



## HAEMATOLOGICAL INDICES OF *Heterobranchus longifilis* JUVENILES VAL. (PISCES: 1840) EXPOSED TO AQUEOUS BARK EXTRACT OF *Tephrosia vogelii*

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### ABSTRACT

*This study was carried out to assess the changes in haematological indices in Heterobranchus longifilis juveniles exposed to aqueous bark extract of Tephrosia vogelii. The experimental fish were obtained from the wild and transported to the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi, Nigeria for two weeks acclimatization. Ten (10) fish of average weight  $115.25 \pm 25.00$ g were selected randomly, injected intramuscularly (IM) with aqueous bark extract of T. vogelii using five concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 g/l with replicates. Control fish were injected with distilled water. Blood was collected and analyzed for changes in some haematological indices. Result obtained showed no significant ( $P > 0.05$ ) changes in the Haematological indices considered.*

**Key words:** Haematological changes, *T. vogelii*, *Heterobranchus longifilis*

### INTRODUCTION

The rapid growth of the aquaculture industry has placed an increasing demand on the various inputs such as feeds, drugs, chemicals and anesthetics. Anesthetics have been used to immobilize fish in the management of fish stocks to minimize stress and physical damage during tagging, weighing, measuring, clipping and collection of scales and in aquaculture during egg and milt stripping, and to calm brood fish during transportation in order to reduce mortality from excitement and hyperactivity under confinement (Agoke *et al.*, 2010; Omoniyi *et al.*, 2002; Ramanayaka and Atapatu, 2006; Ayuba and Ofejekwu, 2004; FAO, 1997).

Chemical substances currently in use as fish anesthetics have several disadvantages: poor solubility in water, long induction time, acute toxicological effects at high concentrations, bioaccumulation in tissues (Marking, and Meyer, 1985; Oshode *et al.*, 2008; Solomon and Amali, 2004). The use of natural plant extracts as anesthetics is cheaper, safer and is biodegradable. For these reasons, there has been an increase in the exploration of various plant extracts as anesthetics in aquaculture. Some of these plants include clove oil from clove plant *Eugenia*

*caryophylla* (Soto and Burhanuddin 1995), *Datura innoxia ugenia aniophylun*, *Clotalaria sp*, *Derriss candens*, *Barringtonia raecemosa*, *Eryagau npoetidany*, *Anamirla cucculus*, *Caryphaembra culifera* (Ramanayaka and Atapatu, 2006). *Lepidogathisa lopecuriodes*, *Nicotiana tobaccum* (Agokei and Adebisi, 2010; Jegede (2014), *Acorus calamus* (Agokei and Adebisi, 2010); *Ocimum gratissimum* (Jegede, 2014).

The use of the Fish Poison Bean, *T. vogelii* vary from one part of the world to another. The leaves of *T. ephrosia vogelii* have long been used by the Tiv people of Benue State and in other parts of Nigeria to sedate fish when fishing in natural water bodies. The leaves are also used for the treatment of dyspepsia and are highly toxic to cold blooded animals like mollusks, frogs, toads, worms and insects. They are highly effective fish poison for killing fish (Michael, 2002; Lungu, 1987). In Southern and Eastern Africa, it is widely cultivated for its use in crop protection, soil enrichment and as pesticide (Stevenson, *et al.*, 2012). This study aims to determine hematological changes in *Heterobranchus longifilis* exposed to aqueous bark extract of *T. vogelii*

## Materials and Methods

The plant was collected at Tofi village in Mbagen, Buruku Local Government Area, Benue State of Nigeria. The fresh, wet samples of *T. vogelii* bark were air-dried for 21 days to remove the moisture content, then oven-dried to crisp dry at 60°C for 3-4 hours to constant weight to make them pliable. The dried samples were crushed to powder using an electric kitchen blender and stored in air-tight bottles.

The experimental fish, *H. longifilis* were purchased from fishermen of the River Benue and taken to the General Purpose Laboratory of the Department of Fisheries and Aquaculture of the University of Agriculture, Makurdi for two weeks acclimatization. During the period of acclimatization, the fish were fed once daily at 09.00 hours at 4% of their body weight with a commercial fish diet.

### Preparation of aqueous bark extract

Known weights of the dry bark of *T. vogelii* were dissolved in 1 liter of de-ionized water in 2.5 litre air-tight laboratory bottles at 27.0±0.4°C room temperature. The mixture was shaken to ensure that they were properly mixed with the water allowed to stand for 24 hours. The settled portion was decanted and filtered through No.1 Whatman filter paper. The filtrate was kept in air tight bottles and used for exposure.

### The Administration of *T. vogelii* Aqueous Bark Extract.

The fish were starved for 24 hours before the exposure. Ten (10) fish of average weight 115.25±25.00 g were placed in tanks and each injected intramuscularly (IM) with the aqueous bark extract of *T. vogelii* using 0.5ml of the extract

corresponding to five concentrations of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 g/l with replicates in 20-liter capacity tanks. Control fish were injected with distilled water. The injection was done with a No 23 needle and a 2 mL syringe. Water was changed daily. Water quality parameters of temperature, dissolved oxygen, pH, and alkalinity were measured using a Hanna multi-parameter water tester model HI98129.

### Collection of Blood Samples

Blood was collected from the heart of a stunned fish with a No 23 needle and a heparinized syringe and placed into EDTA bottles. They were then taken to the PERFAR Laboratory of Federal Medical Center, Makurdi where they were analyzed for the haematological indices of White blood cell count(WBC), Red blood cell count(RBC), Haemoglobin(HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin(MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) using a Mindray Auto Hematology Analyzer.

### Statistical Analysis

The statistical analysis was carried out using Genstat Discovery Edition 4 for one-way Analysis of variance (ANOVA).

## RESULTS

The haematological indices of *H. longifilis* injected with various concentrations of *T. vogelii* aqueous bark extract. The values of RBC, Hb and Hct were below the control, but they did not differ significantly ( $P < 0.05$ ) among the treatments with the control (Table 1).

The water quality parameters measured were not significantly different with the control and among the treatments (Table 2).

**Table 1: Mean Haematological indices of *H. longifilis* injected various concentrations of *T. vogelii* aqueous bark**

Conc. . (g/l)	Fish Weight (g)	Haematological Indices						
		WBC (x10 <sup>11</sup> /L)	RBC (X10 <sup>12</sup> /L)	Hg g/Dl	HCT (%)	MCV (FL)	MCH (Pg)	MCHC g/dL
0.00 (Control)	77.50±2.50 <sup>a</sup>	1.608±8.0x10 <sup>9</sup> <sup>a</sup>	1.525±6.50x10 <sup>10</sup> <sup>a</sup>	7.50±1.10 <sup>a</sup>	22.60±4.20 <sup>a</sup>	115.40±0.30 <sup>a</sup>	42.35±3.95 <sup>a</sup>	36.55±1.75 <sup>a</sup>
0.01	74.00±1.00 <sup>a</sup>	1.588±1.32x10 <sup>10</sup> <sup>a</sup>	1.485±6.50x10 <sup>10</sup> <sup>a</sup>	6.500±0.40 <sup>a</sup>	18.30±1.10 <sup>a</sup>	112.90±1.90 <sup>a</sup>	40.10±0.70 <sup>a</sup>	35.55±0.05 <sup>a</sup>
0.02	77.50±2.50 <sup>a</sup>	1.452±1.64x10 <sup>10</sup> <sup>a</sup>	1.395±1.65x10 <sup>11</sup> <sup>a</sup>	7.300±0.90 <sup>a</sup>	20.25±2.95 <sup>a</sup>	136.00±0.70 <sup>a</sup>	40.10±8.10 <sup>a</sup>	35.350±0.05 <sup>a</sup>
0.03	83.50±3.50 <sup>a</sup>	1.295±3.79x10 <sup>10</sup> <sup>a</sup>	1.310±4.50x10 <sup>11</sup> <sup>a</sup>	6.20±1.80 <sup>a</sup>	21.15±1.35 <sup>a</sup>	137.20±0.30 <sup>a</sup>	39.10±6.30 <sup>a</sup>	34.35±1.15 <sup>a</sup>
0.04	71.50±3.50 <sup>a</sup>	1.323±1.53x10 <sup>10</sup> <sup>a</sup>	1.255±1.50x10 <sup>10</sup> <sup>a</sup>	6.000±0.90 <sup>a</sup>	22.650±0.45 <sup>a</sup>	138.60±24.0 <sup>a</sup>	37.45±2.75 <sup>a</sup>	33.25±2.95 <sup>a</sup>
0.05	72.50±2.50 <sup>a</sup>	1.442±1.85x10 <sup>10</sup> <sup>a</sup>	1.330±2.10x10 <sup>11</sup> <sup>a</sup>	5.250±0.85 <sup>a</sup>	23.400±0.20 <sup>a</sup>	140.90±23.3 <sup>a</sup>	35.95±6.30 <sup>a</sup>	31.90±1.80 <sup>a</sup>
0.06	77.50±2.50 <sup>a</sup>	1.597±1.14x10 <sup>10</sup> <sup>a</sup>	1.485±5.50x10 <sup>10</sup> <sup>a</sup>	6.750±0.45 <sup>a</sup>	24.20±3.40 <sup>a</sup>	142.40±4.20 <sup>a</sup>	40.40±6.30 <sup>a</sup>	31.40±3.20 <sup>a</sup>

Means in the same Colum with the same superscript are not significantly different (P<0.05)

WBC - White Blood Cells, MCV - Mean Corpuscular Volume, HC – Haematocrit; RBC - Red Blood Cells; MCH - Mean Corpuscular Haemoglobin  
Hg – Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

**Table 2: Mean Water Quality Parameters in exposure of *H. longifilis* to *T. vogelii* Aqueous Bark Extract**

Conc. (G/l)	Water Quality Parameters			
	Temperature °C	Dissolved Oxygen (MG/ l)	PH	Alkalinity (MG/ l)
0.00	25.41±0.02 <sup>a</sup>	6.81±0.02 <sup>b</sup>	7.05±0.50 <sup>c</sup>	30.18±0.01 <sup>d</sup>
0.01	25.38±0.02 <sup>a</sup>	6.82±0.02 <sup>b</sup>	7.17±0.02 <sup>c</sup>	30.21±0.03 <sup>d</sup>
0.02	25.36±0.02 <sup>a</sup>	6.88±0.01 <sup>b</sup>	7.24±0.01 <sup>c</sup>	30.34±0.01 <sup>d</sup>
0.03	25.35±0.01 <sup>a</sup>	6.90±0.91 <sup>b</sup>	7.30±0.01 <sup>c</sup>	30.38±0.02 <sup>d</sup>
0.04	25.33±0.01 <sup>a</sup>	6.91±0.01 <sup>b</sup>	7.29±0.01 <sup>c</sup>	30.42±0.02 <sup>d</sup>
0.05	25.34±0.00 <sup>a</sup>	6.95±0.02 <sup>b</sup>	7.32±0.01 <sup>c</sup>	30.39±0.02 <sup>d</sup>
0.06	25.32±0.01 <sup>a</sup>	6.93±0.02 <sup>b</sup>	7.30±0.03 <sup>c</sup>	30.45±0.03 <sup>d</sup>

Means in the same column with the same superscript not significantly different (P<0.05)

## DISCUSSION

Similar reductions in RBC and Hg values have been reported by (Tilak, *et al.*, 2006; Sudagara *et al* 2009; Atamanalp *et al.*, 2008) also reported decrease in the values of RBC in Sheatfish (*Silurus glanis*) immediately after treatment with 2-phenoxyethanol anesthetic without significant differences. It is known that plant-derived toxins cause changes in blood variables associated with oxygen transport (Agbon *et al.* 2002; Omoniyi *et al* 2002). In some cases, this may lead to anaemia which could result from the lysis of erythrocytes by the active ingredients (Brown, 1980). Gabriel *et al.*, (2009) reported the absence of significant changes in the blood variables associated with oxygen transport when catfish hybrids were exposed to aqueous leaf extract of *Lepidagathisa lopecuroides* and suggested that the levels of toxicant used did not interfere with erythropoiesis nor cause hemolysis. Consequently, in the present investigation the absence of changes in the haematological variables associated with oxygen transport in *H. longifilis* following treatment with the different concentrations of aqueous bark extracts of *T. vogelii* could be attributed to absence of negative impact of the anesthetic substance on the experimental fish which did not lead to erythropoiesis or hemolysis.

MCV values obtained with the aqueous bark extracts declined with increasing concentration without significant differences. The MCH values obtained with aqueous bark extracts did not assume a definite pattern but rather showed fluctuations without significant changes. Mean values of MCHC obtained with aqueous bark extract all declined with increasing concentration of anesthetic extracts without significant

differences (P>0.05). This result is similar to the finding of (Gabriel *et al* 2011) in *Clarias gariepinus* following anesthesia with metomidate where neither a definite pattern nor significant differences were found in values of MCH and MCHC. Ucar and Atamanalp (2010) reported that no significant differences were recorded in the values of MCV, MCH and MCHC in Rainbow trout (*Onchorynchus mykiss*) and Brown trout (*Salmo trutta furio*) treated with clove oil anesthetic. Similarly, in (Sudagara *et al* 2009) Roach (*Rutilus rutilus*) anesthetized with clove powder did not show significant differences (P>0.05) in the values of MCV, MCH and MCHC in both experimental subjects' groups B (after 7 minutes of anesthesia at concentrations 175, 225, 275 and 350mgL<sup>-1</sup>) and experiment subjects group C (24hours after 7 minutes of anesthesia at the same concentrations). Significant increase in the values of MCV and MCH were reported in Siberian sturgeon (*Acipenser baerii*) treated with both eugenol and MS-222 immediately after anesthesia compared with controls (Imanpoor. *et al.*, 2012) In this case, the researchers suggested erythrocyte swelling due to significant increase in MCV. Ayuba and Ofojekwu (2004) reported significant decrease in MCV in *Clarias gariepinus* exposed to *Datura innoxia* extract and attributed the result to anaemic condition resulting from erythrocyte destruction. Therefore, the absence of significant changes in the absolute red blood indices (MCV, MCH and MCHC) obtained with the aqueous bark extract of *T. vogelii* seems to.

## CONCLUSION

This research work investigated the effect of the aqueous bark extract of *T. vogelii* on the haematological indices in *H. longifilis* juveniles.

The result obtained revealed that all the haematological indices studied had no significant difference ( $P>0.05$ ). This implies that the use of

aqueous bark extract of *T. vogelii* did not impact negatively on the experimental subjects.

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