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Research Article

The Efficacy of Clove Seed Extracts as an Anaesthetic Agent and Its Effect on Haematological Parameters of African Catfish (*Clarias Gariepinus*)

Abstract

Background and Aim: The intensive nature of aquaculture has subjected fish to a number of stressors in the culture medium, anesthetics are widely used to minimize the issue of stress during farming operations and activities. Clove oil is a well known, established and acceptable anesthetics commonly used in aquaculture, this anesthetics is not readily available in developing countries, thus leaving the fish farmers with option of using the locally available plant extracts as anesthetic agent in fish culture. This study therefore assessed the efficacy of aqueous extracts of clove seed and its effect on hematological parameters of the fish.

Methods: A total of 300 *Clarias gariepinus* comprises of 150 juveniles and 150 adult fish were exposed in triplicates to aqueous extracts of clove seed at concentrations of 5.0, 10.0, 15.0, 20.0 and 25.0mg/l at the rate of 10 fish per tank for both juveniles and adult fish. Induction time (Time taken for the fish to be completely anaesthetized) and recovery time (Time taken for the fish to resume normal swimming) was monitored using stop watch. Also, the effect of the extracts on water quality, survival and hematological parameters of the fish were evaluated using standard methods.

Results: The result obtained revealed that the induction time indicated a size related response with the adult fish significantly ($P<0.05$) higher than the juveniles at all concentration of exposure. However, reverse was the case in recovery time, as the recover times in juveniles were higher than the adult fish. The survival was 100% in both sizes. Significant alterations were recorded in the haematological variables of the fish, which was more pronounced at higher concentrations of the extracts.

Conclusion: The extract was effective in sedating *C.gariepinus* and is recommended for use as an aesthetic agent.

Introduction

The practice of aquaculture has increased tremendously in recent years, making it the fastest growing food producing venture in the world [1]. The intensive nature of aquaculture has subjected farmed fish to a number of stressors which involves various handling procedures from hatchery to the final harvest stage [2]. When stress is induced by these handling procedures in the culture medium, fish react by consuming more energy to compensate for the elevated stress level, this subsequently activate secretion and release of hormones such as catecholamine and cortisol into the system of the fish, these hormones will impact negatively on the maintenance of homeostasis [3-5] and reduced fish performance in the culture medium [6]. Conversely, anesthetics are widely used in aquaculture to minimize the incidence of stress during culture operations and farming activities. The use of anesthetics enhances safety for both fish and farmer during aquaculture operations and management procedures. It allows farm routine practices to be performed with minimum stress for the fish [7-9]. Recently, application of clove oil as anesthetic in farmed fish is on the increase, this is because it is safe, cheap, and nontoxic to the fish and environment. Moreover, it does not require a withdrawal period of 21 days for table fish as other anesthetics of chemical origin such as MS-222, 2 phenoxyethanol, benzocaine, metomidate and quinidine [9,10].

In Nigeria, like other tropical countries of the world, clove plant is commonly cultivated and the leaves used in preparation of drinks, and the seeds are used as food spice in different parts of the country The major component of this seed is eugenol and eugenyl acetate, which are responsible for its sedative properties. It's used as anesthetics in fish culture is not common, thus necessitating the need to evaluate its efficacy as potential anesthetic agent, which can easily be used by the local fish farmers, who cannot accessed the conventional clove oil as a result of its high cost and scarcity.

Hematological parameters are useful in evaluation of internal physiological conditions of fish, this depends on the species of fish, sex, health status and environmental conditions [11-13]. Many authors reported on the use of blood parameters to assess changes associated with stressful conditions, this is because fish is known to be in close association with its immediate environment, and therefore the blood parameters will reveal the internal status of the fish before any outward manifestation of disease [14-16]. Hence, blood can provide substantial diagnostic information in fish, which will be useful for monitoring their health status, in response to application of

chemicals in aquaculture. This study therefore, assessed the efficacy of clove seed extracts as a viable anesthetics agent in *C. gariepinus* and its effect on hematological parameters of the fish.

Materials and Methods

Experimental fish

A total of 300 *C. gariepinus* comprised of 150 juveniles (mean length 26.64cm ± 1.02 SD; mean weight 356.21g ± 1.86 SD) and 150 adults (mean length 52.13cm ± 1.04SD) were collected from the production units, in African Regional Aquaculture Center, Aluu, Port Harcourt, Rivers State, Nigeria and transferred immediately in 40L open basin (half filled with water), to the hatchery unit, where they were acclimated to laboratory conditions for seven days. During this period the fish in acclimation tanks were fed with ARAC fish feed (40.0% C.P) at 5% body weight, the water in these holding tanks was renewed every two days [17].

Experimental procedure

Dried seeds of clove plant (*Syzigium aromaticum)* were purchased from open market. Authentication of the plant was done using the keys of Agbaje [18]. The seeds were later ground in the laboratory using 1.5L kitchen blender (model BL 440 Kenwood, Japan). The milled clove seed were sieved into fine powdery form using 0.1µ nylon mesh [19]. The filtered seed were weighed and applied directly in three replicates at concentrations of 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l to the water in 70L experimental tanks filled to 40L mark, the mixture were stirred vigorously to allow homogenous mixing. The fish were individually introduced to the experimental tanks (30 in number) at the rate of 10 fish per tanks in triplicates for both juveniles and adult fish.

Evaluation of induction and recovery time

Eugenol and eugenyl actate compounds were the major constituents of clove seed and were responsible for its anesthetic ability (Table 1). The efficacy of clove seed extracts as anesthetic agents was evaluated by monitoring the induction time (Time taken for the fish to get sedated) using stop watch according to the methods of Coyle et al. [20], (Table 2). The induction time was obtained when fish lost equilibrium, showed slow opercula movement, and did not react to stimuli when touched. These correspond to 4 stages of anesthesia in fish as described by Coyle et al. [20]. After anesthesia, fish was individually removed from the experimental tanks by using a scoop net and transfer into other tanks containing clean water tank without anesthetics, to determine the recovery time. The recovery time (time taken for the fish to resume normal swimming) was equally taken with stop watch and recorded. Recovery was considered as complete; when after transfer of the fish to clean water, the fish fully regained its equilibrium and resume normal swimming.

Assessment of water quality and blood parameters

During the study, some water quality parameters such as temperature, pH, dissolved oxygen, nitrite, ammonia and sulphide were evaluated in the experimental tanks using the method of APHA [21]. After the fish was immobilized with the clove seed extracts,

Table 1: Chemical Composition of Clove Seed Extracts. Nassar et al. [28].

No.	Compound	Concentration (%)
1.	p-cymene	0.9
2.	S-Hexene-2-one	0.67
3	Thymol	0.87
4	Eugenol	71.56
5	Eugenyl acetate	8.99
6	Caryophyllene oxide	1.67
7	Guaiol	0.90
8	Benzene-1-butyheptyl	0.55
9	Nootkatin	1.05
10	Isolongifolanone	0.86
11	Hexadecanoic acid	0.50
12	9,17 Octdeca-dienal	0.24
13	Octadecanoic acid butyl ester	0.33
14	Phenol	0.98
15	Dodecatrienoic acid-3,7,11 trimethyl ester	0.38
16	Vitamin E acetate	0.43

Table 2: Anesthetic Stages in Fish.

Stages of	Anesthesia	Description
Induction	**I**	**Slow swimming**
	II	Slight increase in opercula beat frequency
	III	Loss of equilibrium
	IV	Loss of reflexes and movement
	V	Deep anaesthesia, fish lies on one side
Recovery	**I**	**Reappearance of opercula movement**
	II	Partial recovery of equilibrium
	III	Irregular balance
	IV	Total recovery of equilibrium
	V	Normal swimming
Adopted from [20].		

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. Various hematological parameters were evaluated thus: Hemoglobin (Hb) concentration was done using cyanmethemoglobin method [22] and packed cell volume (PCV) was evaluated using microhaematocrit method of Snieszko [23]. The Red Blood cell (RBC) was estimated using haemocytometer (Improved Neubauer Weber, Scientific ltd) according to Wintrobe [24]. Also, the total white Blood cell counts (WBC) was evaluated with an improved Neubauer Haemocytometer using shaw's diluting fluid [25]. Differential counts: lymphocytes, monocytes and neutrophils were done on blood film stained with May Grumwald-Giemsa stain. Thromobcytes Count was done by Ress and Ecker method [26]. The Red blood cell indices which include: mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated using the formula described by Dacie and Lewis [27].

Ethical standards

The experiments were conducted with ethical standards in handling of the experimental animals. The fish used were handled with care, which involved high consideration of standard fish rearing and good facilities. There was no ethical issues or reservations encountered during the experiment.

Data analysis

The data obtained were collated and analyzed statistically by one way analysis of variance (ANOVA) and differences among means were determined by Tuckey's multiple comparison tests by SPSS Software for determining the significance of change from the control.

Results

The water quality parameters in the experimental tanks of the fish exposed to clove seed extracts revealed that the parameters were within the same range, with no significant variation comparable to the control (Table 3). Stages of induction time in juveniles adult stages of *C.gariepinus* exposed to various concentrations of clove seed extracts are presented in Tables 4, 5. The various stages of induction time which include slow swimming, loss of equilibrium, opercula movement and loss of movement reduced significantly (P<0.05) as the concentrations of the clove extracts increased. (Tables 4, 5). Various stages of recovery time in *C.gariepinus* and adult size of the fish are shown in Tables 6, 7. The various stages of recovery time which include: reappearance of opercula balance, recovery of equilibrium and resumption of normal swimming increased significantly (P<0.05) with increasing concentration of the clove seed extracts (Tables 6, 7).

Table 3: Water Quality Parameters in Experimental Tanks of *C.gariepinus* Exposed to Clove Powder (Mean ± SD).

	Concentrations (mg/l)				
Parameter	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Temperature (°C)	28.80 ± 0.34^{a}	28.70 ± 0.036^{a}	29.10 ± 1.64^{ab}	28.73 ± 0.41^{a}	29.06 ± 0.032^{a}
PH	6.81 0.17^{a}	6.80 ± 0.16^{a}	6.82 ± 0.13^{ab}	6.08 ± 0.15^{a}	6.93 ± 0.36^{c}
Dissolved Oxygen (mgL^{-1})	6.89 ± 0.14^{a}	6.93 ± 0.35^{a}	6.77 ± 0.025^{a}	6.89 ± 0.46^{a}	6.79 ± 0.66^{c}
Nitrite (mgL^{-1})	0.0047 ± 0.02^{a}	0.053 ± 0.02^{a}	0.060 ± 0.03^{a}	0.0057 ± 0.01^{a}	0.0047 ± 0.03^{a}
Ammonia (mgL^{-1})	0.31 ± 0.05^{a}	0.32 ± 0.02^{a}	0.31 ± 0.02^{a}	0.32 ± 0.004^{a}	0.36 ± 0.05^{a}
Sulphide (mgL^{-1})	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.14 ± 0.03^{a}
Mean within the row with different superscripts are significant (P<0.05).					

Table 4: Stages of Induction Time (Sec) in *C.gariepinus* Juveniles Exposed To Various Concentrations of Clove Seed Extracts (Mean ± S.D).

	Behavioural Descriptions (Sec)			
Concentration(mg/L)	**Slow Swimming**	**Loss of Equilibrium**	**Loss of Opercula Movement**	**Loss of Movement**
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50.00	35.6 1 ± 0.21	47.81 ± 1.21	62.67 ± 1.02	75.73 ± 8.90
100.00	30.12 ± 0.44	42.61 ± 0.81	50.21 ± 0.41	58.33 ± 3.21
150.00	25.61 ± 0.21	30.22 ± 0.31	36.12 ± 0.21	43.33 ± 2.88
200.00	10.21 ± 0.21	14. 68 ± 0.32	17.88 ± 047	19.67 ± 0.57
Means within the column with different superscripts are significantly different (P<0.005).				

Table 5: Stages of Induction Time (Sec) in *C.gariepinus* adult exposed to various concentrations of clove seed extracts (mean ± SD).

	Behavioural Descriptions (Sec)			
Concentration (mg/L)	**Slow Swimming**	**Loss of Equilibrium**	**Loss of Opercula Movement**	**Loss of Movement**
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50.00	120.11 ± 11.21	180.61 ± 8.11	196.71 ± 7.87	238.00 ± 15.39
100.00	86.24 ± 6.24	121.61 ± 8.11	196.71 ± 7.87	238.00 ± 15.39
150.00	61.11 ± 5.11	100.24 ± 6.12	151.67 ± 71.24	180. 67 ± 0.21
200.00	57.21 ± 3.21	86.14 ± 7.14	100.66 ± 9.11	122.67 ± 6.43

Table 6: Stages of Recovery time in *C.gariepinus* juveniles exposed to various concentrations of clove seed extracts (mean ± SD).

	Behavioural Descriptions (Sec)			
Concentration (mg/L)	**Reappearance of Opercula**	**Irregular Balance**	**Equilibrium Recovery**	**Normal Swimming**
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50.00	45.61 ± 1.16	68.72 ± 1.21	81.61 ± 2.62	104.66 ± 6.42
100.00	72.88 ± 2.61	96.84 ± 4.28	120. 72 ± 11.11	158.66 ± 12.08
150.00	108.61 ± 4.81	156.74 ± 1.61	176.12 ± 12.13	190.00 ± 10.00
200.00	146.21 ± 7.81	178.77 ± 6.21	201.14 ± 9.11	233.33 ± 15.27

Table 7: Stages of Recovery time in *C.gariepinus* Adult fish exposed to various concentrations of clove seed extracts (mean ± SD).

	Behavioral Description (Sec)			
Concentration(mg/l)	**re-appearance of opercula**	**Irregular Balance**	**Equilibrium Recovery**	**Normal Swimming**
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50.00	35.66 ± 1.81	47.81 ± 2.61	61.21 ± 6.11	90.00 ± 10.01
100.00	46.28 ± 3.12	64.22 ± 3.11	89.61 ± 3.41	1.11.00 ± 18.19
150.00	67.28 ± 4.21	81.12 ± 2.66	97.88 ± 4.86	137.10 ± 4.58
200.00	91.33 ± 6.11	121.61 ± 3.11	159.66 ± 10.06	190.67 ± 20.00

The induction time both juvenile and adult size of *C.gariepinus* exposed to clove seed extracts indicated a size related response, with the induction time in the adult consistently higher than the juveniles at all concentrations of exposure (Table 8). However, a reverse situation in recovery time, where the juveniles were observed to be significantly ($p<0.05$) higher than the adult sizes. Interestingly, the survival was 100% in both sizes, as no mortality was recorded in all concentrations of clove powder extracts (Table 8).

Changes in heamatological variables exposed to clove seed extracts in both juveniles and adult size of *C.gariepinus* are presented in Table 9 and 10 respectively. Significant reduction ($p<0.05$) were recorded in the values of PCV, Hb, RBC, OCC, Thrombocytes and Lymphocytes, the reduction was more noticeable in thrombocytes in adult fish (Table 9) which reduced from 193.35 ± 7.21% to 148.01 ± 7.21. Moreover, WBC and some differential counts such as MCV also increased as the concentration of the clove extracts increased. This was more pronounced in the juvenile fish (Table 9) than in the adult (Table 10). There was no definite trend, in the response or MCH and MCHC, as all the values were within the same range in both size groups.

Discussion

Induction time is the time taken for the fish to be sedated and it is usually depends on the concentration of the anaesthetics [29]. The results in this study showed that increasing concentrations of the clove seed extracts significantly reduced with induction time. This was in line with the reports of Akinrotimi et al. [30], in two species of mullets, *Liza falcipinis* and *Liza grandisquamis* exposed to clove seed extracts, but differs from that of Akinrotimi et al. [31], in exposure of *C.gariepinus* to aqueous extracts of Indian almond tree leaf, which required a higher concentration to induce the fish. This difference could be as a result of variations in biological and environmental factors that influences the efficacy of botanicals as an anesthetic agent. Anesthesia is influenced by the concentration of the anesthetic in the central nervous system (CNS) of the organism. Therefore, in the present investigation, the shorter induction time taken to sedate the experimental fish *C.gariepinus,* with increased concentration of the clove extracts, may be attributed to the accumulation of the active ingredients such as eugenol and eugenyl acetate in the body system of the fish which impaired the activity of CNS at a much faster rate. Conversely, biological factors such as age, species, life stage, size, lipid content body condition and disease status, have been reported by many authors to have profound effect on metabolic rate and consequently' the pharmacokinetics of the anesthetic agents [32-34]. Similarly, environmental indices namely, salinity, dissolved oxygen and p^H could affect the anesthetic rate as well as its uptake across the gills [33]. The induction time in this study were found to be lower in juveniles' fish when compared to adult fish. This agrees with the findings of Hseu et al. [35], in gold lined sea bream, (*Sparus sarba)* and that of Pawar et al. [36], in yellow horse (*Hippocampus kuda).* The shorter induction time in juvenile fish when compared to adult may be due to small body size and reduced surface area of the gill in juvenile fish [37].

The recovery time was directly proportional to increasing

Table 8: Summary of induction, recovery and survival of *C.gariepinus* exposed to clove seed extract (mean ± SD).

		Concentrations (mg/l)				
life stage	**Parameters**	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Juvenile	Induction time (S)	0.00 ± 0.00^{ad}	75.73 ± 8.9^{d}	$58.333.21^{c}$	43.33 ± 2.88^{b}	19.67 ± 0.57^{a}
	Recovery time (S)	0.00 ± 0.00^{a}	104.66 ± 6.42^{b}	$158.6612.05^{c}$	190.00 ± 10.00^{d}	233.33 ± 15.27^{e}
	Survival (%)	100.0 ± 0.0^{a}	$100.0 \pm 0.10_{a}$	$100.000.01^{a}$	100.0 ± 1.20^{a}	100.0 ± 1.20^{b}
Adult	Induction time (S)	0.00 ± 0.00^{ad}	356.00 ± 17.77^{d}	$238.0015.39^{c}$	180.67 ± 9.021^{b}	122.67 ± 6.43^{a}
	Recovery time(S)	0.00 ± 0.00^{ad}	90.00 ± 10.01^{b}	$111.0018.19^{ab}$	137.10 ± 4.58^{c}	190.67 ± 20.03^{d}
	Survival (%)	100.0 ± 0.00^{a}	100.00 ± 0.01^{a}	$100.00\ 0.00^{a}$	100.00 ± 0.1^{a}	100.00 ± 0.01^{a}
Mean within the row with different superscripts are significant ($p<0.05$).						

Table 9: Haematological Response in Juveniles of *C.gariepinus* Treated With Clove Seed Extracts (Mean ± SD).

	Concentrations (mg/L)				
Variables	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Packed Cell Volume (%)	37.33 ± 2.08^{b}	$33.3322.08^{ad}$	32.00 ± 1.73^{ad}	30.33 ± 1.52^{ab}	27.67 ± 2.04^{a}
Haemoglobin (g /d1)	8.93 ± 0.25^{ab}	8.20 ± 0.81^{ab}	8.03 ± 0.45^{ab}	158.66 ± 12.05^{c}	6.07 ± 0.75^{a}
Red Blood Cell (cells x 10^{12})	4.63 ± 0.50^{b}	4.27 ± 0.50^{b}	$3.93 \pm 0.25_{a}$	3.33 ± 0.31^{a}	3.23 ± 0.04^{a}
Oxygen carrying capacity (vol. %)	10.97 ± 0.31^{c}	$10.70V0.52^{ab}$	9.31 ± 1.02^{ab}	$9.000.72^{ab}$	8.31 ± 2.20^{a}
White Blood Cell (cells x 10^{9}/1)	15.66 ± 0.68^{a}	$16.171.01^{a}$	18.40 ± 0.79^{ab}	20.53 ± 0.60^{a}	22.86 ± 1.92^{a}
Thrombocytes (%)	95.00 ± 3.60^{b}	90.00 ± 6.00^{b}	85.00 ± 8.667^{b}	81.00 ± 1.00^{a}	79.00 ± 1.00^{a}
Lymphocytes (%)	66.33 ± 2.51^{ab}	64.66 ± 3.05^{ab}	62.33 ± 2.08^{b}	59.33 ± 3.21^{a}	55.61 ± 1.51^{b}
Monocytes (%)	5.33 ± 1.15^{a}	6.33 ± 1.14^{a}	7.00 ± 1.00^{ab}	7.67 ± 0.14^{b}	9.00 ± 0.01^{b}
Neutrophi l (%)	29.00 ± 1.73^{a}	29.00 ± 2.00^{a}	30.52 ± 2.44^{b}	33.00 ± 3.46^{b}	35.33 ± 1.53^{b}
Mean corpuscular Haemoglobin (pg)	19.41 ± 1.88^{b}	19.43 ± 4.18^{a}	20.52 ± 2.44^{b}	22.74 ± 4.04^{ab}	18.72 ± 1.33^{a}
Mean corpuscular Haemoglobin conc. (g/d1)	23.96 ± 1.03^{a}	24.73 ± 4.00^{ab}	25.20 ± 2.87^{ab}	$24.76 \pm 2.41_{ab}$	21.90 ± 1.85^{a}
Mean corpuscular volume (FL)	80.87 ± 4.47^{a}	78.57 ± 6.76^{a}	81.42 ± 3.03^{a}	91.55 ± 9.97^{ab}	85.58 ± 13.15^{a}
Mean within the row with different superscripts are significant ($p<0.05$).					

Table 10: Haematological Response in Adult of *C.gariepinus* anaesthetized with Clove Seed Extracts (Mean ±SD).

	Concentrations (mg/L)				
Variables	**0.00**	**50.00**	**100.00**	**150.00**	**200.00**
Packed Cell Volume (%)	40.33±0.5^{ab}	39.67±0.62^{a}	39.00±1.00^{a}	38.41±1.52^{a}	37.00±1.01^{a}
Haemoglobin (g /d1)	13.70±0.44^{ab}	12.80v1.26^{ab}	39.00±1.00^{a}	12.00±0.45^{ab}	10.20±0.40^{a}
Red Blood Cell (cells x 10^{12})	6.06±0.30^{b}	5.53±0.55^{ab}	12.03±0.61^{ab}	4.900.95^{a}	4.67±0.56^{a}
Oxygen carrying capacity (vol.%)	15.46±0.14^{b}	15.67±0.32^{ab}	5.30±0.51^{ab}	14.70±0.58^{ab}	12.52±0.49^{a}
White Blood Cell (cells x 10^{9}/1)	21.96±1.28^{a}	22.802.40$_{a}$	14.74±0.72^{ab}	29.46±2.11^{ab}	36.70±3.05^{b}
Thrombocytes (%)	193.33±10.40^{c}	170.00±10.11^{b}	25.25±3.51.00^{b}	158.33±2.82^{ab}	148.01±7.21^{a}
Lymphocytes (%)	70.33v2.08^{b}	68.00±2.64^{ab}	170.00v5.00^{b}	61.041.73^{ab}	57.33±1.15^{a}
Monocytes (%)	2.00±1.00^{a}	3.33±0.62^{a}	66.00±2.01^{ab}	5.000.01^{ab}	60.4±0.2^{ab}
Neutr0phil (%)	27.67±2.08^{a}	28.66±2.14$_{a}$	3.67±0.64+	34.00±1.73^{b}	36.211.22^{b}
Mean corpuscular Haemoglobin (pg)	22.61±1.32^{a}	21.81±0.61^{a}	30.14±2.24^{ab}	25.07±4.71^{a}	22.03±2.13^{a}
Mean corpuscular Haemoglobin conc. (g/d1)	33.96±0.08^{ab}	32.27±0.93^{a}	22.77±1.25^{a}	31.231.25^{a}	28.33±0.93^{a}
Mean corpuscular volume (FL)	66.60±4.08$_{a}$	72.11v6.50^{ab}	74.02±6.87^{ab}	79.82±12.36^{ab}	80.33±12.94^{ab}
Mean within the row with different superscripts are significant (p<0.05).					

concentration of the extracts. In this study the adult fish recover faster from the effects of the anesthetics than the juveniles. A similar result was observed in redline torpedo fish (*Sahyadria denisonii)* exposed to 2 - phenoxy ethanol [38] contrary view was recorded by Mylonass et al. [33], in *Dicentrarchus* labrax and *Sparus aurata* treated with clove oil, such differences may be related to variation in species, and physiological status of the fish [39]. In the present study where different concentrations of the anesthetics was used to tranquilized the fish, the differences in recovery time observed, may be explained by the fact that more of the active ingredients of the anesthetic extracts accumulated in the CNS of the fish at higher concentrations, thus suppressing the activity of the CNS to a greater degree than at lower concentrations and consequently prolonging the recovery time of the fish from the effects of anesthetics.

Haematological parameters are closely related to fish response to adverse environmental conditions [40]. In this study significant variations were observed at higher concentrations (150 and 200 mg/l) of the clove extracts. This result support the findings of Prashar et al. [41], in roach, (*Rutilus rutilus)* exposed to clove powder. These alterations in blood variables may be due to cytotoxicity of clove extracts at higher concentrations. These caused a significant decrease in red blood cell, hemoglobin and PCV of the fish at higher concentrations of the extracts. This reduction may be attributed to hemolysis which results in haemodilution, a means of diluting the haemoconcentration of the extracts thus reducing the effect of the chemicals in its system. The white blood cells in experimental fish increased notably at higher concentrations of the clove seed extracts. Thus, increasing or decreasing numbers of white blood cells are normal reaction on the exposure of fish to chemical irritants. In the present investigation, the increase in WBC (leukocytosis) may have resulted from the excitation of defense mechanism of the fish to counter the effect of the higher concentrations of the extracts. Abdolazizi et al. [42], in exposure of gold fish (*Carassius auratus)* to clove oil did not report any alteration in the haematological variables of the fish, this may be due to the exposure of fish to ideal (safe) concentrations of clove. In this study, the ideal concentration is 100mg/l. According to Stetter [43], an ideal concentration for an anesthetic agent should induce a rapid induction time of 3-5 minutes and recovery time of 5 to 10 minutes, with little or no effects on its haematological variables.

Conclusion

Based on the findings of this study, aqueous extracts from clove seed could effectively be used to sedate fish for different farm operations in aquaculture. This study further revealed that the extracts could be safely applied at 100.0 mg/l, which is sufficient to anaesthetize the fish with little or no changes on its heamatological parameters.

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