

Haematological Reaction Of *Clarias Gariepinus* (Burchell 1822) Juveniles Exposed To *Tetrapleura Tetraptera* Leaf Powder

T Jegede

Citation

T Jegede. *Haematological Reaction Of Clarias Gariepinus (Burchell 1822) Juveniles Exposed To Tetrapleura Tetraptera Leaf Powder*. The Internet Journal of Toxicology. 2013 Volume 10 Number 1.

Abstract

Acute toxicity of *Tetrapleura tetraptera* leaf powder on *Clarias gariepinus* juveniles (46.68 ± 0.62 g) was conducted using static bioassay tests over a period of 96 hour. The range finding test was conducted to determine the lethal concentration of the botanical on *C. gariepinus* juveniles and was found to induce varying behavioral response in the fish. The 96 h median lethal concentration, LC₅₀ of 1.60 g L⁻¹ was determined graphically. Percentage mortality of the test organisms followed a regular pattern, increasing with decreasing concentration. Prior to death, fish exhibited marked behavioural changes like hyperventilation, erratic swimming (vertical/spiral uncoordinated swimming movement), irregular operculum and tail frequencies, loss of reflex and settling at the bottom. Haematological analysis carried out after experiment showed significant haematological variations, the Pack Cell Volume (PCV), White Blood Cell (WBC), Red Blood Cell (RBC), Platelet and Lymphocyte decreases as concentration of *Tetrapleura tetraptera* leaf dust increases. The Dissolved oxygen (DO₂), pH and temperature values of the water were within tolerable limits for fish culture.

INTRODUCTION

The use of ichthyotoxic plants for capturing fish is a common practice worldwide (Ayotunde et al., 2011).

Fisher folks in Africa extensively use plants and plant products for capturing fish (Neuwinger, 2004; Fafioye et al., 2004). Indiscriminate use of these natural biocides in Nigeria water bodies is now increasing at an alarming rate (Fafioye et al., 2004). Fish farmers and fisher folks haphazardly use various kinds and parts of these plants due to their narcotic, pesticidal and molluscidal properties in other to stupefy fish for easy catch also to clean up the aquatic ecosystems off some pests (Ologe and Sogbesan 2007). Studies have been conducted on the response of fish to some plant toxicants (Jegede and Olanrewaju 2012; Olufayo 2009; Fafioye et al 2004; Wade et al., 2002; Ayuba and Ofojekwu, 2002; Ufodike and Omoregie, 1994; Omoregie and Okpanachi, 1992; Omoregie and Ufodike, 1994); but the piscicidal effect of *Tetrapleura tetraptera* leaf powder on fish has not been given much attention.

Tetrapleura tetraptera is a species of flowering plant in the pea family native to Western Africa, (Steentoft 1988). The plant is called Prekese in the Twi language of Ghana, (Osei-tutu et al., 2011)

Tetrapleura tetraptera belongs to the family Fabaceae,

commonly known as Aridan in the south-western Nigeria (Aladesanmi 2007). It is a single stemmed, robust, perennial tree of about 30m. It has a grey-brown, smooth-rough bark with glabrous branchlets. The flower is yellow or pink and racemes is white, while the fruit is dark brown, with four winged pods 25 x 6.5cm. It is generally found in the lowland forest of tropical Africa. The fruit consists of a fleshy pulp with small, brownish-black seeds. The fruit possesses a pungent aromatic odour, which is attributed to its insect repellent property (Aladesanmi 2007). It is used as spices and aroma (exotic tropical scents) and fish poisoning. *Tetrapleura tetraptera* is a known piscicides (Fafioye 2005) and a molluscicides (Lekana-Douki et al., 2011; Aladesanmi 2007; Adewunmi 2001). The leave contains active ingredients like aridanin, tannins, flavonoids, umbelliferone and ferulic acid (Aladesanmi 2007).

Clariid catfish constitute the main food fish family of economic value in Africa (Adebayo and Fagbenro 2004). It's one of the vital genera in the family Clariidae. *Clarias gariepinus* is the most cultured fish in Nigeria and indeed Africa and the third in the world (Haylor 1992). It is hardy: due to the presence of arborescent air breathing organ, omnivorous feeding habit, better growth rate, better feed conversion, ability to withstand adverse environmental

condition, high fecundity and ease of culture (Hecht et al., 1996). The fish is of a high demand because of its high quality and better taste of its flesh (Sogbesan and Ugwumba, 2008). Hence this study was undertaken to determine the median lethal concentration of *Tetrapleura tetraptera* leaf powder on *Clarias gariepinus* juveniles during the exposure period (96 hours) and to establish the influence of *Tetrapleura tetraptera* leaf powder on the blood parameters of *C. gariepinus* juveniles.

MATERIALS AND METHODS

Two hundred apparently healthy fingerlings of *Clarias gariepinus* juvenile mixed sex and the same brood stock, mean weight (46.68 ± 0.62 g) were procured from Ministry of Agriculture, Forestry and Fisheries Resources, Alagbaka, Akure, Ondo State.

They were transported live to Fisheries Management laboratory of Ekiti State University, Ado Ekiti, Ekiti State in a 50 litre capacity plastic container, half filled with pond water between 1700-1730h. They were later stocked in rectangular fibre tanks (75 x 40 x 40) cm, 60-litre capacity where they were allowed to acclimatize for 7 days. Ten *C. gariepinus* juveniles (46.68 ± 0.62 g) were stocked into each tank, with three replicates per treatment. *Tetrapleura tetraptera* leaves were collected along Ilokun village settlement, Ekiti State, Nigeria, it was shade-dried at ambient and milled into fine particle size ($< 250 \mu\text{m}$); and kept in a dry, clean, air-tight transparent plastic container. The treatments are: Treatment 1, 1.0g *T. tetraptera* /L-1 of water; Treatment 2, 1.6g *T. tetraptera* /L-1 of water; Treatment 3, 2.2g *T. tetraptera* /L-1 of water; Treatment 4, 2.8g *T. tetraptera* /L-1 of water and Control, 0 g *T. tetraptera* /L-1 of water. Prior to the commencement of the experiment, the fish were starved for 2 days, to minimize waste in the test media and to prevent organic decomposition and oxygen depletion during blood collection. Temperature, pH, dissolved oxygen, and conductivity level were determined using standard methods and readings were taken at 24 h interval for 96 h.

Fish samples were gently removed (with care) per treatment using a hand net in order to avoid stress; they were anaesthetized and weighed using a Metler Top Loading balance (Model P13 8001); the fish showed no symptoms of stress or diseases. Blood samples were collected from each treatment group. About 5-10ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant; it was properly mixed and kept in

the refrigerator for haematological analysis. Packed-Cell Volume (PCV) was determined thus: heparinized micro-haematocrit capillary tubes were filled with blood and centrifuged for 5 minutes at 15,000 rpm. PCV was calculated using a micro-haematocrit reader and it was expressed as a percentage (Svobodova et al., 1991). Haemoglobin concentration was determined using the cyanomethaemoglobin method. 2 ml of blood was pipetted and mixed with the diluent; the mixture was centrifuged to remove suspended cellular materials and the readings were made in a spectrophotometer. Erythrocyte count was made using the methods of (Svobodova et al., 1991); plasma obtained from the samples used in PCV determination, was put into Goldberg's Refractometer (Model 10400A) at 200C and the total plasma protein was determined by direct reading (gm/100 ml). Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing the haemoglobin content in g/100ml by the PCV/100ml of blood. MCH was determined from the haemoglobin value (Hb) and from the erythrocyte count (Bouck and Ball (1966).

RESULTS

The following behaviours were exhibited during the definitive test; loss of balance, respiratory distress (hyperventilation), erratic swimming (vertical/spiral uncoordinated swimming movement), irregular operculum and tail frequencies, loss of reflex and settling at the bottom which revealed sensitive indicators of physiological stress in fish.

Fish mortality at varying concentrations increased with increasing concentration of *Tetrapleura tetraptera* leaf powder. All fish in the control tank survived the experimental duration of 96 hours.

There was significant loss of fish with increase in *Tetrapleura tetraptera* leaf concentration ($P < 0.05$). The LC50 was determined graphically to be 1.60g *Tetrapleura tetraptera* leaf powder / L- 1 of water.

Water samples were collected weekly at a depth from each fibre tank. Dissolve oxygen (DO₂) and temperature were measured using oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Spring, OH, USA) and mercury in glass thermometer, respectively. pH was measured with pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). In all treatments, DO₂ concentrations decrease with the increase in the concentration of *Tetrapleura tetraptera* leaf powder from 0.1-2.8 mg/L, water temperature average was 27.6 °C, pH value ranged from 6.3 - 8.6. All the water quality parameters

were within the acceptable range for fish growth (Environment Protection Authority, EPA (2003). There was significant reduction ($p < 0.05$) in the values of the blood parameters of *C. gariepinus* juveniles after exposure to *Tetrapleura tetraptera* leaf powder concentration for 96 h. Pack cell volume reduces from 26.50 ± 1.50 in the control to 15.02 ± 0.05 in Treatment 4(T4), white blood cell reduces from 25.45 ± 3.80 g mm⁻¹ in the control to 20.03 ± 0.03 g mm⁻¹ in Treatment 4(T4), which is the highest concentration, 2.8 g L^{-1} *Tetrapleura tetraptera* leaf powder concentration, the red blood cell reduces from 3.21 ± 0.40 in the control to 1.55 ± 0.35 in T4, platelet decreased from 91.00 ± 5.00 in control to 76.02 ± 0.03 in Treatment 4(T4). While, Neutrophil increase from $33.00 \pm 2.00\%$ in the control to $64.50 \pm 0.05\%$ in Treatment 4(T4), Lymphocyte decreases from 66.5 ± 2.50 (%) in control to 34.00 ± 0.02 (%) in Treatment 4 (T4) which is the highest concentration. Finally, monocyte increases from 1.50 ± 0.50 (%) in the control to 2.02 ± 0.03 (%) in Treatment 4(T4).

Figure 1

Effect of *Tetrapleura tetraptera* leaf powder on mortality of *Clarias gariepinus* juveniles

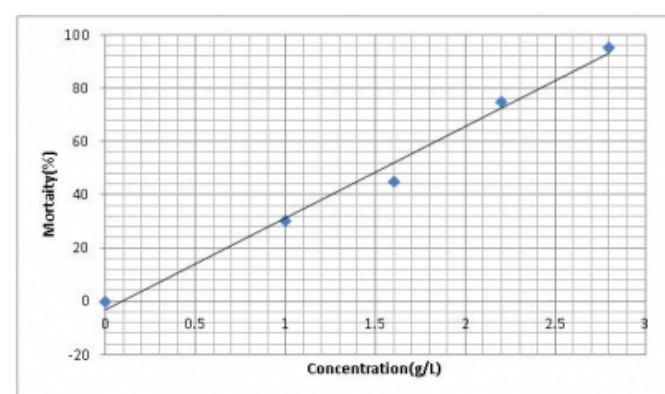


Table 1

Haematological parameters of *Clarias gariepinus* juvenile subjected to *Tetrapleura tetraptera* leaf powder concentration for 96 hours.

HAEMATOLOGICAL PARAMETERS	Control	T1(1.6g/L)	T2(1.6g/L)	T3(2.2g/L)	T4(2.8g/L)
PCV (%)	26.50 \pm 1.50	17.00 \pm 0.01	16.10 \pm 0.1	15.05 \pm 0.05	15.02 \pm 0.05
WBC($\times 10^9$ /L)	25.45 \pm 3.80	21.63 \pm 0.03	20.63 \pm 0.03	20.02 \pm 0.03	20.03 \pm 0.03
RBC($\times 10^{12}$ /L)	3.21 \pm 0.40	2.05 \pm 0.05	1.63 \pm 0.01	1.60 \pm 0.01	1.55 \pm 0.35
Platelets ($\times 10^9$ /L)	91.00 \pm 5.00	80.03 \pm 0.03	82.10 \pm 0.10	76.02 \pm 0.03	76.02 \pm 0.03
Neutrophil (%)	33.00 \pm 2.00	54.05 \pm 0.05	55.10 \pm 0.10	64.50 \pm 0.50	64.50 \pm 0.05
Lymphocyte (%)	66.50 \pm 2.50	44.02 \pm 0.03	44.02 \pm 0.02	34.00 \pm 0.00	34.00 \pm 0.02
Monocyte (%)	1.50 \pm 0.50	2.00 \pm 0.00	2.00 \pm 0.00	2.00 \pm 0.00	2.02 \pm 0.03

*Mean \pm Standard Error

Key

PCV: Pack Cell Volume

WBC: White Blood Cell

RBC: Red Blood Cell

DISCUSSION

This study revealed that *Clarias gariepinus* juveniles exposed to *Tetrapleura tetraptera* leaf powder exhibits marked behavioural changes like hyperventilation, erratic swimming (vertical/spiral uncoordinated swimming movement), irregular operculum and tail frequencies, loss of reflex and settling at the bottom which are indicators of physiological stress in fish. In a similar study, Kori-Siakpere and Oviroh (2011) reported similar behavioural changes in *Clarias gariepinus* subjected to *Nicotiana tobaccum* leaf dust toxicity. Also in similar study by Dan Ologe and Sogbesan (2007) where the piscicidal potential of *Euphorbia heterophylla* was tested on *Barbus Occidentalis* fingerlings. The dried *Euphorbia heterophylla* stem water extract was found to induce varying behavioral response in the fish. An akin research by Ayoola et al., (2011) reported agitated behaviours, respiratory distress and abnormal nervous behaviors when *Oreochromis niloticus* juveniles was exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf at varying concentrations.

Fish mortality increases with increase in concentration of *Tetrapleura tetraptera* leaf powder. This study corroborates the study by Jegede and Olanrewaju (2012) where the piscicidal effect of *Nicotiana tobaccum* leaf dust on African giant catfish fingerling was investigated. The result revealed that the percentage mortality of the test organisms (African

giant catfish) fingerlings followed a regular pattern, as concentration of *Nicotiana tobaccum* increases, mortality also increase.

Exposure of *C. gariepinus* juveniles to *Tetrapleura tetraptera* leaf powder concentrations for 96 h clearly destroys the order in the blood parameters when compared with the control. Pack cell volume reduces from 26.50+ 1.50 in the control to 15.02+0.05 in Treatment 4(T4), white blood cell reduces from 25.45+ 3.80 g mm⁻¹ in the control to 20.03+0.03 g mm⁻¹ in Treatment 4(T4), which is the highest concentration, 2.8 g L⁻¹ *Tetrapleura tetraptera* leaf powder concentration, the red blood cell reduces from 3.21+0.40 in the control to 1.55+0.35 in T4, platelet decreased from 91.00+5.00 in control to 76.02+0.03 in Treatment 4(T4), while lymphocyte also decreases with increase in *Tetrapleura tetraptera* leaf powder concentration. In a similar study by Olufayo and Jatto(2011) where the haematology response of *Oreochromis niloticus* juveniles were exposed to *Nicotiana tobaccum* leaf dust was investigated. It was reported that Erythrocyte, Haemoglobin values and Pack Cell Volume (PCV) values decreased with increasing concentrations of tobacco leaf dust. The reduction in some of the blood parameters is an indication of anemia, which is a condition characterized by deficiency of haemoglobin, PCV and erythrocyte (Mason et al., 1994). Kecceci et al., (1998) also corroborated this by reporting that haematological values are indirect pointers to the health of live stocks (broiler chicken).

CONCLUSION

Finally, this study revealed the median lethal level (LC50) of *Clarias gariepinus* juveniles exposed to *Tetrapleura tetraptera* leaf dust toxicity to be 0.60g/L and has also shown the various alterations in haematological parameters, hence knowledge of this could help in fish health management and water quality management.

References

- Adebayo O.T. and Fagbenro O. A. (2004) Induced ovulation and spawning of pond raised African giant catfish, *Heterobranchus bidorsalis* by exogenous hormones. *Aquaculture* 242:229-236.
- Adewunmi C. O. (2001) Aridan - Success in fighting Bilharzia the natural way. *Science in Africa*. Africa's First On line Science Magazine
- Aladesanmi A. J. (2007) *Tetrapleura Tetraptera*: Molluscicidal Activity and Chemical Constituents. *Afr J. Tradit Complement Altern Med*. 4(1): 23–36.
- Ayoola S. O., Kuton M.P., Idowu A. A. and Adekun, A. B. (2011) Acute Toxicity of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed to Aqueous and Ethanolic Extracts of *Ipomoea aquatica* Leaf. *Nature and Science*; 9(3):91-99].
- Ayotunde E.O., Offem B.O. and Bekeh A. F. (2011) Toxicity of *Carica papaya* Linn: Haematological and Piscicidal Effect on Adult Catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*, 6: 291-308.
- Ayuba, V.O. and Ofojekwu, P.C. (2002). Acute toxicity of the root of Jimson's weed, *Datura innoxia* to the African catfish, *Clarias gariepinus* fingerlings. *J. Aquat. Sci.*, 17: 131-133.
- Bouck R. G and R. C (1966) Ball Influence of capture methods on blood characteristics and mortality in rainbow trout (*Salmo gardneri*) *Trans Am. Fish Soc.* p 16
- Dan Ologe I. A. and Sogbesan O. A. 2007. Piscicidal Potential of Dried *Euphorbia heterophylla* (L.) Stem Water Extract on *Barbus Occidentalis* (Pisces: Cyprinidae) (Boulenger, 1920) Fingerlings. *Research Journal of Environmental Toxicology*, 1: 191-197.
- Lekana-Douki J. B., Liabagui S. L. O., Bongui J. B. , Zatra R., Lebibi J. and Toure-Ndouo F. S (2011) In vitro antiplasmodial activity of crude extracts of *Tetrapleura tetraptera* and *Copaifera religiosa*. *BioMed Central Research Notes*, 4: 506. <http://www.biomedcentral.com/1756-0500/4/506>
- Haylor, G. S. (1992) The case of the African Catfish. *Aquaculture and Fisheries Management*. 20(3): 279-285.
- Hecht J., Oellermann L and Verheust L. (1996) Perspectives on clariid catfish culture in Africa. *Aquat. Living Resour.* ; 9: 197-206 (Hors Serie).
- Fafioye O. O. (2005) Plants with Piscicidal Activities in Southwestern Nigeria. *Turkish Journal of Fisheries and Aquatic Sciences* 5: 91-97.
- Fafioye, O.O., Adebisi, A.A. and Fagade, S.O. (2004). Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. *African Journal of Biotechnology* 3(10) Available on line at <http://www.academicjournals.org/AJB>.
- Jegade T. and Olanrewaju B. (2012) Piscicidal effect of tobacco (*Nicotiana tobaccum*) leaf dust on African giant catfish (*Heterobranchus bidorsalis*) fingerlings. *Agriculture and Biology Journal of North America* 3(11): 435-438
- Kecceci, T., Oguz, H., Kurtoglu V. and Demet O. (1998) Effect of polyvinylpyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological character of broiler chickens during aflatoxicosis. *Br. Poult. Sci.*, 39: 152-158.
- Neuwinger, H.D. (2004). Plants used for poison fishing in tropical Africa. *Toxicon*, 44: 417-430.
- Olufayo M. O. (2009) Haematological characteristics of *Clarias gariepinus* (Burchell 1822) juveniles exposed to *Derris elliptica* root powder. *African Journal of Food, Nutrition, Agriculture and Development*. 9(3):921-933.
- Ologe, I. A. D. and Sogbesan O. A. (2007). Piscicidal Potential of Dried *Euphorbia heterophylla* (L.) Stem Water Extract on *Barbus Occidentalis* (Pisces: Cyprinidae) (Boulenger, 1920) Fingerlings, *Journal of Environmental Toxicology*, 1: 191-197.
- Omoriege, E. and Ufodike E. B. C. (1994) Acute toxicity of water extract of apple *Bligha spida* with suasage plant

Kigelia africana on African catfish *Clarias gariepinus* (Teugels). J. Aqua. Sci., 9: 35-41.

Omorieg, E. and Okpanachi M. A. (1992). Growth of *Tilapia zillii* exposed to sublethal Acta. Hydrobiol., 34: 281-286.

Osei-Tutu P., Nketiah K., Kyereh B., Owusu-Ansah M. and Faniyan J. (2011) Hidden forestry revealed: Characteristics, constraint and opportunities for small and medium forest enterprises, IIED, ISBN 978-1-84369-454-0, Prekese (*Tetrapleura tetraptera*) – prekese tea bags, syrup as medicine and spices

Sogbesan AO, Ugwumba A. A. A. (2008). Nutritional Evaluation of Termite (*Macrotermes subhyalinus*) Meal as animal protein Supplements in the Diets of

Heterobranchus longifilis (Valenciennes, 1840)

Fingerlings. Turk. J. Fish. Aquat. Sci. 8: 149-157.

Steentoft, M. (1998) Flowering plants in West Africa, Cambridge University Press, ISBN 978-0-521-26192-aidan tree (*Tetrapleura tetraptera*) fruits are similarly useful, the seeds being rich in oil ..."

Svobodova Z, Pravda D and Palackova J. (1991) Unified Methods of haematological examination of fish. Research Inst. of Fish Culture and Hydrobiology, Czecholovakia, p 31.

Ufodike E. B. C. and Omorieg E. (1994) Acute toxicity of water extracts of barks of *Blancifera aeghptiaca* and *Kigelia africana* to *Oreochromis niloticus*. Aquaculture and Fisheries Management. 25: 873- 879.

Author Information

Temitope Jegede

Department of Forestry, Wildlife and Fisheries Management,
Ekiti State, Nigeria