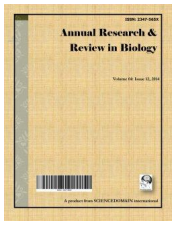

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The Effects of Raffia Palm Mesocarp on Haematological Parameters of *Clarias gariepinus*, a Common Niger Delta Wetland Fish

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Indigenous people of the Niger Delta region in Nigeria depend on fisheries as the main protein source. The mesocarp of raffia palm is used by the indigenous people to stupefy and catch fishes. The mechanism of action of the raffia palm mesocarp is not well understood. Hence, this study investigated the effect of raffia palm mesocarp on the haematological properties of African catfish. Thirty two juveniles of *C. gariepinus* (eight in each treatment) were exposed to graded sub-lethal doses (0.4 – 0.6 g/l) of *Raphia hookeri* fruit mesocarp in 4 plastic aquaria. Renewal of test media was done every 48 hours for 10 days. Results show that increasing concentration of raffia led to decreasing red blood cells, platelets and haemoglobin levels while increasing white blood cells, eosinophils and monocytes. Water quality analysis results corroborated the haematological analysis. Alkalinity and pH increased, while dissolved oxygen decreased as the concentration of raffia palm fruit mesocarp increases. We therefore conclude that the mode of action of raffia palm fruit mesocarp involved increased alkalinity and decreased oxygen tension in the water which temporarily stupefies fishes for easy catch.

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1. INTRODUCTION

Fish is a very important and healthy source of animal protein. Fish are obtained from the wild and cultured fishes from ponds. Various methods have been used to harvest fish from the wild such as the use of traps and various types of nets. One method of fish harvesting from the wild that is quite damaging to the ecosystem, is the use of piscicides (chemicals for killing or stupefying fish). Some of the commonly used piscicides are either chemically synthesized or of biological origin mostly botanicals. The use of fish poisons is an old practice. Heda and Kulkarni [1] cited instances in 1212 AD and 15th century where the use of fish poisons were prohibited. Evidence from literature suggests that the use of pisicicides is worldwide [2-5].

Various parts of plants have been extracted and used as poisons including bark, flower, seeds, fruits, leaves, roots, stems, pulp and even the entire plant [6,7]. Many authors ascribed the piscicide activities of plants to be due to the presence of phytochemicals such as tannis, glycosides, resins, flavonoids, anthraquinones, saponins, nicotine, pipanne, ricin, amide, carbazole, cardol, counanin, curcin, akuammine, pyrethrum and diosgenin [4,6,8,9]. These botanicals contain chemicals that stun or stupefy fish for easy catch or kill them out rightly [7,8,10]. Ekpendu et al. [3] listed some of the botanical piscicides in Nigeria including *Raphia vinefera*. Sogbesan and Emmanuel [4] listed 13 piscicidal plants found in Adamawa state, Nigeria. While Fafioye [6] listed 40 piscicidal plants in south western Nigeria including *Raphia vinefera*. Neuwinger [11] listed 325 fish poisoning plants spread among 71 plant families with 183 genera used for poison fishing in tropical Africa.

Raphia (raffia) palm is one of the most important palms found in southern Nigeria and also in the tropical forests of Africa, America and Asia. The palm is underutilized. The palm is mostly exploited for the production of palm wine, which is commonly fermented into ethanol [12-17] and less reported for their ethnobotanical uses for the production of building materials and crafts [9,10,18] and oil [10,19]. In Bayelsa state and many coastal communities in the Niger Delta, the fruits of raffia palm are milled and used as bait to stupefy and poison fish for easy catching.

Physicochemical screening of raffia palm root extract suggest that the plant has high concentration of tannins, flavonoids, sterols and triterpenes, saponins and polyphenol; moderate in alkaloids and cardiac glycosides; low in cynogenetic glycoside deoxy sugar and reducing sugar and lack chlorogenic acid, phlobatannins and anthraquinone glycoside [20]. Ukwubile et al. [9] reported that raffia fruits are very high in saponins, high in flavoids, moderate in alkaloids and tannins but low in anthraquinones. While some authors attributed the ichthyotoxicity of raffia palm to the presence of alkaloids [4,6], others attributed it to saponins [9,21,22]. The mechanism of action of the bioactive compound is still not clear. Ukwubile et al. [9] demonstrated that most of the fishes that were killed by raffia palm fruit extracts were mostly surface dwelling families whose operculum was not tightly closed, which suggest that the mode may be related to respiration. The authors also reported a change in the colour of the gills of succumbed fishes from red to pale red initially to almost whitish within 20 minutes. The authors also suggested two possible mechanisms of action first the extract cause the blockage of the blood capillaries supplying blood to the gills and other respiratory organs of the fish, which resulted in the change of the colour of the gills. Secondly, phytochemicals particularly tannins, saponins and alkaloids in aqueous solution complex with basic salts, which react with oxygen to form fish poison and/or decrease oxygen tension, which could suffocate fishes. Several authors have used haematological parameters to assess the physiological conditions of catfish exposed to different botanicals [8,23-30]. Ukwubile et al. [9] assessed the ichthyotoxicity of grind raffia palm (*Raphia farinifera*) fruit extracts on several fish species including *Clarias*. Afalayan et al. [22] reported the sub lethal effects of methanolic extract of *R. hookeri* on the reproductive capacity of African catfish, *Clarias gariepinus*, but Adeogun [21] reported the haematological profile of African catfish exposed to methanolic extract of *R. hookeri*. Ekelemu [31] presented preliminary studies on the use of raffia palm pulp for the harvest of fish. Hence, this study is aimed at investigating the effect of sub lethal doses of raffia palm, *Raphia hookeri* on haemotological characteristics of the African catfish *Clarias gariepinus* and selected water quality properties.

2. MATERIALS AND METHODS

2.1 Test Fish

Clarias gariepinus (Burchell, 1822) juveniles (mean length: 17.5±0.30 cm and mean weight 270±0.30 g) were purchased from a hatchery in Yenagoa, Bayelsa State and transported in aerated tanks to the Laboratory. Thirty two fish juveniles were held in four circular plastic tanks (40 L) for a 14 day period of acclimation. The tanks were filled to half their capacities with tap water (without chlorine), which had been allowed to stand for 2 hours. Feeding was administered using 6 mm Chi® Pelletized Fish Feed from Ajanla Farm (Table 1) twice daily. Change of used water was done every other day to avoid pollution by fish exudes and food remnants.

Table 1. Proximate composition of commercial feed used in the study

Composition	Concentration
Crude protein	38%
Crude fibre	3%
Crude fat	8%
Crude ash	8%
Phosphorus	0.8%
Metabolizable energy	2985 Kcal/kg

2.2 Preparation of Raffia Fruit Mesocarp

Ripe fruits of *Raphia hookeri* were obtained from Wilberforce Island (N, 4.97926, E, 6.1064). Its mesocarp was removed manually and oven dried for 3 days at 250 C. The dried mesocarp was later milled with mechanical blender. 50 g of the milled dried messocarp was soaked in 10 L of water. The prepared aqueous solution resulting from the mixture of ground dried mesocarp of *Raphia hookeri* and water was directly used for the sub-lethal toxicity testing following standard procedure of FAO [32] after it was allowed to stand for two hours.

2.3 Test Procedure

Range finding test was carried out prior to the definitive test. Thirty two juveniles of *C. gariepinus* (eight in each treatment) were exposed to sub-lethal doses of aqueous solution of ground *Raphia hookeri* mesocarp soaked in water (i.e. doses that are insufficient to cause death to the organism but significant enough to result in certain physiological changes). Test fishes for each treatment group were placed in a 30 L aquarium covered with netting material to prevent the fish from jumping out. Three graded concentration for the three treatment groups respectively (0.4 g/l, 0.5 g/l, and 0.6 g/l) were prepared by injecting 2.2 ml (T2), 3.0 ml (T3), and 3.6 ml (T4) of the aqueous solution into the corresponding 30 L aquaria. The *control* (T1) did not contain any mesocarp (i.e. 0.0 g/l). Feeding of fish was stopped 24 hours prior to toxicity testing. Renewal of test media was done every 48 hours for 10 days (the test media were replaced with freshly prepared concentrations of the same quality every 48 hours to maintain the requisite level and potency of the concentrations).

2.4 Sample Collection and Analysis

After the test period had elapsed, the fishes were taken out individually using a hand net and placed belly upward on a table. Blood samples of about 4 milliliters was collected from the caudal peduncle according to the method described by Stoskopf [33] with the aid of a 2 cm^3 plastic syringe, the blood was dispensed into ethylene diaminetetraacetic acid (EDTA) anticoagulant for haematological studies. The use of plastic syringes was a necessary precaution because glass results in decreased coagulation time.

Blood samples collected from each Treatment (Control, T1 T2, and T3) were sent to Federal Medical Centre (FMC), Yenagoa, Bayelsa State for analysis of haematological parameters of *C. gariepinus* exposed to sub-lethal levels of *R. hookeri* mesocarp for 10 days. The parameters analyzed were Haemoglobin (Hb) level, Red Blood Cell (RBC), White Blood Cell (WBC), Eosinophil (EO), Monocytes, and Platelets (PT).

The exposure aquaria for different treatment groups were also obtained and tested for its physicochemical parameters which included pH, Conductivity, Temperature, Turbidity, Dissolved Oxygen and Alkalinity.

2.5 Statistical Analysis

SPSS version 17 (SPSS Inc, Chicago, USA) statistical package was used for descriptive statistics and analysis of variance was carried out, while Duncan Multiple Range Test was used to separate the means at P=0.05.

3. RESULTS

The effect of *Raphia* palm mesocarp on heamatological parameters was investigated in this study. Test fishes were exposed to sub-lethal

concentration of *Raphia hookeri mesocarp* and blood parameters were obtained after the test period.

Table 2 present the effect of graded concentration of raffia palm fruit mesocarp on haematological properties of catfish. Haemoglobin decreased significantly (P=0.000) as the concentration of raffia palm fruit mesocarp increased. Haemoglobin was highest in the control (11.20 g/dl) and least in treatment 4 (7.01 g/dl) where raffia palm mesocarp was highest (0.6 g/l). Similarly, red blood cells (RBC) declined significantly as the concentration of raffia palm fruit mesocarp increases. RBC was 148 x $10^3/m^3$ in the control, 144.2 x $10^3/m^3$ at 0.4 g/l AERP, 121.01 x $10^3/m^3$ at 0.5g/l and 100 x $10^3/m^3$ at 0.6 g/l.

In the three different treatments, white blood cells (WBC) increased significantly (P=0.000) as the concentration of raffia palm increased, which is opposite to the pattern observed in RBC and haemoglobin.

This result showed that the raffia palm mesocarp might have caused the reduction in haemoglobin and RBC. Physically, the test fish became more erratic and a general decrease in swimming was observed as the concentration of raffia palm mesocarp increases (Table 3).

While platelets decreased significantly (P=0.000) as the concentration of raffia palm mesocarp increases, eosinophils and monocytes increased as the concentration of raffia palm mesocarp increases. Platelets decreased from 23 x$10^3/m^3$ in the control to 20 x$10^3/m^3$ at 0.4g/l, 18.x$10^3/m^3$ at 0.5 g/l and 15.32 x$10^3/m^3$ at 0.6 g/l of raffia palm fruits mesocarp.

Physicochemical properties of the water in the aquaria for the different concentration of raffia palm fruit mesocarp are presented in Table 4.

4. DISCUSSION

The findings of this study are similar to the observations of Ukwubile et al. [9] who reported that at increased concentration of raffia palm fruit mesocarp result in the blockage of blood capillaries supplying blood to the gills and other respiratory organs. On the contrary, Adeogun [21] reported significant increases in RBC and haemoglobin in fish exposed to raffia palm mesocarp relative to the control. Ukwubile et al. [9] also reported that saponins cause the conversion of the aglycone portion of glycoside to genins, which in turn combine with the haem portion of RBC to form partial or total clot, thus reducing haemoglobin and RBC levels. Generally, the values of haemoglobin recorded during this study is higher than the values recorded by other authors; 7.03 g/dl [24], 8.43g/100 ml [23], 7.55 g/dl [26], 6.04 g/dl [34]. But values recorded in this study were comparable to those reported by the following authors: 10.63 g/100 ml [25], 10.14 g/dl [30] and 13.4 g/dl [28]. RBC levels followed the same pattern as haemoglobin.

Values of WBC reported in control was comparable to those reported by other authors; 4.4 x10^3/µl [26], 4.08 mm^3 [24], 16.13x10^3/ml [23]. But some other authors reported higher values close to the values obtained when the fish were exposed to graded levels of raffia palm fruit mesocarp in our experiment. WBC levels were 65.3x$10^3/m^3$ at 0.4 g/l, 84.5x$10^3/m^3$ at 0.5 g/l and 90.35 x$10^3/m^3$ at 0.6 g/l, which were by far lower than 10.23 x$10^3/m^3$ recorded in the control. Osuigwe et al. [25] recorded a WBC of 20.68 x$10^3/mm^3$, while Erhunmwunse and Ainerus [34] reported 41.1 x10^3/µl. Sotolu and Faturoti [23] reported WBC of 16.13 x10^3/ml while Mahmond and Ahmed [30] reported 19.191 ml x 10^3.

Table 2. Haematological parameters of *C. gariepinus* exposed to chronic levels of raffia palm mesocarp for 10 days

Raffia fruit mesocarp, g/l (Treatment)	Haemoglobin level (Hbg/dl)	Red blood cell count ($10^3/m^3$)	White Blood cell count ($10^3/m^3$)	Platelet count (PT) ($10^3/m^3$)	Eosinophil (EO) ($10^3/µL$)	Monocytes (%)
0 (T1)	11.20±0.00d	148±6.30d	10.2±0.34a	23.00±0.00d	1.40±0.00a	12.00±0.01a
0.4 (T2)	11.10±0.003c	144.20±3.20c	65.3±2.00b	20.00±0.03c	3.02±0.01b	15.00±0.01b
0.5 (T3)	10.52±0.01b	121.01±1.30b	84.5±2.10c	18.31±0.01b	5.00±0.01c	17.02±0.02c
0.6 (T4)	7.01±0.01a	100.00±1.20a	90.35±4.4d	15.32±0.04a	8.02±0.02d	17.50±0.01c

Mean±standard deviation (n=3) with different alphabets are significantly different (P<0.05) according to Duncan multiple range test

Table 3. Physical observations during the experiment

Conc. of raffia fruit mesocarp, g/l (Treatment)	Physical observations
0 (T1)	Swimming activity, eye colour and feeding pattern were normal throughout the procedure.
0.4 (T2)	Slight reduction in feeding pattern was observed during the last 5 days of experimentation.
0.5 (T3)	Erratic swimming was observed in test fishes present in this group from day 8 to day 10 of the test procedure.
0.6 (T4)	Significantly decreased swimming activity was observed in this group. Some of the test fishes also had slight skin ulceration.

Table 4. Physicochemical properties of water media during the experiment

Conc. Raffia fruit mesocarp, g/l (Treatment)	pH	Conductivity, µS/cm	Temperature, ºC	Turbidity, NTU	Dissolved Oxygen, mg/l	Alkalinity, mg/l
0 (T1)	6.27±0.08a	136.12±14.04a	26.00±0.00a	0.50±0.57a	6.90±0.01d	12.30±0.75a
0.4 (T2)	6.37±0.07b	136.12±14.04a	26.20±0.00a	0.50±0.50a	6.60±0.06c	12.35±0.20b
0.5 (T3)	6.37±0.07b	136.12±14.04a	26.60±0.13a	0.50±0.60a	6.20±0.80b	12.60±0.80c
0.6 (T4)	6.37±0.07b	136.12±14.04a	26.00±0.30a	0.50±0.32a	5.89±0.40a	13.01±0.20d

Mean±standard deviation (n=3) with different alphabets are significantly different (P<0.05) according to Duncan multiple range test

Other authors have reported varying values of platelets in *Clarias gariepinus*; 5.5 ml x 10^3 [28], 91 x 10^3/l [25] and 41.99 x 10^3/µl [30]. Monocytes and Eosinophils recorded during, this study was 12.0% and 1.40 x 10^3/µL respectively in the control and increased significantly (P<0.05) as the concentration of raffia palm mesocarp increases. Agbabiaka et al. [28] reported eosinophils in *Clarias gariepinus*. Erhunmwunse and Ainerus [34] reported monocytes levels of 1.675 x 10^3/µl. Jedege [27] reported monocyte levels of 1.5% in African catfish.

Conductivity, temperature and turbidity of the containing aquarium were not significantly different (P>0.05) at the different concentration of palm mesocarp. Other authors have recorded similar temperature values during toxicological experiments with *Clarias gariepinus*; 25.87ºC [7] and 23.0 – 25.0ºC [24]. Musa et al. [24] while studying the haematological response of *Clarias gariepinus* fingerlings exposed to different concentration of tobacco (*Nicotiana tobaccum*) leaf dust recorded temperature of 22.3ºC and conductivity of 102.3 µS/cm.

The pH of the control was significantly lower (P=0.000) than all the other treatments, the alkalinity was also significantly different (P=0.000) in all the treatments. The result show that the pH increased from 6.27 to 6.37 when the mesocarp were added. Alkalinity increased significantly as the concentration of palm mesocarp increases. The control had total alkalinity of 12.3 mg/l which increased to 12.35 mg/l at 0.4 g/l, 12.60 mg/l at 0.5 g/l and 13.01 mg/l at 0.6 g/l aqueous raffia palm mesocarp. This result suggests that pH has a role to play in the toxicity of the palm mesocarp. Other authors have similarly reported changes in pH and alkalinity in catfish ponds. Ochang et al. [26] reported pH in the range of 5.76 – 6.86, Abalaka [8] recorded 7.34 while Musa et al. [23] reported a pH of 6.8 and total alkalinity of 73.8 mg/l. Ekelemu [31] reported an increase in pH from 6.8 to between 11.9 and 12.9 when raffia palm fruit was added at a concentration of 1-7 g/l.

In this study, Dissolved oxygen (DO) tends to have an inverse relationship with concentration of raffia palm in the tanks. Dissolved oxygen decreased as the concentration of raffia palm mesocarp increased. In the control, DO was 6.9 mg/l, but decreased to 6.60 mg/l at 0.4 g/l, 6.20 mg/l at 0.5 g/l and 5.89 mg/l at 0.6g/l of the raffia palm fruit mesocarp. It therefore seems that the piscicidal effect of raffia palm mesocarp involves the depression of oxygen tension in the water, which stupefies or kills fish. Ukwubile et al. [9] reported that the sharp decrease in DO when raffia palm mesocarp is added to catfish ponds/ tanks is due to the presence of saponins, alkaloids and tannins in the mesocarp, which form soluble complexes of basic salts that react with oxygen in the water to form poison. Other authors have similarly reported oxygen levels in

catfish ponds; 6.23 mg/l [8], 5.3 – 6.5 mg/l [26] and 8.7 mg/l [24].

5. CONCLUSION

The mesocarp of raffia palm is used by indigenous people in the Niger Delta of Nigeria to stupefy and catch fishes. This old practiced have not been well demonstrated scientifically. In this study, we investigated the effect of raffia palm mesocarp on the haematological characteristics of a common African catfish, *Clarias gariepinus*. Results show that the raffia palm mesocarp induced increased alkalinity and reduced oxygen tension in the water which affected the blood characteristics of the catfish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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