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Journal of Aquatic Sciences and Oceanographye

Reasearch Article

Haematological and Biochemical responses in African catfish, (*Clarias gariepinus*) juveniles immobilized with clove basil, (*Ocimum gratissimum*) powder Anaesthetic

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Citation: IB Okey, MR Igiri, JJ Ekpenyong, FU Inya (2022) Haematological and Biochemical responses in African catfish, (*Clarias gariepinus*) juveniles immobilized with clove basil, (*Ocimum gratissimum*) powder Anaesthetic. J Aquat Sci Oceanogr 3: 102

Abstract

Clarias gariepinus is the most suitable aquaculture fish species in Nigeria and are very active therefore must be anaesthetized during handling and transport to reduce mortality due to stress. Haematological and biochemical parameters have been employed to assess the health status of fishes. Information on the use of these parameters to evaluate the health status of African catfish treated with plant-based anaesthetics is still limited. Therefore this present study evaluated the effects of haematological and biochemical parameters of African catfish treated with *Ocimum gratissimum* (scent leaf) powder. One hundred and fifty (150) African catfish juveniles of mean body weight (34.50g ± 4.25) and total length (17.60cm ± 5.70) were treated with (0, 100, 120, 140, 160 and 180mg/l) of scent leaf powder in triplicates. The time to attain deep anaesthesia (completely unconscious) and full recovery (normal swimming) was noted and recorded using a stop watch. Blood samples were collected from fish for haematological and biochemical analyses following standard methods. The result showed that fish treated with 100mg/l of were not completely immobilized while shorter induction (deep anaesthesia) of 2.25 minutes and a longer recovery time of 18.50 minutes were achieved with the highest concentration of 180mg/l. There was a strong linear relationship between induction time (R2 =0.998) and recovery time (R2 =0.997) against concentration. The red blood cell (RBC), haemoglobin (Hb), pack cell volume (PCV) and mean cell haemoglobin concentration (MCHC) decreased slightly while white blood cell (WBC), platelets (Plt) and all the differential counts increased significantly ($p < 0.05$) at the highest increasing concentrations of scent leaf. A slight decrease in plasma levels of cholinesterase (Che), lactate dehydrogenase (LDH), creatinine kinase (CK) and calcium (Ca) and a significant increase in aspartic aminotransferase (AST), sodium (Na) and unchanged values of the Hydrogen bicarbonate (HCO3) were recorded with increasing concentrations of the anaesthetics. All the metabolites did not increase significantly except triglycerides while cholesterol decreased significantly ($p < 0.05$) in the treated fish. Since there was no mortality observed in the anaesthetized fish and with minimal changes recorded in some of the haematological and biochemical parameter, Ocimum gratissimun is recommended as suitable anaesthetic for African catfish used within the range of 140 – 180mg/L.

Keywords: *Clarias gariepinus*; Scent Leaf; Anaesthesia; Haematological Parameters; Biochemical Parameters

Introduction

Sedative and anaesthetic agents are very useful for reducing the stress caused by handling, sorting, transportation, artificial reproduction, tagging, administration of vaccines and surgical procedures in fishes [1]. There are also used to immobilize fish so they can be handled more easily by biologists during blood sampling and research experiments [2, 3]. Some researchers have worked on plant extracts as a natural anaesthetic because it is cheaper, safer and more effective at lower concentrations when compared with synthetic anaesthetics [4,5,6,7,8,9] reported that Clove oil induced anaesthesia faster and at lower concentrations than MS -222, although the efficacy of anaesthetics can be affected by species, body size, the density of fish in the bath as well as water quality [10]. The powder produced from the clove plant (Eugenia spp) have also been used for short-term immobilization of fish, Roach (*Rutilus rutilus*) in Iran (Sudagara et al.2009) and clove powder on *C. gariepinus* in Nigeria [13,5,14,5]. Studies have also been reported on the use of certain plant materials to anaesthetize African catfish [16] (Olufayo and Ojo 2018) although with some degrees of side effects on the exposed fish. Extracts from clove basil have been used for short term immobilization of African catfish [6], Nile tilapia, *Oreochromis niloticus* [9] and silver catfish (*Rhamdia quelen*) juveniles [103]. Researchers have stated that sizes, body weight, species, environmental conditions and pharmacokinetics of the anaesthetic agent influence its efficacy and effectiveness [98] (Mitjana et al 2014). Plant materials are shown to produce genotoxic effects in fishes by changing their enzymes profile and immune stimulation of exposed fish [18]. They have also been reported to cause the death of fish and changes in behavioural, haematological, biochemical responses [19,20] and even histopathological changes [21,22] on clariids. Knowledge about the ideal and optimum concentration of plant anaesthetic for various fish species is necessary because inappropriate concentrations may lead to adverse effects on the blood chemistry resulting in stress and mortality [23] (Hoseini and Ghelichpour 2012).

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec et al., 2000). Haematological characteristics of most fish have been studied to establish a normal value range and deviation from it may indicate a disturbance in the physiological process (Rainza-paiva et al., 2000). Several researchers have reported significant changes in haematological parameters of various fish species exposed to xenobiotics [62,114,23]. Studies have also shown changes in haematological indices of African clariids exposed to various toxicants under laboratory conditions [25,26]. 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). The immune status of fish is related to haematological parameters such as white blood cell, platelet and total and differential counts are effective tools that can be used to evaluate physiological, biochemical and pathological changes in fish. Biochemical parameters reflect the condition of fish more quickly than other common measures parameters since they respond quickly to changes in the environmental conditions [27]. They have been widely used for the assessment of fish health, monitoring stress responses, predicting the systematic relationship and physiological adaptions of animals [28,20] (Ralio and Mikinman, 1985). Cells naturally contain enzymes for their functions such that damages to the cellular membrane lead to their escape into the blood where their presence or activities can be measured as an index of cell integrity [30]. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) are normally found within the cells of the liver, heart, gills, kidneys, muscles and organs [31] but their increase in the plasma indicate tissue injury or organ dysfunctio [32]. Changes in enzymes profiles are important toxicity indices (bio-markers) and have been used to assess the biochemical and physiological health of vital organs (tissues) in fishes [33]. According to [33], the antioxidant enzyme is responsible for preventing cellular damage and improving immune competence.

African Catfish is widely cultured in Africa, Europe and some parts of Asia for its hard nature. It has been a suitable candidate for aquaculture because of its high prolificacy, simplicity of culture, possession of arborescent air-breathing organ, omnivorous feeding habit, repaid growth rate and high feed conversion rate (Hecht et al 1996). *Clarias graiepinus* is in great demand in Nigeria because of its striking attributes and palatability [34].

Ocimum gratsissimum (Lamiaceae), commonly known as scent leaf or African clove basil is found in many tropical countries of the world including Nigeria [35]. In Nigeria, the plant grows in all regions, found in many farms, residential and industrial areas (Effraim et al 2000). Many authors have reported its phytochemical properties to include eugenol, methyl cinnamate, camphor

flavonoid, saponins and thymol [37,38,39] have also reported its major constituents to include eugenol (42.3%), cineole (20.4%), caryophyllene (5%) among other compounds. The plant has been used for many purposes ranging from human consumption to its application in traditional medicine in Nigeria. It is used as a condiment and as sedative for the treatment of stress, headache and other diseases including diarrhea, conjunctivitis, skin diseases and pneumonia (IIori et al 1996). Several studies have also shown various effects of Ocimum species to include bactericidal, anti-inflammatory or, anti-fungal, anti-oxidative, antiulcer, hypoglycemic, nervous stimulation, chemopreventive and radiation protection. According to Silva [36], eugenol is the main compound of scent leaf have been reported to cause anaesthesia, analgesic, antimicrobial, antifungal and immunostimulant activity in exposed organisms. (Meneses et al 2018) have reported its phytotherapeutic efficacy against monogenean, *Cichlidogyrus tilapiae* in the gills of Nile tilapia. The essential oil (eugenol) extracts from the clove basil have been reportedly used in short-term immobilization of silver catfish [36], Tambaqui, *Colossoma* macropomum [38], matrinxa, *Brycon cephlus* [98] and Nile tilapia [9]. Recently Okey and Igiri (2021) reported the efficacy of scent leaf powder for immobilization of African catfish juveniles. In Cross River State and most part of Nigeria, it is mostly used to prepare "pepper soup" pottage plantain and yam in various ceremonies. According to [9], the good aroma has become a delicacy and serves as a spice for fish and meat products such as "Kilishi", "Dembu" and "Yaji". Despite the enormous uses of *O. gratissimum*, there is a paucity of information on possible effects of short–term immobilization in haematological and biochemical parameters of African catfish. The study is aimed at investigating the effects of short-term induction and recovery time on the blood profile of African catfish exposed to *Ocimum gratissimum* powder. The findings will shed more light and act as a guide on the management in fish.

Materials and Methods

Study location, Fish and Scent leaf powder

This research was carried out at the Wet Laboratory, Department of Fisheries and Aquatic Science, Cross River University of Science and Technology (CRUTECH), Obubra Campus. Fresh leaves of *O. gratissimum* was sourced within the premises of the University campus, identified in Forestry Department air-dried for 5 days. It was then pulverized with a sterile manual blender and sieved with a 100-micron net to obtain a fine powder. One hundred and fifty (150) healthy juveniles of *Clarias gariepinus* mean body weight (34.50g ± 4.25) and mean total length (17.60 cm ± 5.70) were procured from the University of Calabar (UNICAL) Fish Farm Calabar, acclimated for 2 weeks in groups of 10 fish per rectangular glass aquaria and fed twice daily with a commercial feed (Coppen) of 40% crude protein at 1% body weight. The fish were starved 24 hours before the commencement of the experiment to avoid contamination of the test solution. A stock solution of 200mg/l of the powder was prepared by dissolving 2g into 10 litres of water using the appropriate formula and concentrations of 100, 120, 140, 160 and 180mg/l for the bioassay were obtained by serial dilution of the stock solution.

Experimental procedure

Anaesthesia bioassay

Thirty (30) glass aquaria were cleaned and randomly labelled and each filled with water to the 25 litres mark for induction test and 30 litres mark for recovery in each of the experiments. The mixtures were stirred thoroughly to ensure homogeneity of the test solution. Ten (10) fish were randomly selected into the test aquaria and monitored for the onset of the various stages of induction and recovery, recorded for 30 minutes according to [16]. Any test fish that lost balance and no longer responded to external stimulus (Deep anaesthesia) was removed immediately and transferred to 30 litres of powder-free water for Recovery. At the recovery tank any test fish that regained equilibrium, responses to tactile stimulation and pre-anaesthetic appearance was considered to have fully recovered. The time of induction (deep anaesthesia) and recovery (full recovery) from the scent leaf powder solution (anaesthetic) were noted and recorded using a stopped clock. These behavioural changes of the fish in response to the effects of the scent leaf are observed according to [67]. The number of fish that were completely immobilized was computed as Number of fish completely anaesthetized ÷ number of fish in the tank

Blood sampling

After 30 minutes of anaesthesia, 2ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes into sample bottles containing sodium ethylene diamitetraacetictic acid (EDTA) as an anticoagulant for haematological parameters and the other into a tube containing Lithium heparinised anticoagulant to obtained plasma for biochemical parameters analysis. The samples fish were mopped with tissue paper to prevent haemolysis due to dilution of oozing blood with any other fluid and the blood sample was rocked gently in the tube to allow thorough mixing of its contents. Thereafter, the blood samples were taken to the Departments of Haematology and Biochemistry, University of Calabar Teaching Hospital (UCTH) for haematological and biochemical analyses respectively.

Haematological parameter

The direct measurement of erythrocyte value (Packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC), platelet (Plt) and White blood cell (WBC) were done using an Automated haematological analyzer. The absolute erythrocyte indices (MCH, MCV and MCHC) were calculated using the formulae according to Lee et al. (1998).

Mean cell volume (MCV) expressed in femtolitre (10^{-15})

$$MCV\ (fl) = \frac{PCV\ (\%)}{RBC\ (1012\ Cells/\ L)} \times 10$$

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

$$MCH\ (pg) = \frac{Hb\ (g/l)}{RBC\ (10^{12}\ Cells/\ L)} \times 10$$

Mean cell haemoglobin concentration (MCHC) indicates the haemoglobin concentration in 100ml of packed red blood cells. It is expressed in grams per 100ml MCHC (g/100ml)

$$MCHC\ (\%) = \frac{Hb\ (g/l)}{PCV\ (\%)} \times 100$$

The differential white blood count (eosinophil, basophils, neutrophils monocytes and lymphocytes) were analyzed as described by Davie and Lewis (2001). The various differentiated cells identified were counted and expressed as a percentage of the total WBC in the sample.

Biochemical parameter

The clotted blood was centrifuged for 15 minutes at 3500 revolutions per minute (rpm). A clear fluid which is the plasma was pipetted out into clean sterilize bottle for further analysis. The stored serum was used for the analysis of some metabolites, enzymes and electrolytes using a commercial kit, VetTest Biochemical Analyzer (Idexx Lab., USA). The metabolites determined were glucose (Gluc), protein (Prt), cholesterol (Chol), urea (Urea) and triacylglycerols (Trgly) while enzymes were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Cholinesterase (CHOS) and creatinine kinase (CTK). The plasma electrolytes also determined were sodium (Na+), potassium (K+), chloride (Cl-), bicarbonate salt (HCO3-), Phosphorus (P3-) and Calcium (Ca2+).

Data analysis

The data obtained from the experiment were subjected to multivariate analysis using a statistical software SPSS version 25 to compute for the mean value of the variables of scent leaf powder deep anaesthesia, full recovery, haematological and biochemical parameters of the experimental fish according to [6]. The differences among the means were compared using Turkey's multiple comparison test at 5% significance level. Regression analysis was computed to determine the linear relationship between independent variable (concentration) and dependent variables (deep anaesthesia and full recovery time) according to [53]. Linear equations was predicted for time to achieve deep anaesthesia and regain full recovery from the anaesthetic.

Results

Deep anaesthesia and full recovery

Table 1 shows the number (%) and time (min) taken for African catfish to attain deep anaesthesia and full recovery respectively from scent leaf anaesthetic. The result revealed that fish treated with 100mg/l did not achieved deep anaesthesia while those treated with 120 and 140mg/l had 60.25 and 83.33% respectively of the fish anaesthetized. Fish treated with higher dosage of 160 and 180mg/l had 100% complete immobilization (deep anesthesia). The time to achieved deep anaesthesia shows that, higher concentration tends to reduce the time to achieve deep anaesthesia. The induction time reduced from 11.08 min to 2.25 min as concentration increase from 120 – 180mg/l. Faster induction time of 5.48 and 2.25 min was achieved with higher dosages of 160 and 180mg/l respectively of scent leaf powder. There was significant variation in the time to attained complete immobilization for fish treated with 120mg/l compared with other dosages at $p < 0.05$. The time to regain full equilibrium and normal swimming increased from 4.08 – 18.50 min as concentration increase from 120 – 180mg/l. Fish treated with higher dosages 160 and 180mg/l took longer time of 13.28 and 18.50 min to fully recover. There were also significant variations ($p < 0.05$) in the recovery time for fish treated 120mg/l compared to other dosages of the powder.

Item	Concentration (mg/l)				
	100	120	140	160	180
Number of fish anaesthetized (%) = $\frac{NFI}{NFT} \times 100$	-	60.25 ± 3.67^{b}	83.33 ± 2.25^{ab}	100 ± 0.00^{a}	100 ± 0.00^{a}
Number of fish recovered (%) = $\frac{NFI}{NFR} \times 100$	-	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
Time of complete immobilization (min)	-	11.08 ± 3.55^{a}	8.03 ± 0.28^{b}	5.48 ± 0.55^{c}	2.25 ± 0.47^{d}
Time of full recovery (min)	-	4.08 ± 1.20^{d}	8.20 ± 0.47^{c}	13.28 ± 1.18^{b}	18.50 ± 1.61^{a}
NFI = Number of fish completely immobilized, NFR = Number of fish fully recovered, NFT = number of fish treated (30), Mean with the same superscript under the same row are not significant at $p < 0.05$					

Table 1: The number (%) and time (min) of deep anaesthesia and full recovery in *C. gariepinus* treated with scent leaf powder solution

The relationship between concentration of the scent leaf powder and time (min) required to attain deep anaesthesia is shown in Figure 1. The result showed the predicted equation ($y = -0.145x + 28.48$, $R2 = 0.997$) indicating a decrease in the time to achieve deep anaesthesia as concentration increase. For any unit rise in concentration there was -0.145 decrease in the time of induction with a significant linear relationship ($R2 = 0.998$) between concentration and induction time. The predicted equation for full recovery ($y = 0.241x – 25.24$, $R2 = 0.998$) shows a 0.241 increased in time to regain full recovery for any unit increase in concentration of scent leaf powder with also a higher linear relationship (100%) between concentration and time to regain full recovery. However, the

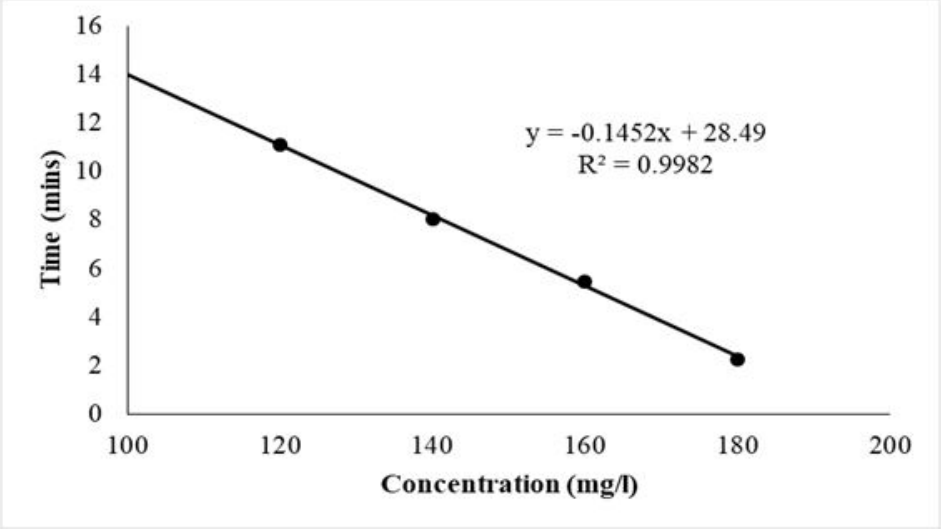

Figure 1: Relationship between concentration (mg/l) and time (min) of *C. gariepinus* to attain full recovery from scent leaf powder solution

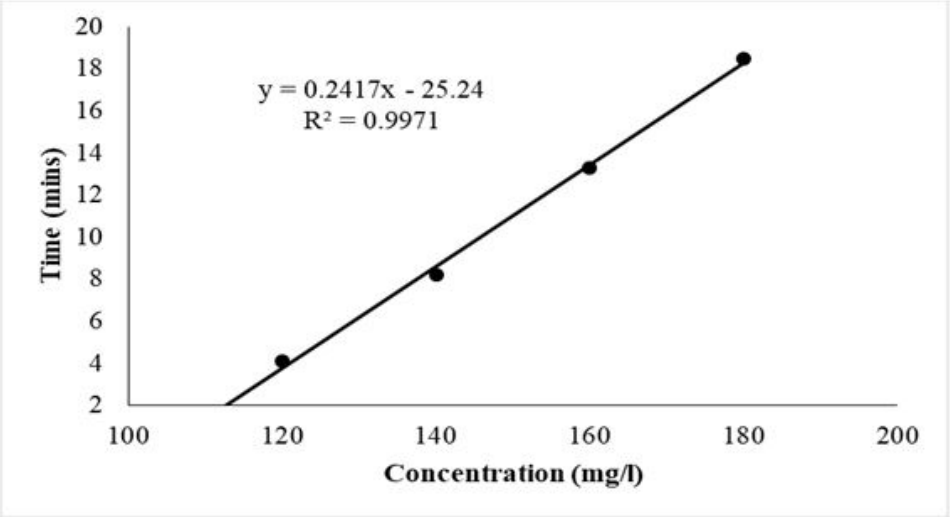

Figure 2: Relationship between concentration (mg/l) and time (min) of *C. gariepinus* to attain full recovery from scent leaf powder solution.

Heamatological parameters

Table 2 shows the haematological parameters of *C. gariepinus* treated with *O. gratissimum* leaf's powder anaesthetic at different levels (0, 100, 120, 140, 160, and 180mg/l). The result revealed that the mean values of the RBC (3.12± 0.64 – 1.06 ± 0.04 x 1012cells/L), PCV (40.60 ± 1.41 – 34.05 ±1.05%), Hb (17.12± 1.82 – 11.58 ± 0.50g/dl) and MCHC (42.17 ±4.65 – 34.01 ± 1.55%) decreased while those of erythrocytes indices MCV (128.21 ± 2.50 – 193.46 ± 3.06fl) and MCH (54. 87 ± 1.37 – 69.57 ± 1.36pg) increased from the control as concentrations increases. Fish treated with higher concentrations (160 and 180mg/l) had significantly lower mean values of RBC, PCV, Hb and MCHC with higher values of MCV and MCH than the control at ($P < 0.05$). However, those treated with 100 – 140mg/l of the anaesthetic had mean values that were not significantly higher ($p > 0.05$) while the values of WBC (27.06 ± 1.24 - 31.73 ± 1.78 x 109cells/L) and platelets (38.35 ± 0.47 and 39.58 ± 1.25 x 109cells/L) in fish exposed 160 and 180mg/l were significantly higher ($p < 0.05$) than those of the control. More so, all the values of the differential white blood counts of lymphocytes (17.04 ± 1.02 - 19.65 ± 1.32%), eosinophil (1.61 ± 0.23 – 2.80 ± 0.54%), basophils (1.07 ± 0.17 – 2.27 ± 0.35%), neutrophils (4.03 ± 0.34 – 5.37

± 1.04%) and monocytes (3.31 ± 0.55 – 3.98 ± 1.05%) increased from the control as concentrations increases. Lymphocytes were recorded as the most populated followed by neutrophils while basophils and eosinophils were the least cells. Fish exposed to the

Parameter	Concentration (mg/l)					
	0.00	100	120	140	160	180
RBC(x 10^{12}cells/L)	3.12 ±0.64^{a}	2.35 ±0.35^{ab}	2.26 ±0.08^{ab}	2.16 ± 0.67^{ab}	1.87 ± 0.17^{b}	1.76± 0.04^{b}
PCV (%)	40.60 ± 1.41^{a}	38.46 ± 0.66^{ab}	36.90 ± 1.14^{b}	36.65 ±0.95^{b}	34.85 ±2.18^{b}	34.05± 1.05^{b}
Hb (g/dl)	17.12 ± 1.82^{a}	16.35 ±0.43^{a}	14.87 ± 0.44^{ab}	14.45 ± 0.33^{ab}	12.96 ± 0.09^{b}	11.58 ± 0.50^{b}
MCV (fl)	128.21 ±2.56^{d}	163.66 ± 1.57^{c}	163.27±2.61^{c}	169.68±2.68^{bc}	186.36±2.04^{ab}	193.46 ± 3.06^{a}
MCH (pg)	54.86 ±1.37^{b}	69.56 ± 2.30^{a}	65.80 ±0.54^{ab}	66.45± 2.36^{ab}	69.57 ± 3.13^{a}	65.80 ± 1.36^{ab}
MCHC (%)	42.17 ±4.65^{a}	42.51 ± 1.09^{a}	40.30 ±1.57^{a}	39.43± 1.19^{a}	37.19 ± 1.20^{ab}	34.21 ± 1.55^{ab}
WBC (x10^{9}cells/L)	27.06± 1.24^{b}	27.64 ±1.29^{b}	28.42 ±0.47^{ab}	29.75± 0.31^{ab}	31.36 ±0.65^{a}	32.73 ±1.78^{a}
Plt (x 10^{9}cells/L)	36.04 ± 0.45^{b}	36.93 ± 0.44^{b}	37.34 ± 1.91^{ab}	37.58 ±0.39^{ab}	38.35 ±0.47^{a}	39.58 ± 1.25^{a}
Differential White blood cell counts (%)						
Lymphocytes	17.04 ±1.02^{c}	17.63 ± 0.74^{bc}	17.67 ±0.65^{bc}	18.48± .46^{ab}	19.25 ±0.38^{ab}	19.65 ± 1.32^{a}
Eosinophil	1.61 ± 0.23^{c}	1.75 ± 0.07^{bc}	2.04 ± 0.08^{bc}	2.26 ± 0.09^{ab}	2.57 ± 0.44^{ab}	2.80 ± 0.54^{a}
Basophils	1.07 ± 0.17^{c}	1.13 ± 0.15^{bc}	1.58 ± 0.30^{bc}	1.96 ± 0.13^{bc}	2.05 ± 0.49^{ab}	2.27 ± 0.35^{a}
Neutrophils	4.03± 0.34^{b}	4.50 ± 1.10 ^{ab}	4.61± 0.25^{ab}	4.96 ± 0.50^{ab}	5.10 ±1.02^{a}	5.37 ±1.04^{a}
Monocytes	3.31± 0.55^{b}	3.37 ± 0.29^{ab}	3.41 ± 0.23^{ab}	3.66 ± 0.22^{a}	3.81 ± 0.52^{a}	3.98 ± 1.05^{a}
Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), platelet (Plt), White blood cell (WBC), Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) Mean with the same superscript in each parameter is not significant at p< 0.05.						

Table 2: The mean values of the haematological parameters of *C. gariepinus* juveniles treated with *O. gratissimum* leaf powder

Biochemical parameters

The results of the mean values of the biochemical (plasma enzymes, electrolytes and metabolites) parameters of African catfish treated with *O. gratissimum* powder solution is presented in Table 3. The mean values of plasma enzymes Che, LDH, AST, ALT, ALP and Ck of African catfish treated with clove basil powder anaesthetic revealed a decreased in Che (51.68 ± 2.25 – 37.45 ± 0.65 IU/L), LDH (99.55 ± 1.58 – 85.27 ± 2.09IU/L), ALT (65.55 ± 5.04 – 53.66 ± 1.05 IU/L) ALP (75.57 ± 4.73 – 59.91 ± 1.15 IU/L) and CK (136.07 ± 2.45 – 59.91 ± 1.07 IU/L) while AST (48.44 ± 0.87 – 64.68 ± 2.16 IU/L) increased as concentrations increases from those of the control. Creatinine kinase had the highest mean value of 136.07IU/L followed by LDH (99.55IU/L) while 37.45 and 48.44 IU/L values were recorded for Che and AST enzymes respectively. The enzyme Che was not significant ($p > 0.05$) from those of the control across all the treatments except at the highest concentration whereas ALT and CK were only significantly lower than those of the control at the highest concentration (180mg/l) of scent leaf powder solution. The values of LDH, AST and ALP enzymes in fish treated with 100, 120 and 140mg/l were not significantly different from those of the control at $p < 0.05$.

The mean values of the plasma electrolytes sodium, potassium, phosphorus, calcium, chloride and bicarbonate of African catfish treated with *O. gratissimum* is shown in Table 3 The values of Na+ (94.80 ± 6.07 – 118.20 ±2.35 mmol/dl), K+ (21.35 ± 0.10 – 33.08 ±1.33 mmol/dl), Cl (57.13 ± 4.56 – 76.70 ± 2.25 mmol/dl) and HCO3 (16.97 ± 0.40 – 21.06 ± 0.61 mmol/dl) increased while Ca2+ (19.21 ± 0.86 – 14.84 ±1.32 mmol/dl) decreased from the control with increasing concentrations of scent leaf powder solution. Sodium (118.20mmol/dl) appears to be the highest electrolyte followed by Cl (76.76mmol/dl) while Ca2+ (14.84mmpl/dl) and P (11.31mmol/dl) were the least recorded in this study. The values of potassium and bicarbonates in the treated fish across all the concentrations did not differ significantly while phosphorus and chlorides differ only at the highest concentration (180mg/l) from

the control at a 5% significant level.

The mean values of the plasma metabolites glucose (Glu), total protein (TP), triacylglycerol (Trgly), cholesterol (Cho) and urea (Ure) of fish treated with clove basil is presented in the table 3. The result showed that Glu (22.73 ± 0.37 – 25.97 ± 0.07 mg/dl), TP (31.78 ± 0.29 – 33.97 ± 0.15 mg/dl), Trgly (48.98 ± 2.45 – 68.82 ± 3.21 mg/dl), and Ure (26. 30 ± 0.33 – 27.96 ± 0.75 mg/dl) all increased while Cho (54.12 ± 0.33 – 41.18 ± 1.95 mg/dl) decreased from the control as concentrations increases. Triacylglycerol (68.82g/dl) had the highest value of the metabolites followed by cholesterol (54.12mg/dl) while Glu (22.73mg/dl) and Ure (26.30mg/dl) have the lowest levels in the treated fish. The values of Glu, TP, and Ure of the treated fish were not significant ($p > 0.05$) from those of the control across all the levels of treatment. Fish treated with concentrations above 140mg/l had a significantly higher and lower Trgly and Cho level at $p < 0.05$ respectively.

Parameter	Concentration (mg/l)					
	0.00	100	120	140	160	180
Plasma Enzymes (IU/L)						
Che	51.68 ±2.25^{a}	49.22 ±1.24^{a}	49.84 ±0.65^{a}	45.05 ±0.75^{ab}	41.92 ±1.06^{ab}	37.45 ±0.65^{b}
LDH	99.55± 1.58^{a}	96.99 ± 0.39^{a}	94.38 ± 0.99^{ab}	91.36 ±0.59^{ab}	87.41 ± 1.00^{b}	85.27 ±2.09^{b}
AST	48.44 ±0.87^{c}	50.63 ±1.19^{bc}	53.27 ±0.58^{bc}	57.79 ±3.23^{ab}	63.01 ±2.53^{a}	64.68 ±2.61^{a}
ALT	65.55 ±5.04^{a}	63.77 ±7.61^{a}	59.56 ±2.04^{a}	56.44 ±0.96^{ab}	55.19 ±1.28^{ab}	53.66 ±1.05^{b}
ALP	75.57 ±4.73^{a}	73.40 ±4.61^{a}	68.23 ±3.45^{ab}	65.68 ±0.86^{ab}	61.41 ±3.56^{b}	59.91 ±1.15^{b}
CK	136. 07 ±2.45^{a}	135.06 ±2.05^{a}	135.01 ±0.75^{a}	133.85 ±1.12^{ab}	133.36 ±0.51^{ab}	132.75 ±1.07^{b}
Plasma electrolytes (mmol/dl)						
Na	94.80 ±6.07^{c}	103.76 ±1.12^{bc}	107.36 ±1.08^{ab}	113.33 ±0.81^{ab}	116.57 ±1.57^{a}	118.20±2.35^{a}
K	21.35 ±0.10^{ab}	22.54 ±0.63^{ab}	32.04 ±0.06^{a}	32.23 ±0.08^{a}	32.84 ±0.41^{a}	33.08 ±1.33^{a}
P	11.31 ±0.13^{b}	12.14 ±0.10^{ab}	12.78 ±0.06^{ab}	13.06 ±1.11^{a}	13.26 ±0.15^{a}	13.45 ±1.06^{a}
Ca	19.21 ±0.86^{a}	16.87 ±0.31^{ab}	16.25 ±0.16^{ab}	15.92 ±0.11^{b}	15.23 ±0.14^{b}	14.84±1.32^{bc}
Cl	57.13 ±4.56^{b}	63.59 ±3.69^{ab}	66.98 ±2.62^{ab}	70.44 ±1.80^{ab}	75.50 ±2.30^{a}	76.70 ±2.25^{a}
HCO_3	16.97 ±0.40^{ab}	17.55 ±0.15^{ab}	17.73 ±0.59^{ab}	19.13 ±0.25^{a}	20.58 ±1.12^{a}	21.06 ±0.61^{a}
Plasma metabolites (mg/dl)						
Glu	22.73 ±0.37^{ab}	22.96 ±0.03^{ab}	23.50 ±0.28^{ab}	24.09 ±0.14^{a}	25.06 ±1.03^{a}	25.97 ±0.07^{a}
TP	31.78 ±0.29^{ab}	32.22 ±0.14^{a}	32.45 ±0.05^{a}	32.87 ±0.18^{a}	33.83 ±0.21^{a}	33.97 ±0.15^{a}
(Trgly)	48.98 ±2.45^{b}	53.02 ±2.66^{b}	58.17 ±1.45^{ab}	60.83 ±1.54^{a}	64.81 ±1.27^{a}	68.82 ±3.21^{a}
Cho	54.12 ±2.14^{a}	53.41 ±1.43^{a}	51.53 ±2.12^{a}	49.03 ±1.89^{ab}	44.61 ±1.98^{b}	41.18 ±1.95^{b}
Ure	26.30 ±0.33^{ab}	26.58 ±0.56^{ab}	26.88 ±0.78^{ab}	27.05 ±0.15^{a}	27.56 ±0.51^{a}	27.96 ±0.75^{a}
alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), Cholinesterase (CHOS) and creatinine kinase (CK), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), bicarbonate salt (HCO_3^{-}), Phosphorus (P^{3-}), Calcium (Ca^{2+}), glucose (Gluc), protein (Pt), cholesterol (Chol), Ure (Urea), triacylglycerols (Trgly), Mean with the same superscript in each parameter is not significant at $p< 0.05$						

Table 3: The mean values of the plasma biochemistry of *C. gariepinus* juveniles treated with *O. gratissimum* leaf powder.

Discussion

Deep Anaesthesia and Full Recovery

Anaesthetics are often used to minimize hyper- motility which is a considerable source of injuries and mortality to fish during handling and transportation procedures [57]. The decrease in the time to be completely immobilized with concentration reflects a direct proportional functionality between concentration of the scent leaf powder and the induction time. However, this study shows an inverse proportionality between concentrations and time of recovery. Thisfindings were in agreement with the works of

[4,14,40,20,15]. Shorter immobilization time of 5.48 and 2.25 min were achieved with higher concentrations of 160 and 180mg/l respectively. Shorter immobilization has been reported by several researchers using plant based anaesthetics (Teixeira et al 2017) [54,117,6]. The induction time of 5.48 and 2.25 min was within the ranged of 3 – 5 min recorded at higher concentrations of essential oil of *Lippa* alba on silver catfish (Cunha et al 2010), E. caryophyllata on hybrid catfish [41] and *H. bidorsalis juveniles* [20]. In this study 120mg/l scent leaf powder was required to completely immobilize *C. gariepinus* juveniles in 11.08min. This induction time was lower than the 22.32 min of 120mg/l of clove powder on H. bidorsalis juveniles (Okey 2019) and higher than 4.07 and 4.38 min for catfish hybrid and *C. gariepinus* juveniles respectively [41,20]. This differences in induction time could be attributed to species and variations in biological and environmental factors that influences the efficacy of botanicals used as anaesthetic agents. Shorter induction time was achieved with higher concentration whereas recovery time increase with higher concentrations. The size and life cycle status of anaesthetized fish is also recognized as a factor influencing the concentration of anaesthetic needed to induce anaesthesia within an acceptable time [42]. The recommended treatment concentrations of clove oil on *Danio rerio* and *Poecilia* reticulate vary according to the species, size, exposure time, bath quality and temperature (Doleželová et al., 2011). This finding conforms to those of several workers investigating the effects of anaesthetics on fishes [40,10,78,12,20] reported the ranged of 140 – 180mg/l of clove powder to induce a rapid anaesthesia of less than 5 min and longer recovery of more than 15 min. (Sudagara et al 2009) reported that a ranged of 225-350mg/l of clove powder was required to completely immobilized Roach 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 6-12 min. According *to* (Mylonas et al 2005), an ideal anaesthetic ought to induce anaesthesia in less than 3 min, permit fast recovery of in 10 min, produce no poison to the fish, caused no hazard to human and inexpensive. According to Treves-Brown, (2000) ideal anaesthetic possess several attributes such as non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery. However longer recovery time recorded in this study was in line with those of many other researchers [43,44,45] using various plant extracts as anaesthetic agent to fishes and is important during surgical operations [104]. The high R2 values of induction and recovery (0.997 and 0.998) in this study were higher than the 0.907 and 0.921 reported by [41] and implies that the anaesthetic (scent leaf) works effectively and can be dependent upon. An appropriate anaesthetic depends mainly on its effectiveness in immobilizing fish with good recovery rates [64] (Burka et al., 1997). One of the criteria that proper anaesthetic in fish to meet is its safety at treatment concentration [85]. It is often advisable to identify the lowest effective concentration of different anaesthetics in a specified species, as the responses to the same anaesthetic may vary considerably among different species (Pawar et al., 2011). Fish restarted feeding nearly 8h after the experiment and no fish mortality was observed even one month after the tests suggesting that *O. gratissimum* is safe.

Haematology

Exposure to scent leaf anaesthetic moderately affected the haematological parameters of *C. gariepinus* juveniles. The parameters are often use to evaluate the health status and provide information about the internal environment including metabolic disorders and chronic stress of fish [48] (Adeyemo 2003; Velisek et al 2007). There were significant ($p < 0.05$) variations in some blood parameters from the control of fish treated with higher concentration of between 160 – 180mg/l. this study also showed a decreased in the values of RBC, PCV, Hb and MCHC while those of MCV, MCH, WBC and Plt increased from the control. This observation was in conformity with the findings of previous researchers on the effects of anaesthetics on African catfishes (Olufayo and Ojo 2018) [40,4,20]. The trend recorded in this study and many other studies contradicted [41] who rather reported an increased in PCV, Hb, RCB, MCHC and a decreased in WBC, MCH and MCV in *C. gariepinus* treated with clove oil. Similar findings of a decreased in RBC, Hb and PCV with increased in MCV, MCH, and WBC in Matrinxa juveniles treated with *O. gratissimum* was reported by [98]. However, [6] reported a decrease in all the erythrocyte indices (MCV, MCH and MCHC) of *C. gariepinus* exposed to paraquat herbicide. Slight reduction in the values of RBC, Hb and PCV when exposure to higher concentration is a symptom of commencement of anaemia resulting from inhibition of haemapoietic process. This may also result to immune suppression induced by higher and continuous maintaining the fish in the anaesthetic solution leading to euthanasia of the treated fish. Hashemi et al (2017) reported that lower PCV values of *C. gariepinus* were attributed to anemia resulting from shrunken red blood cells, asphyxiation and death. The red blood indices such as MCV, MCH and MCHC are important in the diagnosis of anaemia in most

animals including fish. A significant increase or decrease in these indices may indicate macrocytic and microcytic anaemia [59]. According to [79] reduction in size and quantity of haemoglobin of red blood cells is measured by the indices MCV, MCH, MCHC which can also be a sign of anaemia in fish. The presence of a large percentage of immature red blood cells in the bloodstream may be a reason for slight increase in MCV, MCH and a reduced MCHC from the control which may be due to decreased production of haemoglobin in fish treated with 180mg/l in this study. During the anaemia, MCHC values reduced because large cells had less haemoglobin concentration (Okomada et al 2013). According to [84] mean cell haemoglobin concentration reduction resulted from increased production and secretion of reticulocytes that had a larger size but less haemoglobin content compared to mature red blood cells.

The increased in the WBC, Plt and the differential counts reported in this study agreed with those of [40] who worked on clove seed, [41] clove oil and [20], clove powder as anaesthetic to African catfishes. This increase may be due to the physiological reactions inform of self- defense mechanism against stress induced by the anaesthetic to counter the effect of the increasing concentration of the scent leaf powder. The increase in the lymphocytes and other WBC indices may also be due to increase production of antibodies to defense against the cellular destruction. Similar observation was reported in, *C. gariepinus* treated with clove seed extracts [40], *H. bidorsalis* juveniles anaesthetized with clove buds powder [20] and *C. gariepinus* juveniles exposed to paraquat [6]. According to (Ainsworth et al. 1991), acute stress in fish is usually followed by a decrease of the percentage of lymphocytes and eosinophiles and an increase in neutrophiles contribution in circulating blood. Cortisol, secreted during stress reaction, shortens the life span of lymphocytes and promotes their apoptosis [118,115]. Thus a decreased lymphocyte count is often observed effect of stress. The increase in these parameters in the present study inferred that scent leaf anaesthetic did not induce stress on *C. gariepinus*. Platelets are one of the indispensable components of blood playing a major role in the clotting of blood by absorbing various factors for blood clotting and delivering them to the site of injury of hemorrhage [96]. Increase in quantity of platelets depict injury caused by the xenobiotics to the cells of the exposed fish. However, [47,107] both reported no significant changes in the haematological indices of gold fish (Carasius auratus) and burbot fish (Lota lotu) respectively. According to Stetter (2001) an effective concentration is that which should have a rapid induction of 3 – 5 min with little or no effects on the haematological parameters of the treated fish. In this study shorter immobilization of less than 3 min required concentration of more than 160mg/l which however had slight changes in some blood parameters of the treated fish. [12,66] have both reported a reversal in significant changes in blood parameters recorded from clove powder on *Rutilus rutilus*, propofol and eugenol on Russian sturgeon respectively 24 hours after recovery from the anaesthetics. The ranged values of the haematological parameters reported in this study were within the optimal values reported for healthy *C. gariepinus* under culture condition [40]

Biochemical indices

Plasma enzymes (Che, LDH, AST, ALT, ALP and CK), electrolytes (Na, K, P, Ca, Cl and HCO3) and metabolites (Glu, TP, Trgly, Cho and Ure) are useful to evaluate the stress condition and health status of fishes treated with anaesthetics [66,41,98,20] According to Ishikawa et al (2007) Lactate dehydrogenase, CK, ALT and AST are major indicators of stress and also give specific information about organ dysfunction. In this study, higher plasma levels of AST lower levels of Che, ALP, LDH, ALT and CK were recorded as concentration of the anaesthetic increased. This was in agreement with the findings of some researchers who use anaesthetics to immobilized fishes [66,98,41]. The significant decrease in plasma Che at the highest concentration was in line with the findings of [116] on *Channa puntatus* exposed to diazonon. The decrease with increasing concentrations could be the reason for direct proportionality of induction time on concentration. This is because as concentration increase, the levels of Che enzymes available in the CNS decreased thereby blocking the hydrolysis of the acetylcholine (cholinergic neurotransmitters) into choline and acetic acid to allow the neurons to return to resting stage after activities, hence the reason for unconsciousness and immobilization. Decrease in activities of Che enzyme results in excess acetylcholine at the synapses of the nerve endings leading to overstimulation of the nerves. Voet and Voetova (1990) reported that a decrease due to inactivation in Che causes a blockage of the cholinergic transfer of nerve signals, paralyses and death due to asphyxia of *Channa punctatus*. This study also recorded slight increase in AST and decrease in LDH, ALT, ALP and CK only significant at the highest concentration of 180mg/l of scent leaf powder. High levels

of AST is an indication of greater energy demand associated with synthesizing activities of cells [107,89] reported that decrease in plasma enzymes could be attributed to their inhibition or reduction in the rate of their synthesis in the liver and cellular activities. Plasma LDH levels can be influenced by exercise, and it increase has been suggested to be a factor in the mortality during fish capture and transport. [82] indicated that the increased level of lactate may have a functional role in sustaining elevated glucose levels in response to stress as a readily available energy source. The decrease in this study is in agreement with [72] who observed a decrease in plasma lactate in matrinxãs anesthetized with 60 mg L-1 benzocaine and 600 mg L-1 phenoxyethanol for 10 min. Transaminases are important enzymes for monitoring the health status of fish and is used to in the diagnosis of damages caused by xenobiotics to various tissues [60]. Alanine aminotransferase is known to play a key role in mobilizing L- amono acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental conditions [100]. According to [9] higher levels of ALT is indication of efficient utilization amino acids for metabolic purposes. The low levels reported in this study could be attributed to the reduced metabolic rate due to immobilization of the treated fish. However elevated levels of ALT and unchanged values of AST, ALP, and CK have been reported for common carp treated with 2 – phenoxyethanol for 24 hours [113,66] also reported an unchanged activity of AST, ALT and CK which reflects no tissue damage following both propofol and eugenol anaesthesia in Russian sturgeon. The fact that treated fish regained consciousness and with no mortality recorded during recovery is an indication that Che, AST and other enzymes returned to normal after scent leaf powder anaesthesia.

Electrolytes function in controlling fluid distribution, intra and extracellular acidobasic equilibrium, maintaining osmotic pressure of body fluids and normal neuro-muscular irritability. The increase in the concentration of Na+ and K+ in the blood of the *C. gariepinus* exposed to scent leaf powder and decrease in Ca+ agreed with the findings of [12,73] in *C. gariepinus* exposed to diazinon and [112] in European catfish exposed to Clove oil anaesthetic. This was also in agreement with the findings of [98] on matrinxas, Brycon amazonicus treated with essential oil from *O. gratissimum* and [66].
on Russian sturgeon treated with propofol and eugenol anaesthetics. Calcium ion and inorganic Phosphorus functionally participate in maintaining normal irritability of the heart, muscles and nerves, as well as the selective permeability of cell membranes. According to Ghosh and Joshi (2008) Increased level of both P and Ca following anesthesia leads to acute respiratory acidosis while decrease in both indices will cause respiratory alkalosis which was not the case in this study. [12] stated that the increase in Na+ and K+ in blood plasma of catfish, in combination with the decrease in cholinesterase indicates inhibition of the heart function and a neurotoxic damage to the central nervous system (CNS). This may probably be why anaesthesia was induced on the *C. gariepinus*. A decrease in Na and Cl levels could explain increases blood water content McDonald and Milligan (1997), but this was also not found in the present study.

Cortisol and some plasma metabolites are physiological indicators of stress in fishes when exposed to handling and xenobiotics (Wagner et al., 2002). Glucose is considered as the main source of energy for fish cells and rapid increase of blood glucose follows acute stress in fish [51]. In fish, proteins are among the main energy sources which play an important role in the maintenance of blood glucose [102]. Triglycerides are synthesized from carbohydrates in liver and stored in fat tissue as an energy source [110]. The significant ($p < 0.05$) elevation in plasma Glu, TP, Trgly and Ure at the higher concentrations (160 and 180mg/l) from the control were in line with the findings of [56] *Channa punctatus*, [107] on Lota lota both treated with clove oil and Ribeiro et al (2015) on *Bryzon amazonicus* anaesthetized with essential oil of *O. gratissimum*. 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. The non – significant ($p > 0.05$) changes in glucose in fish treated with concentration below 160mg/l in this study also corroborated the studies of Iversen et al (2003) on Atlantic salmon treated with clove oil, [113].on common carp anaesthetized with 2 – phenoxyethanol and [66] on Russian sturgeon anaesthetized with propofol and eugenol. The rise in glucose is due to increase demand for energy resulting in the increase in catecholamine and corticosteroids known to induce excessive secretion of adrenaline, which stimulates breakdown of glycogen to glucose to satisfy new energy demand (Pickering et al 1982). Since no mortality was recorded during and after anaesthesia in this study, the rise in glucose could be because of incomplete metabolism of blood sugar due to increased muscular activities before deep anaesthesia. The increased plasma protein may lead to increased osmotic pressure and osmolality of the plasma and resulting from the movement of protein into the cellular compartment (Velisek et al., 2006). Hyperproteinaemia in fish exposed to toxicants

may be due to water loss in plasma, elevated de novo synthesis or relative changes in blood protein mobilization (Al-Attar, 2005). The slight increase recorded in this study could be an attempt of the treated fish to meet up increasing demand to detoxification, immune response and physiological reaction to xenobiotics (Mommsen et al 1999). The slight increase in the levels of triglycerides agrees with the findings of Okey (2019) on *H. bidorsalis* treated with clove powder and Gomulka et al (2008) on Siberian sturgeon exposed to clove oil. However, unchanged triglyceride level was found in rainbow trout and common carp anesthetized with both eugenol and 2-phenoxyetanol (Velišek et al., 2005, 113]. According to Iwama et al (1989) fish under stress mobilized triglycerides and proteins to fulfil an increased energy demand to sustained increase physical activities, biotransformation and excretion of the toxicant. Cholesterols level in this study decreased slight as concentration of scent leaf powder anaeasthetic increased. This finding disagrees with several researchers who reported increase in cholesterol level in *C. gariepinus* exposed various to toxicants [19,80,25]. Increase in plasma cholesterol level is an indication of stress and increase lipid mobilization due to decrease lipoprotein lipase activity (Sharma et al 1982). Bayea et al. (2006) and Gomułka et al. (2008) suggested that hyperlipidemia is an alternative pathway of energy stores mobilization in sturgeons under stress conditions. Most workers who use biochemical indices to assessed stress in fish have reported that cholesterol and triglyceride did not differ ($P > 0.05$) when exposed to anaesthetics [113]; Velisek et al., 2006). Hypercholestrolemia observed may be due impairment of liver and inhibition of enzymes which convert cholesterol into bile acid (Kori-Siakpere et al., 2011) which was not the case in this study. The decrease cholesterol level is an indication that the treated fish were not under stress and lipid were not mobilized. However increased lipoprotein lipase activity plays a role in the reduction of plasma lipid (Sharma et al., 1982). The non- significant increase in urea of fish treated with scent leaf anaesthetic is in line with the findings of several researchers who used anaesthetic on fish (Velisek et al 2005; 2006; Okey, 2019). This implies that fish anaesthesia have reduced metabolic activities hence generate little or no nitrogenous waste and is evidence in the non-significant change observed in the plasma. [66] reported that increased level of urea can be attributed to protein catabolism and gluconeogenesis which is activated to meet the demand for glucose in response to stress. According to [109] increase in urea could be due to protein being used to meet the energy demand during xenobiotics intoxication. The unchanged values recorded in this study is an indication that scent leaf was not toxic to the cell of the African catfish.

Conclusion

This research work investigated the effects of scent leaf powder on the haematological and biochemical profiles of African catfish juveniles. The result obtained revealed that shorter immobilization of 2.25 min can be achieve with higher concentration (180mg/l) but with longer full recovery time of 18.50 min. Minimal changes were recorded in some haematological and biochemical parameters with no mortality recorded during and after exposure. However, *C. gariepinus* anaesthetized with scent leaf powder of 120 – 180mg/l had no negative effects on the haematolgical and biochemical parameters of the treated fish, hence can be recommended as save anaesthetic for aquaculture species.

Acknowledgements

Many thanks to Tertiary Education Fund (TetFund) for sponsoring this research through the Directorate of Research and Development Cross River University of Technology (CRUTECH), Calabar. We also thank the Head, Department of Fisheries and Aquatic Science, CRUTECH Obubra Campus for the logistic support.

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