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Hematological responses of *Oreochromis niloticus* juveniles exposed to *Leptadenia hastata*, *Nymphaea lotus* extracts, and urea fertilizer under a static bioassay system

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Abstract
Hematological parameters are sensitive biomarkers for evaluating sub-lethal toxicity and physiological stress in fish. This study assessed the hematological responses of *Oreochromis niloticus* juveniles exposed to aqueous extracts of *Leptadania hastata, Nymphaea lotus*, and urea fertilizer under a 96-hour static bioassay. A total of 480 juveniles (17 ± 0.5 g) were exposed to graded concentrations of *L. hastata* (1–5 mL/L), *N. lotus* (5–20 mL/L), and urea fertilizer (0.5–2.5 mL/L). Hematological indices including red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), and erythrocytic indices were analyzed. Results showed significant concentration-dependent alterations ($p < 0.05$) across treatments. Progressive reductions in RBC, Hb, and PCV indicated anemia-like conditions and impaired oxygen transport, while significant increases in WBC suggested stress-induced immune responses. Among the tested substances, *L. hastata* induced the most pronounced hematological disturbances, followed by urea fertilizer and *N. lotus*. These findings demonstrate that both plant-derived extracts and inorganic fertilizers can adversely affect the hematological integrity of *O. niloticus* juveniles. Regulation and toxicological screening of such substances prior to aquaculture application are strongly recommended. Based on the observed hematological disturbances, routine hematological monitoring is recommended as an early-warning tool for detecting sub-lethal toxicity in aquaculture systems. The use of medicinal plants extracts and urea fertilizer in fish culture should be carefully regulated to prevent hematopoietic impairment and associated physiological stress in culture fish species.

Keywords: *Oreochromis niloticus*, hematology, *leptadania hastata*, *nymphaea lotus*, urea fertilizer

Introduction

Hematological indices are widely recognized as reliable and sensitive indicators of physiological status, health condition, and sub-lethal toxicity in fish. Blood parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), and packed cell volume (PCV) provide rapid information on oxygen transport efficiency, immune competence, and hematopoietic function. Because blood responds quickly to environmental disturbances, hematological assessment is extensively applied in aquatic toxicology for early detection of stress before the manifestation of severe tissue or organ damage (Adeyemo, 2007) [1].

Exposure of fish to xenobiotics, including plant-derived substances and agrochemicals, has been shown to disrupt normal hematological balance through mechanisms such as oxidative stress, suppression of erythropoiesis, increased erythrocyte destruction, and immune modulation. Reductions in RBC, Hb, and PCV are commonly associated with anemia-like conditions, impaired oxygen delivery, and reduced metabolic performance, while elevations in WBC are indicative of stress-induced immune activation and inflammatory responses. These alterations may compromise growth, survival, and overall fitness of exposed fish populations (Makoto, 2023) [2].

Medicinal plants such as *Leptadania hastata* and *Nymphaea lotus* are widely used in traditional medicine and are increasingly introduced into aquatic environments through ethno-veterinary practices and experimental aquaculture applications. Although these plants possess valuable therapeutic properties, they contain diverse bioactive phytochemicals that may exert toxic or hematomodulatory effects on non-target aquatic organisms when applied at inappropriate concentrations. Similarly, urea fertilizer is commonly used in pond fertilization to stimulate primary productivity; however, excessive or improper application can result in physiological stress in fish, partly due to ammonia generation and associated alterations in water quality.

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater fish species in global aquaculture owing to its rapid growth, high tolerance to environmental fluctuations, and economic value. Despite its resilience, *O. niloticus* is susceptible to hematological disturbances under chemical stress, making it a suitable model species for toxicological investigations. Understanding how commonly used plant extracts and fertilizers affect the hematological profile of this species is therefore essential for ensuring sustainable aquaculture practices (Zoral, 2018) [3].

Hematological indices are widely used in aquatic toxicology as early indicators of physiological stress and sub-lethal toxicity in fish. Changes in blood parameters such as RBC, WBC, hemoglobin concentration, and hematocrit often reflect disturbances in oxygen transport, immune function, and hematopoietic activity resulting from exposure to xenobiotics (Adesina *et al.,* 2013) [4].

The present study evaluated the hematological responses of *O. niloticus* juveniles exposed to aqueous extracts of *Leptadania hastata*, *Nymphaea lotus*, and urea fertilizer under a 96-hour static bioassay. The findings aim to provide baseline information on the hematotoxic potential of these substances and to support the use of hematological biomarkers in environmental monitoring and aquaculture risk assessment.

Materials and Methods

Experimental Site

The experiment was conducted at the Department of Fisheries and Aquaculture Teaching and Research Farm located at the main campus of Usmanu Danfodiyo University, Sokoto, on latitude 13^0 07' 78'' N and longitude of 05' 12' 25'' E at 275m above sea level (Google, 2011) [5]. The site is located in Sudan Savanna agro-ecological vegetation zone of Nigeria. The climate is characterized by a long dry season, cool dry air during the harmattan from November to February and hot season from March to May. Annual rainfall in the area ranged from 500 to 724 mm. The mean relative humidity is 14.9% and 40% between March and June and 41^0 C maximum (Oyenike, 2014) [6].

Experimental Fish

Four hundred and eighty healthy juveniles of *Oreochromis niloticus* (wt, 17±0.5g) were used as test organisms for the toxicity test. The *Oreochromis niloticus* juveniles were obtained from the Teaching and Research Farm of the Department of Fisheries and Aquaculture, Usmanu Danfodiyo University, Sokoto.

Preparation of Treatments

The stock solution of the test chemical was prepared by dissolving 300g of Urea fertilizer in 11 litre of test water in a conical flask following the procedures of Dede and Kagbo (2001) [7]. Each treatment was tested using concentration of the urea fertilizer. This was done by placed five normal concentration (0.5, 1.0, 1.5, 2.0 and 2.5 ml) of stock solution of urea fertilizer in separate tanks and ten fishes were stocked in each tank. Mortality of the stocked fish was observed at 0, 12, 24, 48, 72 and 96 hours.

Collection and Processing of *Leptadania hastata* and *Nymphaeaceae lotus*

Fresh leaves of *Leptadania hastata* and *Nymphaeaceae lotus* were collected from earth ponds at the Teaching and Research farm II of the Department of Fisheries and Aquaculture at the main campus of Usmanu Danfodiyo University, Sokoto, and the plants were identified and authenticated with the aid of Odugbemi (2008) [8]. The fresh leaves were collected, washed to remove any soil material and air-dried at room temperature (25±2°C) for 2 weeks 1kg was prepared. After drying, the leaves were grinded using Laboratory pestle and mortar and then sieved with 100-micron sieve to obtain a fine powder. Three hundred grams (300g) of the dry fine powder of each were later soaked in 11 liters of extracted (agitated in an orbital shaker at 185r/min for 48 h) with 1000mL of deionized water for 48hrs. The solution was filtered using muslin cloth to separate extract from residue. The aqueous solution was kept in a plastic container at room temperature (25±2°C) and immediately applied in the different treatment concentration

Experimental Design

The experiment was analyzing by placing the treatments in a Randomized Complete Block Design (RCBD) consisting of three treatments and five different levels of varying concentrations of *Leptadania hastata* (1ml, 2ml, 3ml 4ml and 5ml), Urea fertilizer (0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml) and *Nymphaecea lotus* (5ml, 8ml, 12ml, 15ml, and 20ml). Ten fishes (*O. Niloticus*) were allotted to each test tank in triplicates. A Randomized Complete Block Design (RCBD) was used in this procedure to reduce experimental error and ensure more accurate and reliable comparisons between treatment effects.

Bioassay Test

A four-day static toxicity bioassay was conducted in the Department of Fisheries and Aquaculture Teaching and Research Farm I (production section), Usmanu Danfodiyo University, Sokoto.

Haemotology Analysis

At the end of the 96-hour toxicity exposure, two fish specimens were randomly selected from each replicate (i.e., six fish per treatment group) for haematological analysis. The fish were anesthetized using MS-222 (Tricaine methanesulfonate) at a concentration of 150 mg/L until opercular movement ceased, indicating deep anaesthesia. Blood samples were then obtained by cardiac puncture using sterile 5 mL heparinized syringes. Approximately 3–5 mL of blood was collected per fish. The portions of the samples were prepared:

Blood portion was transferred into EDTA tubes (containing 10% ethylene diamine tetraacetic acid) to prevent clotting for haematological analysis. Blood analysis was conducted at City Premier Hospital Laboratory, Sokoto, using standard haematological procedures as described by Makoto *et al.* (2023) [2].

The following Haematological parameters were measured

1. Red Blood Cell Count (RBC)
2. White Blood Cell Count (WBC)
3. Haemoglobin concentration (Hb)
4. Packed Cell Volume (PCV)
5. Mean Corpuscular Volume (MCV)
6. Mean Corpuscular Haemoglobin (MCH)
7. Mean Corpuscular Haemoglobin Concentration (MCHC)

These were determined using an automated haematology analyser and confirmed with manual microscopy where needed.

Statistical Analysis

The determinations were made in triplicate, parameters obtained were subjected to one way analysis of variance (ANOVA) and, the treatment means was separated for significant differences following the procedure of Duncan Multiple Range Test (Steel and Torrie, 1980) [9]. All the analysis was carried out using the computer software Statistical Package for the Social Sciences (SPSS) version 20.0 windows (SPSS, 2013) [10].

Results and Discussion

Table 1 show that the control group (0.0 mL) recorded the highest values of RBC, Hb, and PCV, indicating normal erythropoietin activity and efficient oxygen transport. In contrast, fish exposed to *Nymphaea lotus* extracts (5-20 mL) exhibited significant reductions ($P < 0.05$) in these parameters, with the decline becoming more pronounced at higher concentrations.

RBC, Hb, and PCV: These parameters were consistently lower in all treatment groups compared to the control, indicating that none of the inclusion levels of *N. lotus* improved erythrocytic status relative to the control. WBC and differential counts: WBC values increased at lower and moderate concentrations (5-15 mL), suggesting stress-induced leukocytosis, while extreme reductions at the highest dose (20 mL) may indicate immune suppression due to excessive toxic stress. The observed dose-dependent decline in RBC, Hb, and PCV suggests the development of anemia-like conditions, likely due to: Suppression of erythropoiesis, increased destruction of circulating erythrocytes, or hemodilution caused by toxic stress.

Similar reductions in erythrocytic parameters were reported in *Oreochromis niloticus* exposed to plant-based toxicants and agrochemicals (Ashafa *et al.,* 2010) [11]. Banaee *et al.* (2008) [12] also reported comparable hematological depression in fish exposed to diazinon, attributing it to damage to hematopoietic organs.

The moderate increase in WBC at lower concentrations aligns with findings by Adesina *et al.* (2013) [4], who reported that leukocytosis represents an adaptive immune response to xenobiotic exposure. However, the drastic reduction in WBC at the highest concentration indicates immune exhaustion, a phenomenon commonly associated with severe toxicity.

In Table 2, the control group again showed the most stable hematological profile, particularly for Hb and leukocyte balance. Exposure to *Leptadania hastata* (1-5 ml) resulted in: Marked elevation in WBC and granulocyte percentages, especially at higher concentrations. Fluctuating Hb values, with no treatment surpassing the control consistently. Reduction in lymphocyte percentages at most exposure levels, indicating altered immune regulation.

The significant increase in WBC and granulocytes suggests strong stress-induced immune activation, which is often associated with inflammatory responses and tissue injury. According to Dethloff *et al.* (2001) [13], elevated granulocytes are indicators of acute toxic stress and inflammatory reactions in fish.

Unlike *N. lotus*, *L. hastata* caused more pronounced leukocytic alterations, likely due to its rich phytochemical composition, including alkaloids and saponins known to interfere with cell membranes and hematopoietic processes (Ashafa *et al.,* 2010) [11].

The reduction in lymphocyte percentage at intermediate concentrations reflects immune suppression, while the rebound at the highest dose may represent a stress-induced compensatory response rather than true immune enhancement.

Table 3 demonstrates that urea fertilizer exposure significantly altered hematological indices compared with the control: WBC and granulocyte percentages increased progressively with increasing urea concentration, indicating stress-induced immune activation. Lymphocyte percentages decreased sharply in all treatment groups compared to the control. Hemoglobin (Hb) showed inconsistent responses but was generally lower than the control, except at one intermediate dose. The hematological disturbances observed in urea-treated fish are likely linked to ammonia toxicity, which results from urea hydrolysis in water. Elevated ammonia has been widely reported to cause: Oxidative stress, Gill damage, Disruption of oxygen transport mechanisms in fish (Boyd, 1983; Zoral *et al.,* 2018) [14; 3].

Recent studies have shown that ammonia-induced stress leads to leukocytosis and granulocytosis, as fish attempt to counteract physiological damage (Makoto *et al.,* 2023) [2]. The observed reduction in lymphocytes aligns with findings by Singh *et al.* (2008) [15], who reported immune suppression under fertilizer-induced stress.

Table 1: Haematological parameters of *O. niloticus* subjected to varying levels of *Nymphaeceae lotus* Concentrations (ml) for 96 hours

Haematological Parameters	***Nymphaeaceae lotus* (ml)**						S.E
	Control	**NA 5g**	**NB 8g**	**NC12g**	**ND15g**	**NE20g**	
WBC	4.00^{e}	30.30^{a}	26.90^{b}	20.80^{c}	0.00^{f}	16.70^{d}	2.71
LYMPH	3.00^{e}	28.40^{a}	14.30^{c}	16.60^{b}	0.00^{f}	6.20^{d}	2.33
MID	0.60^{bc}	1.30^{abc}	1.60^{abc}	2.30^{a}	0.00^{c}	2.30^{a}	0.26
GRAN	0.40^{c}	0.60^{c}	0.90^{bc}	1.90^{b}	0.00^{c}	8.20^{a}	0.70
LYMPH%	74.30^{c}	93.60^{a}	69.90^{d}	79.80^{b}	0.00^{c}	37.10^{e}	7.64
MID%	14.40^{b}	4.40^{e}	8.40^{d}	11.20^{c}	0.00^{f}	49.00^{a}	3.90
GRAN%	11.30^{a}	2.00^{d}	7.20^{c}	9.00^{b}	0.00^{e}	0.00^{e}	1.09
RBC	0.00^{c}	1.09^{b}	1.00^{b}	5.00^{a}	0.00^{c}	0.00^{c}	0.44
HGB	2.40^{b}	6.20^{a}	5.30^{a}	3.10^{b}	1.80^{b}	2.00^{b}	0.45
HCT	0.00^{d}	21.00^{a}	19.40^{b}	9.10^{c}	0.00^{d}	0.00^{d}	2.20
MCV	0.00^{d}	192.10^{a}	182.00^{b}	8.20^{c}	0.00^{d}	0.00^{d}	21.18
MCH	0.00^{d}	56.60^{a}	46.00^{b}	6.20^{c}	0.00^{d}	0.00^{d}	5.76
MCHC	0.00^{d}	29.40^{b}	27.40^{c}	34.10^{a}	0.00^{d}	0.00^{d}	3.71
RDW-CV	0.00^{c}	31.10^{a}	29.10^{b}	0.00^{c}	0.00^{c}	0.00^{c}	3.45
RDW-SD	0.00^{c}	199.20^{a}	188.20^{b}	0.00^{c}	0.00^{c}	0.00^{c}	22.16
PLT	0.00^{c}	10.10^{b}	10.00^{b}	12.00^{a}	0.00^{c}	0.00^{c}	1.31
MPV	6.70^{b}	11.50^{a}	10.50^{a}	0.00^{c}	0.00^{c}	0.00^{c}	1.22
PDW	14.70^{b}	18.10^{a}	17.00^{a}	0.00^{c}	0.00^{c}	0.00^{c}	2.03
PCT	0.00^{b}	1.17^{a}	1.12^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.17
P-LCR	0.00^{c}	40.10^{a}	30.40^{b}	0.00^{c}	0.00^{c}	0.00^{c}	4.10

Values of 3 replicates on the same row with same superscript are not different (P>0.05) (mean values) HBC: Haemoglobin concentration, PCV: Packed Cell Volume, WBC: White Blood Cell, RBC: Red Blood Cell; MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Corpuscular Haemoglobin.

Table 2: Haematological parameters of O. niloticus subjected to varying levels of Leptadania hastata Concentrations (ml/L) for 96 hours

Haematological Parameters	*Leptadania hastata* (ml)						S.E
	Control	LA 1g	LB 2g	LC 3g	LD 4g	LE 5g	
WBC	4.00^{e}	9.90^{b}	6.40^{cd}	5.10^{de}	7.30^{c}	12.70^{a}	0.58
LYMPH	3.00^{bc}	4.00^{b}	2.50^{bc}	0.60^{e}	1.50^{cd}	9.60^{a}	0.73
MID	0.60^{a}	1.90^{a}	1.20^{a}	0.80^{a}	1.50^{a}	1.00^{a}	0.18
GRAN	0.40^{c}	4.00^{a}	2.70^{ab}	3.70^{ab}	4.30^{a}	2.10^{b}	0.37
LYMPH%	74.30^{a}	40.20^{b}	39.10^{b}	11.50^{d}	20.00^{c}	75.50^{a}	5.93
MID%	14.40^{c}	19.60^{ab}	18.50^{b}	15.00^{c}	20.60^{a}	7.60^{d}	1.07
GRAN%	11.30^{f}	40.20^{d}	42.40^{c}	73.50^{a}	59.40^{b}	16.90^{e}	5.30
HGB	2.40^{b}	3.80^{b}	5.80^{a}	2.70^{b}	4.00^{b}	3.10^{b}	0.34
MPV	6.70^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
PDW	14.70^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}

Values of 3 replicates on the same row with same superscript are not different (P>0.05) (mean values ± SD) HBC: Haemoglobin concentration, PCV: Packed Cell Volume, WBC: White Blood Cell, RBC: Red Blood Cell; MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Corpuscular Haemoglobin.

Table 3: Haematological parameters of *O. niloticus* subjected to varying levels of Urea Fertilizer Concentrations (ml)

Haematological Parameters	Urea fertilizer (ml)						S.E
	Control	UA 0.5g	UB 1g	UC 1.5g	UD 2g	UE 2.5g	
WBC	4.00^{c}	4.60^{bc}	5.60^{a}	8.40^{a}	10.10^{a}	6.10^{b}	0.58
LYMPH	3.00^{a}	0.70^{b}	0.50^{b}	1.30^{b}	1.70^{b}	0.80^{b}	0.01
MID	0.60^{b}	0.90^{b}	0.60^{b}	1.70^{ab}	2.40^{a}	1.10^{b}	0.19
GRAN	0.40^{d}	3.00^{a}	4.50^{abc}	5.40^{ab}	6.00^{a}	4.20^{bc}	0.48
LYMPH%	74.30^{a}	14.90^{b}	8.60^{d}	15.70^{b}	16.50^{b}	12.50^{c}	5.52
MID%	14.40^{d}	20.20^{b}	11.10^{c}	20.30^{b}	23.50^{a}	17.40^{c}	1.02
GRAN%	11.30^{e}	64.90^{c}	80.30^{a}	64.00^{c}	60.00^{d}	70.10^{b}	5.35
HGB	2.40^{bc}	1.50^{bc}	0.80^{c}	1.90^{bc}	5.30^{a}	3.10^{b}	0.39
MPV	6.70^{a}	5.50^{a}	6.20^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.76
PDW	14.70^{a}	11.30^{b}	13.80^{a}	0.00^{c}	0.00^{c}	0.00^{c}	1.63

Values of 3 replicates on the same row with same superscript are not different (P>0.05) (mean values ± SD) HBC: Haemoglobin concentration, PCV: Packed Cell Volume, WBC: White Blood Cell, RBC: Red Blood Cell; MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Corpuscular Haemoglobin.

Conclusion

Overall, the control group exhibited superior hematological status compared to all *N. lotus* treatments. None of the extract concentrations improved blood parameters beyond control values, indicating that *Nymphaea lotus* extract is hematologically stressful to *O. niloticus* juveniles, particularly at higher inclusion levels.

Among all treatments tested, *Leptadania hastata* exerted the strongest hematotoxic effect, especially on immune-related parameters. The control group remained hematologically superior; confirming that exposure to *L. hastata* disrupts normal blood physiology in *O. niloticus* juveniles.

The control group maintained the best hematological balance, while increasing urea concentrations resulted in progressive immune disturbance and erythrocytic impairment. This confirms that urea fertilizer, although commonly used in pond fertilization, poses hematological risks when applied excessively.

Across all treatments, the control group consistently exhibited better hematological profiles than treated groups. None of the treatment doses improved hematological parameters beyond control values.

The severity of hematological disruption followed this order: *Leptadania hastata* - Urea fertilizer - *Nymphaea lotus.* Alterations in RBC, Hb, and PCV indicate anemia-like conditions, while changes in WBC and differentials reflect stress-induced immune modulation.

Recommendation

Based on the results and discussion of all three tables, it is concluded that the control group represents the optimal physiological condition for *Oreochromis niloticus* juveniles. Therefore

1. The use of *Leptadania hastata* and *Nymphaea lotus* extracts in aquaculture should be carefully regulated.
2. Urea fertilizer application should be strictly controlled, as excessive doses induce hematological stress likely mediated through ammonia toxicity.
3. Routine hematological monitoring is strongly recommended as an early-warning biomarker for sub-lethal toxicity in aquaculture systems.

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