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Haematological Response and Weight Changes of the African Catfish *Clarias gariepinus Exposed* To Sub Lethal Concentrations of *Datura innoxia* Root Extract

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Abstract

A Laboratory study was carried out to assess the sublethal effect of Datura innoxia root extract on the haematology and weight gain of Clarias garienpinus fingerlings using the static bioassay with continuous aeration method. Result indicated significantly (P > 0.05) lower weight gain of 13.30g in exposed fish in concentration 100.00mg/l compared to the control group which was 17.6g. Specific growth rate was 0.63 for the control while in the highest concentration (100.00mg/l) it was 0.33.All the haematological parameters Haemoglobin of 8.88mg/l , Red blood cells 2.28 x $10^6 mm^{-3}$, Erythrocyte sedimentation rate of 2.35 mm/h mm, packed cell volume 44.98%, mean corpuscular volume 197.11 f/l and mean corpuscular haemoglobin concentration 25.87 g/e of the control were higher than in the exposed fish of concentration 100.00mg/l for the following parameters: Haemoglobin 8.57 mg/l, Red blood cells1.93 x $10^6 mm^{-3}$, Erythrocyte sedimentation rate 2.63 mm/h. However white blood cells increased with increase in concentration of toxicant. It is obvious from this study that sublethal concentrations of D. innoxia root extract did not kill C. gariepinus fingerlings but impaired their physiological well being.

***Key words**: Clarias gariepinus, Fingerlings, Datura innoxia, Sublethal, Haematology*

Introduction

The use of haematological techniques for toxicological research, environmental monitoring and assessment of fish health conditions is gaining importance (Shah and Altindag, 2004). Often, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish due to their close association with the environment. Maheswaran *et al.*, (2008) noted that studies on fish blood gives the possibility that fish blood will reveal conditions within the fish long before there is an outward manifestation of diseases. Man has always regarded plants as one of the most valued components of the biosphere because of their uses as food and medicine and their chemical values. Of particular interest is *Datura innoxia,* which belongs to the family Solanaceae. The family is of great economic importance as a source of drug in medicine and pharmacology but can be poisonous if taken in excess. Djibo and Bouzon (2000) reported the use of *Datura innoxia* flowers and seeds for voluntary intoxication in Niger. Ayuba and Ofojekwu (2002) who observed a dose dependent toxic effect of root extract of *D. innoxia* on cat fish fingerlings reported its acute toxicity (LC_{50}) to *Clarias gariepinus* fingerlings to be 204.17mg/l. The safety evaluation of use of *D.*

innoxia root depends greatly on the use of test animals following which inference is drawn about its risk to human health. Since there are numerous interactions between biota and their environment, the complexity of these interactions makes it theoretically impossible to predict the hazard which some plants such as *D. innoxia* may pose to the environment. *Clarias gariepinus,* which is an ideal aquaculture species in Nigeria, thrives in diverse environments. It is hardy and adaptable principally as a result of its air breathing ability, feeds on a wide array of food under diverse conditions, and is able to withstand adverse environmental conditions. It is highly fecund and easily spawned under captive conditions. It is highly resistant to diseases and its potential for intensive culture with relatively poor water quality (FAO, 2000) made it the choice for this study. Literature search revealed limited information on the toxicity of *Datura innoxia* root to fish, the present study was aimed at evaluating the haematological response and weight changes in the African Catfish *Clarias gariepinus* exposed to sub lethal concentrations of *D. innoxia* root extract.

Materials and Methods

Fresh samples of *Datura innoxia* roots were collected from University of Agriculture, Makurdi, Nigeria and brought to Hydrobiology and Fisheries Research Laboratory, University of Jos Nigeria, where they were washed and air dried to constant weight. The dried samples were pounded using a clean laboratory mortar to a fine powder which was then sieve through 0.25mm sieve. Five hundred grams of the resultant powder was dissolved in 2 litres of water at room temperature (23± 0.5^{o}C) for 24hours. The extract was filtered through Whatmanôs filter paper (No 1) with the aid of a vacuum pump. The filtrate was freeze dried and used for the experiment.

The fish*, Clarias gariepinus*, fingerlings weighing 12.3 g (± 0.35) were collected from Rock water fish farm, Jos, Plateau State. The fish were transported in oxygenated bags to the Fisheries and Hydrobiology Research Laboratory, University of Jos. They were kept in glass aquaria tanks 60 x 40 x 80cm (115.2L capacity) where they were acclimated to laboratory conditions for two weeks prior to exposure period. During that period and the exposure period the fish were fed 4% of their body weight twice daily (0900 and 1600 hours) with laboratory formulated fish feed (Table 1). Each aquarium was supplied with dechlorinated, well-aerated municipal tap water, Mortality was less than 2% during acclimation. After the acclimation period, the fish were randomly selected and stocked 10 fish per aquarium in 18 glass aquaria for the experimental runs. The following concentrations were made from the freeze dried samples of the root extract after a test run and delivered into the first six glass aquaria: 100.00,50.00,25.00, 12.50, 6.25 and 0.00mg/l. The 0.00mg/l served as the control experiment while the remaining 12 served as replicates. Using static bioassay method with continous

aeration,,fresh preparations were introduced every 24 hours following the methods of Reish and Oshida, (1987). The fish were weighed individually at the start of the experiment and fortnightly thereafter. Mean weights were computed for each treatment for each weighing period. The amount of feed given was adjusted to the new weights. The specific growth rate (SGR) was computed using the formula by Brown (1957).

$$SGR=(\log_e W_2-\log_e W_1 x100/T_2-T_1)$$

FCR (feed intake/weight gain)

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Some water quality parameters (Temperature, dissolved oxygen, free carbon dioxide,total alkalinity and pH) of the experimental aquaria were monitored every 24 hours using the methods described by APHA *et al*. (1980)

At the end of the exposure period which lasted twelve weeks *D. innoxia* root extract is used as a substitute or inclusion in the feeds ingredients then your experimental design is faulty. the caudal peduncle of exposed fish was cut with a sharp blade and blood collected by means of disposable sterile syringe fitted with insulin needle. Owing to insufficient amount of blood, the haematocrit determination was done on pooled blood sample (from 3 fishes) in sterile heparanised vials. Blood filled heperanized mirohaematocrit capillary tubes were centrifuged at 12000 for 5minutes using a microhaematocrite centrifuge (Hermle model Z320) and the haematocrit (Hct) values were red directly. The haemoglobin concentration was measured by the cyanmethaemoglobin method (Blaxhall and Daisley, 1973) at a wavelength of 540nm.The total red blood cell (RBC) count and Mean Corpuscular Volume (MCV) was obtained by employing a Coulter ï model T540 cell counter. Erythrocyte sedimentation rate (ESR) was measured by an adaptation of the standard Wintrobe method (Blaxhall and Daisley, 1973).The Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration MCHC) were calculated using the methods described by Dacie and Lewis (1963) MCH was calculated in picograms/cell=Hb/RBCx10 and MCHC= (Hb in 100mg blood/Hct) x 100. Blood sampling was completed within 2 minutes to minimize the risk of stressful condition. Data obtained were analyzed using analysis of variance (ANOVA)

Results and Discussion

The mean weight gain by the exposed fish to the various concentrations of *D. innoxia* root extract is presented Fig. 1. The highest concentration (100.00 mg/l) gave the least weight gain (13.30g). Statistical analysis showed that the test fish exposed to the various sub-lethal concentrations of *D. innoxia* root extract had significantly ($P > 0.05$)

lower weight gain compared to the group of fish placed in water devoid of the root extract.

Specific growth rate of *C. gariepinus* exposed to the various sub-lethal concentrations of *D. innoxia* root extract is presented in Fig. 2. The highest specific growth rate (0.62) was observed in the concentration devoid of *D. innoxia* root extract. While the highest concentration (100 mg/l) had the least specific growth rate (0.33). Statistical analysis showed that the test fish exposed to the various sub-lethal concentrations of *D. innoxia* root extract had significantly ($P > 0.05$) lower specific growth rate compared to the group of fish placed in water devoid of the root extract.

Table 2 shows the mean values of the haematological indices recorded from exposing *C. gariepinus* to sublethal concentratios of *D. innoxia* root extracts. Differences measured against the control values showed an increase in extract concentration produced a significant reduction in the number of RBC during the exposure period, fish exposed to maximum concentration (100mg/l). had the lowest RBC count (1.93 ± 0.91).There was a significant ($P<0.05$) increase in WBC compared to the control experiment with the highest concentration (100mg/l) having the highest WBC count (39.75 ± 0.86). On the other hand haemoglobin (Hb) Haematocrit (Ht) and Erythrocyte sedimentation rate (ESR) decreased with increase in *D. innoxia* root extract Mean corpuscular volume, even though decreased with increased concentration, was not significantly ($P>0.05$) different from that of the control. Of particular interest is the fluctuation observed in both MCH and MCHC variables in exposed *C. gariepinus* fingerlings for different concentrations

During the exposure period of the toxicity test, it was observed that the mean values of the water quality parameters were not significantly different ($P > 0.05$) for temperature and pH. Even though, dissolved oxygen, free carbon dioxide and total alkalinity values differ significantly ($P < 0.05$) compared to the control values, they were still within acceptable limits as recommended by Mackereth, (1963). The result of the mean values is presented in Table 3.

Table 1 Ingredient and proximate composition of diet fed to *Clarias gariepinus*

INGREDIENT	DIET
Fish Meal	30
Soyabean Meal	35
Meat & Bone Meal	10
Rice Bran	15
Corn Oil	3
Vitamin & Mineral Mix	5
Starch	2
PROXIMATE COMPOSITION	
Crude Protein	44.5
Crude Fat	8.6
Crude Fibre	5.1
Ash	16.4
NFE	25.5

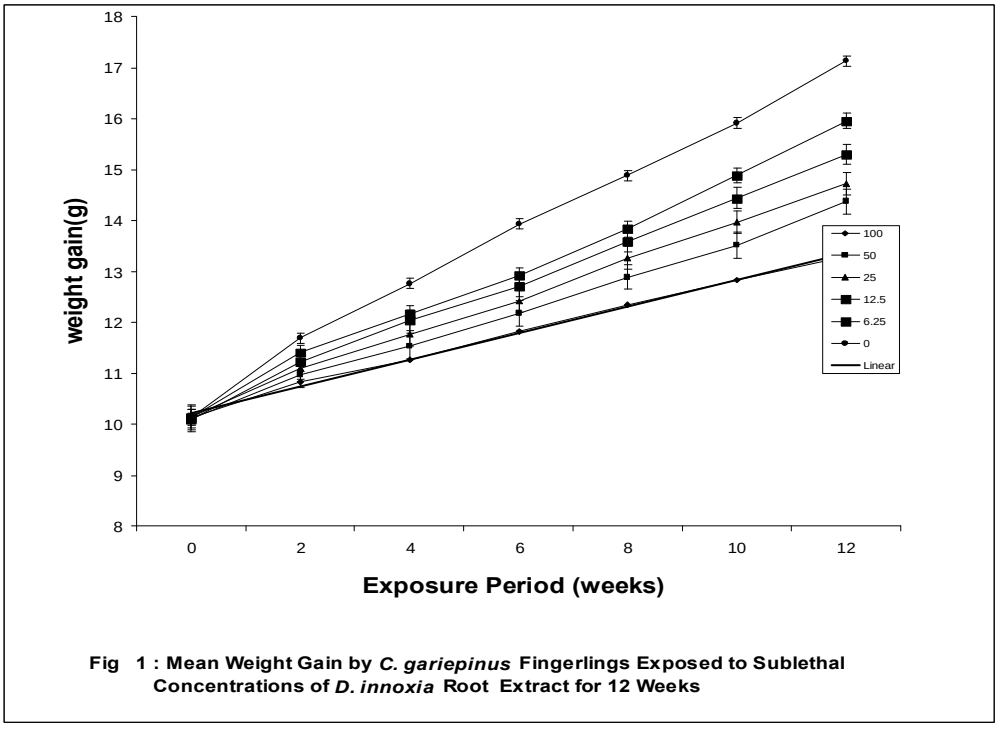

Fig 1 : Mean Weight Gain by *C. gariepinus* Fingerlings Exposed to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks

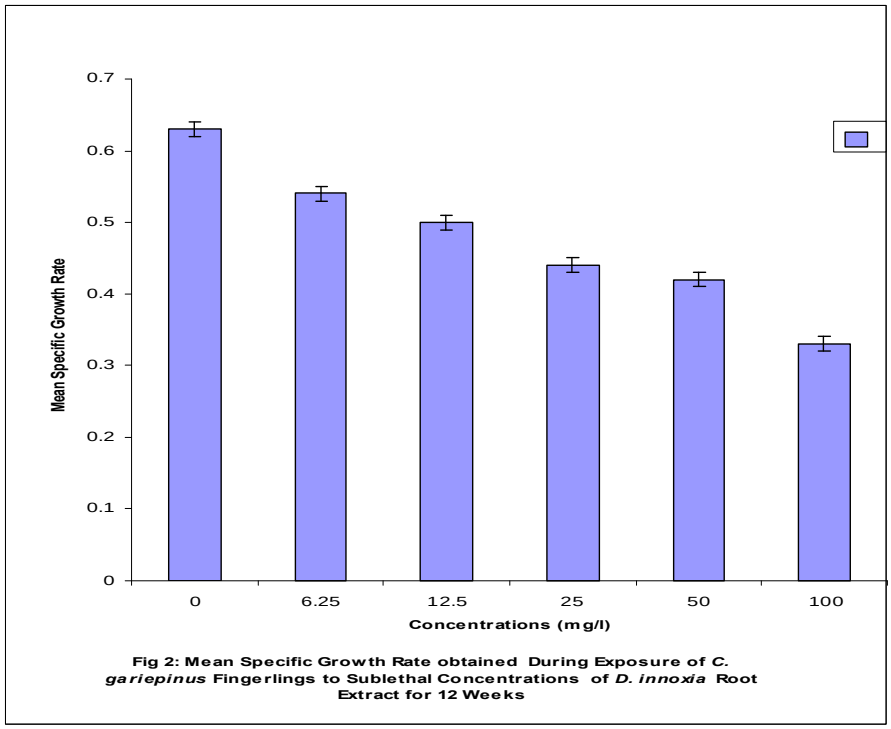

Fig 2: Mean Specific Growth Rate obtained During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Root Extract for 12 Weeks

Table 2: Haematological Parameters of *C. gariepinus* Fingerlings Exposed to Sublethal concentrations of *D. innoxia* Root Extract

Haematological Parameters							
Con(mg/l)	Hb(mg/l)	RBC (x $10^6 mm^{-3}$)	MCHC(g/e)	PCV(%)	MCV(f/l)	ESR(mm/h)	WBC (x $10^3 mm^{-3}$)
0.00	8.88 (1.06)	2.28 (0.86)	25.87(0.10)	44.98 (0.16)	197.11 (0.11)	2.35 (0.30)	38.85 (0.70)
6.25	8.81 (0.07)	2.23 (0.92)	26.05(0.28)	43.33 (0.17)	194.30 (0.14)	2.40 (0.27)	39.10 (0.63)
12.50	8.77 (0.83)	2.16 (0.71)	26.49(0.11)	42.26 (0.10)	195.65 (0.18)	2.44 (0.27)	39.25 (0.41)
25.00	8.69 (0.80)	2.07 (0.53)	26.38(0.16)	41.08 (0.19)	198.45 (0.16)	2.45 (0.33)	39.40 (0.32)
50.00	8.62 (0.75)	2.01 (0.64)	27.09(0.14)	39.00 (0.22)	194.03 (0.10)	2.52 (0.26)	39.50 (0.61)
100.00	8.57 (0.83)	1.93 (0.91)	28.44(0.26)	38.12 (0.16)	197.51 (0.13)	2.63 (0.21)	39.75 (0.86)

($P > 0.05$)

Table 3: Water Quality Parameters During Exposure of *C. gariepinus* Fingerlings to Sublethal Concentrations of *D. innoxia* Root Extract

Parameters	**Concentrations (mg/l)**					
	100.00	50.00	25.00	12.50	6.25	0.00
Temperature (^{o}C)	20.13	20.13	20.13	20.13	20.13	20.13
	(0.26)	(0.26)	(0.26)	(0.26)	(0.26)	(0.26)
Dissolved oxygen (mg/l)	6.32	6.49	6.96	7.20	7.52	7.88
	(0.26)	(0.30)	(0.43)	(0.44)	(0.36)	(0.58)
Free carbondioxide (mg/l)	5.29	5.14	4.83	4.38	3.87	3.46
	(0.34)	(0.35)	(0.34)	(0.33)	(0.31)	(0.24)
Total alkalinity (mg/l)	30.33	29.25	28.08	27.08	26.25	26.08
	(1.12)	(1.09)	(1.32)	(1.40)	(1.30)	(1.27)
$_{p}$H	6.98	7.01	7.05	7.05	7.04	7.03
	(0.04)	(0.04)	(0.02)	(0.02)	(0.02)	(0.02)
	0.21	0.21	0.21	0.21	0.21	0.21
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

($P > 0.05$)

Growth responses of fish to most toxicants have been observed to be due the effect of the chemical, their concentrations, species size and specific environmental condition. The result of this study indicates that *D. innoxia* root extract had dose ï dependent effect on the weight gain of *C gariepinus* fingerlings. Similar results were obtained by Onusiriuka (2002), Ayuba and Ofojekwu (2005) when *C. gariepinus* fingerlings were exposed to sub-lethal concentrations of formalin and *D. innoxia* leaf extract respectively. Mean Specific growth rate with the highest value of 0.63 (±0.01) was observed in the control group and the lowest value of 0.33 (±0.01) in the highest concentration of 100mg//l. Significantly better growths were observed by some authors in control groups of certain fish than those exposed to toxicants; Omoregie and Onuogu (2000) in *Aphyyosemion gairdnerin,* Omoniyi *et al.,* (2002) in *C. gariepinus* fingerlings, Aderolu *et al.,* (2010) in *C gariepinus* fingerlings, Ayuba and Ofojekwu (2010) in *C. gariepinus* fingerlings.

Exposure of *C. gariepinus* fingerlings to sub-lethal concentration of *D. innoxia* root extract for 12 weeks caused a significant decrease in RBC, Hb, and MCV values. There was a gradual decrease in the mean levels of PCV from 44.98% in the control to 38.12% in the100 mg/L *D. innoxia* exposure group. The significant reduction in RBC and Hb content on exposure to sublethal concentration of root extract caused anaemia in *C gariepinus* fingerlings, the decline of MCV with increase in concentrations suggests that anaemic effect could be attributed the destruction of the erythrocytes or inhibition of erythrocyte production, Similar trends in RBC in fishes exposed to various toxicants have been observed and reported other workers (, Bhagwant and Bhikajee 2000.

Adeyemo 2005, Kori Siakpere *et al* .,2009, Gaafar *et al.,* 2010, Ololade and Oginni 2010). Saponin, which is known to haemolyse RBC was found to be present in *D. innoxia* (Eltohami 2002, Ayuba *et.,* 2011) which might explain the decline in haemoglobin content. Possibly the toxicants' (*D. innoxia* root extract) stress in the present study could have caused the anaemic condition in *C. gariepinus* fingerlings by haemolysis of the fish Red Blood Cells and thus reducing the Red Blood Cells count.

Conclusion

The exposure of *C. gariepinus* fingerlings to sub-lethal concentration of *D. innoxia* root extract for 12 weeks did not cause outright mortality but had effect on the physiology of the fish by significant reduction in growth rate as well as negative changes in haematological parameters.

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