



The effect of anaesthetic agents on the haematological parameters of adult African Catfish (*Heterobranchus bidorsalis*)

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ABSTRACT

Introduction: The haematological parameters of giant African catfish exposed to different anaesthetic agents, such as lidocaine, clove oil, and ice, and the control with no exposure were examined.

Methods: Ten fish each from the control and treatment groups had their blood drawn and the samples were examined immediately for haematological parameters. One-way analysis of variance was used to compare each of the parameters among the treatment groups and the control.

Results: The highest red blood cell (RBC), Haemoglobin, and packed cell volume (PCV) were observed in the control and they were different significantly ($P < 0.05$) from the fish exposed to clove oil. Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin (MCHC) were significantly higher ($P < 0.05$) in the control than in all the treatment groups. The highest heterophil was observed in the fish exposed to ice and it was different significantly ($P < 0.05$) from the control treatment. Platelet was significantly higher in the control and lidocaine treatments than in clove oil. The least heterophil-lymphocyte ratio (HLR) was also observed in the control and it was different significantly ($P < 0.05$) from fish ice treatment. The PLR of lidocaine and ice treatments were higher significantly ($P < 0.05$) than the control, while that of clove oil was much lower than the control.

Significance: The findings from the research showed that all the anaesthetics experimented with had considerable negative impacts on the fish's haematological parameters, with clove oil tending to be the worst. Hence, extra care is required in using any of these treatments on fish, and the recommended dosages must be followed.

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Introduction

Aquaculture deals with the process of breeding, raising, and harvesting animals and plants of aquatic origin (National Ocean Service, 2019). An increase in global fish production is essential to meet the increased demands by man for protein-rich, palatable, nutritive, and easily digestible food for consumption and other purposes (Reverter *et al.*, 2014; Dauda 2020). The activities involved in aquaculture from breeding to harvesting, predispose fish to stress, attendant mortalities, and a huge loss. Stress is described as any condition resulting in psychological or physical discomfort to the animal that causes the release of stress-related hormones or some specific psychological trauma on the animal (Foster and Smith, 2007). Fish are frequently exposed to stresses in the wild or artificial settings like aquaculture or labs (Iwama *et al.*, 2006). Fish are usually stressed during transportation, breeding, and other farm activities such as handling, sorting, and counting. Therefore, anaesthetic agents are employed to minimize stress during those activities and may also be used to sedate bigger fish

during breeding (Gabriel and Akinrotimi, 2011). Anaesthetics could also be used to stabilize fish during an outbreak of any disease, before proper treatment may be instituted (Gabriel and Akinrotimi, 2011).

As may be observed in terrestrial animals, fish undergo anaesthesia to eliminate pain and sedate them. This is followed by a loss of balance, mobility, and consciousness (Summerfelt *et al.*, 1990). To reduce motility during handling and transit in fish farms, anaesthetics are utilised (Argungu *et al.*, 2017). Also, these substances can be applied during artificial spawning, grading, weighing, tagging, blood sampling, and surgical operations (Weber *et al.*, 2009). This might reduce vulnerability to infection and pathogens (Woody *et al.*, 2002; Weber *et al.*, 2009).

An ideal anaesthetic should have a wide margin of safety, be simple to administer, retain the animal in an optimum state, quickly induce anaesthesia with the least amount of hyperactivity, help with a speedy recovery, and be effective at low dosages. The sort of activities to be performed, the species of fish, the effectiveness, cost, availability, ease of use, fish toxicity, and environmental friendliness are all important considerations when

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using anaesthetics (Munday and Wilson, 1997; Akbulut *et al.*, 2011).

Tricaine methane sulphonate (MS-222), benzocaine, quinine, phenoxyethanol, clove oil, aqualunol, carbon dioxide, lidocaine hydrochloride, ice, and other anaesthetics are frequently used on fish (Woody *et al.*, 2002; Davis *et al.*, 2015; Collymore *et al.*, 2016). However, clove oil, lidocaine hydrochloride, and ice are more economical and readily available, especially in developing countries like Nigeria.

The route of administration commonly employed in fish anaesthesia (MS222; benzocaine hydrochloride) is either topical or by immersion because the procedures require less or no skill to carry out. On the other hand, the parenteral routes including intramuscular, intravenous, or intracoelomic require skills and expertise to be carried out and may also be time-consuming where a large number of fish is involved (Ferreira *et al.*, 1984; Harms, 1999; Torreilles *et al.*, 2009).

Anaesthetic agents are essential for the evaluation of fish, it is, however, very important to assess the state of health of fish using haematological indicators. Therefore, this study investigated the haematological alterations that may arise through the exposure of fish (*Heterobranchus bidorsalis*) to lidocaine hydrochloride, clove oil, and ice as anaesthetic agents.

Materials and methods

Study Location

The study was conducted at the Department of Veterinary Pathology's Aquatic Animal Health and Disease Unit of the University of Ilorin, Kwara State, Nigeria. Four plastic tanks of 100 litres capacity each were used to acclimatize and keep the fish. The translucent tanks were positioned in a well-ventilated environment with complete protection from direct sunlight, where humans and animals interact.

Experimental Animals

A total of 100 apparently healthy adult African Catfish (*Heterobranchus bidorsalis*) of average weight 260 ± 20 g were collected from a reputable fish farm situated at Egbejila, Ilorin, Nigeria. The fish were chosen randomly immediately after sorting and were transported using plastic bags to the University of Ilorin Veterinary Teaching Hospital (UIVTH) where they were divided into four plastic tanks. The fish were acclimatized for two weeks.

The study was approved by the University of Ilorin's Animal Use in Research's Ethics Committee (UERC/FVM/2021/021) and conducted by their guidelines for the care and welfare of animals.

Sources of anaesthetic agents

Three different anaesthetic agents were used including clove oil, lidocaine hydrochloride HCl and ice. The clove oil and lidocaine were purchased from reputable stores in Ilorin, Kwara state, Nigeria. The ice was obtained from the freezer at the pharmacy section of the UIVTH.

Length and Weight measurement of the fish

The total and standard lengths of the fish were measured with a metre rule and recorded in centimetre. The weights of the fish were determined using a sensitive weighing balance and were recorded in grams.

Methods of fish induction with anaesthetics

Anaesthesia was done through immersion. The clove oil was used to anaesthetise the fish at 0.5ml per litre of water following the method earlier described by (Argungu *et al.*, 2017). A total of 2.5ml of clove oil (solution) was diluted in water (5 L) and mixed with a stirrer for 4 to 6mins. After mixing thoroughly, ten fish were immersed completely in the solution and observed until the fish lost equilibrium and complete sensation after 7 minutes and 30seconds. Thereafter, blood was obtained with a needle (23G) and syringe (2 ml) into an anticoagulant bottle (EDTA). The fish were then transferred to the recovery section with running fresh water. Lidocaine was used at 3ml per litre of water, 15 ml was added to water (5 L), and was completely homogenized before 10 fish were immersed (Collymore *et al.*, 2016). The blood samples of the fish were collected using a 23G needle and 2ml syringe when the fish had lost equilibrium at about 16 minutes of induction of the anaesthesia and the blood samples were transferred into an EDTA bottle. This was followed by the transfer of fish to the recovery section containing running fresh water. Ice was gradually introduced into the container containing ten fish and a thermometer was used to monitor the temperature of the water at which the fish lost equilibrium (Shawn *et al.*, 2004). Upon loss of equilibrium by the fish, blood was collected with a needle (23G) and syringe (2 ml) and transferred into an EDTA bottle. The fish were returned to the recovery section with running fresh water. Normal saline at 3ml per litre of water was used for the control and the fish were well restrained for blood collection.

Haematological analysis

Following the collection of blood into anticoagulated bottles, the haematocrit percentage was determined through the microhaematocrit method. Red blood cell count was evaluated with a haemocytometer using PBS as the diluent, and total and differential leucocyte and platelets counts were evaluated through an indirect method, using a blood smear stained with Giemsa stain. The concentration of haemoglobin (Hb) was obtained using the method of cyanmethaemoglobin. The haematocrit or packed cell volume (PCV), and the values of the haemoglobin and RBC were used to calculate erythrocyte indices including the mean corpuscular volume ($MCV = (10/RBC \times PCV)$ in femtoliter, mean corpuscular haemoglobin ($MCH = (10/RBC \times Hb)$ in picogram and mean corpuscular haemoglobin concentration ($MCHC = (100/PCV \times Hb)$ in g/dl (Adamu and Solomon, 2015).

Heterophil Lymphocyte Ratio and Platelet Lymphocyte Ratio (HLR and PLR)

HLR is derived by dividing the absolute values of heterophils by that of the lymphocytes while PLR is obtained from the division of platelet counts by the lymphocyte counts.

$$\text{HLR} = \frac{\text{Heterophils}}{\text{Lymphocytes}}$$

$$\text{PLR} = \frac{\text{Platelets}}{\text{Lymphocytes}}$$

Statistical Analysis

The data was presented using descriptive statistics (Mean±SE). The data was subjected to normality and homogeneity tests, after which One-way analysis of variance (ANOVA) was used to compare the length, weight, and haematological parameters among the treatments. Where a significant difference was observed at a 95% confidence interval ($p < 0.05$), the Duncan Multiple Range Test (DMRT) was used to separate the means. All analysis was done with IBM SPSS Statistics version 23.0 software.

Results

Length and weight

There was no significant difference ($p > 0.05$) in either total or standard lengths among the treatments (Fig. 1), and the weight of the fish among the treatments was also not different significantly ($p < 0.05$) among the treatments (Fig. 2).

Haematological analysis

The result of the haematological parameters is shown in Table 1. There was a decrease in the values of RBC, Hb, and PCV of the groups treated with anaesthetics compared with the control and they were significantly lower ($p < 0.05$) in clove oil treatment than in the control. MCH and MCHC were higher significantly ($p < 0.05$) in the control than in all the treatment groups. The percentage of heterophil was significantly higher ($p < 0.05$) in the ice treatment than in the control. The least platelet was observed in the clove oil and it was significantly different from the control and lidocaine treatments. The highest HLR (Fig. 3) was observed in the ice and it was significantly different from the control group who had the least. The PLR was significantly higher ($p < 0.05$) in the lidocaine and ice than in the control, while the least was observed in the clove oil treatment (Fig. 4).

Table 1: Haematological parameters of *Heterobranchius bidorsalis* subjected to different anaesthetic agents and control

Parameters	Clove oil	Ice	Lidocaine	Control
RBC ($\times 10^{12}$ L)	4.44±0.41	5.03±0.45	4.96±0.32	5.64±0.20
Haemoglobin (g/dl)	8.79±0.80	9.93±0.91	9.63±0.54	11.51±0.52
PCV (%)	27.22±2.42	30.60±2.78	29.70±1.57	34.63±1.39
MCV (fl)	61.47±0.59	60.83±0.43	61.16±0.36	61.39±0.30
MCH (pg)	19.81±0.18 ^a	19.62±0.10 ^a	19.84±0.08 ^a	20.33±0.17 ^b
MCHC (g/dl)	32.12±0.16 ^a	32.33±0.16 ^a	32.37±0.11 ^a	33.14±0.20 ^b
Heterophil (%)	41.44±2.72	47.00±3.37	41.50±1.92	37.88±2.95
Lymphocyte (%)	56.11±2.35	50.20±3.45	55.50±1.81	58.38±2.75
Monocyte (%)	1.89±0.48	1.90±0.38	2.40±0.37	2.50±0.50
Eosinophil (%)	0.56±0.24	0.80±0.29	0.60±0.27	1.13±0.35
Basophil (%)	0.11±0.11	0.10±0.10	0.00±0.00	0.13±0.13
Platelet ($\times 10^9$ /L)	313.11±36.13 ^a	379.60±21.87 ^{ab}	405.50±14.30 ^b	411.13±27.29 ^b
WBC ($\times 10^9$ /L)	10.08±0.66	11.22±0.57	10.27±0.70	11.19±0.58
Lymphocyte ($\times 10^9$ /L)	5.70±0.43	5.72±0.62	5.72±4.79	6.49±0.36
Heterophil ($\times 10^9$ /L)	4.18±0.41	5.19±0.35	4.26±0.38	4.28±0.48

Different letters as superscripts across each row indicate significant differences ($p < 0.05$)

RBC – Red blood cell, WBC- White blood cell, PCV- Packed cell volume.

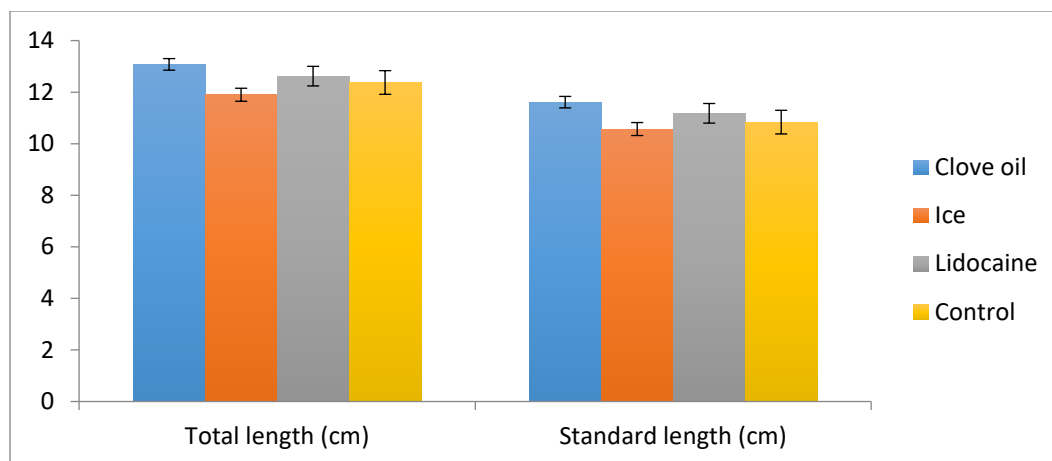


Figure 1: Length (cm) of *Heterobranchus bidorsalis* subjected to different anaesthetic agents

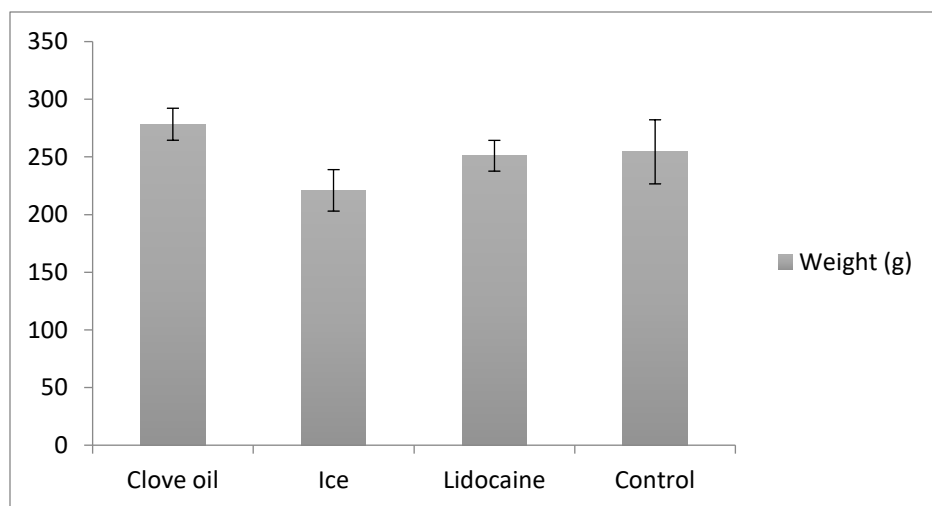


Figure 2: Weight (g) of *Heterobranchus bidorsalis* subjected to different anaesthetic agents

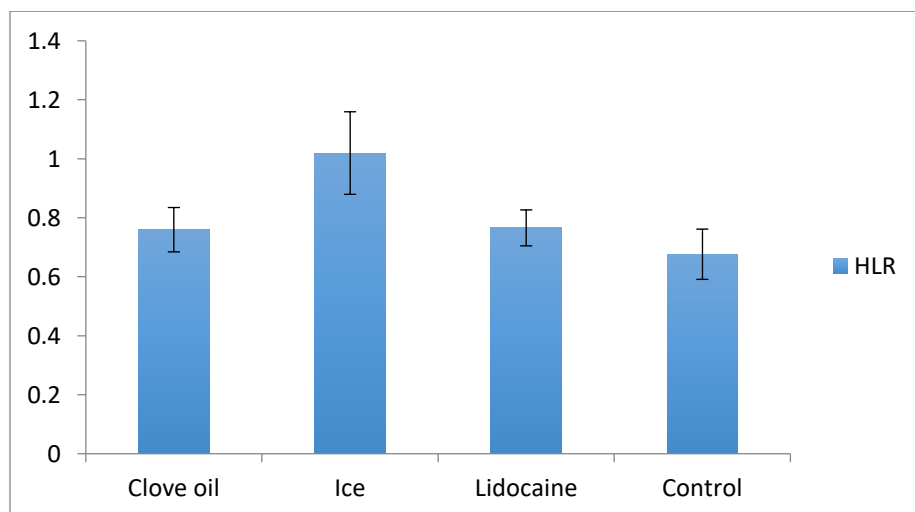


Figure 3: HLR- Heterophil-Lymphocyte ratio of *Heterobranchus bidorsalis* treated with different anaesthetics.

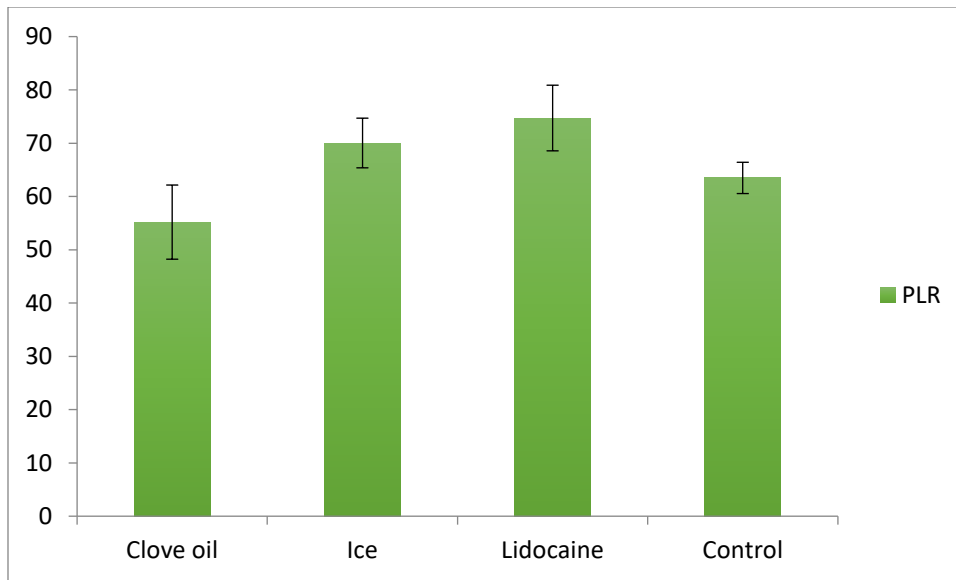


Figure 4: Platelet lymphocyte ratio of *Heterobranchus bidorsalis* subjected to different anaesthetic agents.

Discussion

Anaesthetic agents are employed for light sedation and certain procedures to avoid or reduce pain afflictions during surgery and other interventions. Management practices like sizing, grading, weighing, stripping, blood collection, and transportation are strenuous exercises and stressful to the fish (Wagner, 2002). It is, therefore, pertinent to embrace methods in which stress in fish would be reduced and production would not be negatively altered. Some chemicals such as lidocaine hydrochloride, MS222, and clove oil are employed as anaesthetic agents to reduce stress in fish before handling. Iversen *et al.* (2003) reported that the application of anesthetic agents such as clove oil and metomidate is a common practice to handle fish stress and prevent injuries during all aquaculture procedures. Haematological parameters are an essential indicator of health status in fish and any alteration in these values is a function of systemic abnormality in the fish (Dauda *et al.*, 2018). The analysis of haematological parameters has been described as one of the essential procedures accessible for modern diagnostics (Anver Celik, 2004). Therefore, the relationship between the anaesthetic agents and blood parameters is necessary for evaluation, to determine their safety for use. Biometric data including the length and weight of the fish, have a significant impact on haematological parameters in several species of fish (Adamu and Solomon, 2015; Fazio *et al.*, 2017). The similarities in the length and weight of various groups in this study indicate that the significant differences that existed in the haematological parameters of the various groups, would be attributed to the anaesthetic agents and not due to variations in lengths and weights. The total erythrocyte count, haemoglobin concentration, PCV, and erythrocyte indices were significantly

lower in the group of clove oil than those in the control group. This is an indication that anaemia could result from the use of clove oil. However, this finding is contrary to the report of Mohammadzarejabad *et al.* (2009) where an increase in haematocrit, RBC, and Hb and no changes were observed in the levels of MCV, MCHC, and MCH in great sturgeon when exposed to various concentrations of clove powder in water over a period (constant) of 10 minutes was reported. On the other hand, Velisek *et al.* (2005) stated that the exposure of common carp to clove oil at a concentration of 30 mg/L for 10 min did not affect haematological indices. The differences may be attributed to a variant form of clove oil, species of fish, and age of fish. The total leukocyte, as well as the differentials and thrombocyte counts, are also commonly used as indicators of physiological parameters because of the key role they play in immune defense and are also involved in the control of immunological processes and coagulation respectively in aquatic animals, such as fish (Ballarin *et al.*, 2004). Prior research on anaesthesia revealed that clove oil or clove solution did not significantly alter the total WBC and differential leukocyte counts (Velisek *et al.*, 2005b; Mohammadzarejabad, 2009), which is similar to the observation in this study. It is pertinent that baseline data for haematological parameters in aquatic organisms are generated to be able to appreciate the sequelae of the abnormality associated with variations in values among different groups of fish. Although the anaesthetic materials are supposed to physically reduce induced stress in fish, the leukogram obtained in this study shows a stress leukogram characterized by insignificant mild heterophilia and lymphopaenia. The HLR of all the anaesthetic agents is higher than the control significantly, which may indicate an inflammatory response associated with exposure to anaesthetic agents. Neutrophil lymphocyte ratio (NLR), an analog of heterophil lymphocyte ratio (HLR) in fish has not only been used as a

marker of inflammation (Zahorec, 2001; Nada *et al.*, 2008) but also as a prognostic index for various kinds of disease condition (Halazun *et al.*, 2008; Kao *et al.*, 2010). Kalay (2012) reported that NLR can unconventionally predict the incidence, progression as well as apoptosis of stable atherosclerosis. The platelet lymphocyte count (PLR) of ice and lidocaine anaesthetized groups is higher than control whereas that clove oil is lower significantly than control. The prognosis is good in this case for clove oil. The lower the PLR, the better the prognosis. Platelets play a vital role both in inflammatory and immunomodulatory processes by inducing the release of inflammatory cytokines (Azab *et al.*, 2012; Cho *et al.*, 2014; Kim *et al.*, 2015). PLR is regarded as a unique inflammatory marker that is applied to many diseases for the prediction of inflammation and mortality (Gui-lian *et al.*, 2019).

Conclusion

Ice is the best of the 3 studied anaesthetic agents, with the least haematological alterations in African catfish. It is recommended to be cautious in the use of these anaesthetic agents and their introduction to fish water should be gradual and not in abrupt high concentration.

Conflict of interest

The authors declare that they have no known conflict of interest that could have appeared to influence the work reported in this manuscript.

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