ACUTE TOXICITY OF TRAMADOL IN AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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ABSTRACT

Tramadol is among the most famous analgesic drugs used for the management, treatment and relief of moderate to severe pain condition. The present study investigated the effect of acute toxicity (LC_{50}) of tramadol on juvenile Clarias gariepinus. A total number of one hundred and eighty healthy juveniles of freshwater catfish C. gariepinus with total mean weight and length of (455.3 ± 50 g and 163.9 ± 18.2 cm) were used for the study. The 24, 48, 72 and 96 hour LC_{50} of C. gariepinus exposed to tramadol were 213.01(151.94 – 613.23), 109.86 (66.36 – 936.04), 74.02(4.51 – 205.10) and 63.34(29.50 – 95.83) mg/L respectively. The safe levels of tramadol in C. gariepinus varied from $6.33x10^{-1}$ to $6.33x10^{-4}$mg/L. The toxic unit of tramadol is 0.63 indicating that the drug is toxic to C. gariepinus. Fish exposed to tramadol showed some significance abnormal behavioural responses such as, reduced agility, abnormal mucus secretion, skin coloration, opercula movement and air gulping, very poor swimming rate and mortality increased with increase in the exposure duration and concentrations except for the control. The results of the present study demonstrated that tramadol is toxic to fish and its use should be monitored in the aquatic biota to safeguard non-target organisms.

Keywords: Tramadol, Toxicity, Behaviour, Catfish, Pharmaceuticals, Aquatic pollution

INTRODUCTION

Tramadol is a prescription opioid painkiller for moderate pain. It is often used for pain relief after surgery or for treatment of chronic pain from conditions like fibromyalgia. Tramadol most often comes in 50, 100, 150, 200 and 300 mg tablets and is taken orally. Tramadol, sold under the brand name Ultram among others, is an opioid pain medication used to treat moderate to moderately severe pain. When taken by mouth in an immediate-release formulation, the onset of pain relief usually begins within an hour. It is also available by injection. It may be sold in combination with paracetamol (acetaminophen) or as longer-acting formulations (Dhillon, 2010). Its analgesic effects take about one hour to manifest and between two to four hours to peak after oral administration with an immediate-release formulation (Nakhaee *et al.*, 2021). On a dose-by-dose basis, tramadol has about one-tenth the potency of morphine (thus 100 mg is commensurate with 10 mg morphine but may vary) and is practically equally potent when compared with pethidine and codeine (Grond and Sablotzki, 2012). For pain moderate in severity, its effectiveness is equivalent to that of codeine at low doses, and hydrocodone at very high doses; for severe pain it is less effective than morphine (Grond and Sablotzki, 2012).

These painkilling effects last about six hours. The potency of analgesia varies considerably as it depends on an individual's

genetics. Tramadol induces analgesic effects through a variety of different targets on the noradrenergic system, serotoninergic system and opioid receptors system. Tramadol exists as a racemic mixture, the positive enantiomer inhibits serotonin reuptake, while the negative enantiomer inhibits noradrenaline re-uptake, by binding to and blocking the transporters (Baldo and Rose, 2020). Tramadol has also been shown to act as a serotonin releasing agent. Both enantiomers of tramadol are agonists of the μ-opioid receptor and its m1 metabolite, o-desmetramadol, are also a μ-opioid receptor agonist but are 6 times more potent than tramadol itself. All these effects work synergistically to induce analgesia in human and animals like fish (Baldo and Rose, 2020). The abuse and adverse effects of tramadol has been reportedly on increase. The adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time (usually less than 24 hours (Coecke *et al.,* 2006) is described as acute toxicity. Toxicity data comes from animal testing or, more recently, *in vitro* testing methods and inference from data on similar substances (Coecke *et al.,* 2006).

Fish are very commonly kept as pets and have become increasingly popular animal models in research. Fish are among largest group of all animals used in research. As such, fish frequently undergo potentially painful surgical procedures particularly the African catfish – *Clarias gariepinus* (Chatigny *et al.,* 2017). *C. gariepinus* is fairly insensitive to diseases, has no demanding nutritional requirements, and is fairly easy to propagate. And that is why it is the most farmed fish in many regions of the world (Dauda *et al*., 2018). In Nigeria, it constitutes one of the largest cultured species which grow well under various culture systems and is usually farmed to provide income and valuable sources of protein (Adewumi and Olaleye, 2011).

The excessive discharge of chemical compounds and drugs such as tramadol into the water directly affects not only aquatic organisms like fish but can change the physicochemical and biological characteristics of the aquatic ecosystem (Ebele *et al*., 2017; Kayode-Afolayan *et al*., 2022). Though tramadol has been used over the years as analgesics for routine pain management in both human and veterinary animals, the published work on its effect on fish is limited, thus there is need to investigate its toxicity and the behavioural responses of *C. gariepinus* to this drug for proper understanding of the toxicology in fishes. Since there is obviously not much information or any documented work on the effects that lethal accumulation of tramadol may have on the behavioral, physiological and in body conditions of fishes, the need for this research work became paramount. Hence, this study investigates the survival and mortality rate as well as the behavioural response of juvenile *C. gariepinus* on exposure to tramadol.

MATERIALS AND METHODS

Experimental Fish Specimen and Drug: This research was carried out at the Wet Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria Nsukka, Enugu State, Nigeria. Two hundred and sixty five (265) juveniles of freshwater *C. gariepinus* of mean length of 163.9 ± 18.2 cm and mean weight of 455.30 ± 50.59 g were sourced from Freedom Fisheries Limited, Nsukka, Enugu State, Nigeria. The fishes were transported to the fisheries Wet Laboratory where they were acclimatized for two weeks. Less than 1 % mortality was recorded during acclimatization period. The leftover was fed twice daily with commercial feed (Coppens Commercial Feed of 4 mm, Coppens International, Helmond, Netherlands) with 45 % crude protein, 15 % lipids, 0.8 % crude fiber, 9.4 % ash, 20.9 MJ/kg gross, and 18.6 MJ/kg digestible energy.

Tramadol stock solutions were prepared using commercial formulations of Canesten V6 manufactured by Bayer Pharmaceuticals Private Limited, Malpur, Baddi -173 205, Tehsil Nalagarh, with Manufacturer's License Number L/07/471/mnb. Each uncoated tablet contained Tramadol IP 100 mg as the active ingredients.

Experimental Design for Acute Exposure: Acute toxicity test were carried out in 18 plastic

aquaria. 180 fish (455.3 ± 50.59 g and 163.9 ± 18.2 cm) from the acclimatized batch were randomly introduced in the plastic aquaria at the rate of 10 fish per aquarium. Each aquarium was covered with net mesh tied with rubber to prevent the fishes from jumping out. In the range findings test of tramadol, on *C. gariepinus*, the percentage mortalities of 0 and 100 % lied between 35 and 44 mg/L respectively. Thus, the fish were treated to the following concentrations of tramadol: 30, 60, 90, 120 and 150 mg/L, and a control containing 10 liters of water and no drug. Three replicates were maintained for each group and the control for a robust statistical analysis. The percentage mortality/survival of fish in control and treated groups was recorded at 24, 48, 72 and 96 hour intervals respectively. Fishes were considered dead when their operculum stops beating and were carefully removed with plastic forceps to avoid deterioration of the test media. The toxic unit was calculated by dividing the 96 h LC_{50} by 100. The average mean water qualities of the test solution determined in the experimental tanks were: dissolved oxygen 7.14 mg/l, temperature 27.83℃, pH 12.3, and conductivity 247.50 μScm^{-1}. The behavioral responses of the fish in the experimental aquaria and the control aquarium were observed and recorded daily. The 24 – 96 hour LC_{10-90} and confidence intervals of tramadol were determined by Probit analysis. The concentrations of tramadol, on which the lethal concentrations calculations were based, are the nominal concentrations.

Determination of Safe Levels: The safe levels of tramadol were estimated by multiplying the 96 hour LC_{50} with different application factor (AF) given in Table 1 (Hart *et al*., 1948; Committee on Water Quality Criteria (CWQC, 1972) and Canadian Council of Resources and Environmental Ministry (CCREM, 1991).

Observation of Behavioural Responses: Observations of behavioural and morphological responses of *C. gariepinus* juveniles exposed to tramadol were conducted at 24, 48, 72 and 92 hour during the acute toxicity test. The methods developed by Drummond *et al.* (1986) were used for this study. Controls without toxicant exposure were monitored, along with the acute concentrations to offer a reference for the assessment of any behavioural and phenotypic changes. Responses were recorded if they differed from the controls and if it occurred in 10 % of the fish within each test tank. Each test chamber was observed for 5 – 10 minutes. Startled responses were monitored by light touching the fish with a plastic applicator stick (tactile stimulus).

Statistical Analysis: Data was analyzed using Statistical Packages for social sciences (SPSS) version 23.0 (IBM Corporation, Armonk, USA. Data obtained were subjected to one-way analysis of variance (ANOVA) and Duncan's new multiple range tests to determine the significance. The level of significance was set at $p<0.05$.

RESULTS

Safe Level: The safe level of tramadol ranged from 6.33×10^{-4} to 6.33 using NAS/NAE (1973) application factor (AF). According to Hart *et al.* (1948) procedure, the safe level for tramadol to *C. gariepinus* was 0.88 mg/L. Safe level for all the methods used did not exceed 6.33 mg/L (Table 1).

Behavioural Responses of *C. gariepinus* Exposed to Tramadol: There were observed behavioural responses during the 96h sub lethal exposure of *C. gariepinus* to tramadol at various concentrations. These responses include loss of balance, skin coloration, abnormal mucus secretion, changes in mode of nutrition, poor swimming rate, changes in opercula movement, air gulping, reduced agility and dizziness (Table 2).

Cumulative Mortality of *C. gariepinus*: The cumulative mortality of *C. gariepinus* after 96 hours of exposure to tramadol summarized in Table 3 indicated that mortality increased cumulatively as the concentration of tramadol increased and as duration of exposure extends from 24 to 96 hours. At the end of 96 hours, 100 % mortality occurred at 150 mg/L tramadol, and 20 % at 30 mg/L tramadol.

Table 1: Estimates of safe level of tramadol after 96 hours of exposure of *Clarias gariepinus*

96 hour LC_{50} (mg/L)	Methods	AF	Safe level (mg/L)
63.34	Hart *et al.* (1948)*	-	0.88
	Sprague (1971)	0.1	6.33
	CWQC (1972)	0.01	6.33×10^{-1}
	NAS/NAE (1973)	0.1 – 0.00001	$6.33 – 6.33 \times 10^{-4}$
	CCREM (1991)	0.05	3.17
	IJC (1977)	5% LC_{50}	3.17

**C = 48h LC_{50} x 0.03/S^2, where C = presumable harmless concentration and S = 24 h LC_{50}/48 h LC_5*

Table 2: Behavioral changes in *Clarias gariepinus* exposed to different concentrations of tramadol at 24, 48, 72 and 96 hours

Tramadol Concentration (mg/L)	Equilibrium status	Skin coloration	Mucus secretion	Feeding rate	Opercula movement	Air gulping	Dizziness
24 hours							
Control	+++	-	-	+++	+	+	-
30	+++	_	_	+++	++	++	-
60	++	-	+	+++	++	++	-
90	++	+	+	++	+++	+++	+
120	+	++	++	++	+++	++	++
150	+	+++	+++	+	+	+	+++
48 hours							
Control	+++	-	-	+++	+	+	-
30	+++	-	-	+++	++	+	-
60	++	+	+	++	++	++	+
90	+	+++	+++	+	+++	+++	++
120	+	+++	+++	+	+++	++	++
150	+	+++	+++	+	++	+	+++
72 hours							
Control	+++	-	-	+++	+	+	-
30	+++	-	-	+++	++	+	-
60	++	+	+	++	++	++	+
90	+	++	++	++	+++	+++	++
120	+	+++	+++	+	++	++	+++
150	+	+++	+++	+	+	+	++
96 hours							
Control	+++	-	-	+++	+	+	-
30	+++	-	-	+++	++	+	-
60	+	+	+	++	+++	++	++
90	+	+++	+++	++	++	++	+++
120	+	+++	+++	+	++	+	+++
150	+	+++	+++	+	+	+	++

-, None; +, mild; ++, moderate; +++, strong

Table 3: Cumulative mortality of *Clarias gariepinus* juvenile exposed to varied concentrations of tramadol

Concentration (mg/L)	Number of fish exposed	Cumulative mortality at time intervals				% survival	% mortality
		24h	48h	72h	96h		
Control	30	0	0	0	0	100	0
30	30	0	2	4	6	80	20
60	30	4	8	12	12	60	40
90	30	6	10	16	16	40	60
120	30	8	12	18	18	20	80
150	30	10	18	24	30	0	100

No mortality occurred in the control for the exposure duration. The response curve of the mortality as the duration of exposure increases from 24 to 96 hours have been presented in Figure 1.

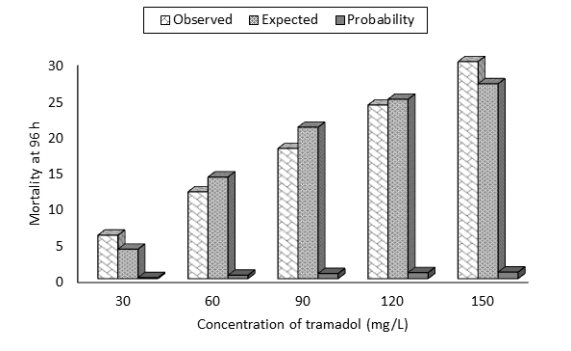

Figure 1: Response bar chart of *Clarias gariepinus* after 96 h exposure to tramadol

The estimated lethal concentration ($LC_{10 - 90}$) of tramadol in *C. gariepinus* shown in Table 4 indicated that the 96 h LC_{50} was 63.34 mg/L (95 % CI = 29.50 – 95.83 mg/L). Approximately 213 mg/L of tramadol caused 50 % mortality of *C. gariepinus* in 24 hours. At 96 hours, the estimated concentration that caused 90 % mortality of *C. gariepinus* was 151.17 mg/L (98.90 – 1025 mg/L). The unit toxicity of tramadol on *C. gariepinus* was 0.63. The no effect concentration (NOEC), lowest observed effect concentration (LOEC), and median lethal concentration (LC_{50}) are presented as Figure 2. The LC_{50} declined from 24 to 96 hours.

DISCUSSION

This current study demonstrated the toxic effect of tramadol in juvenile *C. gariepinus.* The toxicity of any compounds to organisms is known to be dependent on concentration, sex, developmental stages and exposure periods. There was increase in mortality in the exposed groups. This was line with the report of Ali *et al.* (2014) in common carp embryos and larvae exposed to a mixture of tramadol hydrochloride and naproxen sodium for 32 days. The observed mortality may also be attributed to the toxic effects of tramadol on *C. gariepinus.* Sehonova *et al.* (2017) obtained similar results when the early life stages of common carp (*Cyprinus carpio*) were exposed to naproxen sodium and its mixture with tramadol hydrochloride.

On lethal concentration ($LC_{10 - 90}$) of tramadol to *C. gariepinus*, it was revealed that tramadol was able to cause 50 % mortality of *C. gariepinus* in 24 hours. Under 96 hour's duration, the estimated concentration caused 90 % mortality of *C. gariepinus.* This was in line with the report of Ogueji *et al.* (2017) on lethal concentration of the drug ibuprofen against *C. gariepinus*. The low toxic unit obtained in the study indicates that tramadol is toxic to fish. Nwani *et al.* (2020) also reported low toxic unit in *C. gariepinus* exposed to Act Force Gold, Butaforce and Atraforce.

There were variations in safe level of tramadol estimated by different methods at 96 hours LC_{50} of exposure. The differences in safe levels reported by different methods may be attributed to differences in size, species, age, and method of estimation and variation of derivatives in the different chemicals (Nwani *et al.*, 2020).

Abnormal behavioral changes, such as swimming near the water surface, loss of equilibrium, erratic swimming, circling movement, hyperactivity and staying motionless on the bottom of aquarium tank were observed in fish exposed to tramadol. The loss of balance and uncoordinated swimming may be attributed to nervous reaction to the toxicant. The air gulping behavior may be as a result of respiratory impairment due to effect of toxicant on the gills. The inability of the gills surface to actively carry out gaseous exchange might be responsible for the recorded mortalities. The result of this study is similar to earlier report by Bachour *et al.* (2020) that monitored the swimming activity of zebrafish larvae and found that 320 g/L tramadol concentration caused significant anxiolytic (hypoactivity during dark conditions) effect on the fish. Similar behavioral responses were reported by Santos *et al.* (2021) that tramadol induced behavioral changes in the European Chub (*Squalius cephalus*) when compared to the control.

Conclusion: The present study shows that tramadol is toxic to fish. Fish exposed to tramadol showed some abnormal behavioral

Table 4: Lethal concentration (LC_{10-90}) of tramadol depending on exposure time (24 – 96 hours) for *Clarias gariepinus*

Points	Concentrations (mg/L) at different exposure time (95% confidence interval)			
	24h	48h	72h	96h
LC_{10}	62.16 (30.67 – 80.98)	39.09 (0.26 – 65.26)	29.66 (0 – 54.52)	26.54 (1.76 – 44.81)
LC_{20}	94.87 (69.38 – 124.13)	55.74 (0.73 – 85.41)	40.60 (0 – 67.62)	35.77 (4.89 – 55.14)
LC_{30}	128.68 (101.44 – 208.28)	71.98 (7.20 – 116.97)	50.91 (0.001 – 82.58)	44.37 (10.01 – 65.28)
LC_{40}	166.98 (126.86 – 358.63)	89.57 (33.86 – 229.66)	61.78 (0.115 – 108.3)	53.33 (17.96 – 77.58)
LC_{50}	213.01 (151.94 – 613.23)	109.86 (66.36 – 936.04)	74.02 (4.51 – 205.10)	63.34 (29.50 – 95.83)
LC_{60}	271.73 (180.02 – 1059.95)	134.77 (88.13 – 5630.11)	88.68 (34.95 – 1969)	75.22 (44.30–129.47)
LC_{70}	352.59 (214.63 – 1914.27)	167.69 (106.77 – 42924)	107.61 (62.96 - 109844)	90.42 (60.37-202.52)
LC_{80}	478.27 (262.66 – 3838.08)	216.57 (127.70 - 484052)	134.96 (83-18323399)	112.15 (77.12-3845)
LC_{90}	729.95 (346.31 – 10107.64)	308.78 (159 - 14350252)	184.73 (106-254655847.74)	151.17 (98.90-1025)

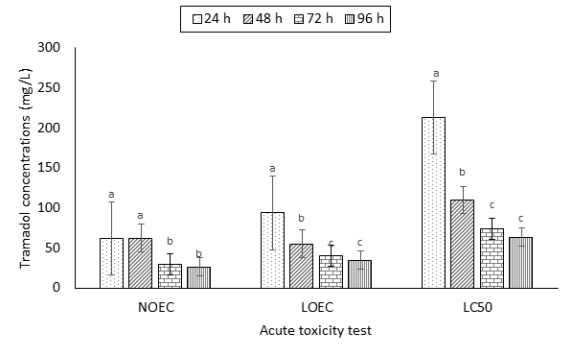

Figure 2: Acute toxicity testing statistical endpoints in *Clarias gariepinus* exposed to tramadol at different durations (24, 48, 72 and 96 hours)

responses and mortality increased with increase in the exposure duration and concentrations except for the control. The results of the present study indicate that tramadol is toxic to fish and its use should be monitored in the aquatic environment. Finally, the use of tramadol should be regulated to avoid toxic effects on non-target aquatic species especially fish.

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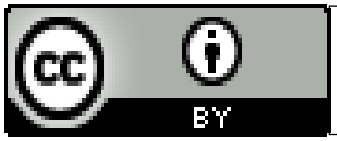

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