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ACUTE TOXICITY AND HAEMATHOLOGICAL RESPONSE OF CLARIAS GARIEPIENUS JUVENILES EXPOSED TO AQUEOUS DATURA INNOXIA LEAF EXTRACT

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ABSTRACT
The acute toxicity of aqueous *Datura innoxia* leaf extract on *Clarias gariepinus* juveniles was accessed, five concentration of the extract where used during the definitive preliminary test as follows 125mg/l, 167mg/l 208mg/l 250mg/l and 292mg/l for a period of 96 hours on *Clarias gariepinus* juveniles of mean weight 31.07 ± 1.23g and mean length of 19.50 ± 0.5cm and stocked 10 fishes per treatment. Acute toxicity was carried out for 96 hours to determine LC_{50}. The 96 hours LC_{50} for *Datura innoxia* leaf extract was 176mg/l with regression coefficient of R^2 is 0.967. The pooled blood samples were collected at the end of the 96 hours of exposure to aqueous *Datura innoxia* leaf extract and were examined using digital analyser. Haematological variables showed significant decreases ($P<0.05$) of blood variables such as HCT, HB, RBC, MCHC and significant increase in values of WBC, PLT, MCH, and MCV during acute toxicity of Datura innoxia leaf extract.

Keywords: *Clarias garipienus*, *Datura innoxia*, water quality parameters, haematological parameters.

INTRODUCTION
The decision of *Datura innoxia* leaf extract over other plant toxicant is because it is the commonest plant species used by fisher men majorly in our inland water. This study was aimed at determining the effects on the physico-chemical parameters of the water, determine the acute toxicity and to investigate the effect of aqueous *Datura innoxia* leaf extract on the haematological parameters of juveniles of *Clarias gariepinus.*

Plants from different families have been applied for catching fish, treatment of diseases, control of predators and reduction of overpopulation in aquaculture ponds all over the world and are considered advantageous when viewed against the backdrop of using persistent chemical (Van Andel, 2000; Tiwari and Singh, 2003). Frequent application of high concentrations of these ichthyotoxins in water may have adverse effects not only on fish species but also on other aquatic fauna. Several studies have shown that plant toxins at very low concentrations are very toxic to all groups of aquatic fauna (Goktepe *et al.*, 2004; Gabriel and Okey, 2009). Different species of plants employed as piscicides have different effects, depending on the species of fish targeted (Van Andel, 2002).

Datura innoxia (thorn-apple, downy thorn-apple, Indian-apple, lovache, moonflower, sacred datura, nacazcul, toloatzin, tolguache or toloache) is a species in the family Solanaceae. It is native to Central and South America, and introduced in Africa, Asia, Australia and Europe. The scientific name is often cited as *D. innoxia* (Preissel *et al.*, 2002).

Datura innoxia, commonly known as downy thorn apple is an herbaceous perennial trailing or bushy herb that can grow up to 2 m high. It has softly gray velvety texture on all parts. The leaves are coarse with whole margin and are gray-velvety turning dark green. Flowers of *D.*

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innoxia are white, solitary, large funnel shaped and up to 200 mm in length (Henderson, 2001). They are monoceious (have both staminate and carpellate flowers on one plant) and are mostly pollinated by night flying sphinx moths, which look and act like small hummingbirds. But honeybees and other insects are attracted to the flowers too, and often manage to squeeze into them before they have opened. The fruits are brown hard spherical- shaped capsules, densely covered in slender spines less than 10mm long. The seed usually germinates in 1-3 weeks at 30°C and there is no need to pre-soak them. The whole plant and seeds are poisonous and can cause irritation of the skin. They are poisonous to people as well as to cattle, horses and sheep, *Datura innoxia* is a cosmopolitan weed occurring in all temperate, subtropical and tropical parts of the world. (Henderson, 2001).

Most *Datura* plants contain tropane alkaloids such as scopolamine, hyoscyamine, and atropine, primarily in their seeds and flowers. Because of the presence of these substances, *Datura* has been used for centuries in some cultures as a poison and hallucinogen (Preissel and Preissel, 2002; Adams *et al*., 2005). Qualitative and quantitative variations in haematological parameters including the red blood cell (RBC) and white blood cell (WBC) numbers, cell proportions of leukocyte, the amount of haemoglobin (Hb), and the size of RBC and WBC are the most significant findings as regards diagnosis.

Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Ateeq *et al.*, 2002).

MATERIALS AND METHODS

Experimental Fish

Juveniles of *Clarias gariepinus* of a mean weight of 31.07±1.23g and mean length of 19.50 + 0.50cm were obtained from Finite fish farm, Makurdi, Benue State. The fish were acclimatized for 14 days in the fish hatchery, Department of Fisheries and Aquaculture, University of Agriculture, Makurdi. The fish were feed twice daily at 0800 and 1600 hours at 5% of their body weight. Prior to and during exposure period fish were starved for 24hrs, to reduce faeces and ammonia in the experimental containers that could act as contaminants to the experiment.

Experimental Site

The study was carried out in the Fisheries Departmental Laboratory in the University of Agriculture Makurdi, Benue State.

Source and Preparation of *Datura innoxia* Leaf

The leaves of *Datura innoxia* was collected from the Makurdi Metropolis. Matured leaves was removed from *Datura* plant and air-dried to a constant weight at (25^{o}C). The dried samples was pounded and grounded separately into fine powder form and sieved through 0.25mm sieve. The powder was stored in airtight bottles for analysis.

Acute Bioassay Test

One hundred and eighty healthy Juveniles of *Clarias gariepinus* of a mean weight of 31.07±1.23g and mean length of 19.50 + 0.50cm were obtained from finite fish farm, Makurdi, Benue State. The fish were acclimatized for 14 days in the fish hatchery Department of Fisheries and Aquaculture, University of Agriculture, Makurdi. The fish were fed twice daily at 0800 and 1600 hours at 5% of their body weight. Prior to and during exposure period fish were starved for 24 hours, to reduce faeces and ammonia in the experimental containers that could act as contaminants to the experiment.

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After the acclimation period, preliminary test were carried out to determine lethal concentrations of *Datura innoxia*. From there, varied concentrations were selected and used for the experiments. Micro-pipette was used to collect the chemical *(Datura innoxia)* into the 18 plastic bowls making it up to 20 liters by using serial dilution. These concentrations were 125 mg/L, 167 mg/L, 208 mg/L, 250 mg/L, 292mg/l and 0 mg/L served as the control. The treatments were triplicated. Ten (10) fish specimens were selected randomly and stocked in each container. The physico-chemical characteristics of the water were analysed according to APHA (1995), Cengiz *et al.* (2001), Adeyemo (2005), and Ayoola (2008). Each concentration was refreshed daily during the experimental period. The exposure period lasted for 96 hours. During this period fish mortality was observed and recorded at 6 hours, 12 hours,18 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, 72 hours, 78 hours, 84 hours, 90 hours and 96 hours. Dead fish were removed and recorded immediately from the test solution to avoid fouling the media. Behavioural changes were observed by visual observation (Kori-siakpere *et al.*, 2007; Ayuba *et. al.*, 2012; Abubakar and Abdulsalami, 2013). The (96hr LC_{50}) was estimated by probit analysis and a slope function, upper and lower confidence limits were calculated.

Haematological Studies

At the end of the acute bioassay test, pooled blood samples were taken from randomly selected fish from the various treatments. This was done by injecting in a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation and analyzed at Tosema Specialist Diagnostic Laboratory, Old Otukpo Road, High Level, Makurdi, Benue State for the following: haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) using an automated haemoglobin analyzer (Cobus U 411) model, while Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were determined by calculations according to Svobodova *et al.* (2001).

Experimental Design

A complete randomized design was used for the experiment. A total of 180 juvenile of *Clarias gariepinus* were randomly distributed into the plastic containers at a stocking rate of 10 fish. The 18 tanks were assigned to 5 treatments with (control inclusive). In order to determine the LC50, the *Clarias gariepinus* were exposed to five different concentrations of *Datura innoxia* for 96hr. LC50 value obtained using EPA Probit Analysis programme version 1.5 which gave 178mg/l.

Method of Data Analysis

Data were analysed using Minitab 14 (Minitab, 2013) for summary statistics; this was carried out to determine if differences exist in water quality parameters for the acute and sub-lethal concentrations. Means in the haematological parameters were analysed using analysis of variance (ANOVA) at 0.05% level by Genstat version 13, to calculate the significant difference between control and experimental means, where differences exists between the mean, the means were separated using least significant difference (LSD)

RESULTS AND DISCUSSION

Analysis of Physico-chemical Parameters of the Test Solutions

The physico-chemical parameters of the test solutions in the acute bioassay are shown in Table 1. The physico-chemical parameters of the test water used for the acute and sub lethal bioassay shows significant difference in the pH, conductivity (ES), Total dissolved solids (TDS) and dissolved oxygen (DO). During the bioassay test, the pH, conductivity, Total

dissolved solids and dissolved oxygen increased with increase in the concentration of the extract. This is similar to Ayuba *et al.* (2012) and Ayuba and Ofojekwu (2002) who worked on *Datura innoxia* root extracts. Generally toxicity increased with reduced oxygen concentration as reported by Adigun (2003) and Kolo *et al.* (2008; 2009).

With regards to the acute bioassay, the abnormal behaviours which increased with increase in the concentration of *Datura innoxia* leaf extract on *Clarias gariepinus* juveniles were excessive gulping for air, erratic swimming behaviour, and restlessness, loss of equilibrium, and excessive secretion of mucus, skin hemorrhage, paralysis and finally death. The pattern of behavioural changes observed above in this study compared favorably with the records of Fafioye *et al.* (2004) when African catfish (*C. gariepinus*) was exposed to *Parkia biglobosa* and *Raphia vinefera*leaf extracts and catfish hybrid fingerlings treated with cassava mill effluents (Oti, 2002). These behaviours also are similar to the ones reported by Omitoyin *et al.* (2006), Omitoyin (2002), Ayuba and Ofojekwu, (2002), Ladikpo and Doherty (2011), Aderolu *et al.* (2010) and Okomoda and Ataguba, (2011). The increase in behavioural responses above as the concentration increases agrees with reported work by Bobmanuel *et al.* (2006) who stated that "behavioural response of fish to toxicants and different reaction time are due to the effect of chemicals, their concentrations, species, size of fish and specific environmental conditions". The mucus secretion of the fish during the acute bioassay is in line with that reported by Annune *et al.* (1994) and Ayuba and Ofojekwu, (2002) which stated that plant toxins even at low concentrations are known to produce mucus on the body and gills of fish. The observed air gulping and surfacing phenomenon attempts by the exposed fish to cope with the increasing demand for oxygen (Schmidt *et al.*, 2005). The irregular, erratic and darting movements coupled with the observed loss of balance and the adoption of different postures by the exposed fish might be due to tannins concentration of the extract. This movement agrees with the earlier work of Oti (2002), Oshode *et al.* (2008), Ezike and Ufodike (2008), when they exposed fish to acute concentrations of cassava mill effluent, leach from land fill and petrol toxicant, respectively. These behaviours suggest respiratory impairment, probably due to the effect of the toxicants on the gills and general metabolism as reported by Omoniyi *et al.* (2002) and Usman *et al.* (2010). Similar signs were reported in *C. gariepinus* exposed to the aqueous extract of *N. Tobaccum* leaf dust (Kori-Siakpere and Oviroh, 2011). The stressed ailment of respiratory impairment due to the toxic effects of *Daturainnoxia* leaf extract on gills was similar to the work of Omotoyin *et al.* (2006). Konar (1970) reported that accumulation of mucus on the gills reduces respiratory activity in fish. The inability of the gills surface to actively carryout gaseous exchange might be responsible for the observed mortalities in this study. This is similar with reports by Fafioye (2001) and Omotoyin *et al.* (2002).

Mortality was observed to be concentration and time dependent in this study. These were similarly observed by Ateeq *et al.* (2005) and Olurin *et al.* (2006) who worked on 2, 4-D butachlor and glyphosphate herbicides, respectively. Substances could be poisonous when exposed to organisms beyond certain concentrations and time limit according to Jauncy and Ross (1982), Ogundele *et al.* (2004) and Wikes (2010).

The LC_{50} of 178mg/L found in this study is lower than 204.17mg/l obtained by Ayuba and Ofojikwu (2002) for *Daturainnoxia*. However, the LC_{50} found in the study is higher to that obtained by Abalaka and Auta (2010) who reported 105.83mg/L for *Parkiabiglobosa*pods and 129mg/l for Lindane on Clarias gariepinus. The difference may be due to difference in age, parts of the plants used (toxicants) and environmental conditions (Botes *et al.*, 2008 and Borokini and Ayodele, 2012).

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Table 1: Water Quality Parameters of Experimental Units during the Acute Toxicity Test of *Daturainnoxia* Leaf Extract on *Clarias gariepinus* Juveniles

Treatment(mg/l)	Water Quality Parameters				
	Ph	Temperature (°C)	EC (µS/cm)	TDS (mg/l)	DO (mg/l)
0.0	7.22± 0.02^{f}	26.07± 0.07	831.0±0.57^{f}	415.67±0.33^{f}	4.62± 0.02^{a}
125	7.39± 0.02^{e}	26.07± 0.07	847.0± 2.52^{e}	423.67±1.33^{e}	4.53± 0.02^{b}
167	7.47± 0.02^{d}	26.10 ± 0.06	878.67±1.33^{d}	439.67±0.88^{d}	4.38± 0.02^{c}
208	7.73± 0.02^{c}	26.10± 0.06	894.67±0.33^{c}	447.33±0.33^{c}	4.31± 0.01^{d}
250	8.06± 0.01^{b}	26.10± 0.06	915.33±0.33^{b}	457.67±0.33^{b}	4.16± 0.00^{e}
292	8.14± 0.01^{a}	26.07± 0.07	931.33±0.67^{a}	465.67±0.33^{a}	4.07± 0.01^{f}

Mean in the same column with different superscripts differ significantly ($P<0.05$); Mean Numbers are Means ± Standard Errors, where; EC = Electric conductivity, TDS = Total Dissolve Solid and DO = Dissolved Oxygen Temperature, Dissolved Oxygen (DO), pH, Total Dissolved Solids (TDS) and electrical conductivity varied significantly ($P<0.05$) from the control.

There was no significant difference in the temperature of the control and the other treatments. The control had the lowest pH (7.22) and it increase with increase in concentration with TRT 5 (292mg/L) having the highest (8.14). Similarly, the control had the lowest conductivity (EC) of 831µS and total dissolved solid (TDS) of 415.67ppm while TRT 5 (292mg/L) had the highest EC and TDS of 931.33 µS and 465.67ppm respectively. The dissolved oxygen (DO) decrease with increase in concentration from the control (4.62mg/L) to TRT 5 (4.07mg/L). There was no significant difference between the control and treatments for temperature.

The result of mortality of *Clarias gariepinus* juveniles after 96 hours of exposure to *Datura innoxia* leaf extract are shown in Figure 1.

The highest mortality (83%) was recorded in Trt 5 (292mg/L) while the least mortality (27%) was recorded in Trt 1 (125mg/L). There was no mortality in the control treatment throughout the 96 hrs exposure.

From Figure 1, the 96hr LC_{50} of Datura inoxia leaf extract is 178 mg/L while the upper and lower limits are 201.23mg/L and 157.45mg/L respectively. The regression equation for the relationship was calculated to be Probit= 4.200Log of Conc - 4.466, log concentration and on R-square value, R^2=0.987. The expression, R^2 value indicates that, mortality rate of fish increased with increase in concentration of Datura innoxia leaf extract.

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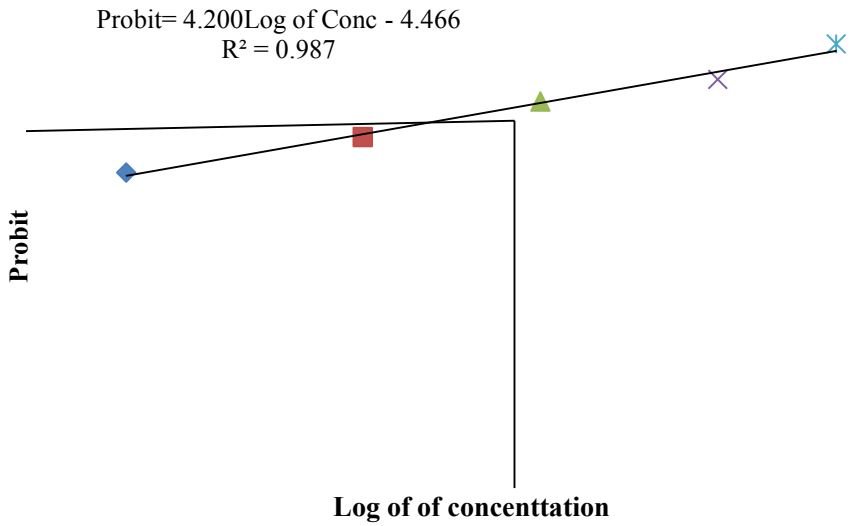

Figure 1: *Linear relationship between mean probit mortality and log concentration of clarias gariepinus juveniles exposed to acute concentrations of datura innoxia leaf extract for 96 hours.*

Analysis of Haematology Parameters of Juveniles of *Clarias*

Haematological parameters of juveniles of *Clarias gariepinus* exposed to acute concentration of *Datura innoxia* leaf extract are shown on Table 2. The results of the haematological analysis of *Clarias gariepinus* exposed to aqueous extract of *Datura innoxia* leaf showed a significant decrease in the Packed cell volume (PCV), Haemoglobin (HG), Red Blood cell (RBC) and Mean cell haemoglobin concentration (MCHC) in both the acute and sub-lethal bioassay but the decrease is much lower in the sub-lethal bioassay as shown in the result. Karuppaswamy (2005) found a significant decreased in total erythrocytes count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish *Channapunctatus* after exposure to sub-lethal dose of *Datura innoxia* leaf extract. Also, Saponin which is known to hemolyse Red Blood cell (RBC) was found to be present in *D. innoxia* which might explain the decline in haemoglobin content. Possibly the toxicants' (*D. innoxia* extracts) stress in the present study (acute and sub-lethal) could have caused the anaemic condition in *C. gariepinus* juveniles by haemolysis of the Red Blood Cells and thus reducing the RBCs count. Gaafar *et al.* (2010) reported that degeneration of the Red Blood Cell (erythrocytes) could be due to pathological condition in fish exposed to toxicant and prolong reduction in haemoglobin content is deleterious to oxygen transport. The variation and fluctuation of the Mean corpuscular Volume (MCV) between the control and treatments is due to different rate of expansion of unripe RBC (Cavalho and Fernandes, 2006) and resistance of the fish to the toxicant. Bhagwant and Bhikajee (2000) and Ayuba *et al.* (2012) observed similar fluctuations.

The white blood cell (WBC) and platelets count increased with increase in the concentration of the leaf extract. The high increase in the WBC during the acute bioassay (3.90-13.35) unlike the sub-lethal bioassay (2.40-4.20) is as a result of the high concentration of the leaf extract in the acute bioassay. WBC (granulocytes, monocytes, lymphocytes and thrombocytes) plays major role in the defence mechanism of fish. They are involved in the adjustment of immunological work in many organisms and the increase in the WBC count

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from the control to treatment 5 shows a generalized immune reply and a defensive response to the toxicant (Witeska, 2004; Saravanan *et al.*, 2011).

Table 2: *Heamatological Parameters of Clarias gariepinus Juveniles Subjected to Acute Doses of Datura innoxia Leaf Extract for 96 hours*

Parameters	Treatments (mg/L)					
	0.00	**125**	**167**	**208**	**250**	**292**
HCT	32.00±0.00^{a}	30.00±0.00^{a}	27.65±0.15^{b}	25.10±0.10^{c}	23.55±0.05^{d}	20.65±0.15^{d}
HB	10.55±0.05^{a}	9.35±0.05^{b}	8.15±0.05^{c}	7.30±0.10^{d}	6.65±0.05^{d}	5.45± 0.05^{e}
WBC	3.90 ± 0.10^{f}	5.40 ± 0.00^{e}	6.45±0.05^{d}	7.70± 0.00^{c}	9.70± 0.10^{b}	13.35 ±0.05^{a}
RBC	4.15± 0.05^{a}	3.90± 0.00^{b}	3.50±0.00^{c}	3.15 ±0.05^{b}	2.50± 0.00^{d}	1.95± 0.05^{e}
PLT	30.50±0.50^{f}	42.50±0.50^{e}	52.00±1.00^{d}	61.00±1.00^{c}	71.00±1.00^{b}	83.50±1.50^{a}
MCH	25.43±0.43^{c}	23.98±0.13^{d}	23.29±0.15^{d}	23.19±0.69^{d}	26.60±0.20^{b}	27.96±0.46^{a}
MCV	77.12±0.93^{d}	76.92±0.00^{d}	79.00±0.43^{c}	79.70±0.95^{c}	94.20±0.20^{b}	105.99±4.93^{a}
MCHC	32.97±0.16^{a}	31.17±0.16^{b}	29.47±0.34^{c}	29.09±0.52^{c}	28.24±0.27^{d}	24.75±2.06^{e}

Mean in the same row with different superscripts differ significantly (P<0.05); Numbers are Means ± Standard Errors. Haematological indices such as Packed Cells Volume (PCV), Red Blood Cells (RBC), haemoglobin, platelets, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cells (WBC) varied significantly (P<0.05) from the control.

The control had the highest PCV, HB, RBC and MCHC of 32%, 10.55 g/dl, 4.15 x10^{12}/l and 32.97% respectively while TRT 5 (292mg/L) had the lowest PCV (20.65%), HB (5.45g/dl), RBC (1.95 x10^{12}/l) and MCHC (24.75%). The PCV, HB, RBC and MCHC decreases with increase in concentration of the *Datura innoxia* extract. Treatment 5 (292mg/L) had the highest WBC (13.35 x10^{9}/l), PLT (83.50 x10^{9}/l), MCH (27.96 pg) and MCV (113.88 fl) while the control had the lowest WBC, PLT, MCH and MCV of 3.90 x10^{9}/l, 30.50 x10^{9}/l, 25.43pg and 77.12fl, respectively.

CONCLUSION AND RECOMENDATIONS

The study concludes that *Datura innoxia* leaf has been found to be poisonous to *Clarias gariepinus* juveniles at a concentration 178mg/l with upper and lower confidence limit being 201mg/l and 157.45mg/l Haematological changes observed in *C. gariepinus* juveniles expose to *Datura innoxia* leaf for 8 weeks indicated that there was significant difference (P<0.05) between *C. gariepinus* juveniles of the control and those exposed to aqueous concentration of *Datura innoxia* leaf for all the haematological parameters measured. Based on the findings of the research, it recommends that the use of *Datura innoxia* leaf extract in water bodies should be controlled to prevent contamination of the aquatic environments. In this way aquatic organisms could be protected from these kinds of toxic chemicals.

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