TOXICITY PROFILE AND BEHAVIOURAL CHANGES IN AFRICAN CATFISH *CLARIAS GARIEPINUS* FOLLOWING SHORT-TERM EXPOSURE TO IVERMECTIN

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ABSTRACT

Ivermectin is one of human and veterinary medicine's most widely used antiparasitic drugs. Reports of low tolerance margins have accompanied its use in aquaculture. This study assessed the behavioural changes in African catfish - Clarias gariepinus exposed to ivermectin. Further, acute toxicity, no effect concentration, least effect concentration, and safe level of ivermectin were determined. Mortality was recorded for all the ivermectin concentrations used; this ranged from 13% to 100% for concentrations between 1.6 µgl^{-1} and 24.3 µgl^{-1}. There were changes in behavioural profiles of the fish especially at higher concentrations of the drug. The LC_{50} values at 24, 48, 72 and 96 hours were 46.58 (22.45 – 450.81), 38.71 (17.69 – 648.27), 12.38 (8.19 – 24.50) and 6.53 (2.86 – 17.43) µgl^{-1} respectively. The 96-hour estimated safe level of ivermectin based on the NAS/NAE method is 6.53 x 10^{-1} – 6.53 x 10^{-5}. Ivermectin was able to cause 100% mortality in catfish at 24.3 µgl^{-1}. The low margin of safety and low toxic unit of 0.07 of the drugs make it unsuitable for use in aquaculture for the control of parasites.

Keywords: Antiparasitic drug, Toxicity, Behaviour, Catfish, Safety margin

INTRODUCTION

Ivermectin is used for the treatment of multiple livestock and human parasitic diseases. In the aquaculture industry ivermectin is used for the treatment of several ectoparasite species. It is one of the antiparasitic agents for fish. Though its use in aquaculture is off-label, it has never been licensed for use in the control of fish parasites (Horsberg, 2012). Presently, ivermectin is still being explored as an alternative anthelminthic drug against some fish parasites. Despite the wide utility of ivermectin in non-aquatic environments, it has not been widely utilized in the aquaculture industry. Studies that have tested the efficacy of ivermectin as a fish ectoparasites control agent have largely reported a low margin of safety for examined fish (Palmer *et al.*, 1987; Collymore *et al.*, 2014; Orobets *et al.*, 2019; Davies and Rodger, 2000). Except for the low margin of safety, the drug was effective against the ectoparasites.

Ivermectin have been used in aquaculture in several parts of the world such as Ireland (Palmer *et al.*, 1987), Scotland (Roth *et al.*, 1993; Davies and Rodger, 2000) and Canada

(Hamoutene *et al.*, 2022). In these countries, they have been used for the control of ectoparasitic copepods. Ivermectin efficacy in the treatment of nematode infection in zebrafish has also been reported (Collymore *et al.*, 2014). In the fish industry, various ivermectin-induced toxicity-related mortality has been reported (Thiripurasundari *et al.*, 2014). The African catfish, *C. gariepinus* is an important economic fish in Nigeria and Africa. It grows very fast, is widely distributed, can withstand stress and commands high market price due to its good taste (Awe, 2017).

Despite ivermectin occupying a premium position as a choice drug for the treatment of multiple livestock and human parasitic diseases in Nigeria, more studies are needed regarding the toxicity of ivermectin in some indigenous fish species such as *C. gariepinus*. It has been severally stated that fish have a low range of tolerance to ivermectin (Bai and Ogbourne, 2016; Ezenwaji *et al.*, 2017; Orobets *et al.*, 2019). This makes it very important to assess the toxicity profile of ivermectin in different fish species. Such studies could facilitate incorporating it into the treatment regimen in fish culture. Where the drug is used without prior consideration of its toxicity profile, enormous economic loss may be sustained. And where the fish can tolerate the drug, its adoption could greatly improve the fish culture. The present study, therefore, sorts to assess the toxicity profile and behavioural changes in *C. gariepinus* following exposure to various concentrations of ivermectin.

MATERIALS AND METHODS

Ethics: The ethical approval to conduct this study, and the clearance on the use of experimental animals was obtained from the Faculty of Biological Science, University of Nigeria Ethical Committee (UNN-FBS-022-00220) and followed strictly.

Experimental Animal and Chemicals: A total of 300 healthy fish specimens of juvenile *C. gariepinus* (mean weight and length 23.06 ± 1.05 g and 12.50 ± 0.45 cm respectively) were procured from Freedom Fisheries Limited along University Market Road, Nsukka, Enugu State, Nigeria. They were transported in well-aerated containers to the Fisheries Wet Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria. The fish were treated with 0.05% potassium permanganate ($KMnO_4$) for 2 minutes to avoid any dermal infections. They were then acclimatized for three weeks in plastic tanks of 300 litres capacity. They were fed 5% of their body weight *ad libitum* daily with 2 mm Coppens (50% crude protein, 19.5 MJ/kg digestible energy) (AllTechCoppens, 2022). Faecal matter, uneaten feed and other waste materials were siphoned off daily; this helps to maintain hygienic conditions and helps prevent contamination of the water by food and faeces. Also, dead fish were removed with plastic forceps to avoid deterioration and contamination of water. During this period of acclimatization, the water in the tank was renewed with well-aerated tap water once weekly. Feeding was terminated 24 hours before range finding and acute toxicity tests to avoid interference of faeces. The acclimatized fish was used for the experiment. Mectizan (containing 3 g of ivermectin as the active ingredient) manufactured and distributed by Merck and Company Incorporated, New Jersey, USA was used for the study.

Acute Toxicity Test: Determination of lethal concentration of ivermectin was conducted using a total of 250 catfish specimens. Triplicates sets of 10 randomly selected fish each in 10 l of tap water were exposed to ivermectin at six different concentrations (0.0, 1.6, 3.1, 6.1, 12.3 and 24.3 μgl^{-1}) that were obtained after range finding test as recommended by Krishna and Hayashi (2000). Another set of 10 fish was maintained simultaneously in an equal amount of tap water but without the test chemical; this served as a control. Feed was not provided to the fish throughout the test and lethality was the toxicity endpoint. Dead fish were removed and mortality was recorded at intervals of 24, 48, 72 and 96 hours. The LC_{50} of the test chemical for the species at 24, 48, 72 and 96 hours was determined using Probit analysis (Finney, 1971). The behavioural response (equilibrium status, opercula movement, jumping, erratic muscle movement, swimming rate) of *C. gariepinus*

during this toxicity test was observed and recorded at 24, 48, 72 and 96 hours of exposure.

Determination of Lowest Observed Effect and No Observed Effect Concentrations: The lowest observed effect concentration (LOEC) was determined using acute toxicity results. The LOEC was the highest concentration and low mortality was observed. The no-observed effect concentration (NOEC) was determined using acute toxicity results. It was determined as the highest concentration in which there was no mortality observed.

Determination of Toxic Unit: The toxic unit was determined according to the method of USEPA (2000) as: Toxic unit (Active) = $100/LC_{50}$.

Determination of Safe Level: The safe level of the test chemicals was determined by multiplying 96-hour LC_{50} with different application factors. This was based on the methods of Hart *et al.* (1984), Sprague (1969), CWQC (1972), NAS/NAE (1973), IJC (1977) and CCREM (1991).

Statistical Analysis: Data was analysed using Statistical Packages for Social Sciences (SPSS) Version 20.0 (IBM Corporation, Armonk, USA). Estimates for lethal concentrations (LC) were done using Probit regression analysis. The level of significance was set at $p<0.05$.

RESULTS

Acute Toxicity of Ivermectin: The total mortality during the acute mortality tests was summarized in Table 1 and the lethal concentration of the fish was presented in Table 2. For the 96-hour exposure duration, there was no mortality recorded in the control. Mortality was recorded for all the ivermectin concentrations used; this ranged from 13 to 100% for concentrations between 1.6 µg/L and 24.3 µg/L (Table 1). The LC_{50} values (95% confidence interval) at 24, 48, 72 and 96 hours were 46.58 (22.45 – 450.81), 38.71 (17.69 – 648.27), 12.38 (8.19 – 24.50) and 6.53 (2.86 – 17.43) respectively (Table 2). The range of concentration of ivermectin required to cause 90% mortality of *C. gariepinus* at 96 hours exposure was within the range of concentration as that for 50% at the same exposure interval. The NOEC of ivermectin on *C. gariepinus* at 24, 48, 72 and 96 hours were between 2 – 13, 0 – 9, 2 – 7 and 4 – 5 µg/L respectively (Figure 1). The LOEC for the 24, 48, 72 and 96 hours were also presented in Figure 1.

Safe Levels: The estimated safe level of ivermectin after 96-hour exposure to *C. gariepinus* was presented in Table 3. The estimate based on the NAS/NAE (1973) method was $6.53 \times 10^{-1} – 6.53 \times 10^{-5}$. According to Hart *et al.* (1948), the safe level was 8.0×10^{-1} while it was 6.53×10^{-1} following Sprague (1969) and was 6.53×10^{-2} according to CWQC (1972). Following the method of CCREM (1991), the safe level was calculated to be 3.3×10^{-1} but 3.3×10^{-1} according to IJC (1977). The toxic unit was calculated to be 0.07.

Behavioural Changes: The behavioural changes of the fish during the acute toxicity exposure were summarized in Table 4. At the initial exposure to the drug, the fish were alert, reduced swimming and remained static in response to the changes in the aquatic environment. At exposure to higher concentrations, the fish were observed to be agitated with jerking of the body, swam very fast and erratic and tried to jump out of the aquarium. As the time of the experiment progressed, the fish became exhausted and were observed to have lost energy and become weak. They later maintained a vertical position trying to gulp some air and later settled at the bottom of the aquaria. The fish with a high amount of mucus on their skin subsequently died with their bellies turned upside down.

DISCUSSION

The acute toxicity activity of ivermectin on *C. gariepinus* from this study indicated that the drug was toxic to the fish. This supports previous findings that ivermectin has a low margin of safety for fish (Palmer *et al.*, 1987; Athanassopoulou *et al.*, 2002) unlike in other vertebrates where it was well tolerated.

Table 1: Cumulative mortality of *Clarias gariepinus* juveniles exposed to various concentrations of ivermectin

Concentration (µg/L)	Number of fish exposed	Cumulative mortality				% survival	% mortality
		24 h	48 h	72 h	96 h		
Control	30	0	0	0	0	100	0
1.6	30	0	2	4	4	87	13
3.1	30	4	6	8	8	73	27
6.1	30	6	8	10	12	60	40
12.3	30	8	10	14	18	40	60
24.3	30	10	12	20	30	0	100

Table 2: Lethal concentrations of ivermectin (µg/L) for *Clarias gariepinus*

Lethal concentration	Exposure time			
	24 hours	48 hours	72 hours	96 hours
LC_{10}	3.44 (0.87 – 5.91)	1.43 (0.08 – 3.15)	1.09 (0.23 – 2.11)	1.68 (0.05 – 3.50)
LC_{20}	8.42 (4.57 – 15.16)	4.44 (1.30 – 7.91)	2.51 (0.96 – 4.02)	2.68 (0.23 – 5.03)
LC_{30}	16.05 (9.72 – 46.64)	10.05 (5.47 – 26.51)	4.58 (2.49 – 6.87)	3.74 (0.68 – 7.10)
LC_{40}	27.84 (15.39 – 146.70)	20.17 (10.86 – 127.82)	7.65 (4.96 – 12.33)	4.99 (1.55 – 10.32)
LC_{50}	46.58 (22.45 – 450.81)	38.71 (17.69 – 648.27)	12.38 (8.19 – 24.50)	6.53 (2.86 – 17.43)
LC_{60}	77.95 (32.10 – 1413.30)	74.27 (27.59 – 3435.01)	20.01 (12.41 – 53.10)	8.54 (4.41 – 35.14)
LC_{70}	135.24 (46.56 – 4850.65)	149.14 (43.55 – 20845.17)	33.47 (18.56 – 126.66)	11.38 (6.12 – 144.00)
LC_{80}	257.69 (71.42 – 20691.97)	337.26 (73.45 – 173976.27)	61.11 (29.04 – 358.62)	15.93 (8.27 – 262.61)
LC_{90}	630.11 (128.37 – 1598.85)	145.71 (150.12 – 3363.08)	140.82 (53.08 – 1545.91)	25.40 (11.67 – 1338.16)

Number in parenthesis = 95% confidence interval

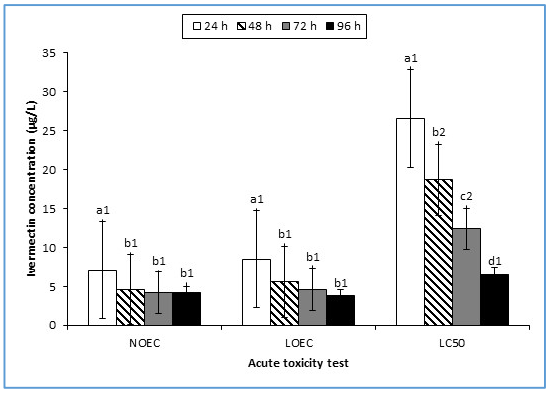

Figure 1: Acute toxicity testing statistical endpoints in *Clarias gariepinus* exposed to ivermectin at different durations

Several authors have noted the tendency of ivermectin to cause toxicity in fish of different species, such as zebrafish (Thiripurasundari *et al.*, 2014) and salmonids (Palmer *et al.*, 1987; Johnson *et al.*, 1993; Athanassopoulou *et al.*, 2002). Though off-license usage of ivermectin in aquaculture for the control of fish arthropod parasites persists (Horsberg, 2012), the low margin of safety negates the possibility of using it in aquaculture. From the present study, as low as 6.53 µg/L (2.86 – 17.43 μgl^{-1}) could cause 50% mortality of *C. gariepinus*. As low as 25.40 µg/L (11.67 – 1338.16 μgl^{-1}) caused 90% mortality of *C. gariepinus* under 96 hours of exposure periods. This poses a significant problem to its use. Thus, the use of ivermectin in *C. gariepinus* culture requires serious precaution.

The acute toxicity effect of ivermectin on *C. gariepinus* in the present study was high compared to other pharmaceuticals that have been tested on different fish species. This 96 hours LC_{50} of 6.53 μgl^{-1} (2.86 – 17.43 μgl^{-1}) for ivermectin was more potent than > 100 mgl^{-1} for

Table 3: Estimates of safe levels of ivermectin after 96 hours of exposure duration of *Clarias gariepinus*

Pesticide	96 h LC_{50} (µg/L)	Methods	AF	Safe level (µg/L)
Ivermectin	6.53	Hart *et al.* (1948) *	-	8.0×10^{-1}
		Sprague (1969)	0.1	6.53×10^{-1}
		CWQC (1972)	0.01	6.53×10^{-2}
		NAS/NAE (1973)	0.1 – 0.00001	6.53×10^{-1} – 6.53×10^{-5}
		CCREM (1991)	0.05	3.3×10^{-1}
		IJC (1977)	5 % LC_{50}	3.3×10^{-1}

**C = 48h LC_{50} x 0.03/S^2, where C = presumable harmless concentration and S = 24 h LC_{50}/48h LC_{50}*

Table 4: Behavioural changes in *Clarias gariepinus* exposed to different concentrations of ivermectin at 24, 48, 72 and 96 hours

Concentration (mg/L)	Equilibrium status	Opercula movement	Jumping	Erratic muscle movement	Swimming rate
24 hours					
Control	+++	+++	-	-	+++
1.6	+++	+++	-	-	+++
3.1	+++	+++	-	-	+++
6.1	++	++	+	+	++
12.3	+	+	+ +	+	++
24.3	+	+	+ +	++	+
48 hours					
Control	+++	+++	-	-	+++
1.6	+++	+++	-	-	+++
3.1	+++	++	-	-	+++
6.1	++	+	+	+	++
12.3	+	+	+ +	++	+
24.3	+	+	++	+++	+
72 hours					
Control	+++	++	-	-	+++
1.6	++	++	-	+	+++
3.1	+	+	+	++	++
6.1	+	+	+	++	+
12.3	+	+	++	++	+
24.3	-	-	+++	+++	-
96 hours					
Control	+++	+++	-	-	+++
1.6	++	++	-	+	++
3.1	++	++	+	++	++
6.1	+	+	+	++	+
12.3	+	+	++	+++	+
24.3	-	-	+++	-	+

Key: -, none; +, mild; ++, moderate; +++, strong

ibuprofen, atenolol, disopyramide, famotidine, fluconazole, erythromycin, clathromycin and levofloxacin, and > 40 mgl^{-1} for indomethacin and carbamazepine as reported by Kim *et al.* (2009) in *Oryzias latipes*. The 96-hour LC_{50} was lower than that of mixed pharmaceuticals

solution (MPS) in *Pseudorasbora parva* and *Cyprinus carpio* (Li and Lin, 2015). The MPS 96 hours LC_{50} was 60.68 mgl^{-1} which is over ten thousand folds higher than 6.53 μgl^{-1} for ivermectin in *C. gariepinus* from the present study. The 96-hour LC_{50} of ivermectin in *C. gariepinus* was comparatively lower than that of praziquantel. Nwani *et al.* (2014) reported a 96 h LC_{50} of 53.32 mg^{-1} for praziquantel compared to 6.53 μgl^{-1} for ivermectin in this study. The marked difference between ivermectin toxicity relative to these other toxicants may best be explained concerning the mode of activity. At micromolar concentration, ivermectin interacts with a wide range of ligand-gated channels that occur in both vertebrates and invertebrates including glycine, histamine, gamma-aminobutyric acid (GABA) and nicotinic acetylcholine receptors (Liang *et al.*, 2017), and may result in significant deleterious consequences.

Safe Levels: The results of the acute toxicity level and the toxic unit indicated that ivermectin was toxic to *C. gariepinus* and hence the need to adhere strictly to the safe levels in veterinary applications of the drug. Similar to the findings of this study, Wang *et al.* (2019) and Madrid *et al.* (2021) had earlier reported various safe levels of ivermectin in *Cyprinus carpio haematopterus* and *Corydoras schwartzi* respectively exposed through oral gavage. Ivermectin was highly toxic to freshwater aquatic species and had a narrow gap (between safe levels and toxic doses) in salmon (Garric *et al.*, 2007; Ucan-Marin *et al.*, 2012). However, the problem of extrapolation of laboratory data to field real data poses some problems of wide acceptance and use of safe levels as obtained from the laboratory studies (Pandey *et al.*, 2005). Variations in safety levels may also be attributed to differences in age, size, species of the animal model and the method of estimation (Ogili *et al.*, 2021).

Behavioural changes in *Clarias gariepinus* exposed to Ivermectin: The behavioural changes may be due to the impairment of the physiological processes in the fish due to the inhibition of acetylcholinesterase (AChE) and the subsequent accumulation in the brain (Muralidharan, 2014). The disruption of AChE activity may lead to nervous impairment and subsequent behavioural abnormality. Similar reports on disruption of behavioural activities in fish due to exposure to pharmaceutical drugs have been reported (Nwani *et al.*, 2014; Odo *et al.*, 2020).

Conclusions: The high toxicity profile of ivermectin to *C. gariepinus* is further corroborated by the very low toxic unit and safe level of the drug. The NAS/NAE (1973) estimated safe level of 6.53×10^{-1} – 6.53×10^{-5} is very low. The behavioural changes in the fish and the low margin of safety imply the need for stiff precautions if ivermectin must be used in aquaculture.

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