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Effect of Castor (*Ricinus communis*) Seed Oilon the Haematological Parameters of Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

Purpose: *Nile tilapia is known to have a high prolific rate of reproduction which impacts the native aquatic bio-diversity. This phenomena and various methods of curtailing have been well studied in few selected places around the world. Hence, this research aims to evaluate the effect of castor oil on haematological parameters of O. niloticus, at sub-lethal doses.*

Research Method: *Sixteen samples of ready to spawn O. niloticus weighing 6.5-8.5g were used for the experiment and castor oil was extracted from the seeds through Soxhlet extraction. Three different doses of castor oil (R. communis) were administered to determine the level of effect of castor oil on the blood parameters.*

Findings: *Packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count, total white blood cell (TWBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using standard methods. MCV, MCH, MCHC, and WBC did not vary significantly (P>0.05). PCV were all within the normal range (40.33-21.00%). Hb values were low and ranged from 6.97 – 13.43 g/dl, and TWBC ranged from 3.90 – 6.70 1012/l.*

Value: *This research revealed castor oil to significantly alter some parameters of O. niloticus and has inverse relationship between WBC and RBC counts of O. niloticus.*

Keywords: *Castor oil, Haematological parameters, Oreochromis niloticus, Ricinus communis*

INTRODUCTION

The castor plant (*Ricinus cummunis*), a plant of the family Euphorbiaceae is the source of castor oil which has a wide variety of uses and contains a poison called ricin which is present in low concentration throughout the plant. The plant is cultivated chiefly for oil and the dried seeds contain approximately 50% oil (Abitogun, 2009; Patel *et al*., 2016). Castor oil has long been used commercially as a highly renewable resource for the chemical industry (Mutlu and Meier, 2010). Castor oil is known to consist of up to 90% ricinoleic, 4% linoleic, 3% oleic, 1% stearic, and less than 1% linolenic fatty acids (Patel *et al*., 2016). The high content of ricinoleic acid (RA) in castor oil has made it valuable, and this oil has a variety of applications in the chemical industry (Dunford, 2012). Reported data show that the median lethal oral dose (LD50) of ricin poisoning in mice is 30ppm of body weight, whereas the lethal oral dose in humans has been estimated to be 1-20 ppm of body weight (He *et al*., 2010). This is approximately the ricin content in 4-8 castor seeds. Castor oil and its derivatives have either served as fuel and biodiesel; polymer materials; soaps, waxes and greases; lubricants, hydraulic, and brake fluids; fertilizers; coatings or; pharmacological and medicinal use (Patel *et*

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al., 2016).It is reported that the presence of *Ricin communis* agglutinin in castor plant has affinity for red blood cells leading to agglutination and subsequently hemolysis. Hematological parameters such as red blood cell count, white blood cell count, Hb concentration etc, are being used for the monitoring of health status of individuals (Clauss *et al*., 2008). Environmental conditions (Arnold *et al*., 2013), nutritional conditions (Honorato *et al*., 2010; Valente *et al*., 2013), animal handling and stress conditions (Sado *et al*., 2013) have been reported to have effect on the hematological characteristics.

There are many factors affecting the hematological parameter and for leukocyte indices can be altered due to the sex, temperature, season, stress, stage of reproductive cycle, and age (Roberts *et al.*, 2013). Haematology can therefore, be considered a useful tool for monitoring health status and stress conditions that can compromise metabolism and growth of fish. There have been several reports on the potentiality of haematological values as indices of diagnosing diseases (Jimoh *et al*., 2014), stress induced condition and also for feed assessment (Akintayo *et al*., 2008; Yue and Zhou, 2008).

Oreochromis niloticus commonly known as Nile Tilapia belongs to the family Cichlidae. It is an omnivorous feeder that feeds on phytoplankton, periphyton, aquatic plants, invertebrates, macro benthic fauna, detritus and other fishes and fish eggs. Nile tilapia is known to have a high prolific rate of reproduction (Suresh and Bhujel, 2012). This research seeks to probe the possible means of controlling the biodiversity of Nile tilapia, thus the use of materials that may have adverse effect on the blood composition of the fish. The impacts of *O. niloticus* on native aquatic bio-diversity has been well studied in few selected places around the world. This research therefore, aims to evaluate the effect of castor oil on haematological parameters of *O. niloticus*, at sub-lethal doses.

MATERIALS AND METHODS

Castor Seed Collection

Dried seeds were bought from Sabo Market and identified in the Herbarium Unit of Department of Botany, Ahmadu Bello University, Zaria. The castor beans were sorted by hand picking and mortar and pestle were used to ground the castor beans into a paste (cake) in order to weaken and rupture the cell walls to release castor fat for extraction (Akpan *et al*., 2006).

Extraction of Castor Oil

Castor oil was extracted by methods described by Akpan *et al.,* (2006). The extract was dried in the oven at 40ºC for 8 hours (Obaroh and Nzeh, 2013), cooled in the desiccator (to reduce the water produced by the steam from the oven dried extract) and weighed again to determine the amount of oil extracted. Further extraction was carried out at 30 minutes interval until the sample weight at further extraction and previous weight became equal. The experiment was repeated by placing 5g of the sample into the thimble again. The weight of oil extracted was determined at each 30 minutes interval. At the end of the extraction, the resulting mixture *(miscella*) containing the oil was heated to separate solvent from the oil.

Determination of Non-lethal Concentration

In a quest to determine the toxicity of *R. communis* seed extract on female Nile tilapia and to select the most suitable concentration and doses to be used for further study, three (3) groups of three (3) female Nile tilapia each were orally gavaged, following Collymore *et al.,* (2013) procedure, with 5000, 2000 and 1000 mg/kg body weight respectively, of 500 mg/mL concentration of oily extract-water solution. The fish were observed for 72 hours for any sign of toxicity and death due to the oily extract, as every other factor was kept at optimum level. At the end of the study, the lethality status and appropriate doses to be used for further studies

were determined. Thirty percent (30%) of the highest non-lethal dose was determined and used as the upper limit of administered doses (OECD, 2018).

Results: A 39.2% oil yield was obtained from 250g intact seeds of *R. communis*. The extract at doses of 5000, 2000 and 1000 mg/kg body weight impacted the sclerae of the eyes of the female fish, as they reddened at varying degrees in a dose-dependent manner, thus, the reddish appearance of the sclerae reduced with reduction in dose. Reduced operculum count and rate and general activity were also observed in the female fish, but mortality was not recorded, even at the highest dose of 5000 mg/1kg body weight.

Experimental Design

A total of 16 sexually mature fish; 4 male and 12 female *O. niloticus* between the ages of 4-5 months weighing 6.5-8.5g were used for the experiment. Prior to this, a pilot study was carried out to determine the lethality status and appropriate doses for the experiment, 30% of the highest no-lethal dose was determined and used as the upper limit of administered doses (OECD, 2018). The experimental fishes were divided into four different groups including control, three female fishes were oral gavaged with 1500, 1250 and 1000mg/kg body weight of 500mg/mL concentration of oily extract water solution while the last group served as the control, so as to make comparison between the blood parameters of fish species in the castor oil treatment groups and the control group.

Blood Sample Collection

After the 5-day exposure period, blood samples were drawn by caudal puncture using 21-gauge hypodermic needle on the fishes, the blood samples were then stored in sample bottles containing ethylene di-amine tetra acetate [Heparin sodium 1%] which acted as anti-coagulant. The collected blood samples were subjected to haematological analysis.

Determination of Haematological Parameters

Haematocrit (PCV) was determined by the Wintrobe and Westergreen method as described by Svobodova *et al.,* (1991). Percentage Haemoglobin (Hb) concentration was determined as described by Mohmoh *et al.,* (2012) using Drabkin's solution and with the aid of a model XF-1C haemoglobinometer. The RBC count was determined using an improved Neubauer haemocytometer under ×40 objective and calculated (Dacie and Lewis, 2001). Total white blood cell count was determined as described using the standard two slide wedge technique to make blood films and the Giemsa's staining technique, counter stained with Leishmann's stain. Total leucocytes were calculated as formulated by Campbell (1995).

Erythrocyte indices which include Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated as follows:

MCV (Mean Corpuscular Volume) =

$$\frac{(\text{Hct})\ (\%)}{\text{RBC}\ (10^6/\text{mm}^3)} \times 10 \qquad (\text{fl})$$

MCH (Mean Corpuscular Hemoglobin) =

$$\frac{\text{Hb}\ (\text{g}/100\ \text{mL})}{\text{RBC}\ (10^6/\text{mm}^3)} \times 10 \qquad (\text{pg})$$

MCHC(Mean Corpuscular Hemoglobin Conc.)=

$$\frac{\text{Hb}\ (\text{g}/100\ \text{mL})}{\text{Hct}\ (\%)} \times 100 \qquad (\%)$$

During the experiment, observations were made on the significant increases in the RBCs, Hct, Hb, RBC indices (MCV, MCH, MCHC) and ESR among the three different treatment groups. Haematological parameters of fish species in the control group were compared with those of the groups containing varying doses of castor oil.

Data Analysis

Differences in the haematological parameters of the fish species in the control group and the treated group containing varying doses of castor oil were statistically analysed using One-way analysis of variance (ANOVA) and ranking

was done where significant difference exists, using Duncan's Multiple Range Test (DMRT). All data analyzed were performed using SPSS statistical software. P0.05 was considered statistically significant for all tests.

RESULTS AND DISCUSSION

The blood parameters of *O. niloticus* treated with varying concentrations of castor oil *R. communis* are presented on Table 01. Among the haematological parameters, MCV, MCH, MCHC and WBC did not vary significantly (P>0.05). The values of the blood parameters reflect physiological response of the fishes to the treatment. Specific variations within each group were observed in all haematological parameters. Haematocrit values vary significantly with decrease in the concentration of treatment. The decrease in the value of Hct from 33% to 21% is probably due to the presence of toxic substance in the castor oil such as ricin and ricinin which led to the malfunctioning of the haemopoietic system. Blaxhall and Daisley (1973) also reported the possibility of using haematocrit in the aquaculture and fishery management for checking anaemic condition in fishes. The experimental fish did not become anaemic on exposure since the least Hct value (21%) recorded was within the usual normal range of between 20-30% and rarely 50% reported by Clark and Walker (2001) for fish.

The RBC also decreased with increase in concentration of the oil and as such indicates a resultant decrease in metabolic demand; as elevated RBC counts are response to a higher metabolic demand and indicate oxygen requirement at a higher metabolic rate as purported by Mozos (2015). Barad and Kulkani (2010) opined that decrease in the RBC counts could be due to haemolysis and haemorrhage due to disturbance from toxic substances in the erythropoiesis.

Clark *et al.,* (2001) reported 12.7-14.0 (g/dL) to be the normal range of values for haemoglobin in fish. From the results, the decrease in the mean values of Hb with increase in the concentration of the treatment denotes the effect of toxic substance present in the castor oil on the Hb level of the fish. The toxicity might have led to a decrease in activity observed in the fishes; as Satheeshkumar *et al.,* (2011) reported that low value of Hb is associated with low active fishes.

White blood cells which are the defensive cells of the body were not affected significantly (p>0.05). This could probably be linked to the fact that the stressor was not pathogenic in nature. Elevated white blood cell counts would have implied immune response of fish to fight infection as opined by Douglass and Jane (2010). However, in addition to the toxin within the oil, physical processes involved in handling the fish during treatment and blood sample collection, may also be contributory to the observed differences in the physiological and chemical properties of some blood parameters.

Table 01: Haematological parameters of *O. niloticus* treated with castor oil *R. communis*

	Hct (%)	Hb (g/dl)	TRBC × 10^{12}/l	MCV (fl)	MCH (pg)	MCHC (%)	TWBC × 10^{9}/l
0 mg/l	40.33 ± 0.88^{a}	13.43 ± 0.30^{a}	6.70 ± 0.21^{a}	60.23 ± 0.55^{a}	20.06 ± 0.18^{a}	33.30 ± 0.03^{a}	12.30 ± 1.14^{a}
1,500 mg/l	21.00 ± 3.21^{c}	6.97 ±1.05^{c}	3.90 ± 0.67^{c}	54.66 ± 4.49^{a}	18.14 ± 1.49^{a}	33.19 ± 0.08^{a}	19.53 ± 1.90^{a}
1,250 mg/l	29.33 ± 0.88^{b}	9.73 ± 0.30^{b}	4.90 ± 0.15^{bc}	59.88 ± 0.94^{a}	19.87 ± 0.33^{a}	33.18 ± 0.04^{a}	15.50 ± 2.84^{a}
1,000 mg/l	33.00 ± 1.73^{b}	11.00 ± 0.58^{b}	5.73 ± 0.27^{ab}	57.56 ± 1.44^{a}	19.19 ± 0.48^{a}	33.33 ± 0.00^{a}	17.60 ± 2.99^{a}
P Value	0.001	0.001	0.005	0.394	0.385	0.094	0.234

Means are presented. Means with the same superscript along columns do not vary significantly (P>0.05).

***Note:** Hct = Haematocrit, TRBC = Total Red blood cells, Hb = Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Cell Haemoglobin and MCHC = Mean Cell Haemoglobin Concentration.*

CONCLUSIONS

This research revealed castor oil to significantly alter the Hct, Hb, and TRBC of *O. niloticus* and there is also inverse relationship between WBC and RBC counts of *O. niloticus*.

Data Availability Statement

The datasets generated and analysed during the current study are not publicly available. They are available from the corresponding author on reasonable request.

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