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Effects of different anticoagulants on the haematology of African catfish (*Clarias gariepinus*)

Abdullateef Abiodun Ajadi[1*],

Abdullateef Damilola Usman[1],

Jamila Abdulhamid Atata[1],

Mohammed Adam[1],

Abdulrauf Adekunle Usman[1],

Akeem Babatunde Dauda[2],

Onyeka Chidiebele Nwufoh[3],

Benedict Olurotimi Muyiwa[4],

Besong Paul Nyenti[1],

Bisi Olajumoke Adeoye[5]

[1] *Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria*

[2] *Department of Fisheries and Aquaculture, Federal University Dutsinma, Dutsin-ma, Katsina State, Nigeria*

[3] *Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Nigeria*

[4] *Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[5] *Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria*

Blood preservatives (anticoagulants) prevent blood from clotting while ensuring that the concentration of the component to be analysed stays constant prior to the analytical procedures. The study was aimed to evaluate the effects of different anticoagulants, such as ethylenediaminetetraacetic acid (EDTA), fluoride oxalate, sodium citrate and lithium heparin, on the haematological parameters of African catfish (*Clarias gariepinus*). Forty fish were obtained and separated into four groups representing the four treatments. Blood samples were collected from ten fish from each group and stored in separate anticoagulant laden bottles. Lithium heparin was used as control in this experiment. Each sample was processed, and values of the parameters were recorded. The RBC, haemoglobin, and PCV of the anticoagulants used were similar as no significant difference ($P > 0.05$) was revealed among the treatments. The heterophil levels of fluoride oxalate treatment group was significantly higher than control, this in turn led to an increase in HLR (heterophil-lymphocyte ratio). Therefore, the haematologic alteration observed in this study, which include heterophilia associated with fluoride oxalate anticoagulant, should be put into consideration when making a choice of anticoagulants for haematological analysis in *C. gariepinus*.

Keywords: *Clarias gariepinus*, haematology, blood, anticoagulants, haemostasis

* Corresponding author. Email: ajadi.aa@unilorin.edu.ng

INTRODUCTION

Aquaculture is a sustainable practice of fish harvest that strives to keep aquatic biodiversity and ecosystems intact. It is the culture of organisms of aquatic origin including molluscs, fish, crustaceans, and plants. Aquaculture also includes the cultivation of freshwater and saltwater organisms under controlled conditions, and should be distinguished from commercial fishing, which is associated with harvesting of wild fish (Marshall, 2017).

The African catfish (*Clarias gariepinus*) is the most cultivable species in Nigeria. The species is hardy with high fecundity, flexible phenotypic and genetic combination, and rapid growth. It has an air-breathing structure and can tolerate very low oxygen levels in any culturing environment (earthen ponds, concrete tanks, or movable plastic tanks) (Walencik, Witeska, 2007).

Owing to the internal and external variants, haematological analysis is quite tasking in aquatic species. It is fully established that blood sampling, collection, storage, handling, and processing of a blood sample can strappingly impact results obtained from haematological analyses (Faggio et al., 2014). The haematological analysis is employed to evaluate fish health status in cultivable species of fish, observe physiological changes associated with stress conditions such as exposure to heavy metals, pollutants, disease or hypoxia (Faggio et al., 2014; Walencik, Witeska, 2007). To obtain error free haematological data, it is pertinent that the right quantity of the right anticoagulant is obtained for the right volume of blood (Faggio et al., 2014).

Anticoagulants are agents that prevent blood, plasma, and/or its derivatives from clotting, ensuring that the constituent to be analysed is unaffected by the analytical procedure. Anticoagulation is attained through the inhibition of thrombin activity (heparinates, hirudin) or binding of calcium ions (EDTA, citrate) (MacNeil, 2015).

Anticoagulants are chemical substances that are used to prevent the formation of blood clots. Anticoagulants intended for use in haematological analysis must possess certain characteristics, which include the following: they must (1) elicit no effect on the size of cells, (2) be water and blood soluble, (3) minimise disruption of staining and morphology of leucocytes, and (4) be able to minimise platelet aggregation (Glade Weiser, 2012).

The anticoagulants under study, which include ethylenediaminetetraacetic acid (EDTA), lithium heparin, sodium citrate, and fluoride oxalate, have different uses, advantages, and disadvantages, and they all elicit different effects on the complete blood count of aquatic species.

EDTA is the anticoagulant of choice for most haematological analyses. Its primary use is for the complete blood count and individual components of the CBC. EDTA preserves the blood cells (morphology) for about 4 h and so it is mostly selected for peripheral smear preparations and cell counts (Ciepliński et al., 2019). However, EDTA causes shrinkage of RBCs (red blood cells). This cellular membrane destruction can result in speciously increased MCHC and decreased haematocrit and a significant decrease in MCV. This also results from excess EDTA and less blood volume. Excess EDTA can also lead to platelets disintegration and can result in speciously increased values. Thus, it is not apposite for coagulation studies (Ciepliński et al., 2019).

Lithium heparin is a mucopolysaccharide agent with affinity for blood (plasma) proteins and act as antithromboplastin and antithrombin. It activates antithrombin, thereby inhibiting coagulation by inhibiting thrombin. Lithium heparin is the anticoagulant selected for use in measuring blood pH, blood gasses, electrolytes and ionized calcium. It is also used for clinical chemistry tests such as cholesterol, C-reactive protein (CRP) and hormones (Sunil, 2017). However, lithium heparin interferes with polymerase chain reaction (PCR) and cannot be used as anticoagulant for cell count and blood smear examinations as it causes clumping of cells and results in staining artefact respectively (Thrall et al., 2012).

Sodium citrate is used during blood collection. It binds and reacts with calcium (free), resulting in Sodium-citrate complex, causing depletion of calcium and percentage coagulation. Sodium citrate is suitable for coagulation studies and as a constituent of acid citrate dextrose (ACD) solution in blood banking. However, citrated blood cannot be used for packed cell volume (PCV), haemoglobin (Hb) estimation, total leukocyte count (TLC), and differential leucocyte count (DLC) because it is used as a solution and it alters the concentration of blood (Thrall et al., 2012).

Fluoride-laden tubes are used when considering the reduction of glycolysis, especially for the accurate glucose analysis. Fluoride of various types is contained in grey top tubes. It may contain sodium fluoride only, sodium fluoride with sodium heparin or with potassium oxalate. Sodium fluoride prevents utilisation of glucose by blood cells and acts as the glycolytic inhibitor. It preserves glucose, but not as an anticoagulant. Potassium oxalate acts as an anticoagulant by binding calcium, enabling the glucose determination to be performed on plasma (Cerón et al., 2004).

This study evaluated the effect of anticoagulants including EDTA, lithium heparin, sodium citrate and fluoride oxalate on haematological parameters such as red blood cell count (RBC), pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count.

MATERIALS AND METHODS

Study location

This study was conducted at the Clinical Pathology Unit, University of Ilorin Veterinary Teaching Hospital (UIVTH), Sabo-Oke, Ilorin, Kwara State, Nigeria.

Experimental animals and housing

Four sets of 100-litre plastic tanks were used. The tanks were positioned in a well-ventilated environment with complete protection from direct sunlight, human and animal interaction. A total of 40 seemingly healthy post juvenile African catfish (*C. gariepinus*) with average weight of 1 kg were collected from a reputable earthen pond farm in Ilorin. The fish were chosen randomly, immediately after sorting, and were divided into ten in four different plastic tanks each. Each of the tanks was represented with letters ET (EDTA), LH (lithium heparin), SC (sodium citrate), and FO (fluoride oxalate). This research used lithium heparin as the control, because it is the most commonly used anticoagulant in aquaculture and reference value.

Using water from a borehole, the fish were acclimatised for two weeks. Four times every week, the temperature, pH, salinity, hardness, and dissolved oxygen were measured using thermometer, pH meter, hygrometer, and dissolved oxygen meter, respectively.

Sampling

Using the metre ruler, the total length and standard length of the fish were measured and recorded in inches. The overall length of the catfish was determined, from the rostral point of the head to the end of the tail fin/caudal fin. The standard length was expressed in inches and measured from the top of the head to the beginning of the caudal fin.

The weights of the fish were determined using a weighing balance and were recorded in grams. The fish were immobilised by gradual introduction of ice into the fish holding containers; a thermometer was used to monitor the temperature of the water at which the fish lost equilibrium. Once the fish lost equilibrium, blood was obtained via the caudal vein using 23G needle and 2ml syringe. The blood samples collected from the fish in tanks ET, LH, SC, and FO were transferred into EDTA, lithium heparin, sodium citrate, and fluoride oxalate sample bottles, respectively. The fish were transferred into the recovery section containing fresh water

Blood samples collected were transported to the laboratory using transportation cooler containing ice pack at 4°C.

Haematological analysis

Complete blood count (CBC) was carried out by methods described by Feldman et al. (2000). The PCV was determined using the micro haematocrit centrifuge and read with the micro haematocrit reader. The haemoglobin concentration was carried out using the cyanmethemoglobin calorimetric method after centrifuging, while differential white blood counts were carried out on blood smears stained with Geimsa as described by Cambell and Ellis (2012).

Heterophil-lymphocyte ratio (HLR) was obtained by dividing the absolute value of heterophils by that of the lymphocytes (Proctor et al., 2012).

$$\text{HLR} = \frac{\text{Absolute peripheral blood cell count of heterophil}}{\text{Lymphosyte}}.$$

Platelets-lymphocyte ratio (PLR) was obtained by dividing the total platelet count by that of the lymphocyte

$$\text{PLR} = \frac{\text{Total platelet count}}{\text{Lymphosyte}}.$$

Statistics

The data were presented using mean ± standard error. All the parameters assessed were compared among the treatment groups using one-way analysis of variance (ANOVA), after the test of homogeneity. Significant difference was observed at $P < 0.05$. Post hoc test was done with Duncan multiple range test. Charts were drawn using Microsoft Office Excel, while ANOVA was done using IBM SPSS v.23.

RESULTS AND DISCUSSION

Length and weight of the fish

The results of the total length, standard length, and weight from the different treatments are shown in Figs 1 and 2. There is no significant difference in any of the three parameters among the treatments.

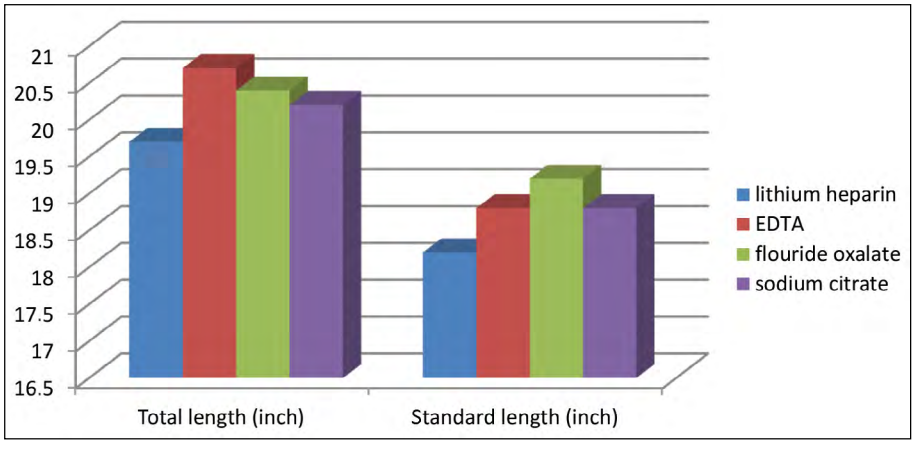

Fig. 1. Mean average of total length and standard length of *C. gariepinus* in four different treatments

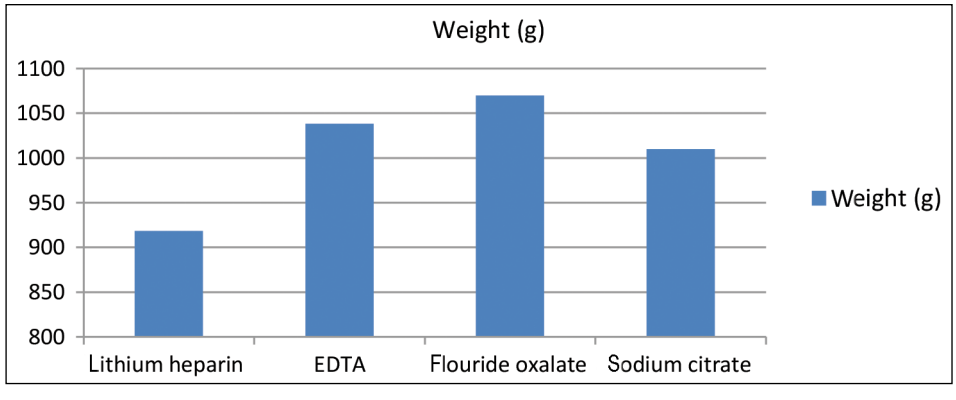

Fig. 2. Mean average of weight of *C. gariepinus* in four different treatments

The following bar chart represents the mean average of weight of the fish sampled for each category of anticoagulant used.

Haematology

The Table below shows the mean ± standard error of various haematological parameters of the fish in different anticoagulant groups. There was a significant increase in heterophil count of FO when compared with the control group.

Heterophil-lymphocyte ratio (HLR)

The results of the heterophil-lymphocyte (HLR) and platelet-lymphocyte (PLR) ratios from the different treatments are shown in Figs 3 and 4. The HLR of the FO group was significantly higher compared with that of the control group. The PLR of the EDTA group was significantly lower than the control.

Blood preservatives called anticoagulants prevent blood from clotting while ensuring that the concentration of the component to be analysed stays constant prior to the analytical procedures (Maqbool et al., 2014). This research adopted lithium heparin as the control because it is the most commonly used anticoagulant in aquaculture and reference value while evaluating the effect of anticoagulants such as sodium citrate, fluoride oxalate, EDTA on the haematology of *C. gariepinus*.

The haematological parameters analysed in this study were to assess each of the anticoagulants in comparison to the control (lithium heparin) in order to monitor and record haematological changes elicited by the interaction of blood with anticoagulants. The RBC, haemoglobin, and PCV of the anticoagulants used were unaffected as it showed no significant differences in the values when compared with the control. This indicated that the erythrogram is uncompromised by the interaction of blood with the respective anticoagulants. This is in contrary to Maqbool et al. (2014), which posited that EDTA induced membrane distortion and haemolysis of erythrocyte in rainbow trout (*Oncorhynchus mykiss*). The study further observed that EDTA resulted in an elevation of PCV and reduced the RBC counts and haemoglobin concentration. It also induced RBC swelling, variations in cell sizes (anisocytosis),

Table. **Haematological parameters of *C. gariepinus* blood sample collected with different anticoagulant bottles**

Parameters	Lithium heparin	EDTA	Fluoride oxalate	Sodium citrate
RBC ($\times10^{12}$/l)	4.37 ± 1.12	4.80 ± 0.74	4.61 ± 1.06	4.23 ± 1.13
PCV (%)	26.60 ± 7.47	29.33 ± 5.13	29.00 ± 5.90	25.00 ± 7.51
Haemoglobin (g/dl)	8.80 ± 1.76	9.60 ± 1.25	9.10 ± 1.98	8.00 ± 2.04
MCV	60.64 ± 3.81	60.95 ± 3.74	63.22 ± 2.49	58.43 ± 4.24
MCH	20.36 ± 1.32	20.02 ± 0.47	19.73 ± 0.90	18.93 ± 0.92
MCHC	33.74 ± 2.87	33.05 ± 2.46	31.32 ± 1.19	32.52 ± 1.91
WBC ($\times10^{9}$/l)	10.38 ± 0.65	14.41 ± 4.53	11.35 ± 1.62	11.26 ± 2.37
Heterophil ($\times10^{9}$/l)	3.59 ± 1.90	4.75 ± 1.16	5.23 ± 0.97[a]	3.82 ± 0.67
Lymphocyte ($\times10^{9}$/l)	4.97 ± 2.50	9.18 ± 3.82	6.36 ± 0.73	7.10 ± 1.94
Monocyte ($\times10^{9}$/l)	0.10 ± 0.05	0.31 ± 0.13	0.21 ± 0.13	0.20 ± 0.10
Eosinophil ($\times10^{9}$/l)	0.00 ± 0.00	0.16 ± 0.14	0.04 ± 0.01	0.18 ± 0.05
Basophil ($\times10^{9}$/l)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Platelets ($\times10^{9}$/l)	185.00 ± 24.11	155.83 ± 18.72	181.72 ± 27.15	193.17 ± 37.27
HLR	0.62 ± 0.36	0.55 ± 0.15	0.83 ± 0.15	0.56 ± 0.15
PLR	26.18 ± 13.72	18.56 ± 5.83	28.97 ± 6.53	28.30 ± 7.40

* as superscripts indicates a significant difference

P value is set at <0.05.

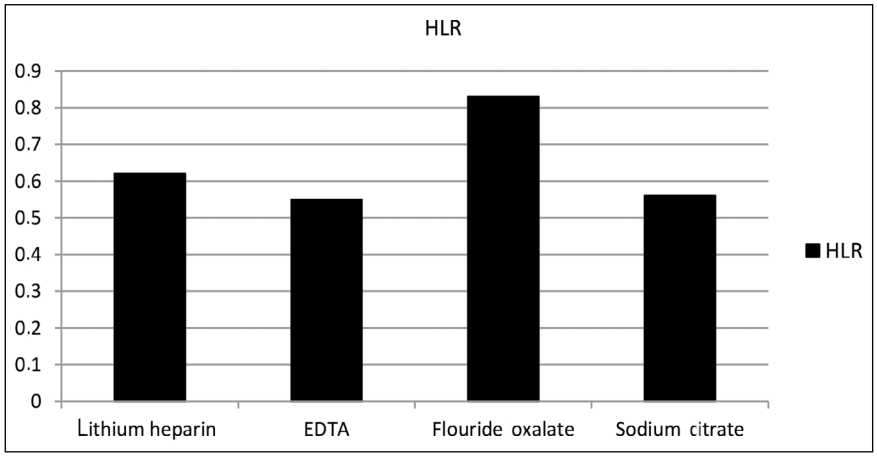

Fig. 3. Heterophil-lymphocyte ratio (HLR) of *C. gariepinus* blood sample collected with different anticoagulants

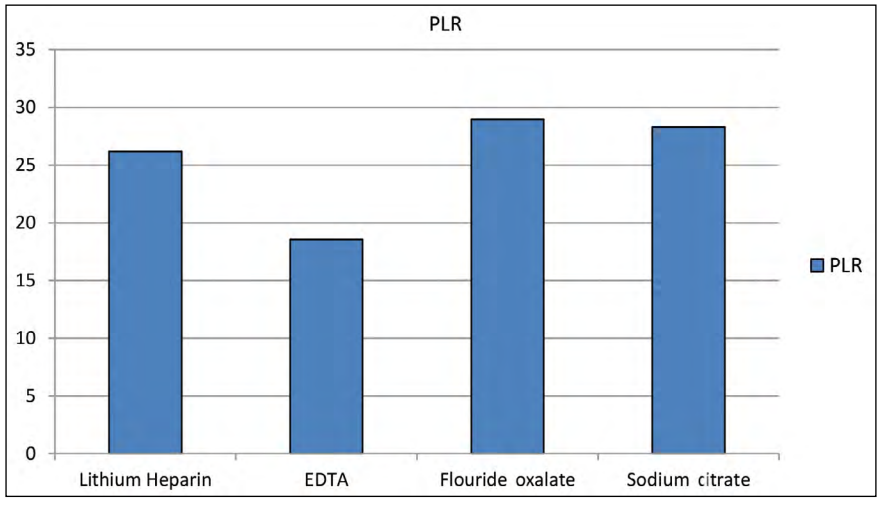

Fig. 4. Platelet-lymphocyte ratio of *C. gariepinus* blood sample collected with different anticoagulants

variations in nuclei sizes (anisonucleosis), and caused lysis of erythrocytes. Meanwhile, no significant alteration was observed in lithium heparin-laden samples.

As leukocytes are important for immune defence and are involved in the control of immunological activities in aquatic creatures, including fish, their numbers, as well as those of thrombocytes, are widely utilised as indicators of health state (Kirschbaum, Denizot, 2011). In this study, the heterophil levels of fluoride oxalate were significantly higher than control. Meanwhile, the previous study by Adeyemo et al. (2009) observed that heterophils of blood treated with EDTA and sodium citrate were higher than the control. The study compared EDTA and lithium heparin as anticoagulating agents for haematological evaluation in cultured and undomesticated African catfish (*C. gariepinus*).

The WBC of EDTA, sodium citrate, and fluoride oxalate showed a significant difference when compared to the control. EDTA elicited a significant difference in the level of lymphocytes, while fluoride oxalate and sodium citrate showed no significant difference when compared to the control. The absolute monocyte counts of EDTA showed a slight difference when compared to the control, while fluoride oxalate and sodium citrate showed no significant difference. The platelet level of EDTA was significantly higher than the control while fluoride oxalate and sodium citrate showed no significant differences. This is in pact with Adeyemo et al. (2009), who observed that the absolute value of monocyte was

significantly higher with the consideration of EDTA in pond cultivated African catfish.

Earlier studies revealed the influence of biometric data, including weight and length, on haematology of several fish species (Adamu, Solomon, 2015; Fazio et al., 2017), although in this study the length and weight of fish had no significant differences. Therefore, the resulting lengths and weights of each fish, which indirectly indicate their sizes, have no significant effect on the haematological results, and any differences seen in the results could be the effect of the anticoagulant bottle used.

CONCLUSIONS

The preservation of blood samples with different anticoagulants has some effects on haematological parameters of fish. This may be associated with the bioactive components of these anticoagulants. Fluoride oxalate causes elevation in heterophil counts and subsequent increase in the total leukocyte count. The haematologic alteration observed in this study should be put into consideration when making a choice of anticoagulants for haematological analysis in *C. gariepinus*.

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Ethics committee approval: Ethical approval was granted by Ethical Review Committee of the University of Ilorin with ethical approval number: UREC/FVM/15/32TA068

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ĮVAIRIŲ ANTIKOAGULIANTŲ POVEIKIS AFRIKINIO ŠAMO (*CLARIAS GARIEPINUS*) HEMATOLOGINIAMS PARAMETRAMS

Santrauka

Kraujo konservantai (antikoaguliantai) neleidžia kraujui krešėti ir užtikrina, kad tiriamo komponento koncentracija iki analizės išliktų pastovi. Tyrimu siekiama įvertinti skirtingų antikoaguliantų, tokių kaip etilendiaminotetraacto rūgštis (EDTA), fluoro oksalatas, natrio citratas ir ličio heparinas, poveikį afrikinio šamo (*Clarias gariepinus*) hematologiniams parametrams. Keturiasdešimt žuvų buvo suskirstyta į keturias grupes, atitinkančias keturis gydymo būdus. Iš kiekvienos grupės buvo paimti 10-ies žuvų kraujo mėginiai ir laikomi atskiruose antikoaguliantų buteliuose. Šio eksperimento metu kaip kontrolė buvo naudojamas ličio heparinas. Kiekvienas mėginys buvo apdorotas ir užregistruotos parametrų reikšmės. Panaudojus antikoaguliantus RBC, hemoglobinas ir PCV buvo panašūs, nes reikšmingų skirtumų ($p > 0,05$) tarp gydymo būdų nenustatyta. Heterofilų lygis fluoro oksalatu gydomoje grupėje buvo žymiai didesnis nei kontrolinėse grupėse, o tai savo ruožtu padidino HLR (heterofilų ir limfocitų santykį). Taigi renkantis antikoaguliantus *Clarias gariepinus* hematologinei analizei, reikia atsižvelgti į šiame tyrime pastebėtus hematologinius pokyčius, įskaitant heterofiliją, susijusią su fluorido oksalato antikoaguliantu.

Raktažodžiai: *Clarias gariepinus*, hematologija, kraujas, antikoaguliantai, hemostazė