	
	72	294.23 ± 2.05^{a}	349.60 ± 39.65^{a}	380.25 ± 3.30^{a}	460.10 ± 75.06^{b}	503.73 ± 64.80^{b}	454.77 ± 155.48^{b}	
	96	307.79 ± 0.98^{a}	362.85 ± 11.54^{b}	429.77 ± 13.57^{b}	523.26 ± 60.08^{b}	609.50 ± 15.90^{b}	899.01 ± 19.59^{b}	
Temperature (°C)	24	27.09 ± 0.29^{a}	24.95 ± 0.10^{b}	24.96 ± 0.18^{b}	25.97 ± 0.08^{b}	27.00 ± 0.03^{a}	25.89 ± 0.06^{a}	20 - 32
	48	26.53 ± 0.30^{a}	24.85 ± 0.25a	24.52 ± 0.25^{a}	26.98 ± 0.64^{b}	26.01 ± 0.11^{b}	26.00 ± 0.13^{a}	
	72	26.64 ± 0.21^{a}	24.90 ± 0.08^{a}	24.63 ± 0.23^{a}	25.59 ± 0.41^{a}	25.90 ± 0.50^{a}	25.62 ± 0.44^{a}	
	96	26.01 ± 0.45^{a}	24.79 ± 0.34^{a}	24.74 ± 0.33^{a}	25.70 ± 0.45^{a}	25.00 ± 0.40^{a}	25.78 ± 0.39^{a}	
	24	0.00 ± 0.00^{a}	39.08 ± 0.37^{b}	42.73 ± 0.27^{b}	63.82 ± 0.41^{b}	99.20 ± 0.13^{b}	103.`10 ± 0.80^{b}	

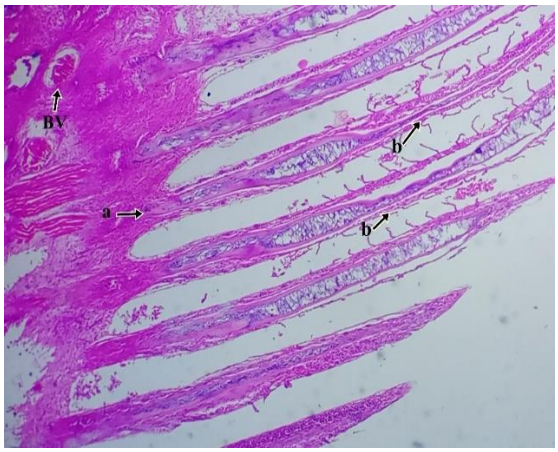
Turbidity (NTU)	48	0.00 ± 0.00^{a}	38.00 ± 0.07^{b}	45.05 ± 0.08^{b}	63.64 ± 0.35^{b}	101.89 ± 0.13^{b}	108.76 ± 0.45^{b}	5
	72	0.00 ± 0.00^{a}	48.17 ± 0.65^{b}	56.73 ± 0.74^{b}	85.63 ± 0.39^{b}	132.65 ± 1.60^{b}	170.91 ± 6.60^{b}	
	96	0.00 ± 0.00^{a}	55.25 ± 0.20^{b}	65.91 ± 0.67^{b}	113.00 ± 1.39^{b}	167.74 ± 0.59^{b}	189.20 ± 1.04^{b}	

Values are presented in mean ± S.D; values bearing different superscript for each concentration category when compared to the control group shows significant different ($p<0.05$)

Histopathology examination

Microscopic assessment of the juvenile *Clarias gariepinus* gills taken from the control treatment group showed an unaltered gill structure, the radiating primary lamellae and bony arch were broad with scantly populated cells. The presence of a short and scanty secondary lamellae with the lining of the surface epithelium still intact shown in Plate 1. The *Clarias gariepinus* gills from concentration of 150mg/L showed a primary lamellae with a spread/thin out central core and a secondary lamellae that is scanty and degenerated. Noticeable crowded blood vessels were observed at the base (Plate 2). The histologic examination of the gills of young *Clarias gariepinus* from the 200mg/L concentrations which killed 50% sample, revealed gills with a declined primary and secondary lamellae. The primary lamellae showed an enlarged paucicellular core with calcification, the surface epithelial lining showed disintegration as shown Plate 3.

X100



X400

PLATE 1: *Clarias gariepinus* gill administered with 0.00mg/l.

a = Bony arch with radiating lamellae consist mainly of the primary lamellae that are broad with sparsely populated cells.

b = Short and scanty secondary lamellae with the lining surface epithelium intact.

X100

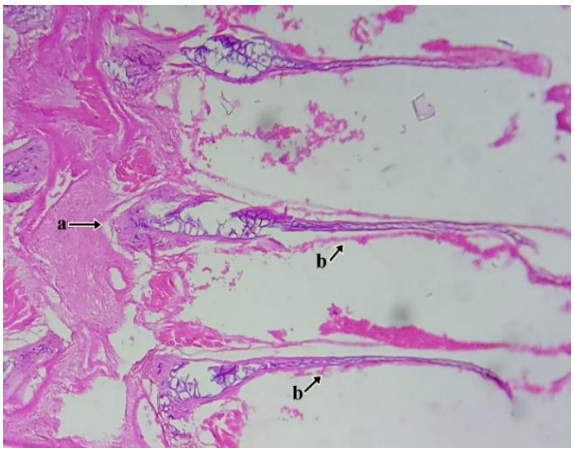


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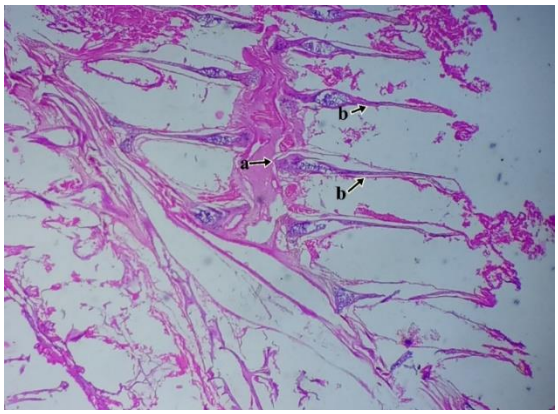
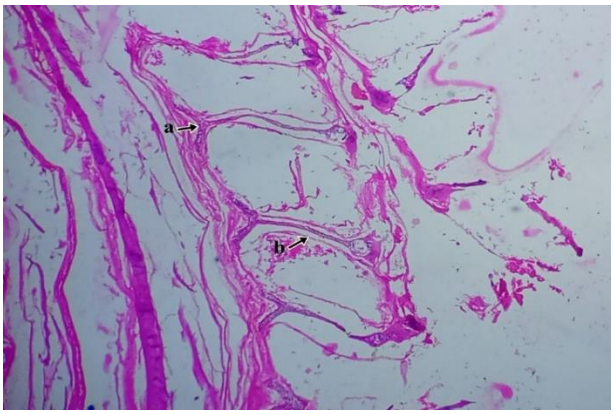
PLATE 2: Gill of *Clarias gariepinus* administered with 150mg/L concentration

a = Primary lamellae showing a spread/thin out central core.

b = Scanty and degenerated secondary lamellae with congested blood vessels noted at the base





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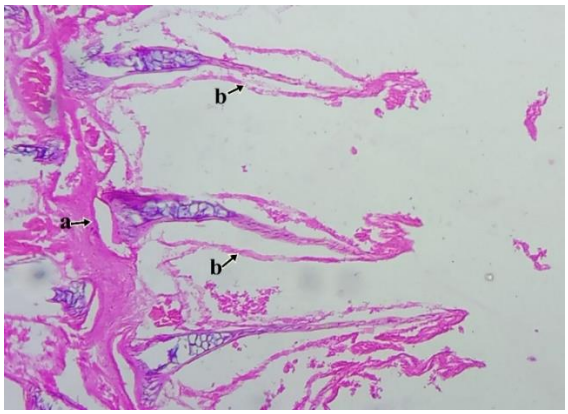

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PLATE 3: Gill of *Clarias gariepinus* administered with 200mg/L.

a = Degenerated primary lamellae displaying an expanded paucicellular core with calcification and eroded lining of surface epithelium.

b = Degenerated secondary lamellae

DISCUSSION

Pharmaceuticals such as trimethoprim cause both lethal and sub-lethal effects to fishes, the findings of this paper revealed that trimethoprim possesses toxic and deleterious potentials on the juveniles of *Clarias gariepinus* which is known from literatures to exhibit a characteristic resistance to pollutants. Noticeable biological behavioral responses to the test chemical included: violent, erratic and slow movement, as well as spume production on the water surface at concentration of ≥150 mg/L. The findings of this paper bears similitude with results of (Carlsson *et al*., 2006; Li and Lin, 2015). Chemical substances of pharmaceutical origination at increased doses have been generally detail to produce physiological, neurological, behavioral, as well as mutagenic disorders in fish and biological elements (Vethaak *et al.,* 2002), such as blocking of nervous impulse and reduction in dissolved oxygen resulting in the sluggish, aggressive and uncoordinated brownian fish movement, in very high doses above tolerable limit could result in mortality as evident in this paper. The concentration threshold of 0.00mg/l earmarked as control category recorded no mortality, which is not the case in categories with elevated doses of trimethoprim (Suhendrayatna *et* al, 2019). This paper provided a valid confirmation of the report of Sogbesan and Aji (2017) on the strength for pharmaceutical substance to disrupt the normal physiological conditions of fishes, thereby causing to death. The LC_{50} value of trimethoprim on the treatment categories of juvenile *Clarias gariepinus* in this paper is higher than that reported by Li and Lin, 2015.

Suhendrayatna *et al*, 2019 findings revealed the toxicity (LC_{50}) of fish exposed to mercury (HgII) concentration to 0.1435mg-Hg (II)/L with observed pathological damages to the internal organs which included pale gills, anemic eyes and whitish body coloration in consonance with this current study. Jùrg 2013 described the dominant pharmaceutical elements as suspects in inducing accidental negative result in ecological units, due to their increased biological role as can be seen in this present paper at high trimethoprim doses. LC_{50} value reported in this paper is lower than from (Carlsson *et al*., 2006). The differences in LC_{50} values in this study to that of other published literatures could be attributed to differences in the applied chemical substance and inherent toxicity. There's still the likelihood of chronic consequences on the sample even at low toxicity ensuing into a physiologic inequality as trimethoprim break down uniquely at a slow pace under innate stellar lighting, roughly 10% in 500 min in demineralized water (Wu, *et al*., 2011). Correlation coefficient (r^2) of 0.75 obtained

during the short acute toxicity test for log-concentration and probit relationship of *Clarias gariepinus* subjected to various trimethoprim concentrations, suggest a linear relationships that is sturdy and positive between trimethoprim concentration and fish mortality (Au-Yeung, *et al*, 2022), hence, mortality of juvenile *Clarias gariepinus* is concluded to be concentration and time-dependent.

The histological examination of gill organ subjected to trimethoprim concentrations ≥150mg/l revealed an adverse level of tissue decline similar to Suhendrayatna *et al*, 2019. This adverse effect arising from concentration of trimethoprim caused impairment in gaseous exchange efficiency of the gills to death, corroborating with the findings of Omitoyin et al., (2006). Pharmaceutical substance are capable of inducing fluctuations in the physico-chemical properties of the experimental design as can be observed in this paper with similar findings reported in Santos *et al.* (2010).

CONCLUSION

The issue of environmental (aquatic and terrestrial) is becoming increasing rampant particularly underdeveloped and developing countries, the findings from this paper will form useful reference and baseline tool in guiding he decisions of policy makers in recognizing the fact that our ecosystem is under great pressure from hundreds to thousands of chemical substance particularly on-the-counter pharmaceutical product that find its way into the environment through improper intake and discard of the substance.

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