Assessment of Haematological Aberrations in *Heterobranchus longifilis* Treated with Sublethal Concentrations of KEMDrill, a Water Based Drilling Fluid

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Abstract

Drilling muds and their additives get easily released into aquatic ecosystems in the Niger Delta area through accidental discharge, poor waste management and handling during drilling process, causing serious environmental impacts on non-target organisms. A completely randomized static renewal bioassay was conducted using 35 juveniles of *Heterobranchus longifilis* in this study, after a 14-day acclimation period. Treatment fishes were exposed to 167 mg/L, 333 mg/L, 400 mg/L and 500 mg/L in a 21-day definitive test after a range finder experiment for 14 days, while maintaining a negative control. After the exposure period, fishes were anaesthetized, and blood samples were collected into EDTA bottles for blood cell analysis. The results revealed a significant decrease in RBC, WBC, platelets and haemoglobin counts compared to the control. Whereas differential counts such as neutrophil, eosinophil, and monocytes did not show any significant difference compared to the control, lymphocytes recorded a significant ($p<0.05$) decline to 7.98±0.20 at 500 mg/L compared to the control (11.64±0.21). The results of this study therefore confirm the toxicity of KEMDrill, a water based drilling fluid on haematological parameters of exposed fish. Hence, the chemical should be used cautiously under strict regulations and continuous environmental monitoring.

Key words: Drilling-fluid, Sublethal, Bioassay, Bentonite, and Clay

1. Introduction

In the Niger Delta ecosystem, oil and gas (petroleum) exploration and production have been on the rise being the main stay of Nigeria economy. Certainly, these operations by the oil and gas companies have exacerbated the destructive influence of human activities on the aquatic ecosystem within the area, thus resulting in negative impact on aquatic lives [1]. These exploration activities are basically made possible through extensive drilling activities using either water based or non-aqueous drilling fluids, thus generating large volumes of drilling wastes within the region [1]. In the course of drilling, most drilling muds and their toxic wastes are left in the environment without adequate treatment. They eventually find their way into water bodies causing severe pollution on aquatic lives, including fish.

Sometimes, these toxic wastes are left in temporary holding-pits during onshore drilling operations or dumped directly into surrounding water bodies such as swamps, rivers and oceans during offshore drilling [2]. These unprofessional practices by the oil and gas companies operating within the Niger Delta has caused debilitating impacts on the ecosystem of the region. These practices also affect fish farming in the area, which has led to a reduction in aquaculture. Common fishes in the Niger Delta are increasingly becoming endangered by the negative impact of drilling operations and pollution. This often results in fish kills arising from pollution of both aquatic and terrestrial environment [3]. Another effect of drilling fluid pollution is smothering which results in oxygen depletion and increase in particle suspension in the aquatic ecosystem [4].

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Recent studies have shown that some of the water based mud (WBM) additives that were acceptable from an environmental point of view decades ago, are no longer acceptable for current and future drilling operations in environmentally sensitive areas [5]. The recent clamour by governments all over the world to improve on environmental laws to curb the menace of air pollution, clean water, hazardous waste disposal, as well as the control of occupational health and safety hazards have charted a new course for the petroleum industry to begin to re-evaluate all aspects of drilling and production [6].

Water based drilling fluids (WBDF) such as KEMDrill, and their additives are readily released into aquatic ecosystems in the Niger Delta area through accidental discharge, poor waste management and handling, as well as poor management of drill cuttings during the drilling process [7]. Over the years, this has proven to be harmful to marine flora and fauna, thus causing contamination in various food chains [8]. All of these attendant effects are believed to have been caused by poor handling and management of drilling mud, its wastes, improper control of hazards due to lack of government guidelines and enforcement, as well as lack of strict adherence to safety standards by oil exploration and production companies during drilling operations [9].

In all of these challenges, the effect of toxic concentrations of WBDF containing Bentonite and clay on haematological indices of *Heterobranchus longifilis* have not been effectively reported in the Niger Delta area. Additionally, there is little, or no studies carried out to assess the environmental impact of these fluids on the ecosystem at the early stage of drilling in the Niger Delta [10]. Therefore, the present study investigates the toxicity of KEMDrill on selected haematological indices, including red blood cells (RBC), white blood cells (WBC), platelets, and haemoglobin (Hb), as well as differential counts (neutrophil, eosinophil, lymphocytes, and monocytes) in juvenile African catfish (*Heterobranchus longifilis*).

2. Materials And Methods

2.1 Experimental Fish and KEMDrill

Experimental fish (n=35) were bought from Ikuligan Fish Farm located at Agudama Epie, within Yenagoa metropolis of Bayelsa State, Nigeria, and transported in 50 L plastic cans covered with fishing nets to the wet laboratory of Department of Biology, Bayelsa Medical University, for the experiment. KEMDrill was collected from a drilling site within Yenagoa metropolis, Bayelsa State, Nigeria for the experiment. The fluid was collected in a plastic bottle and mixed thoroughly to ensure sample homogeneity.

2.2 Acclimatization and Range Finding Test

The experimental fish were acclimatized in four plastic aquaria for a period of fourteen (14) days, following the method described by Inyang [11]. A range finder test was carried out for two weeks during which four sublethal concentrations needed for the definitive test were determined using 5 mL, 10 mL, 12 mL and 15 mL of KEMDrill in a static renewal bioassay [11]. The concentrations obtained during the trial were converted and presented in mg/L in the main definitive test using the formula outlined in Equation 1.

$$C = \frac{V_k\ (ml) \times (mg)}{V_w\ (L)} \qquad (1)$$

Where:
C: concentrations used in the main experiment
V_k: volume of KEMDrill used (in mL); 1 mL = 1000 mg
V_w: volume of water used to make up the test solution
This gave concentrations of 167 mg/L, 333 mg/L, 400 mg/L and 500 mg/L used in the experiment.

2.3 Experimental Set Up

The main experiment was divided into one control and four treatment groups comprising 167 mg/L, 333 mg/L, 400 mg/L and 500 mg/L concentrations with each group having four (4) replicates. A total of twenty (20) circular plastic aquaria were used with each aquarium containing 30 L of tap water and one (1) fish. A completely randomized static renewal bioassay experimental design was adopted which lasted for 21 days. Throughout the experimental period, the tap water and test chemical were renewed daily at 11:00 hours. The holding plastic aquaria were properly aerated and regularly cleaned with a piece of foam while maintaining equal light and dark condition. Fish were also fed with compounded fish feeds, 5% body weight daily at 11:00 hours during the period of the sublethal experiment. This process was discontinued twenty-four (24) hours prior to termination of the experiment.

2.4 Termination of Experiment and Collection of Blood Samples

At the expiration of the 21-day exposure period, the experimental fish were immobilized using a hand towel to cover two-thirds of the body from the head and then anaesthetized with a mild knock on the head using a ceramic pestle. A 10 mL hypothermic needle and syringe was used to collect blood samples from the dorsal aorta into EDTA bottles containing an anti-coagulant. The blood samples were gently mixed with anticoagulant to prevent blood clots before being analyzed for red blood cells (RBC), white blood cells (WBC), platelets, and haemoglobin levels as well as differential counts.

2.5 Statistical Analysis

The data obtained was subjected to one-way analysis of variance (ANOVA) to determine significant differences that exist between the measured parameters. Means and standard deviations were also calculated from the data derived from the analysis of the various experimental groups, as well as a Post Hoc Test (Turkey HSD test) to separate means between groups and determine their interrelatedness. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) statistical tool kit version 20.8.

3. Results

Table 1 shows the levels of haematological indices (RBC, WBC, Platelets and Hb) in *Heterobranchus longifilis* exposed to KEMDrill for 21 days, while Table 2 presents the values of differential counts (lymphocytes, eosinophil, monocytes and neutrophil) measured in *Heterobranchus longifilis* exposed to KEMDrill for 21 days. From the results obtained, there was a significant decline in all the haematological parameters measured relative to the control. Specifically, platelet and white blood cells recorded significant decline ($p<0.05$) at 500 mg/L, compared to the control while RBC and haemoglobin levels slightly dropped across all concentrations. On the other hand, values of differential counts including neutrophil, eosinophil and monocytes recorded in this study did not show any significant difference compared to the control. However, lymphocytes recorded significant ($p<0.05$) decline to 7.98±0.20 at 500 mg/L compared to the control (11.64±0.21).

Table 1: Levels of haematological indices in *H. longifilis* exposed to KEMDrill for 21 days

Concentration of KEMDrill (mg/L)	Red Blood Cells (g/dL) ($x10^{12}$)	White Blood Cells (g/dL) ($x10^{9}$)	Platelets (g/dL) ($x10^{9}$)	Haemoglobin (g/dL)
Control	2.25 ± 0.01^{a}	203.00 ± 2.45^{bc}	53.00 ± 0.08^{a}	9.40 ± 0.03^{a}
167	2.15 ± 0.01^{a}	205.65 ± 2.31^{b}	21.50 ± 0.16^{d}	9.20 ± 0.04^{a}
333	2.30 ± 0.02^{a}	211.30 ± 4.10^{a}	31.50 ± 1.41^{c}	9.30 ± 0.03^{a}
400	2.49 ± 0.05^{a}	201.34 ± 2.09^{c}	33.20 ± 1.33^{b}	8.92 ± 0.02^{b}
500	1.60 ± 0.10^{b}	179.35 ± 3.15^{d}	31.00 ± 2.00^{c}	8.80 ± 0.01^{b}

All data are expressed as Mean ± Standard Deviation. Different superscripts indicate a significant variation ($p<0.05$).

Table 2: Differential Counts in *Heterobranchus longifilis* exposed to KEMDrill for 21 days

Concentration of KEMDrill (mg/L)	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocytes (%)
Control	4.09±0.41^{a}	11.64±0.21^{a}	1.15±0.05^{a}	1.00±0.20^{a}
167	4.10±0.97^{a}	10.53±0.32^{b}	1.12±0.13^{a}	1.00±0.20^{a}
333	4.51±0.21^{a}	8.34±0.11^{c}	1.14±0.10^{a}	1.00±0.10^{a}
400	4.69±0.32^{a}	7.84±0.17^{d}	1.17±0.22^{a}	1.00±0.19^{a}
500	4.85±0.23^{a}	7.98±0.20^{d}	1.21±0.50^{a}	1.00±0.10^{a}

All data are expressed as Mean ± Standard Deviation. Different superscript indicated a significant variation ($p<0.05$).

4. Discussion

Haematological parameters are usually associated with health status, and they represent significant diagnostic tools in routine clinical evaluation of the state of health of fish and other animals. In this study, there were fluctuations in the values of haematological parameters of fish exposed to varying concentrations of KEMDrill. This result is in line with the report of a researcher who also recorded decline in the values of some haematological parameters after exposing fish to xenobiotics [12].

The depreciation in the values of various blood parameters recorded in this study were observed to be non-dose dependent and may have interfered with the processes of neural transmission, blockage of ionic channels as well as inducing histopathological activities in fish as reported in previous research [13]. This effect could interfere with proper functioning of enzyme activities, which may lead to hormonal changes or imbalances as well as inhibition effects as reported in previous studies [14].

Knowing that haemoglobin is responsible for carrying oxygen in the blood, any change in the level of haemoglobin may either directly or indirectly affect the ability of fish to carry oxygen and vice-versa. For example, decrease in haemoglobin has been reported after fish was exposed to toxicants [15]. Similar report has also been published on the lowering of red blood cells in *Sarotherodon mossambicus* following exposure to Diuron [16]. The decline in haematological parameters recorded in experimental fish may also infer that the primitive stem cells responsible for blood production have been hampered due to the toxic effect of the toxicant. An impairment of blood production in probe fish could result in anaemia (low red blood cells) or leucopenia (low white blood cell) in exposed fish [17]. Previous studies also showed that reduction in haematological parameters in exposed organisms is a direct influence of xenobiotic toxicity to some receptor enzymes in exposed fish [18]. Hence, these changes may adversely impact the normal blood cell physiology of the fish.

Thrombocytes (platelets) are nucleated cells responsible for blood clotting in fish and other animals. Therefore, the declining trend of platelets as presented in this study possibly signify the effect of the toxicant on platelets formation by the primitive stem cells responsible for blood production. In line with previous reports, the observed reduction in the values of platelets in the experimental fish indicates haemophilia (continuous bleeding), which could result to death due to reduced clotting factors caused by the toxic effect of xenobiotic on thrombocytes [19].

The reduction in the levels of WBC recorded in this study is in sharp contrast with widely accepted belief that WBC fight against foreign bodies. It is thus expected that its values would have increased as a result of the introduction of toxicant (antigen) or foreign body in the fish. The exposure of fish to relatively low concentrations of toxicant (167 mg/L and 333 mg/L) resulted in a slight increase in WBC number, which shows the defensive response of fish to the presence of the toxicant antigen (foreign body). However, prolonged exposure of fish to KEMDrill at higher concentration (500 mg/L) revealed the toxic potential of the toxicant on the immune system of fish.

Thus, the initial appreciation in the levels of WBC following exposure of fish to KEMDrill may be an indication of host immune system to fight against foreign invasion when the need arises. However, subsequent decline in WBC count clearly shows that KEMDrill is toxic to fish. Hence, exposure of fish to the toxicant may have caused a decline in the established immune response against a variety of antigenic substances, thus capable of causing immune depression. This finding agrees with previous reports that confirmed depreciation in WBC following exposure of fish to xenobiotics [11, 20]. The alterations in leukocyte values may manifest in the form of leukocytosis (heterophilia and lymphopenia), which are characteristics of leukocytic response in fish exhibiting stress [20].

Red Blood Cells (RBC) are responsible for all transportation and circulation of nutrients in the body. Thus, they are essential parameters in fish and suitable for evaluating the effects of chemicals [21]. The recorded decline in the values of RBC of experimental fish suggests an osmotic disturbance and alteration in oxygen carrying capacity of fish exposed to KEMDrill. Previous studies also recorded reduction in the values of some measured blood parameters including RBC, haemoglobin Hb and haematocrit in experimental fish exposed to Diazinon, an organophosphate pesticide [11, 22]. The researchers attributed the declining values of RBC in probe fishes to the destructive effects of toxicants. Therefore, the decline in RBC due to increased concentration of KEMDrill as observed in this study could be attributed to the toxic effect of the toxicant on haematopoietic stem cells (HSC) responsible for blood production. This effect could lead to reduced supply of RBC, either due to less production, increased rate of removal from circulation or rapid destruction due to effect of toxicants. This may equally affect the respiratory potential of the fishes. Significant reduction in RBC of exposed fishes could further result in hypochromic microcytic anaemia, which is in line with previous studies that reported toxic effect of glyphosate-based herbicides on haematological parameters of freshwater fish [15, 19, 23].

Haemoglobin is the chemical molecule responsible for the red pigmentation of blood. Its function is vital as it is responsible for carrying oxygen in the blood. The significant ($p<0.05$) fall in haemoglobin values in the blood of experimental fish may be attributed to the toxic effect of KEMDrill on the synthesis of haemoglobin. The result thus suggests a possible dysfunction of enzyme involved in the synthetic pathway of haemoglobin of experimental fish. In clinical examinations, reduction in haemoglobin level is an indication of low oxygen concentration which may result in anaemia, suffocation and possibly cardiac arrest. In a similar study, it was reported that prolonged low level of haemoglobin is deleterious to oxygen transport and electrolytes degeneration in erythrocytes of experimental animals [24].

Differential count comprises the immunological parameters of white blood cells. Indices such as neutrophil and eosinophil are granulocytes, also known as polymorphonuclear leucocytes [25]. The stability in the value of monocytes recorded in this study suggests that KEMDrill is less toxic to some differential count indices of exposed fish, which is in line with the report of a previous author [26]. The slight increase in eosinophil and neutrophil levels in the treatment groups compared to the control is an indication that the immunological or defensive agents in experimental fish reacted towards invasion of the toxicant, a foreign body.

The recorded decline in lymphocyte levels across the treatment groups compared to the control may be attributed to the pressure of toxicant, thereby breaking its defensive ability [26]. Hence, lymphocytes become overpowered by the high concentration of toxicant causing its reduction in value. The drop in lymphocyte value in this study is possibly due to the toxic effect of KEMDrill on the immune system of *Heterobranchus longifilis* [26].

5. Conclusion

The results of exposure of sublethal concentrations of KEMDrill, a water based drilling fluid to juveniles of *Heterobranchus longifilis* in this study reveals that the drilling fluid (KEMDrill) is toxic to haematological indices of fish at higher concentrations. Therefore, the water based drilling fluids should be used cautiously and in a safe manner around rivers, oceans, lakes, swamps and even flood prone environments to avoid contamination and pollution of the aquatic food chain.

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Ethical Issues
The authors are all aware of ethical issues as stipulated by the laws of the land and thus completely complied with best practices while carrying out this research.

Conflict of Interest
The authors declare that there is no conflict of interest in this work.

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