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Effects of Clove (*Eugenia carophylatta*) Powder Anaesthetic on Some Haematological and Biochemical Parameters of *Heterobranchus bidorsalis* Juveniles.

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Abstract

Anaesthetics are often used to reduce metabolic activities, minimize hypermobility, injuries and mortality during handling procedures in aquaculture practices. Synthetic anaesthetics is considered not ideal due to its residual effects on the test fish. Natural (plant) anaesthetics have been adjudged the most ideal and effective for fish handling. Clove products have been successfully utilized to anaesthetized fishes including the African catfishes. However there is still paucity of information on its effects on the haematological and biochemical parameters especially on *H. bidorsalis*. Therefore this study aims to investigate the use of clove powder as anaesthetic and its effects on the haematological and biochemical parameters of *H. bidorsalis* juveniles. Different concentrations of 80, 100, 120, 140, 160 and 180mg/l of clove powder solution were used in a static anaesthesia bioassay for 30mins to determine the induction and recovery time. The time to attain each stage of induction and recovery was noted and recorded using a stop watch. Blood of fish from each tank was collected into heparinised tubes for haematological and biochemical parameters using appropriate standard methods. Fish exposed to concentrations less than 120mg/l could not attained anaesthesia. Fish exposed to 120mg/l achieved complete anaesthesia (Stage 4) at 22.32mins while those exposed to the highest concentration (180mg/l) took just 2.60 (3) mins to be fully anaesthetized (Stage 4). Fish exposed to 120mg/l regained upright position (stage 2) at 4.56 (5) mins and attained normal swimming (stage 3) at 10.45 (11) mins while at 180mg/l fish attained stage 2 at 11mins and stage 3 at 24 mins. Induction and recovery were concentration dependant. The values of pack cell volume (41.08 – 29.65%), red blood cell (4.45 – 2.66 x 10^{12} cells/l), Haemoglobin (9.04 – 5.73g/l), basophil (2. 89 – 1.59 x 10^{9}cells/l) and monocytes (2.07 – 1.93x 10^{9}cells/l) decreased while white blood cell (65.47 – 135.85x10^{9}cells/l), platelet (62.23 – 136.75x 10^{9}cells/l), mean cell volume (84.44 – 122. 42fl) and Lymphocytes (14.04 – 18.30 x 10^{9}cells/l) increased with increase in concentration of the anaesthetic. The plasma levels of glucose, total protein, cholesterol urea and triglyceride of fish exposed concentration ranged from 80 to 120mg/l were not significant ($p > 0.05$) from those of the control. A reduction in the plasma levels of creatine kinase (146.56 – 136.59 IU/L), lactate dehydrogenase (96.85 – 82.98 IU/L),alanine aminotransferase (31.17 – 20.52 IU/L) and alkaline phosphatase (19.51 – 17.28 IU/L) were decreasing while the aspartate aminotransferase (123.53 – 147.09 IU/L) and cholinesterase (29.19 – 53.23 IU/L), decreased with increasing concentration of clove. All the concentrations of plasma electrolytes decreased with increasing concentration of clove except K and Cl. The results of the induction, recovery and blood profile of clove powder compares favourably with and even better than some plant derived anaesthetics use in fisheries management. Clove powder can be recommended for use anaesthetic for fish when use within the recommended ranged of 140 – 180mg/l.

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Introduction

The need to handle fish without impairing their health or commercial value has led to the development of many techniques to anaesthetize fish (Yildiz *et al*. 2013). Different anaesthetics, synthetic and natural act with various intensity driving fish into general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity, and reduction in skeletal muscle tone (Hajek *et al*. 2006). Anaesthetics are often used during various handlings processes to reduce metabolic activities. Some researchers have worked on some plant extracts as natural anaesthetic, because it is cheaper, safer and more effective at lower concentrations when compared with chemical anaesthetics (Akinbulumo, 2005; Agokei and Adebisi 2010; Okey *et al*, 2013; 2017; Akinrotimi *et al* 2015; Popoola *et al*. 2015; Adebayo and Olufayo 2017). Several plant materials have shown to be toxic to commercial fish species both in the laboratory and field (Singh and Singh, 2002; Tiwari and Singh 2003).

They are shown to produce genotoxic effects in fishes by changing their enzymes profile and immune stimulation of exposed fish (Guha and Khud-Bukhsh, 2003; Logambal and Michael, 2000). They have also cause death of fish and changes in behavioural, haematological, biochemical responses (Agokei and Adebisi, 2010; Abalaka *et al* 2010; Gabriel and Okey 2009) and even histopathological changes (Omoniyi *et al* 2002; Fafioye *et al* 2004) on clariids. *Haematological* and biochemical parameters reflect the condition of fish more quickly than other commonly measures parameters, since they respond quickly to changes in the environmental conditions (Atamanalp and Yaniks 2003). They have been widely used for the assessment of fish health, monitoring stress responses, predicting the systematic relationship and physiological adaptions of animals (Ramesh and Saravana, 2008; Okey et al 2013, Ralio and Mikinman, 1985).

Stress response is characterised by biochemical and physiological changes which may be manifest in both acute and chronic toxicity test and easily assessable by the changes in enzymes activities in functional organs (Tiwaria and Singh, 2004; Dela Torre *et al* 2000). Haematological techniques have been employed as rapid tools in monitoring fish health in agriculture and environmental perturbance (Akinrotimi and Gabriel, 2012). Result from studies of fish blood suggest the possibility that blood will reveal conditions within the body of the fish long before there is any outward manifestation of stress or disease (Gabriel *et al* 2004; Ezeri *et al* 2004). Several workers have reported significant changes in haematological parameters of various fish species exposed to xenobiotic (Gabriel *et al* 2011; Velisek, *et al* 2007; Okey, *et al* 2013; Farahi *et al* 2011). Studies have also shown changes in haematological indices of African clariids exposed to various toxicant under laboratory conditions (Adedeji, *et al* 2009; Aderou *et al* 2010 and Okomada *et al* 2010). Although anaesthetics have positive effects on the fish during transportation and handling by reducing stress, some anaesthetics can pose dangerous problems to the fish organs and the blood parameters (Nicula et al. 2010).

Clove, oil widely utilized as anaesthetic before handling of fish in breeding, blood sampling and some other veterinary operations (Velisek *et al* 2005). Clove oil (eugenol) is reported to have antibacterial, antifungal, antitumor, antioxidant and molluscicidal properties (Lee and Shibamoto, 2001; Garcia-Gomez and Gandara 2002). According to Saroja and Annapoorani (2012), antioxidant enzyme is responsible for preventing cellular damage and improving immune competence. The immune status of fish is related to haematological parameters such as white blood cell, platelet and total and differential counts and are effective tool that can be used to evaluate physiological, biochemical and pathological changes in fish. Sudagara *et al* (2009), reported changes in the blood parameters of Rutilus rutilus exposed to clove powder although reversible after 48 hours.

Olufayo and Ojo (2018) reported a significant reduction in pack cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV) and mean cell haemoglobin (MCH) while white blood cell (WBC) increased with increase in concentration of clove oil on C. gariepinus. Akinrotimi *et al* (2015) also reported similar changes in blood parameters however with a decrease in red blood cell (RBC), mean cell haemoglobin concentration (MCHC) and lymphocytes while the thrombocytes increase with increased in concentration of clove seed extracts. Chelladurai *et al* (2013) reported increase in some biochemical parameters such as glucose and alkaline phosphatase in Channa punctatus with clove oil while Svacina *et al* (2016) reported increase in the levels of total protein, ammonia, glucose, aspartate aminotransferase and calcium without any significant effects on the haematological parameters of burbot (Lota lota) anaesthetized with clove oil. Information of clove powder on African

clariids and its effects especially on the biochemical parameters is unknown at the time of this research. Thus, the current study was designed to evaluate the anaesthetic effects of clove powder on of the haematological and biochemical parameters of *H. bidorsalis* juveniles.

Materials and Methods

Plant material

Dried flower buds of *Eugenia aromatica* were procured from an herbal shop in watt market calabar, Nigeria. The buds were sun dried for 30 minutes and then pulverized with a sterile manual blender sieved to obtain a fine powder at the Fisheries Laboratory CRUTECH, Obubra campus.

Experimental fish

Two hundred (200) apparently healthy juveniles of *Heterobranchus bidorsalis* of average weight of 23.60 ± 4.35g were purchase from University of Calabar (UNICAL) fish farm. They were acclimated in the Fisheries wet laboratory CRUTECH, Obubra for 2weeks prior to the experiment using rectangular glass aquaria. The fishes were fed twice daily with a commercial feed (vital feed) at 5% body weight and the water renewed daily during this acclimation period. Feeding was discontinued 24 hours prior to the commencement of the experiment to minimize the contamination of the test media. Some water quality parameters were measured and recorded before the commencement and renewal of the test solution using water quality test kits.

Experimental procedure (Anaesthesia bioassay)

A stock solution of Clove powder with a concentration of 200mg/L was prepared by dissolved 2g of *E. aromatica* powder into 10 litres of river water. Exposure concentrations 80, 100, 120, 140, 160 and 180mg/l for the bioassay were obtained by serial dilution of the stock solution. Thirty-six glass aquaria were cleaned and randomly labelled and each filled with water to the 15 litres mark for induction test and 20 litres mark for recovery in each of the experiment. The test was conducted to determine induction (Anaesthesia) and recovery time from Clove powder following the methods of (King *et al*., 2005). Four stages of induction and three of recovery time were considered and recorded using a stop watch (Table 1). Each aquarium was stocked with 10 fish each in triplicate and monitored for the onset of induction (anaesthesia) for 30 minutes as periods greater than this were considered impractical for routine fish handling procedures (Agokei and Adebisi, 2010). Any test fish that lost balance and no longer responding to external stimulus (prodding) was removed immediately and transferred to 20 litres of Clove powder free water (Recovery tank). The induction and the recovery time were noted. The induction time was defined as the time taken from the moment the fish was exposed to the anaesthetic to the moment the respiratory movement of the opercula stopped. Recovery time was defined as the time taken from the moment the fish was considered anaesthetized until the moment regular respiratory movements were resumed. None of the revived fish were re-used for further experimentation but were kept in another glass aquaria and plastic buckets to monitor post experimental mortality.

Stages of Anaesthesia	Description
Induction	
I	Slight increase in opercula beat frequency
II	Loss of equilibrium
III	Loss of reflexes and movement
IV	Deep anaesthesia, fish lies on one side
Recovery	
I	Partial recovery of equilibrium
II	Total recovery of equilibrium
III	Normal swimming

Adopted from Coyle *et al* 2004

Table 1: *The various stages of fish anaesthesia and recovery.*

Water Quality Parameter

The temperature of the media were taken using a mercury in glass thermometer, pH values were determined using pH meter, Dissolved Oxygen was determine using Dissolved oxygen meter inserted into the sample glass tanks after standardization in three different buffers.

Statistical Data Analysis

Data obtained from the various parameters of water quality, time (induction and recovery in minutes, haematological and biochemical were analysed with a one – way analysis of variance (ANOVA) using SPSS version 25. The differences among means with in the treatments were separated at 5% significant level ($P< 0.05$) by honest significant difference (HSD).

Blood Sampling

After 30 mins and when the fish was completely immobilized with the clove powder, 2ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes into sample bottles containing 0.5mg ethylate diamine tetracetic acid (EDTA) as anticoagulant for haematological parameters and the other into a tube containing Lithium heparinised anticoagulant to obtained plasma for biochemical parameters analysis. The samples were mopped with tissue paper to prevent haemolysis due to dilution of oozing blood with any other fluid. The haematological parameter determined were red blood cell (RBC), white blood cell (WBC), pack cell volume (PCV), haemoglobin (Hb), platelet and erythrocyte indices such as mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). Red blood cell, WBC and platelet were estimated using a Neubauer haemocytometer as described by Ochei and Kolhatkar (2003). Haemoglobin concentration were estimated using cyan methaemoglobin method as described by Blaker and Silverton (1985), while pack cell volume was done using a micro haematocrit method according to Ochei and Kolhakater (2003). Differential counts of lymphocytes, monocytes, eosinophils, basophils and neutrophils were done on blood film stained with May Gromwell-Giemsa stain according to Ochei and Kolhakater (2003). Other parameter were calculated using the formulae according to Lee et al (1998).

Mean cell volume (MCV) = PCV × 100 / RBC (10 cell/l). Mean cell volume (MCV) expressed in femtolitre (10^{-15})

Mean cell haemoglobin (MCH) indicates the weight of the haemoglobin in the red blood cell and it's expressed in picogram (10^{-12}/g).

MCH = Haemoglobin (g/100mg/l)/ RBC (10^{-12} cell/l) × 10

Mean cell haemoglobin concentration (MCHC) indicates the haemoglobin concentration in 100ml of packed red blood cells. It is express in gram per 100ml.

MCHC (g/100ml) = Haemoglobin (g/l) /PCV% ×100

The stored serum was used for the analysis of some metabolites (glucose, protein, cholesterol, urea and triglyceride) enzymes (ALP, LDH, ALT, AST and CHE) and electrolytes (Na, K, Cl^- , P, and CO_3^{2-}). Serum glucose and triglyceride was estimated by enzymatic method using spectrophotometer, protein and urea (Diacetyl monoxime) by colorimetric method according to Ochei and Kolhattar (2003). Serum electrolyte such as sodium (Na^+), potassium (K^+), chloride (Cl^-), Phosphorus (P^{3-}) and Calcium (Ca^{2+}) were estimated by colorimetric test using commercially available kit (centronic GmbH-Germany, Lot 0603010). Serum enzymes such as AST, ALT, ALP, LDH and CHC were estimated by enzymatic colorimetric method according to Blaker and Silverton (1985) and Ochei and Kohlhatkar (2003).

Results

Water quality parameters

The result of the water quality parameters (Table 2) indicates that the mean values were similar ($p > 0.05$) from the respective control in all the tanks containing the exposed fish. However, dissolved oxygen, temperature, alkalinity and hardness all decreased slightly with increasing concentration. Conductivity increased slightly from 166.07 ± 2.04 in the control to 167.05 ± 2.71 at 180mg/l tank whereas the mean value of pH (6.78 ± 0.28 to 6.77 ± 0.22) was relatively closed. The ranged of Conductivity (166.07-167.05 µScm/l) and alkalinity (37-41-38.51mg/l) shows a slight increase above those of control at 180mg/l of Clove powder atter 30 mins of exposure

Induction and Recovery

The result of the mean values of induction and recovery time of *H. bidorsalis* juveniles from clove powder is shown in Table 3. It revealed that fish exposed to concentrations less than 120mg/l could not attained anaesthesia, however fish exposed to 100mg/l completely loss equilibrium at 21mins with inability to regain upright position (Stage 3) after 30 mins of exposure. Fish exposed to 120mg/l achieved complete anaesthesia (Stage 4) at 22.32mins while those exposed to the highest concentration (180mg/l) took just 2.60 (3) mins to be fully anaesthetized (Stage 4). The higher the concentration the low the time of the fish to be completely immobilized (stage 4). Similar but inverse trends was observed in the recovery time. Fish exposed to 120mg/l regained upright position (stage 2) at 4.56 (5) mins and attained normal swimming (stage 3) at 10.45 (11) mins. At the highest concentration (180mg/l), fish attained stage 2 at 11mins and stage 3 at 24 mins. This is an indication that the higher the concentration and lower the time of anaesthesia the higher the time to regained full recovery.

Conc. (mg/l)	Parameter					
	DO (mg/l)	Tempt. (°C)	pH	Cond. (µS/cm)	Alk. (mg/l)	Hardness (mg/l$CaCO_3$)
0	4.74 ± 0.27[a]	28.13 ± 1.07[a]	6.78 ± 0.28[a]	166.07 ± 2.04[a]	37.41 ± 0.45[a]	39.32 ± 0.81[a]
80	4.29 ± 0.23[a]	27.53 ± 1.18[a]	6.64 ± 0.29[a]	167.17 ± 1.47[a]	37.91 ± 0.49[a]	38.40 ± 0.36[a]
100	4.37 ± 0.35[a]	26.87 ± 1.25[a]	6.83 ± 0.27[a]	166.51 ± 1.31[a]	38.18 ± 1.96[a]	37.61 ± 0.58[a]
120	4.48 ± 0.12[a]	27.37 ± 0.78[a]	6.79 ± 0.41[a]	165.96 ± 1.32[a]	40.76 ± 2.69[a]	38.33 ± 0.54[a]
140	4.38 ± 0.36[a]	26.67 ± 1.02[a]	6.79 ± 0.16[a]	166.79 ± 2.14[a]	36.75 ± 0.85[a]	38.72 ± 0.83[a]
160	4.38 ± 0.38[a]	27.74 ± 0.91[a]	6.72 ± 0.19[a]	167.23 ± 2.50[a]	38.67 ± 1.05[a]	38.26 ± 0.33[a]
180	4.12 ± 0.12[a]	26.88 ± 0.19[a]	6.77 ± 0.22[a]	167.05 ± 2.71[a]	38.51 ± 0.47[a]	38.80 ± 0.85[a]

Mean with the same superscript in the same column are not different (P> 0.05, DO = dissolved oxygen, Tempt. = Temperature, Cond. =Conductivity, Alk. =alkalinity.

Table 2: *Water quality parameters of the test solutions for H. bidorsalis juveniles from Clove powder anaesthetic for 30 minutes (m ± SD).*

Con (mg/l)	Stages of induction (min)				Stages of recovery (min)		
	1	2	3	4	1	2	3
80	11.67 ± 0.12[b]	22.87 ± 0.20[a]	0.00 ± 0.00[c]	0.00 ± 0.00[c]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
100	7.53 ± 0.24[c]	14.07 ± 0.29[b]	20.93 ± 0.41[a]	0.00 ± 0.00[d]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
120	2.60 ± 0.12[d]	4.87 ± 0.29[c]	8.27 ± 0.41[b]	22.32 ± 0.22[a]	1.67 ± 0.19[c]	4.56 ± 0.29[b]	10.45 ± 0.22[a]
140	1.93 ± 0.18[c]	2.93 ± 0.35[c]	5.40 ± 0.31[b]	13.40 ± 0.80[a]	2.00 ± 6.19[c]	6.00 ± 0.86[b]	17.00 ± 0.51[a]
160	0.00 ± 0.00[c]	1.80 ± 0.23[b]	2.87 ± 0.13[b]	4.07 ± 0.13[a]	3.33 ± 0.19[c]	10.56 ± 0.19[b]	21.56 ± 0.79[a]
180	0.00 ± 0.00[a]	0.00 ± 0.00[a]	1.20 ± 0.12[a]	2.60 ± 0.12[a]	5.67 ± 0.38[c]	11.00 ± 0.58[b]	24.11 ± 0.40[a]

Mean with the same superscript under the same rows are not significant at (p> 0.05), Conc.= concentration.

Table 3: *The mean time (min.) induction and recovery of H. bidorsalis juveniles from Clove powder anaesthetic.*

Haematological Parameters

The mean values of the haematological parameters of H. bidorsalis juveniles exposed to clove powder anaesthetic is presented in Tables 4a and 4b). The result revealed that the values of PCV (41.08 – 29.65%), RBC (4.45 – 2.66 x 10^{12} cells/l) and Hb (9.04 – 5.73g/l) decreased with increased concentrations of the anaesthetic. Also decreasing were some differential white blood cell parameters such as basophil (2. 89 – 1.59 x 10^9cells/l), monocytes (2.07 – 1.93 x 10^9cells/l) and eosinophil (8.06 – 6.69 x 10^9cells/l). Others parameters such as WBC (65.47 – 135.85 x 10^9 cells/l), platelet (62.23 – 136.75x 10^9 cells/l), MCV (84.44 – 122. 42fl) and Lymphocytes (14.04 – 18.30 x 10^9cells/l) increased with increase in concentration. The mean separation shows that the values of PCV, RBC, MCV, MCH and MCHC of fish exposed to concentrations between 80 – 160mg/l were significantly different (p > 0.05) from those of the control.

Biochemical Parameters

The results of the mean values of some plasma metabolites, enzymes and electrolytes is presented in Tables 5, 6 and 7. The result of the metabolites showed that the level of glucose (22.10 – 27.06 mg/l), protein (38.26 – 42.48mg/l), cholesterol (55.32 – 62.21mg/l), urea (20.84 – 27.07mg/l) and triglyceride (56.03 – 61.61mg/l) increased with concentrations of clove powder. The levels of glucose, total protein, urea, and triglyceride concentration in the plasma at 160 and 180mg/l were not significant (p> 0.05) but were different from those of the control. The plasma levels of glucose, total protein, cholesterol urea and triglyceride of fish exposed concentration ranged from 80 to 120mg/l were not significant (p> 0.05) from those of the control.

Conc. (mg/l)	PCV (%)	RBCC (10^{12} cells/L)	Hb (g/l)	WBCC (10^9cells/L)	MCV (fl)	MCH (pg)	MCHC (g/l)
0.00	37.09 ± 0.17a	6.04 ± 0.68^{a}	9.18 ± 0.39^{a}	65.47 ± 2.01^{f}	62.84 ± 6.55^{d}	15.65 ± .21^{d}	24.75 ± 1.18^{a}
80.00	33.89 ± 1.60^{a}	4.34 ± 0.08^{b}	8.60 ± 0.18^{ab}	69.05 ± 2.22^{f}	87.19 ± 3.82^{cd}	19.85 ± 0.58^{cd}	25.43 ± 0.64^{a}
100.00	32.99 ± 2.84^{ab}	3.79 ± 0.12^{bc}	7.73 ± 0.12^{bc}	81.08 ± 2.68^{e}	37.90 ± 5.15^{c}	20.45 ± 0.36^{cd}	23.82 ± 2.22^{a}
120.00	32.04 ± 1.46^{ab}	3.41 ± 0.14^{bc}	8.22 ± 0.33^{b}	93.13 ± 2.08^{d}	93.92 ± 0.79^{bc}	23.37 ± 0.63^{c}	24.89 ± 0.78^{a}
140.00	31.41 ± 1.72^{ab}	3.33 ± 0.12^{bc}	7.72 ± 0.15^{bc}	106.95 ± 3.03^{c}	94.89 ± 8.89^{bc}	23.25 ± 1.15^{c}	24.68 ± 8.86^{a}
160.00	27.35 ± 1.35^{bc}	2.44 ± 0.25^{de}	7.16 ± 0.18^{bc}	121.74 ± 6.72^{b}	112.16 ± 5.15^{b}	29.53 ± 2.51^{b}	26.23 ± 1.18^{a}
180.00	24.49 ± 0.92^{c}	1.63 ± 0.00^{e}	6.23 ± 0.33^{d}	135.85 ± 1.35^{a}	125.25 ± 7.89^{a}	38.19 ± 1.99^{a}	25.41 ± 0.38^{a}

Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, PCV = packed cell volume , RBCC= red blood cell counts Hb = haemoglobin, MCV =mean cell volume MCH = mean cell haemoglobin MCHC= mean haemoglobin concentration, WBCC =white blood cell count.

Table 4a: *The mean values of selected haematological indices of Clarias gariepinus juveniles' exposure to Clove powder anaesthetic for 30min.*

Conc. (mg/l)	Cell count (10^9 cells/L)					
	Plt	Neut	Lymp	Baso	Mono	Eosin
0.00	62.23 ± 4.72^{f}	2.57 ± 0.29^{a}	14.87 ± 0.68^{d}	2.89 ± 0.11^{a}	2.07 ± 0.06^{a}	8.06 ± 0.99^{a}
80.00	68.57 ± 3.24^{ef}	2.45 ± 0.21^{a}	15.94 ± 0.51^{c}	2.83 ± 0.13^{a}	1.99 ± 0.07^{a}	7.88 ± 0.58^{a}
100.00	78.78 ± 1.55^{e}	2.33 ± 0.13^{a}	16.15 ± 0.72^{c}	2.75 ± 0.07^{a}	1.97 ± 0.01^{a}	7.41 ± 0.48^{a}
120.00	90.13 ± 2.80^{d}	2.36 ± 0.19^{a}	16.31 ± 0.49^{bc}	2.09 ± 0.08^{b}	2.08 ± 0.05^{a}	7.30 ± 0.14^{a}
140.00	104.10 ± 5.07^{c}	2.30 ± 0.15^{a}	17.17 ± 0.04^{ab}	2.14 ± 0.23^{b}	2.07 ± 0.09^{a}	7.09 ± 0.09^{a}
160.00	125.08 ± 2.77^{b}	2.31 ± 0.27^{a}	17.47 ± 0.67^{a}	1.86 ± 0.09^{bc}	1.84 ± 0.21^{a}	7.14 ± 0.27^{a}
180.00	136.75 ± 1.20^{a}	2.40 ± 0.30^{a}	18.09 ± 0.23^{a}	1.59 ± 0.28^{c}	1.93 ± 0.19^{a}	6.69 ± 0.09^{a}

Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, Neut = neutrophil Lymp= lymphocytes Basop = basophil, Mono = monophil, Eosino = eosinophil, Plt = platelet.

Table 4b: *The mean values of selected haematological indices of Clarias gariepinus juveniles' exposure to Clove powder anaesthetic for 30min.*

Clove powder anaesthetics showed a reduction in the plasma levels of Ck (146.56 – 136.59 IU/L), LDH (96.85 – 82.98 IU/L), ALT (31.17 – 20.52 IU/L) and ALP (19.51 – 17.28 IU/L) were decreasing while the AST (123.53 – 147.09 IU/L) and CHE (29.19 – 53.23 IU/L), decreased with increasing concentration of clove. The level of Ck, AST and ALT at the control were significant ($p < 0.05$) to those of the exposed fish, whereas LDH, ALP and CHE were not significant ($p > 0.05$) at 80mg/l. Fish exposed to the highest concentration (180mg/l) had a significantly different ($p< 0.05$) plasma levels in LDH, ALT and CHE enzymes from other concentrations. The concentrations of plasma electrolytes investigated in this study decreased with increasing concentration of clove except K and Cl which increase slightly with increasing concentration of clove powder. The concentration of K and Cl at the control were not significant ($p > 0.05$) to those exposed to the various concentration of clove powder.

Conc. (mg/l)	Gluc	T.P	Cho	Ure	Trigly
0	23.90 ± 0.28^{c}	41.98 ± 0.73^{c}	62.24 ± 0.14^{c}	27.86 ± 0.88^{b}	64.29 ± 1.21^{d}
80	24.19 ± 0.03^{c}	42.53 ± 0.29^{c}	62.87 ± 0.13^{ab}	27.81 ± 0.77^{ab}	76.19 ± 0.93^{b}
100	25.84 ± 0.17^{bc}	42.34 ± 0.64^{c}	70.00 ± 0.13^{ab}	29.02 ± 1.44^{ab}	65.39 ± 0.51^{cd}
120	26.62 ± 0.20^{ab}	43.26 ± 0.80^{bc}	69.65 ± 0.9^{bc}	29.49 ± 0.83^{ab}	68.59 ± 0.79^{ab}
140	26.82 ± 0.67^{ab}	44.41 ± 0.24^{b}	70.19 ± 0.24^{a}	29.91 ± 0.22^{ab}	69.79 ± 0.49^{a}
160	27.60 ± 0.13^{ab}	44.90 ± 0.27^{b}	72.21 ± 0.18^{a}	26.30 ± 6.43^{ab}	70.07 ± 0.86^{a}
180	28.76 ± 0.19^{ab}	46.64 ± 0.35^{a}	74.22 ± 0.14^{a}	32.15 ± 0.15^{a}	71.20 ± 0.67^{a}

Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, Glucose (Glu), total protein (TP), cholesterol (Cho), urea (Ure) and triglyceride (Trigly)

Table 5: *The mean values of selected plasma metabolites (mg/dl) of C. gariepinus exposure to Clove powder anaesthetic for 30mins.*

Conc. (mg/l)	Selected Plasma Enzymes (IU/L)					
	CK	LDH	AST	ALT	ALP	CHE
0.00	156.71 ± 0.77^{a}	97.94 ± 1.30^{a}	138.66 ± 3.89^{e}	39.32 ± 0.73^{a}	24.09 ± 0.35^{a}	34.09 ± 1.61^{d}
80.00	154.69 ± 0.85^{ab}	97.67 ± 0.67^{a}	141.86 ± 1.72^{e}	37.74 ± 0.42^{ab}	23.51 ± 0.22^{ab}	38.20 ± 1.04^{cd}
100.00	154.56 ± 0.28^{ab}	90.73 ± 0.85^{b}	149.69 ± 0.74^{d}	36.85 ± 0.41^{bc}	23.21 ± 0.11^{ab}	41.19 ± 0.73^{c}
120.00	156.47 ± 0.52^{cd}	83.03 ± 1.16^{c}	154.34 ± 0.41^{cd}	37.05 ± 1.22^{ab}	22.77 ± 0.45^{bc}	65.63 ± 3.77^{b}
140.00	153.78 ± 1.32^{bc}	76.38 ± 3.19^{d}	156.39 ± 1.09^{bc}	34.89 ± 0.29^{c}	21.79 ± 0.09^{c}	71.74 ± 1.66^{b}
160.00	151.58 ± 0.83^{de}	61.71 ± 1.55^{e}	160.39 ± 1.28^{ab}	32.45 ± 0.80^{d}	20.75 ± 0.30^{d}	88.40 ± 2.51^{a}
180.00	150.35 ± 0.41^{e}	52.16 ± 1.83^{f}	162.39 ± 1.69^{a}	31.49 ± 0.83^{d}	19.57 ± 0.32^{f}	93.42 ± 1.42^{a}

Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, CK= Creatine kinase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, CHE = Cholinesterase

Table 6: *The mean values of selected plasma enzymes of C.gariepinus exposure to Clove powder anaesthetic for 30 mins.*

Conc. (mg/l)	Selected Plasma Electrolytes (mmol/dl)					
	Na^{+}	K^{+}	Ca^{2+}	Cl_	P	HCO_3-
0	121.08 ± 3.66^{c}	22.17 ± 0.87^{e}	19.75 ± 0.29^{a}	97.06 ± 0.99^{f}	8.94 ± 0.22^{f}	20.30 ± 0.34^{d}
80	128.62 ± 0.92^{b}	23.96 ± 0.57^{d}	19.19 ± 0.11^{ab}	97.96 ± 0.18^{ef}	9.86 ± 0.15^{e}	21.27 ± 0.38^{cd}
100	128.63 ± 0.88^{b}	25.17 ± 0.15^{d}	18.76 ± 0.17^{bc}	99.11 ± 0.09^{de}	10.60 ± 0.25^{d}	20.90 ± 0.35^{cd}
120	130.44 ± 0.66^{ab}	27.78 ± 0.64^{c}	18.31 ± 0.32^{bc}	100.58 ± 0.62^{cd}	10.87 ± 0.18^{cd}	21.29 ± 0.23^{c}
140	130.90 ± 0.61^{ab}	29.09 ± 0.39	17.85 ± 0.09^{d}	10165 ± 0.17^{c}	11.31 ± 0.13^{bc}	22.12 ± 0.71^{bc}
160	132.32 ± 0.24^{ab}	30.50 ± 0.18^{ab}	18.01 ± 0.03^{cd}	102.44 ± 0.78^{ab}	11.68 ± 0.37^{ab}	22.96 ± 0.3^{6b}
180	133.75 ± 0.98^{a}	31.08 ± 0.50^{a}	17.52 ± 0.38d	103.91 ± 0.54^{a}	12.28 ± 0.17^{a}	24.33 ± 0.29^{a}

Mean with the same superscript are not significantly different at p< 0.05, Conc.= concentration, Na = Sodium, Ca = Calcium, K = Potassium, Cl = Chloride, P = Phosphorus, HCO_3 = Bicarbonate salts

Table 7: *The mean values of selected plasma electrolytes of C.gariepinus exposure to Clove powder anaesthetic for 30 mins.*

Discussion

Water Quality

The physicochemical properties of the test medium did not vary significantly (p> 0.05) from the control (0mg/l). It was within the acceptable ranges for toxicity test tolerance level (APHA, 2009; Boyd, 1981), hence may not have acted in synergy with the behavioural, haematological biochemical changes recorded in this study. Similar observation was recorded by Okey *et al* (2013), Akinrotimi *et al*. (2015), Olufayo and Ojo (2018), Iheanacho and Nworu (2017) using, clove flower bud powder, clove seed, clove oil and Chloromolaena odorata as anaesthetic agents to African catfish species respectively. However, leaf extracts of Datura stramonium according to Adebayo and Olufayo (2017) caused significant changes in temperature, pH and dissolved oxygen in test solution of H. bidorsalis juveniles. According to Adeyemo (2005) and Heath (1991) bad quality water can result to physiological changes which will be reflected in the values of one or more of the haematological, biochemical and swimming activity of fishes.

The induction and recovery time (minutes) of H. bidorsalis was concentration dependent. The result showed that as concentration increase the induction time reduces while the recovery time increase. This was in time with the study of Okey et al (2013, 2018) on catfish hybrid, *C. gariepinu*s and *H. bidorsali*s fingerlings exposed to clove powder Olufayo and Ojo (2013) on *C. gariepinus* exposed to Datura stramonium. Concentrations less than 120mg/l of clove powder could not completely immobilized the fish but showed some behaviour responses such as initial increase opercular beat, sporadic loss of equilibrium, inability to regained up right and decrease in opercular movement but still responding to external stimulus. Similar observation was reported by Akinrotimi *et al* (2015), Okey *et al* (2013) and Adebayo and Olufayo (2017) on African catfishes. In this study 120mg/l was required to completely immobilized the juveniles of H. bidorsalis at 22mins which was higher than the 100mg/l required to completely immobilized catfish hybrid at 22mins (Okey, *et al* 2013), 50mg/l of clove seeds to anaesthetizes juveniles of C.gariepinus at 75.73 seconds Akinrotimi *et al* (2015), 1.0ppm of ethanol extract of D. stramonum at 17.00min for H. bidorsalis (Adebayo and Olufayo 2017) and 8.0min of clove oil at 477.50 Seconds for *C. gariepinu*s (Olufayo and Ojo 2018).

This differences could be attributed to species and variation in biological and environmental factors that influences the efficacy of botanicals used as anaesthetic agents. This findings agreed with Akinrotimi *et al* (2014) who reported a higher concentration of aqueous extract of Indian almond tree than clove seed as anaesthetic agent to *C. gariepinus.* The higher the concentration, the lower the time to achieve complete immobilization and the longer the time to regain normal swimming (full recovery). This finding agrees with the results of many researchers (Keene *et al* 1998; King *et al* 2005; Velisek *et al* 2006; Sudagara *et al* 2009). Similar observation recorded in this study is an indication that clove powder at 120-180mg/l induced anaesthesia in *H. bidorsalis*. However to obtain rapid anaesthesia of less than 5 minutes and longer recovery time of not less than 15 minutes, 140-180mg/l is required. Sudagara et al (2009) reported that a ranged of 225-350mg/l of clove powder was required to completely immobilized Rutilus ratilus in less than 4mm while Cunha et al (2010) reported a range of 100-500mgl essential of oil of Lippa alba to induce deep anaesthesia in sliver catfish within 2-4 minutes and recovers within 6-12minutes. According to Mylonas *et al* (2005), an ideal anaesthetic ought to induce anaesthesia in less than 3 minutes, permit fast recovery of in 10 minutes, produce no poison to the fish, caused no hazard to human and inexpensive. However longer recovery time recorded in this study was in line with those of many other researchers,(Akinrotimi et al 2013; Martin *et al* 2009; Solomon *et al* 2014) using various plant extracts as anaesthetic agent to fishes. Samoes et al (2011) reported that the most appropriate clove oil concentration to induce surgical anaesthesia in Silver catfish was 90mg/l while 50-60mg/l was used for brief handling during biometric studies to provides fast recovery. Modern aquaculture activities such as breeding, transportation, blood sampling and morphometric studies can induce stress and even mortality hence the need for anaesthetic at various concentration depending on the activity to be performed. Summerfelt et al (1990) reported that anaesthetics act on the central nervous system (CNS) and induces calming effects, loss of equilibrium, mobility, consciousness and loss of reflex actions. In this study depending on the aquacultural procedure clove powder of between 120 – 180mg/l is effective to anaesthetized *H. bidorsalis* juveniles.

Haematological parameters

Haematological parameters have been employed in monitoring the responses of fish to stressor and evaluating the health status of fish (Campbell 2007; Ralio and Mikinman (1985). *Haematological* parameters are valid and often used to measure fish heath status and can provide information about the internal environment of fish (Velisek 2007, 2011; Okey *el al* 2013). This study showed a slight variation in the blood parameters recorded especially at higher concentration of 160-180mg/l. The study also showed a decrease values ($p,< 0.05$) in PCV, RBC and Hb while WBC, Plt and MCV increased with increasing concentration. This finding was in agreement with Olufayo and Ojo (2018) who reported a significant decrease in the PCV, Hb RBC with increase in WBC and MCH of H. bidorsalis juveniles exposed to clove oil anaesthetic. Similar result was reported on various African clariids using various clove products (Akinrotimi *el al* 2015; Okey *el al* 2013; Olufayo and Ojo 2018). Slight reduction in RBC, Hb and PCV values is a symptom of anaemia caused by exposure to very high concentration of the anaesthetic.

The increase in WBC, Plt, MCV and lymphocytes reported in this study agreed with the report of Olufayo and Adeyanju (2012) who worked on the haematological effects of neem leaves (Azadirachta indica) on *H. bidorsali*s and Akinrotimi *et al*. (2015) who worked on the blood of C. gariepinus exposed at clove seed anaesthetic. This increase may be as a result of the physiological reaction inform of defense mechanism to the stress induced by the anaesthetic to counter the effects on the increasing concentration of the anaesthetic and also self-mechanism against the destruction of erythrocytes. This was in line with the study of Olufayo and Ojo (2018), Adebayo and Olufayo 2017 on African catfishes. Lymphocytes is the must number of the white blood differential component and functions in the production of anti-bodies and chemical substance serving as defense against infection (Iheanacho *et al*., 2017). The increase in the lymphocytes recorded with increasing concentration could be due to the increase in the production of antibodies to defense the cells against destruction. Savaeina et al (2016) and Addolazizl *et al* (2011) reported that clove oil did not cause any significant alteration in the haematological variables of burbot (Lota lotu) and gold fish (Carasius auratus) respectively. This could be due to the fact that these concentrations were safe (ideal) to the fishes. Stetter (2001) and Okey *et al* (2013) stated that an ideal concentration for an anaesthetic agent should have a rapid induction time of 3-5minutes with little or no effects on its haematological variable. In this study 140 – 160mg/l was considered ideal concentrations to immobilised *H. bidorsalis* juveniles at 3 – 5 minutes without any significant change in blood parameters. For quick anaesthesia of less than 3 minutes, concentration of more than 160mg/l is required, although with significant effects in some haematological parameters however, Sudagara *et al*. (2009) reported a reversal in significant changes recorded in the haematological of *R. rutilus* 24 hour after recovery from clove powder anaesthetic. The values reported in this study were within the range of values reported for apparently healthy African clarrids (Gabriel *et al* 2004; Adeyemo, 2004). This could probably be the reason why no mortality was recorded during and after 30 minutes of anaesthesia bioassay with clove powder.

Biochemical parameters

Biochemical parameters are useful tools to evaluate the stress condition of the fishes (Wagner and Congleton, 2004). Plasma cortisol and sugar are physiological indicators of stress in fishes and their exposed to handling and xenobiotics (Wagner *et al*., 2002). In this study a significant increase in the values of plasma glucose, protein, triglyceride and cholesterol levels with increasing concentration of clove powder was recorded. This corroborates with the studies of Farahi *et al* (2011) using clove essence on Rutulus frisii, Caspian kutum, Chelladurai *et al* (2013) using clove oil on Channa puntatus, and Svacina *et al* (2016) using clove oil on Lota lota, burbot. Increase glucose level although concentration dependent is an indication that the procedure caused some stress in *H. bidorsalis.* According to Inoue *et al* (2005) a rise in glucose concentration is a second order reaction under stress and is mediated by the rise in cortisol concentration by stress. Iversen *et al* (2003) found no change in glucose concentration of Atlantic salmon following clove oil anaesthesia. Similar observation was reported common carp anaesthetized with 2-phenoxyethanol (Velisk and Svobodovo, 2004). The increase recorded in this study may be due to increase demand for energy resulting in increased plasma catecholamine and corticosteroids, known to induce excessive secretion of adrenalin, which stimulate breakdown of glycogen, to glucose by inhibiting the neuroeffector sites in adrenal medulla to satisfy their new energy demand (Picking 1982; Verma *et al*., 1983). This increase may not be due to stress, since it returns to normal soon as the fish fully recovers from the anaesthetics.

The increase in total protein level reported in this study agree with Velisek *et al* (2006) and Savacina *et al* (2016), using various types of anaesthetics on fishes. The increase in plasma protein may lead to increased osmotic pressure, osmolality of the plasma and hyperproteinaemia causing relative changes in blood protein mobilization (velisek *et al*., 2006; A1-Attar, 2005). It could also be an attempt of the exposed fish to meet up increasing demands to detoxification, immune response and body physiological reaction to the toxicant (Gill *et al* 1991; Mommsen et al 1999). The rise in cholesterol is an indication of stress and increase lipid mobilization due to decrease lipoprotein lipase activity (Sharma et al 1982). According to Iwama et al (1989) fish under stress mobilized triglyceride and protein to fulfil an increased energy demand to sustained increased physical activities, biotransformation and excretions of the xenobiotic. This could be the reason for the slight increase reported for *H. bidorsalis* exposed to clove powder in this study. The values of urea in this study did not differ (p >0.05) from those of the control and was in line with the report of Velisek *et al* (2005, 2006) using 30mg/l of clove oil on European catfish and common carp respectively. This implies that fish under anaesthesia have reduced metabolic activities hence generate little or no nitrogenous waste and evidence by the non-significant changes in water quality parameter of the test solutions recorded throughout the duration of the experiment. However, Bamidele (2007) reported an increase level of urea and uric acid on *C. gariepinus* exposed to a toxicant, malachite green. Tiwari and Singh (2004) reported that increased urea could be due to protein being used to meet energy demand during xenobiotics intoxication. With non – significant level of urea recorded in this study is an indication that clove powder was not toxic to the cells of the test fish.

Plasma enzymes

Plasma enzymes (LDH, Ck) and the transaminase (ALT, AST) are indication of stress (Ishikawa *et al*., 2007). They also give specific information about organ dysfunction (Wagner and Congleton 2004). High levels of AST would indicate a greater energy demand which is normally associated with synthesizing activities of the cell (Savacina *et al* 2016). Alanine aminotransferase (ALT) is frequently use in the diagnosis of damage caused by xenobiotics in various tissues of fish (De la Torre *et al*., 2000). In this study, H. bidorsalis had significant (P< 0.05) decreased in the plasma levels of CK, LDH, ALT, ALP while AST increased with exposure to Clove powder anaesthetic for 30 minutes. Velisek and Svobodovo, (2004) reported a significant increased (P< 0.05) in blood plasma enzyme, alanine aminotransferase (ALT) levels from 0.15 µkat/l to 0.35 µkat/l after 24h anaesthesia of common carp with 2- phenoxyethanol while no significant changes (P> 0.05) were reported for alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatine kinase (CK). Alanine aminotransferase is known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental conditions (Sastry and Subhadra 1985). The significant increased (P< 0.05) in ALT observed in this study at higher concentrations may have also contributed to the rise in glucose levels of the exposed fish to Clove powder. Sastry and Sharma (1980), Voet and Voetová (1990) reported that a decrease due to Inactivation of Che enzyme causes a blockage of the cholinergic transfer of nerve signals, paralysis and death due to asphyxia of Channa punctata following an action of diazinon. This could be the reason why anaesthetized H. bidorsalis at stage 4 did respond to external stimulus due to decreased in the plasma level of the enzyme Che with increasing concentration of Clove powder. The fact that the fish regained consciousness during recovery and no mortality recorded during and after exposure to Clove, inferred that the level of Che and other enzymes in the fish returned to normal during recovery.

Conclusion

Fish exposed to concentrations less than 120mg/l could not attained anaesthesia. The induction as well as recovery time were dependant on the concentration of the clove powder. The values of pack cell volume (41.08 – 29.65%), red blood cell (4.45 – 2.66 x 1012 cells/l), Haemoglobin (9.04 – 5.73g/l), basophil (2. 89 – 1.59 x 10^9cells/l) and monocytes (2.07 – 1.93x 10^9cells/l) decreased while white blood cell (65.47 – 135.85x109cells/l), platelet (62.23 – 136.75x 10^9cells/l), mean cell volume (84.44 – 122. 42fl) and Lymphocytes (14.04 – 18.30 x 10^9cells/l) increased with increase in concentration of the anaesthetic. A reduction in the plasma levels of creatine kinase (146.56 – 136.59 IU/L), lactate dehydrogenase (96.85 – 82.98 IU/L),alanine aminotransferase (31.17 – 20.52 IU/L) and alkaline phosphatase (19.51 – 17.28 IU/L) were decreasing while the aspartate aminotransferase (123.53 – 147.09 IU/L) and cholinesterase (29.19 – 53.23 IU/L), increased with increasing concentration of clove. The study shows that clove powder increase stress parameters such as glucose and AST due to the hyper motility of the fish culminating to achieving anaesthesia however, the fast recovery and normal swimming behaviour exhibited by the fish is an indication that is was the stress was temporary. The fact that H.

bidorsalis recovered and exhibited no abnormal behaviour, no mortality and minimal disruption in haematological and biochemical parameters after 30mins is an indication that Clove powder is less toxic. Therefore Clove powder can be recommended as anaesthetic for African catfishes and should be encourage to reduce stress and mortality during handling and transportation of live fish for aquaculture.

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