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TOXICITY AND HAEMATOLOGICAL CHANGES OF *Datura innoxia* STEM ON *Clarias gariepinus* JUVENILES

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ABSTRACT

The acute toxicity and haematological changes of *Datura innoxia* stem on *Clarias gariepinus* juveniles was investigated in a static bioassay to determine the Median Lethal concentrations (LC_{50}) at 96 hrs of exposure. Six graded concentrations of 0, 320, 330,340, 350 and 360mg/l of the aqueous extract were applied to *C. gariepinus* juveniles in plastic bowls. The result obtained revealed the 96hrs LC_{50} values to be 86.67mg/l with an 87% confidence interval between the lower and higher limits of 320 and 360mg/l. Fish exposed to acute toxicity test exhibited several abnormal behaviours, including air gulping/gasping, erratic swimming, discolouration haemorrhage, loss of reflex and molting were observed at the bottom of the bowl just before death The oxygen consumption by juveniles decreased with increased concentrations of *Datura innoxia* stem extract. The packed cell volume (31.34-34.78%), the red blood cell counts (1.75-2.01), haemoglobin (9.03-11.12g/dl) and mean corpuscular haemoglobin concentration were significantly depleted while, white blood cell counts (6.05-7.21) significantly decreases as the concentrations of *Datura innoxia* stem extract increases. The implication of these findings revealed that the stem of *D. innoxia* stem have negative effects on the test fish. The toxic effect of *Datura innoxia* stem on *Clarias gariepinus* juveniles were both time (12hrs) and dose dependent (340mg/l). It was concluded that *Datura innoxia* stem are harmful to *Clarias gariepinus* juveniles with significant haematological alterations.

Keywords: *Clarias gariepinus* juveniles, *Datura innoxia* stem, Acute toxicity, Lethal concentration, Hematology

INTRODUCTION

Plants contain a remarkable array of chemicals that can be detrimental to fungi, bacteria, insects, herbivores, and humans. Yet, this extensive chemical diversity involved other compounds that are useful to humans, such as nutrients, vitamins, antioxidants, anticarcinogens, and some other medicinal substances (Novak and Haslberger, 2015).

Aquatic ecosystems, crucial for sustaining diverse life forms, are increasingly vulnerable to the adverse effects of environmental contaminants. In particular, the contamination of water bodies with toxic substances poses a significant threat to aquatic organisms and, by extension, to the overall health of aquatic ecosystems. Among these contaminants, plant-derived compounds can exert a substantial influence on aquatic life, leading to a range of ecological and physiological responses (Abdelhamid, & Fathy, 2020**).**

Datura innoxia is a highly toxic plant that belongs to the Solanaceae family. It contains a range of active compounds, such as hyoscyamine, scopolamine, and atropine, which are known to have hallucinogenic and toxic effects (Sambasivam *et al*., 2003). Understanding the impact of *Datura innoxia* on fish species is important for managing and protecting aquatic ecosystems and the organisms that rely on them. *Datura innoxia* is a plant species known for its various medicinal properties. It has been widely used in traditional medicine though its numerous medicinal benefits, *Datura innoxia* contains toxic substances that can cause adverse effects on human and animal health (Ayuba *et al*., 2013).

Fish are frequently utilized as sentinel organisms in ecotoxicological research due to their various roles in the trophic web, their ability to accumulate toxic substances, and their sensitivity to low concentrations of mutagens (Cavas and Ergene-Gözükara, 2005). Consequently, fish biomarkers are becoming increasingly important as indicators of pollution effects, enabling the early detection of aquatic environmental issues (Baser *et al*., 2003).

Mortality or bioassay experiments in general present the most preferred way to evaluate the ecological influence of toxic compounds as their effects on fish and ecological risks cannot be determined by chemical analysis (Baser *et al*., 2003; Svobodova *et al*., 2013). Acute toxicity tests provide basis for understanding the limiting effects of various chemicals on organisms.

The impact of *Datura innoxia* on haematological parameters creates the unsettling possibility that these fish's very lifeblood is changing. These modifications could include variations in haemoglobin levels, red blood cell counts, and the delicate balance of different blood components. The resilience, health, and life expectancy of the fish are steadily diminished by these alterations, despite their subtlety.

Therefore, a thorough research into these haematological changes is essential. We can only hope to assess and subsequently safeguard the health of aquatic ecosystems, ensuring that they are resilient and capable of supporting a variety of life forms over time, through a profound understanding of the complexities surrounding *Datura innoxia's* impact on *Clarias gariepinus* juveniles' blood.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the Fish Hatchery Lab, Department of Fisheries and Aquaculture, Bayero University, Kano, Kano is located between latitude $12^{0}00$N and 12.000^{0}N and between longitude $8^{0}31$'E and 8.517^{0}E in the Savanna, south of the Sahel. Kano is a major route of the trans-Saharan trade, having been a trade and human settlement for millennia. The State lies in North-West geopolitical zone of Nigeria and shares common borders with Katsina, Jigawa, Kaduna and Plateau State.

Experimental fish
The study utilized 180 juvenile *C. gariepinus* with average weights of 19.56 ± 0.7g and 31.07 ± 1.23g. These fish were obtained from Gidan-Kifi fish farm Kano, Kano State.

Source and Preparation of *Datura innoxia Stem*
Fresh samples of *D. innoxia* stem plants were collected from Ankpa Local Government Area in Kogi State.The samples were air-dried to a constant weight in the lab and then finely grinded to fine powder using a clean mortar and pestle. The powder were sieved through a 0.2 mm mesh and kept in airtight, wide-mouth bottles for analysis. Each 500g sample was dissolved in 2 liters of distilled water at room temperature (27 ± 0.3°C) for 24 hours (Omoregie and Onuogwu, 2015). The aqueous solution were decanted, filtered via Whatman filter paper using a vacuum pump, and the filtrates were freeze-dried and kept in a refrigerator (10°C) for later use.

Experimental Design
Completely randomized design was employed for the study. Eighteen plastic tanks, each with dimensions of 60cm x 40cm x 40cm, were used. These tanks were thoroughly cleaned and filled with 20 liters of water. Each tank was labeled accordingly. Fish were weighed and allocated at a rate of 10 fish per tank. A total of 180 *C. gariepinus* juveniles were randomly distributed among the tanks, with each tank containing 10 fish, repeated in triplicate.

Phytochemical Screening of *Datura innoxia* stem
Phytochemical screening for major constituents was carried out on *Datura innoxia* stem in the Department of Plant Biology, Bayero University Kano. The *Datura innoxia* stem was tested for the presence of active ingredients following the procedures of (Ushie *et al*., 2013).

Range finding test
A preliminary 96-hour range-finding test was separately conducted for *C. gariepinus* juveniles using a static bioassay to determine the toxic range of *Datura innoxia* stem, following the method described by Parrish (1985). For this test, concentrations of 300mg, 330mg, 360mg, 390mg, and 420mg of *Datura innoxia* stem per liter of water were used. Ten *C. gariepinus* juveniles were individually weighed with a sensitive electronic scale (Mettler Toledo FB602) and placed into each of the 18 tanks filled with 20 liters of tap water with different concentration of toxicant. The fish's response to slight stimuli was used as an indicator of toxicity, while a lack of response to touch indicated death. The LC50 of *Datura innoxia* stem was determined after 96 hours of exposing the test fish to the plant.

Definitive Test
Results from the range-finding tests guided the concentration levels used in the definitive test. For the definitive test, eighteen plastic tanks were each filled with 20 liters of water. The concentrations of *Datura innoxia* stem used, determined from the range-finding test, were 320mg, 330mg, 340mg, 350mg, and 360mg per liter of water. These concentrations were accurately prepared using a sensitive weighing balance. The fish's response to slight stimuli served as an indicator of toxicity, and a lack of response indicated lethality, estimated to affect 50% of the test organisms after 96 hours of exposure.

Haematological examination of fish
Blood samples were collected by randomly selecting two fish from each treatment group. Using a 2mm needle and syringe, blood was drawn from the dorsal aorta and placed in EDTA-treated bottles to prevent clotting. These samples were then analyzed at Aminu Kano Teaching Hospital in Kano, Kano State, for hemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), and White Blood Cell (WBC) counts using an automated hemoglobin analyzer (Cobus U 411). Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Volume (MCV) were calculated according to the method described by Svobodova *et al.*, (2013).

Data Analysis
Data were analyzed with Minitab 16 (Minitab, 2016) to generate summary statistics and assess differences in water quality parameters at acute concentrations. Hematological parameter means were evaluated using analysis of variance (ANOVA) at a 0.05 significance level with SPSS version 23, to detect significant differences between control and experimental groups. Observed mean differences were further compared using the least significant difference (LSD) method.

RESULTS AND DISCUSSION
The water quality parameters after 96-hours acute toxicity test of *Clarias gariepinus* juveniles expose to *Datura innoxia* stem is shown in Table 1.

Table 1: Water quality parameters of experimental units during acute toxicity test of *Clarias gariepinus* exposed to *Datura innoxia* stem

S/N	Treatment Mg/L	pH	TEMP (^{0}C)	TDS (ppm)	EC (µS/cm)	DO (mg/L)
1	T0 (control)	6.57±0.01^a	25.87±0.03^{ab}	306.20±0.05^a	613.40±0.10^a	4.57±0.03^e
2	T1 (320)	6.63±0.03^a	25.30±0.06^a	314.52±0.17^b	620.03±0.35^b	4.47±0.01^d
3	T2 (330)	5.87±0.02^b	25.27±0.03^{ab}	320.00±0.05^c	635.00±0.10^c	4.42±0.01^c
4	T3 (340)	5.57±0.03^b	25.32±0.03^b	327.55±0.15^d	650.10±0.31d	4.37±0.00^c
5	T4 (350)	5.33±0.03^b	25.35±0.03^b	365.40±0.28^e	736.80±0.56^e	4.31±0.01^b
6	T5 (360)	4.67±0.02^c	25.27±0.03^{ab}	401.90±0.06^f	799.80±0.12^f	4.18±0.00^a
	P-Value	0.104	0.061	0.001	0.000	0.001

Mean in the same column with different superscripts differ significantly (P<0.05).
DO: Dissolved Oxygen; pH: Hydrogen Ion Concentration; Temp: Temperature; EC: Electrical Conductivity; TDS: Total Dissolved Solids.

The water quality parameters recorded during the acute toxicity bioassay were within suitable ranges for the survival and normal growth of *C. gariepinus* Therefore, any observed changes in fish behavior and mortality were unlikely to be due to poor water quality. According to Badiru (2005) optimal pH scale for fish growth, the pH range of 4.67-6.57 in this study does not meet the preferred range of 6.50-9.0 for fish production. However, the dissolved oxygen levels of 4.18-4.57 mg/L fall within the range associated with slow growth from long-term exposure (1-5 mg/L) for warm water fish

according to Badiru's scale. Additionally, the temperature range of 25.27-25.87°C is within the typical tropical range for fish adaptation (25-32°C), as reported by Ayuba *et al.*, (2013). It was noted that both pH and dissolved oxygen levels declined, likely due to the impact of *Datura innoxia* stem on water quality. This observation is in line with the findings from Ayuba and Ofojekwu (2002) and Ayuba *et al.*, (2012) on *Datura innoxia* root extracts. Although total dissolved solids (TDS) and electrical conductivity (EC) values were significantly different ($P<0.05$) from control values, they remained within acceptable limits as per Isiyaku *et al*., (2015). This finding is consistent with reports by Adigun (2003), Kolo *et al*., (2009), and Ayuba *et al.*, (2012) on other toxicants. These results corroborate Warren's (1997) assertion that introducing a toxicant into an aquatic system can lower dissolved oxygen levels, impair respiration, and cause asphyxiation. Increased temperature and other physiological stressors in fish can contribute to mortality in acute toxicity scenarios, as highlighted by Olaifa *et al*., (2003), Mekkawy *et al.*, (2013), and Isiyaku *et al*., (2015).

The fact that mortality of fish during acute bioassays was concentration dependent as shown in (Table 2) could be directly linked with the direct effect of the toxicant. This is similar to the findings of Obiezue *et al*. (2014) and Isiyaku *et al*. (2021) in which a direct relationship between mortality in *C. gariepinus* and concentration of diethyl phthalate and Tamarind seed husk powder recorded.

Table 2: Mortality of *Clarias gariepinus* juveniles exposed to *Datura innoxia* stem over the 96-hr period

Treatments/ Concentration (mg/L)	Log Concentration	Number Stocked	Mortality							Probit
			12 hrs	24 hrs	48 hrs	72 hrs	96 hrs	Total	%	
T0 (Control)	0	30	0	0	0	0	0	0	0	0
T1 (320)	2.51	30	0	0	3	3	2	8	26.67	4.39
T2 (330)	2.52	30	0	3	5	3	2	13	43.33	4.82
T3 (340)	2.53	30	3	4	5	3	3	18	60	5.25
T4 (350)	2.54	30	6	5	5	2	4	22	73.33	5.61
T5 (360)	2.56	30	9	7	4	3	3	26	86.67	6.13

The LC50 identified in this study is 334.37 mg/l (Fig. 1), which contrasts with the 180 mg/l LC50 reported by Bamidele *et al.*, (2018) for *Clarias gariepinus* exposed to acute concentrations of *Datura innoxia* root. On the other hand, Isiyaku *et al.*, (2021) found an LC_{50} of 3.78 mg/l for *Oreochromis niloticus* exposed to tamarind seed husk powder, significantly lower than the value found in this study. Slabbert and Venter (1999) reported an LC_{50} of 0.20 mg/l for *Poecilia reticulata* exposed to mercuric chloride, while in Malaysia, Shyong and Chen (2000) found LC_{50} values of 0.168 mg/l and 0.161 mg/l for *Variocorhinus barbatulus* and *Zacco barbara* respectively. Isiyaku *et al.*, (2015) recorded an LC50 of 1.52 mg/l for *Clarias gariepinus* in an acute mercury toxicity test. The LC_{50} in this study is notably higher than those reported by Ayuba and Ofojekwu (2002) and Ezike and Ufodike (2008) for *Clarias gariepinus*, which were 204.17 mg/l for *Datura innoxia* conducted in Makurdi and 34 mg/l for petrol conducted in Anambra respectively. These variations may be due to differences in the substances used and the specific environmental conditions of each study.

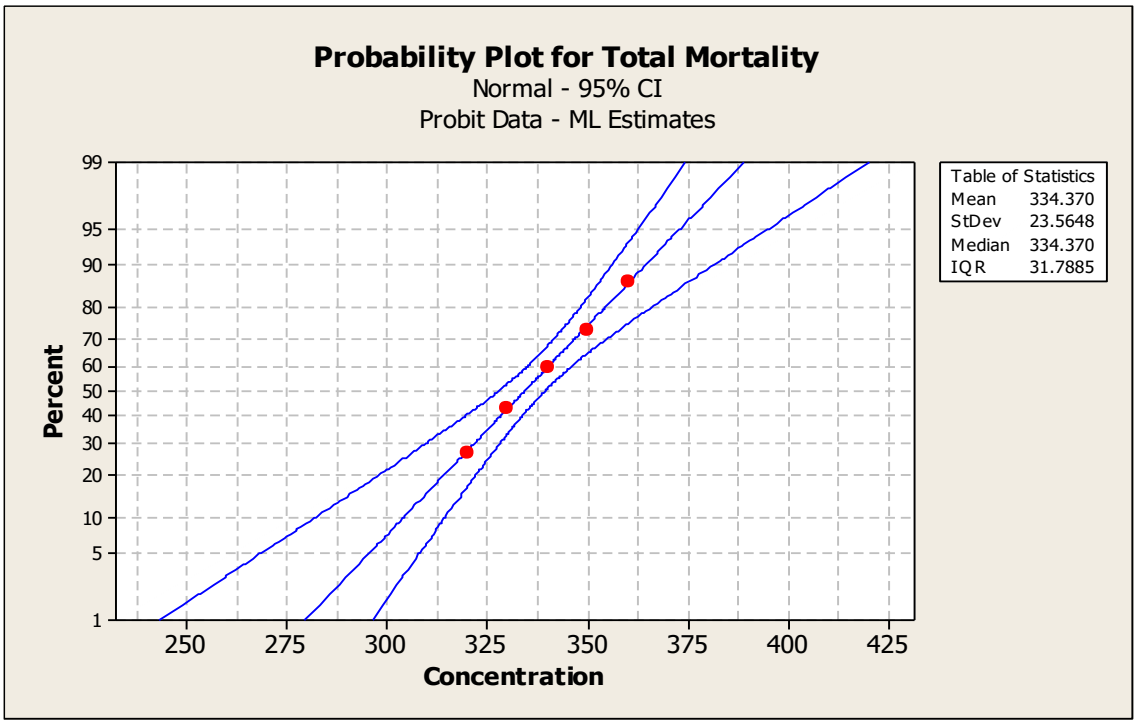

Figure 1: Linear relationship between mean probit mortality and log concentration of *Clarias gariepinus* exposed to various concentrations of *Datura innoxia* stem for 96 hrs

Table 3 presents the haematological parameters of Clari*as gariepinus* juveniles exposed to *Datura innoxia* stem. Hematological and blood biochemical parameters are influenced by a variety of environmental stressors, they have the potential to be used as biomarkers of the detected water pollution Summarwar (2012). Evaluation in fish has become an important means of understanding the toxicological impacts of exposure hazards (Borges *et al.*, 2007). The results align with the findings from Mekkawy *et al*. (2013), that reported decreases in erythrocyte counts, hemoglobin, total protein, and packed cell volume in *C. gariepinus* due to *Datura innoxia* stem exposure. These reductions were attributed to the destruction of mature red blood cells and inhibition of erythrocyte production caused by impaired haem synthesis due to the pollutant. The findings also agree with Jayaprakash and Shettu (2013), who observed decreases in hemoglobin content, total erythrocyte count, packed cell volume, mean corpuscular volume, and mean corpuscular concentration in *Channa punctatus* exposed to deltamethrin. They attributed these changes to stress, hypoxia, or swelling of erythrocytes leading to red blood cell lysis. Similarly, Karuppaswamy (2005) noted significant decreases in total erythrocyte count, hemoglobin content, hematocrit value, and mean corpuscular hemoglobin concentration in the air-breathing fish *Channa punctatus* following exposure to a sub-lethal dose of *Datura innoxia* leaf extract.

Table 3: Haematological parameters of *Clarias gariepinus* juveniles exposed to Aqueous stem extract of *Datura innoxia*

Parameters	Treatment (mg/L)					
	0.00(T1)	**320(T2)**	**330(T3)**	**340(T4)**	**350(T5)**	**360(T6)**
PCV (%)	34.78 ± 0.03^{e}	32.03 ± 0.03^{d}	32.69 ± 0.01^{d}	31.34 ± 0.02^{c}	30.82 ± 0.04^{b}	31.43 ± 0.01^{a}
HB (g/dl)	11.12 ± 0.03^{e}	10.32 ± 0.00^{d}	10.25 ± 0.00^{d}	9.94 ± 0.02^{c}	9.26 ± 0.04^{b}	9.03 ± 0.01^{a}
WBC ($x10^{9}$/L)	7.21 ± 0.05^{a}	7.50 ± 0.00^{b}	6.43 ± 0.03^{b}	6.38 ± 0.03^{c}	6.15 ± 0.10^{d}	6.05 ± 0.05^{e}
RBC($x10^{12}$/L)	2.01 ± 0.05^{e}	1.97 ± 0.03^{d}	1.90 ± 0.00^{d}	1.86 ± 0.06^{c}	1.90 ± 0.03^{b}	1.75 ± 0.05^{a}
PLT ($x10^{9}$/L)	121.65 ± 0.50^{a}	116.57 ± 0.50^{b}	118.47 ± 1.00^{c}	120.50 ± 1.00^{d}	118.30 ± 1.00^{e}	118.50 ± 1.50^{f}
MCH (Pg)	64.56 ± 0.35^{a}	53.13 ± 0.21^{b}	53.11 ± 0.00^{b}	48.51 ± 0.47^{b}	52.64 ± 0.11^{b}	47.20 ± 0.85^{c}
MCV (fL)	144.31 ± 0.77^{a}	138.75 ± 0.54^{ab}	142.37 ± 0.03^{c}	140.27 ± 1.49^{b}	141.53 ± 0.71^{c}	137.7 ± 2.59^{d}
MCHC (%)	48.26 ± 0.13^{e}	45.24 ± 0.04^{c}	39.78 ± 0.01^{c}	36.92 ± 0.05^{d}	40.46 ± 0.13^{b}	41.27 ± 0.06^{a}

Mean in the same column with different superscripts differ significantly (P< 0.05)

Agbo-Hegab *et al.* (1993) and Isiyaku *et al*., (2015) attributed the stress-induced decreases in hemoglobin and hematocrit values to blood heam dilution, red blood cell loss, and imbalances in osmotic pressure inside and outside the blood cells. These findings are consistent with Elbialy, *et al.* (2015), which reported significant reductions in red blood cell count, packed cell volume, hemoglobin, as well as increases in neutrophilia, lymphopenia, and monocytosis in Nile Tilapia (*Oreochromis niloticus*) exposed to various organochloride and organophosphate pesticides. However, these results differ from Velisek *et al*. (2008), who found significantly higher values (P<0.01) of erythrocyte count, hematocrit, and hemoglobin in rainbow trout (*Oncorhynchus mykiss*) exposed to the herbicide metribuzin. Additionally, the findings of this study contradict those of Isiyaku *et al.* (2021), which showed increased packed cell volume in *C. gariepinus* juveniles exposed to tamarind seed husk powder.

The present study reports changes in MCV, MCH, and MCHC values induced by *Datura innoxia* stem, either increasing or decreasing. Since these values are derived from total erythrocyte counts, hemoglobin, and PCV, a significant decrease in total erythrocyte counts can directly affect MCV and MCH values. This suggests that the toxicant interfered with the normal physiology of erythrocytes. MCV provides insight into the size and condition of erythrocytes (Nussey *et al*., 1995). Adeyemo (2007) reported a significant increase in MCV, MCH, and MCHC values in *Clarias gariepinus* exposed to lead. Similarly, Bhagwant and Bhikajee (2000), Siang *et al.* (2007), and *Ayuba et al.,* (2012) observed a decrease in MCH and MCHC values and an increase in MCV values in *Oreochromis niloticus*, *Monopterus albus* and *Clarias gariepinus* exposed to jimson weed, endosulfan, and *Datura innoxia* leaf extract. Shah (2006) and Isiyaku *et al.* (2021) noted that chemical-induced changes in MCV, MCH, and MCHC were due to direct or feedback responses to structural damage of red blood cell membranes, resulting in hemolysis, impaired hemoglobin synthesis, stress-induced release of red blood cells from the spleen, and hypoxia.

CONCLUSION

It was concluded that *Datura innoxia* stem are harmful to *Clarias gariepinus* juveniles. Significant haematological alterations were noted in *C. gariepinus* juveniles exposed to *Datura innoxia* stem for 96 hours, with statistically significant differences (P<0.05) observed between the control and those exposed to various stem concentrations across all haematological parameters assessed.

RECOMMENDATION

The research suggests regulating the use of *Datura innoxia* stem in aquatic environments to prevent contamination and safeguard aquatic organisms from such toxic substances**.**

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