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Comparative Effects of Clove Oil and 2-phenoxyl ethanol on the Anaesthetic and Haematological Properties of *Clarias gariepinus* Juveniles

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ABSTRACT

This study was carried out to determine the effect of two anaesthetic agents; clove oil and 2-phenoxyethanol on Clarias gariepinus juvenile. Clove oil was mixed with ethanol in ratio 1:1, to aid its solubility in water. Also, 5gm of 2-phenoxyethanol was mixed with 5ml ethanol to make it miscible in water. A total of 240 juvenile were used, sixty fishes were anaesthetized at different concentration of clove oil (0, 0.5, 1.0, 1.5, 2.0, 2.5ml/l) and another sixty anaesthetized at different concentration of 2-phenoxyethanol (0, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l). The physio-chemical properties of water and haematological parameters of fish exposed to anaesthetics were measured to know the effects of the anaesthetic agents on the test fish blood. Single factor analysis of variance (ANOVA) was used to analyze the variables studied. The induction time decreased with increased concentration while the recovery time increased with increasing concentration. The effective concentration that gives induction in 194.50± 20.51s and recovery time in 298.00±7.07s for clove oil with no mortality was 2.0ml. Also, 2.5 ml of 2-phenoxyethanol gives 185.00±35.36s induction time and recovery time of 295.00±42.43s. The result shows significant difference ($p<0.05$) in haematological variables across the samples. From the experiment, increase in the concentration of these agents decrease the level of PCV, RBC and Hb while the level of WBC increases with increasing concentration. In conclusion, clove oil is the most suitable agent for juvenile African catfish when compared 2-phenoxyethanol based on the quicker induction and recovery time when clove oil was administered.

Key words: clove oil, phenoxyl ethanol, anaesthetic, haematology, *Clarias gariepinus*.

INTRODUCTION

Rapid growth in aquaculture with high demand for fish as a cheap source of food and engagement of the technological advances has placed more demand on the necessary chemicals. These chemicals are grouped based on their uses in various operations both in aquacultural practices and fisheries managements. One of the groups is anaesthetics or anti-stress agents. Anaesthetics are used with increasing frequency in aquaculture, mainly to reduce the stress and to prevent mechanical damage to fish during handling, thus reducing stress-induced problems such as decreases in feeding and immune functions (Ross and Ross, 1999). Anaesthesia is achieved mostly by introduction of the fish into an anaesthetic solution which is absorbed through the gills and enters the arterial blood, from where it acts on the central nervous system (Ross and Ross 1999). Some anaesthetics had been reported to reduce or block the activation of the hypothalamic-pituitary-interrenal (HPI) axis associated with stressors and thus decrease or prevent the release of the stress hormone cortisol to the bloodstream of fish (Hoskonen and Pirhonen 2006). After return of the anaesthetised fish to the fresh water, the anaesthetics or their metabolites are excreted via the gills (Ross and Ross 1999). The anaesthetics most generally used in aquaculture are MS-222, benzocaine, quinaldine sulphate, methomidate, clove oil and 2-phenoxyethanol (Brown, 1988; Svoboda and Kolarova, 1999; Waterstrat, 1999), with anaesthesia usually induced by immersing the fish in an anaesthetic solution.

Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Soto and Burhanuddin, 1995). Clove oil is also distilled from stems, leaves and flower buds of Eugenia caryophyllata, and its active ingredient, i.e. eugenol (4-allyl-2-methoxyphenol), makes up 70 to 90% by weight (Isaacs, 1983; Briozzo*et al*., 1989; Keene *et al*., 1998). Clove oil is highly effective even at low concentrations. Keene *et al*, (1998) reported that it induced anaesthesia faster and at lower concentrations than MS-222 while

Munday and Wilson (1997) found clove oil only marginally less effective than quinidine. Clove oil provides a much calmer induction to anaesthesia and longer recovery time compared with the other chemicals. It is also very much less expensive compared to other anaesthetics (Keene *et al.,* 1998).

The efficacy of 2-phenoxyethanol has been documented for many fish species: platyfish (Guo *et al*., 1992), grass carp and silver carp (McCarter, 1992), guppy (Teo and Chen, 1993), black porgy (Hseu*et al*., 1996), goldfish (Weyl *et al*., 1996; Kaiser and Vine, 1998), perch (Hamackova *et al*., 2001), sea bream (Tort *et al*., 2002), tench (Myszkowski *et al*., 2003; Hamackova *et al*., 2004).
The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants, toxicants and effluents etc. (Adamek, *et al*, 1993).Haematological and biochemical profile in fish is a sensitive index for evaluation of fish metabolism under pollutant stress. Blood is a good bio- indicator to study the problem in organ function. The measurement of haematological changes of fish under exposure to any toxicant may be used to predict its effect upon chronic exposure. The blood parameters get affected on account of chemical toxicity. (Fernandes and Mazon, 2003).
In recent years, haematological variables were used more when clinical diagnosis of fish physiology was applied to determine the external stressors and toxic substances as a result of close association between the circulatory system and external environment. Hence, the present study aims at investigating the changes in different haematological parameters of the *Clarias garipenius* exposed to various concentration of clove oil and 2-Phenoxyethanol.

MATERIALS AND METHODS

Study area and experimental design

The study was undertaken in the Department of Fisheries and Aquaculture, Federal University of Technology, Akure, Nigeria. The study was designed to test the effect of two anaesthics agent on *Clarias gariepinus* and their effects on heamatological properties on *C. gariepinus*. During the experiment, the beginnings of individual phases of anaesthesia and recovery rates were studied. Four consecutive phases according to Thienpoint and Niemegeers (1965), and Yoshikawa *et al*., (1988) were used in evaluating the phases. (1) acceleration and subsequent deceleration of opercular movements, a partial loss of reactivity to external stimuli, (2) loss of equilibrium, opercular movements very slow, fish still reactive to strong stimuli, (3) total loss of reactivity, fish are lying at the tank bottom and do not respond to handling and (4) complete cessation of opercular movements, fish dies if left in the bath for too long.

Experimental Fish

A total of 240 cultured *C. gariepinus* of average weight of 38.76±0.51g and length of 14.97±6.78 were examined in this study. The fish were purchased from Ministry of Agriculture fish farm Adegbemile area in Akure, Nigeria and transported live to the Department of Fisheries and Aquaculture Technology, Federal University of Technology, Akure, Nigeria.

Anaesthetics

The experiment involved the use of clove oil (Fine Chem. Limited, Biosar) and 2-phenolxyethanol (purchased from Collins Scientific Laboratory, Lagos, Nigeria. A total of 10 ml of clove oil and ethanol was prepared in the ratio 1:1. Different concentrations of clove oil (0, 0.5, 1.0, 1.5, 2.0 and 2.5ml) were then dissolved in 10 L of bore hole water. Also 2- phenoxyethanol was prepared by dissolving 5gof 2- phenoxyethanol in 5mL of ethanol to form stock solution. Five different concentrations (0, 1.0, 1.5, 2.0, 2.5 and 3.0ml) were then obtained from the prepared stock solution and dissolved in 10 L of water from bore hole in glass tank of 45 x 60 x 40cm size.These concentrations used were based on the safe concentration obtained through range finding test. Each concentration was replicated twice to total of 12 tanks per trial. Experimental fish, 10 pieces were exposed to each glass tank containing prepared concentrations of clove oil and 2-phenoxyethanol and controls. The fish and its behaviour, water temperature, pH and oxygen saturation were monitored throughout the tests period of 96 hours in each tank.

Haematological analysis

The exposure period lasted for 96 hours, after which blood samples were obtained from the control and experimental fish. Two fish were picked from each concentration and approximately 2 mL of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA), an anticoagulant; it was properly mixed and used for haematological analysis (Svobodova and Vykusova, 1991; Aziz *et al*., 1993; Atamanalp *et al*., 2002). The indices used to evaluate the haematological profile include the erythrocyte count (ER), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), White blood cell count and Erythrocyte Sedimentation Rate (ESR). The derived hematological indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using formulae described by Jain (1986): MCV

was calculated in femtoliters = PCV/RBC x 10; MCH was calculated in picograms = Hb/RBC x 10; and MCHC = (Hb in 100 mg blood / Hct) x 100.

Statistical analysis

Linear regression was used to determine the relationship between concentrations, induction and recovery to anaesthesia. The results were presented as means ± standard deviation. Difference between parameters were analysed by one way analysis of Variance and significant means were subjected to a multiple comparison test (Duncan) at $\alpha = 0.05$ level. All analysis was performed using SPSS software (version 17.0).

RESULTS

Table 1 and 2 show the induction and recovery time for *C. gariepinus* exposed to various concentration of clove oil and 2-phenoxylethanol respectively. In each of the table, the induction time decrease with increase concentration of the anaesthetics. Unlike the recovery time, that exhibit linear relationship with the anaesthetics concentrations.

Table 1: Induction and recovery time of *C. gariepinus* exposed to various concentration of clove oil

Clove oil concentration (ml/L)	Induction Time (s)	Recovery Time (s)
0	0	0
0.5	587.00±94.75	95.00±7.07
1.0	396.50±30.41	170.00±28.28
1.5	230.00±28.28	225.00±8.33
2.0	194.50±20.51	298.00±7.07
2.5	82.00±11.31	564.00±49.50

Induction and recovery time are presented in Mean ± standard deviation

Table 2: Induction and Recovery time of *C. gariepinus* exposed to various concentration of 2-phenoxyethanol

2-phenolxyethanol concentration (ml/L)	Induction Time (s)	Recovery Time (s)
0	0	0
1.0	455.00±35.36	77.00±28.28
1.5	385.00±35.36	107.00±14.14
2.0	325.00±35.36	223.00±35.36
2.5	185.00±35.36	295.00±42.43
3.0	95.00±21.21	443.00±21.21

Induction and recovery time are presented in Mean ± standard deviation

Table 3 and 4 show the physico-chemical parameters of the test media (clove oil) before and after the experiment. It shows from the table that the dissolved oxygen (DO) decreases with increase in concentration (Table 4). However, conductivity and pH display direct relationship with concentrations of the test solutions. Slight decrease was also noticed in temperature values in the test media also.

Comparative analysis of physico-chemical analysis of 2-phenoxylethanol before and after the exposure are presented in Table 5 and 6. Conductivity and pH show increase proportion with the test concentrations. This was different with DO and Temperature that decreased with increases in test concentration.

Haematological parameter of *C. gariepinus* exposed to various concentration of clove oil is shown in Table 7. Significant differences exist among the concentrations of the test solution and with the control. There is no significant difference between concentration 0.5 and 1.0 in the value of ESR. Also, noticed is non-significant difference in concentrations 2.0 and 2.5 with clove oil, caused a progressive decrease ($p < 0.05$) in the values of PCV, Hb, RBC and NEU. But there was a progressive increase in ESR and WBC, while the responses of MCH, MCHC, MCV, relative to clove oil concentrations varied with no definite pattern.

There is significant different in the haematological parameters of *C. gariepinus* exposed to various concentrations of 2-phenoxyl ethanol. No significant different exist between control and the first three concentrations (Table 8). Treatment with 2-phenoxyethanol caused a progressive decrease ($p < 0.05$) in the values of PCV, Hb, RBC with increase in concentration. But there was a noticeable increase in ESR, and WBC while the fish responses to MCH, MCHC, and MCV, fluctuates with concentration.

DISCUSSION

The result from the study showed the anaesthetic property of clove oil and 2-phenoxyethanol for *C. gariepinus* juvenile. Anaesthesia of the tested organism could be attributed to the effect of the test solutions of the fish respiration during which the behavioural changes on the test organism was noticed. The lowest effective concentration causing general anaesthesia was 2.0ml/l in clove oil with induction time was 194.50sec (3.2min). The induction time was noticed to decrease with increasing concentration of clove oil used while the recovery time was noticed to increase with increasing concentration. The results are in agreement with previous studies in teleost fish (Hseu *et al*., 1998; Mylonas *et al*., 2005; Gullian and Villanueva, 2009; Weber *et al*., 2009; Heo and Shin,

2010). Result of over exposue to high concentration of clove oil showed that recovery time was dependent of the anaesthesia duration. This is in agreement with (Woody *et al*.2002) who reported that, prolonged recovery with increased anaesthetic dosage was found in sockeye salmon (Woody *et al*., 2002) and cobia (Gullian and Villanueva, 2009). However, decreasing recovery times with an increase in concentration of clove oil for European sea bass and gilthead seabream has been reported by Mylonas *et al*. (2005). Also stages of anaesthesia were observed to be concentration dependent in the trial, except that the concentration used produced no mortality. The induction time also decreases with concentration of 2-phenoxyethanol, the lowest effective concentration of 2-phenoxyethanol causing general anaesthesia was 2.5ml/l with induction time of 185sec (3.1min). The induction time was noticed to decrease with increasing concentration of 2-phenoxyethanol used (from 455-95sec) while the recovery time was noticed to increase with increasing concentration (77-443sec) (Woody *et al*., 2002). Also stages of anaesthesia were observed to be concentration dependent in the trial, except that the concentration used produced no mortality. Recovery time observed with 2-phenoxyethanol was shorter compared with that of clove oil. The reason for this may be traced to solubility of phenoxyethanol in water. Also clove oil is possible to be eliminated in the tissue of fish; this is supported by Kildea *et al*., (2004) who reported that elimination of clove oil could take place in the tissue of *Bidyanus bidyanus*. The induction time is inversely related to concentrations of clove oil used. (Gulian and Villanueva, 2009) observed the same effect on *Clarias garipenius.*

It was observed that the recovery time was concentration dependent with prolong recovery with increased anaesthetic dosage. Similar observation was made by Woody *et al*., (2002) on juvenile Clarias exposed to different concentration of clove oil. Clove oil was effective in using much lower concentration than other anaesthetic used. From this study, 0.5ml/l of clove oil produce anaesthesia at temperature of 26^0C, this is in agreement with Faith *et al*., (2007) who stated that lower concentration of 0.3-0.35 ml/l of 2-phenoxyethanol at temperature of 15-27^0C was enough to anaesthetize Sea bass. The reason for this could be from the geological location and biological responses of different species of fish. 2-phenoxyethanol was effective at higher concentration. In this study , 2.0 and 2.5ml are effective concentrations for anaesthesia with induction time 185 and 95secs, recovery time of 295 and 443 secs , but this disagree with Faith *et al*., 2007 who stated that lower concentration of 0.3-0.35 ml/l of 2-phenoxyethanol at temperature of 15-27^oC was enough to anaesthetize Sea bass. The concentration of anaesthetic in the body remains in equilibrium with their concentration in water under general anaesthetisia, meaning after transferring fish to freshwater, the anaesthetics leave the body through a concentration gradient (Myszkowki *et al*., 2003).

Table3: Physico-chemical analysis of water before subjecting to various concentrations of clove oil.

Parameters (ml/L)	0	0.5	1.0	1.5	2.0	2.5
Conductivity (S/m)	202.50±0.71	201.50±0.71	200.50±0.71	201.00±0.00	203.00±1.41	201.50±2.12
D.O (mg/L)	7.29±0.04	7.49±0.04	7.49±0.04	7.51±0.03	7.51±0.04	7.41±0.05
Temperature (^{o}C)	27.75±1.06	27.05±0.21	26.45±0.64	26.35±0.78	27.60±0.28	27.05±0.35
pH	6.27±0.06	6.50±0.02	6.34±0.16	6.45±0.04	6.42±0.01	6.56±0.08

Values are presented in Mean ± standard deviation

Table 4: Physico-chemical analysis of water after subjecting to various concentrations of clove oil.

Parameters (ml/L)	0	0.5	1.0	1.5	2.0	2.5
Conductivity (S/m)	201.50 ± 0.35	206.00 ± 0.00	209.40 ± 0.11	212.50 ± 0.00	214.60 ± 1.40	216.40 ± 1.40
D.O (mg/L)	6.45 ± 0.25	2.70 ± 0.28	2.50 ± 0.28	2.40 ± 0.70	1.00 ± 0.00	1.00 ± 0.07
Temperature (^{o}C)	28.1 ± 0.00	26.00 ± 0.28	25.85 ± 0.11	25.90 ± 0.07	26.05 ± 0.18	25.85 ± 0.11
pH	6.88 ± 0.01	7.28 ± 0.07	7.77 ± 0.16	8.13 ± 0.08	8.02 ± 0.21	7.86 ± 0.28

Values are presented in Mean ± standard deviation

Table 5: Physico-chemical analysis of water before subjecting to various concentrations of 2-phenoxyethanol.

Parameters (ml/L)	0	1.0	1.5	2.0	2.5	3.0
Conductivity (S/m)	200.50±0.71	202.00± 1.41	201.00± 1.41	201.00±0.00	201.50± 2.12	202.50± 0.71
D.O (mg/L)	7.36 ±0.06	7.49±0.06	7.47 ±0.09	7.58 ±0.05	7.38 ±0.07	7.50 ±0.04
Temperature (^{o}C)	27.10 ± 0.28	26.80 ±0.14	26.60 ±0.42	27.50 ±0.71	26.90±0.14	26.75±0.35
pH	6.51 ±0.04	6.47 ±0.02	6.49 ±0.03	6.53 ±0.05	6.51 ±0.02	6.48 ±0.04

Values are presented in Mean ± standard deviation

Table 6: Physico-chemical analysis of water after subjecting to various concentrations of 2-phenoxyethanol.

Parameters (ml/L)	0	1.0	1.5	2.0	2.5	3.0
Conductivity (S/m)	201.50 ± 0.35	206.00 ± 0.01	209.00 ± 0.41	212.50 ± 0.00	214.60 ± 1.40	216.40 ± 1.40
D.O (mg/L)	6.45 ± 0.25	2.95 ± 0.04	3.15 ± 0.53	3.75 ± 0.04	3.63 ± 0.18	2.35 ± 0.11
Temperature (^{o}C)	28.1 ± 0.00	25.35 ± 0.11	25.60 ± 0.11	25.30 ± 0.07	25.40 ± 0.07	25.50 ± 0.07
pH	6.88 ± 0.01	7.04 ± 0.01	7.38 ± 0.00	8.00 ± 0.04	8.15 ± 0.06	8.20 ± 0.08

Values are presented in Mean ± standard deviation

Table 7: Haematological parameters of *Clarias garipenius* exposed to Clove oil of different concentrations.

Parameters (ml/L)	0	0.5	1	1.5	2	2.5
PCV (%)	26.50±0.71^{a}	24.50±0.71^{b}	23.00±1.41^{c}	20.50±0.71^{d}	17.50±0.71^{e}	18.00±0.00^{e}
ESR (mm/h)	7.50±0.71^{a}	9.50±0.71^{b}	10.00±0.00^{b}	12.02±0.04^{c}	12.19±1.41^{c}	13.00±0.00^{d}
RBC (10^{12} cells/L)	2.95±0.07^{a}	2.65±0.07^{b}	2.50±0.14^{c}	2.20±0.14^{d}	1.90±0.00^{f}	2.00±0.00^{e}
WBC (10^{9}/L)	20.50±0.71^{a}	22.00±1.41^{b}	23.50±0.71^{c}	24.50±0.71^{d}	25.00±2.83^{d}	28.50±2.12^{e}
Hb (g/L)	8.70±0.14^{a}	8.15±0.35^{b}	7.55±0. .21^{c}	7.20±0.28^{d}	5.90±0.42^{e}	6.10±0.14^{e}
MCH (pg/cell)	29.55±1.20^{a}	30.75±0.49^{b}	30.20±0.85^{c}	32.80±3.39^{d}	31.05±2.19^{d}	30.50±0.71^{e}
MCHC (mmol/L)	32.85±0.35^{a}	33.30±0.42^{a}	32.85±1.06^{b}	35.15±2.61^{c}	33.65±1.06^{d}	33.85±0.78^{e}
MCV (fL)	89.90±4.53^{a}	92.45±0.21^{b}	92.00±0 .42^{c}	93.25±2.76^{d}	92.10±3.68^{e}	90.00±0.00^{a}

The values are expressed as the mean ± S.E.Means in the same horizontal column followed by different superscript are significantly different at $P < 0.05$ according to Duncan's New Multiple Range Test.

The study showed that recovery and induction time is dependent on concentration of 2-phenoxyethanol .This is in relation with Weyl *et al*., (1996) that reported the effect of 2-phenolxyethanol on goldfish, that the recovery and induction time depends on concentration of anaesthetics. Hematological parameters in fish can significantly change in response towards chemical stressors; however, these alterations are non-specific to a wide range of substances. Some of these changes may be the result activation of protective mechanisms (Cazenave *et al*, 2005) such as the blood parameters observed in the present work. The PCV values always decrease when a fish loses appetite or become diseased or stressed Monteiro, *et al.,* (2005). The decrease in the values of PCV and Hb could be attributed to haemolysis resulting in haemodilution, a mechanism for diluting the concentration of the anaesthetics in the circulatory system of the fish (Smith, *et al*., 1979). Similar reductions have been reported by Musa and Omoregie (1999) when exposed fish to polluted environment under laboratory conditions. The exposure of *C. gariepinus* to various levels of clove oil and 2-phenoxylethanol, led to a decrease in the values of packed cell volume (PCV) and haemoglobin (Hb) in brood *C. gariepinus.*

Table 8: Haematological parameters of *Clarias garipenius* exposed to 2-phenoxyethanol of different concentrations.

Parameters (ml/L)	0	1.0	1.5	2.0	2.5	3
PCV (%)	24.00±1.41[a]	20.50±0.71[b]	18.00±1.41[c]	14.00±1.41[d]	13.00±1.41[e]	11.00±0.00[f]
ESR (mm/h)	8.00±1.41[a]	10.50±0.71[b]	12.00±0.00[c]	13.50±0.71[d]	16.50±0.71[e]	17.50±0.71[f]
RBC (10^{12} cells/L)	3.85±0.35[a]	3.20±0.00[b]	3.05±0.071[c]	2.80±0.14[d]	2.35±0.35[e]	1.95±0.071[f]
WBC (10^9/L)	50.50±3.54[a]	55.50±2.12[b]	59.00±1.41[c]	62.00±1.41[d]	56.50±2.12[e]	62.50±2.121[f]
Hb (g/L)	7.95±0.49[a]	6.85±0.21[b]	6.00±0.42[c]	4.80±0.28[d]	3.95±0.35[e]	3.40±0.14[f]
MCH (pg/cell)	20.65±0.64[a]	21.40±0.71[b]	19.70±1.84[c]	17.20±1.84[d]	16.90±0.99[e]	17.45±0.07[f]
MCHC (mmol/L)	33.10±0.14[a]	33.40±0.14[a]	33.35±0.21[a]	34.35±1.48[a]	30.40±0.57[b]	32.70±1.27[b]
MCV (fL)	62.45±2.05[a]	64.05±2.19[b]	59.05±6.01[c]	50.20±7.64[d]	55.45±2.33[e]	56.45±2.05[f]

The values are expressed as the mean ± S.E.Means in the same horizontal column followed by different superscript are significantly different at P < 0.05according to Duncan's New Multiple Range Test

Thiswas similar to the one reported by Sudagar *et al*. (2009) in poach, (*Rutilus rutilus*) on application of 2-phenoxyethanol. Increase in the values of WBC, can be the result of the activation of immune system in the presence of pollutant, which in turn may be an adaptive response of the organism resulting in a more effective immune defence (Barreto- Medeiros *et al*., 2005) in brood *C. gariepinus*. The value of RBC decreases in both anaesthesia. This is in agreement with Mishra and Srivastava (1979) that observed a decrease in the RBC in *Colisa fasciatus* followed an exposure to zinc also Domaity (1987) observed decrease in RBC in *Claria slazera* expose to clove oil. The decrease observed in the RBC may be due to the swelling of the red cells, which may lead to anaemia. Haemolysis of ESR has also been described in rainbow trout expose to zinc (Koyama and Ozaki, 1982).The increase in the number of ESR may be due to increase protein carbon dioxide level in the blood. These changes could be a result of hypoxia or stress caused by anaesthetic substances. The observed increase in WBC in the present study could be attributed to several factors like increase in thrombocytes, lymphocytes or squeezing of WBC's in peripheral blood. This increase in WBC count can be correlated with an increase in antibody production which aids in survival and recovery of fishes exposed to anaesthetics. The increase level of WBC observed in this study was similar to the report of Banerjee and Banerjee (1988) in *Channa punctatus* due to Copper sulphate and Potassium dichromate induced toxicity (Singh, 1995; Anand *et al*., 2006) in *Channa punctatus* exposed to Copper.

CONCLUSION

The study established the anaesthetic properties of clove oil and 2-phenoxylethanol. The various concentrations of the anaesthetic used resulted into zero mortality and sustained the recovery of the fish. It is also observed that the anaesthetics were absorbed into blood and can be concluded that the haematological parameter of *Clarias gariepinus* juveniles is significantly (p>0.05) changed with increase in concentration of the anaesthetics used.

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