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Erythrocyte and Platelet Counts Analysis and Effect in Female Wistar Rats after Prolonged Administration of Aqueous Fruit Pulp Extract of *Raphia Hookeri Plant*

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Abstract: *This research aimed to analysis the effects of the prolonged administration of aqueous fruit pulp extract of Raphia hookeri fruit on erythrocyte indices and platelet counts in female Wistar rats. A total of 28 female Wistar rats, weighing 130g to 200g, were grouped into four (4) groups, with Group 1 as the control. The control group was provided standard feed and water, while Group 2 received 1000 mg/kg, Group 3 received 2000 mg/kg, and Group 4 received 3000 mg/kg body weight daily for 28 days. After the treatment period, blood samples were collected, and parameters such as erythrocyte count and platelet count were analyzed using standard haematological techniques. Statistical analysis was done with SPSS version 21.0, and results were expressed as mean ± standard error of the mean (SEM), with $p<0.05$ considered statistically significant. Results showed erythrocyte counts of 5.80 ± 0.12 in the control group, with a significant decrease in treated groups 2, (5.00 ± 0.58), 3 (4.40 ± 0.17) and 4 (4.80 ± 0.12). Platelet counts also showed a dose-dependent decrease, with values of 250.00 ± 1.15 in the control and significantly lower counts of 120.00 ± 2.89, 100.00 ± 1.73, and 94.00 ± 2.31 in Groups 2, 3, and 4, respectively. These findings indicate that higher doses may significantly impact erythrocyte and platelet parameters, suggesting potential risks associated with prolonged high-dose administration.*

Keywords: Erythrocyte, Platelet, Raphia Hookeri, Fruit Pulp,Wistar Rat

I. INTRODUCTION

Throughout history, humankind has faced significant health challenges that have spurred both medical practitioners and traditional healers to seek out natural, plant-based solutions. Among the numerous plants with notable medicinal applications, *Raphia hookeri* has garnered significant interest for its wide-ranging therapeutic properties, known for its potential in managing ailments such as inflammation, cardiovascular issues, and liver conditions, *Raphia hookeri* is utilized in various indigenous treatments across West and Central Africa (Egbono *et al.,* 2023; Altiok *et al.,* 2010). This interest reflects a broader trend toward plant-based medicines as complementary therapies to conventional medical practices, highlighting *Raphia hookeri*'s role in holistic health management (Abimbola *et al.,* 2018). Moreover, as modern science increasingly investigates the physiological impacts of *Raphia hookeri*, attention has turned to its potential benefits on blood health, particularly in enhancing erythrocyte indices and platelet counts (Egbono *et al*.,

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2023), which are essential markers in hematology and overall health assessment. Erythrocyte indices are key indicators of red blood cell (RBC) health and function (Rodak *et al.,* 2016). These metrics are useful in identifying several types of anaemia and evaluating oxygen-carrying capacity, which is essential for maintaining cellular energy and health throughout the body (Roberts & Fulwood, 2018). A healthy erythrocyte index reflects sufficient oxygen transport to tissues, while abnormalities may indicate conditions such as iron deficiency anaemia (often microcytic), folate or B12 deficiency anaemia (often macrocytic), and hereditary or acquired disorders that influence RBC morphology (Hoffbrand et al., 2011).

The significance of these indices extends beyond diagnosing anaemia, as they are also valuable markers for assessing overall health and managing chronic conditions influenced by oxidative stress. Oxidative stress, known to shorten erythrocyte lifespan and impair erythrocyte functionality, is especially impactful in conditions like diabetes, cardiovascular disease, and chronic inflammation (Sies, Berndt, & Jones, 2017). Studies show that the antioxidants found in *Raphia hookeri*, such as vitamins C and E, flavonoids, and phenolic compounds, can help protect erythrocytes from oxidative damage. By stabilizing erythrocyte indices, these antioxidants can potentially mitigate oxidative stress, thereby preserving erythrocyte stability, improving oxygen transport, and enhancing cellular health (Hebbel *et al.,* 1982; Egbono *et al.,* 2023). Platelets, or thrombocytes, play a crucial role in blood clotting and wound healing, as they are responsible for forming clots to prevent excessive bleeding (Harrison & Keeling, 2005). A complete blood count typically includes a platelet count, which provides essential information on coagulation capacity. An ideal platelet count helps maintain a delicate balance between clot formation and breakdown, ensuring that the blood remains fluid while being capable of forming clots when necessary. Abnormal platelet counts can lead to various health complications: thrombocytopenia (low platelet count) is associated with bleeding disorders, while thrombocytosis (high platelet count) can lead to an increased risk of thrombosis, which may result in cardiovascular events such as stroke or myocardial infarction (Schulman &Kearon, 2005). Oxidative stress can negatively impact platelet function, making antioxidants from plant sources like *Raphia hookeri* particularly valuable. By combating oxidative damage, *Raphia hookeri*'s antioxidant-rich profile may help preserve platelet function, thus aiding in the prevention of abnormal clotting or bleeding tendencies (Tao *et al.,* 2019). Studies suggest that antioxidants like vitamins C and E in *Raphia hookeri* contribute to platelet stability and support cardiovascular health, as oxidative damage to platelets is linked to conditions like atherosclerosis and thrombosis (Sies *et al.,* 2017). This protective effect of *Raphia hookeri* makes it a promising natural adjunct in managing conditions related to platelet function and cardiovascular health (Oluwaniyi *et al.,* 2014).

The importance of these blood health parameters—erythrocyte indices and platelet counts—is well-established in medical practice, where they provide crucial insights into immune health, anaemia, coagulation disorders, and overall physiological status (Roberts & Fulwood, 2018). Studies on *Raphia hookeri* have primarily focused on its lipid profile effects, vitamin and phytochemical composition, and toxic elements (Ogbuagu, 2008; Bassey, 1985). However, targeted research into its impacts on erythrocyte indices and platelet counts remains limited, revealing a promising avenue for future studies that could enhance applications in both hematology and the health sector. Despite the abundance of natural remedies available in Nigeria, including the *Raphia hookeri* plant, these resources remain significantly underutilized. This lack of widespread knowledge about their medicinal properties contributes to the low consumption of these natural treatments. Many Nigerians, regardless of socioeconomic status, tend to rely on expensive synthetic drugs, unaware that affordable and accessible alternatives like *Raphia hookeri* can offer substantial health benefits, including the improvement of blood cell parameters and overall well-being. The underuse of these natural remedies highlights a gap in awareness and education, underscoring the need for greater recognition of *Raphia hookeri* and its therapeutic potential warranted this scientific investigation.

II. MATERIALS

The materials involved in this study includes Syringe, Hand Gloves, Cages, Dissecting Blade, Dissecting Board, Permanent Marker, Animal Feeds, Water, Chloroform, EDTA Bottles, Cannula, matured female Wistar rats, Lab coats, Disinfectants, Dry saw dust etc.

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Animal Preparations

A total of twenty (20) healthy female Wistar rats, weighing between 130g and 200g, were utilized for this study. These rats were housed in the Experimental Pharmacology and Toxicology Animal House of the Faculty of Pharmaceutical Sciences at the University of Port Harcourt, Nigeria. The animals were kept in a well-ventilated environment with optimal humidity and temperature conditions, and a natural light-dark cycle. They were provided with unrestricted access to food and water.

Acclimatization of Animals

Following identification, the animals were weighed using a precise weighing balance. They were then housed in clean plastic cages for a period of two weeks. This acclimatization period was designed to ensure the animals adjusted to the environmental conditions of the Animal House, which included factors such as temperature, humidity, and light-dark cycles.

Experimental Extract and Preparation

The extract of *Raphia hookeri* fruit pulp was utilized for the experiment. The preparation of the extract was carried out using the maceration method. Initially, the fruit pulp was carefully air-dried to avoid degrading the active ingredients. After drying, the pulp was thoroughly crushed and then placed in a maceration jar for soaking. Approximately 1000 grams of the dried pulp were mixed with 2000 ml of water. This mixture was allowed to stand for a period of 72 hours, during which it was continuously agitated to maximize the extraction yield. Following this maceration period, the mixture was filtered to separate the solid residues from the liquid extract. The obtained filtrate was then transferred to a water bath, where it was heated to a temperature of 65 degrees Celsius to evaporate the liquid content. This evaporation process concentrated the extract. After the evaporation was completed, the final weight of the extract was measured. The concentrated extract was then stored properly for subsequent use in the experimental procedures.

Study Design

A total of twenty-eight healthy female Wistar rats were used for this study. The rats were divided into four groups: a control group consisting of 7 animals and three experimental groups with 7 animals each. Following a 14-day acclimatization period in the Experimental Pharmacology and Toxicology Animal House of the Faculty of Pharmaceutical Sciences at the University of Port Harcourt, Nigeria, the experimental groups (Groups 2, 3, and 4) were administered the extract for 28 days.

Sample Collection

The *Raphia hookeri* fruit pulp used for this study was purchased from the Ayeezilocal market in Abua/Odual LGA, Rivers State, Nigeria.

Mode of Administration of Extract

Aqueous pulp extract of *Raphia hookeri* was administered orally in low, medium, and high doses daily for 28 days. During this period, the following doses were given to each group, except the control group. The lethal dose (LD50) of the aqueous pulp extract of *Raphia hookeri* fruit was calculated using Lorke's method, with 5000 mg/kg body weight of female Wistar rats being established as the maximum. Therefore, the female Wistar rats were not given doses exceeding 5000 mg/kg body weight:

- Group 1 (Control Group): Received standard animal feed and water.
- Group 2 (Low dose): Received 1000 mg/kg body weight of the extract.
- Group 3 (Medium dose): Received 2000 mg/kg body weight of the extract.
- Group 4 (High dose): Received 3000 mg/kg body weight of the extract.

Analysis of sample blood cell parameters

Estimation of the erythrocyte indices

Materials: Microscope and Slide

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Procedures:

- Place a small drop of blood on a clean glass slide.
- Use another slide to spread the drop by holding it at a 30° – 45° angle and swiftly moving it across the first slide to create a thin smear.
- Allow the smear to air dry.
- Fix the cells by dipping the slide in methanol.
- Stain the smear using a Romanowsky stain (e.g., Wright-Giemsa stain). This process will make the erythrocytes more visible under the microscope.
- Place the stained slide under the microscope.
- Start with a low magnification to locate the optimal area where the cells are evenly spread and not overlapping.
- Switch to a higher magnification (usually 100x with oil immersion) to closely examine the erythrocytes.

Estimation of erythrocyte shape

- Observe normal erythrocytes.
- Identify erythrocytes with shapes differing from the normal.
- Estimate the proportion of the abnormally shaped cells.
- Note the distribution of the abnormally shaped cells.

Estimation of Platelet Count

Materials: Microscope and Slide

Procedures:

- Place a small drop of blood on one end of a glass slide.
- Use another slide to spread the drop by holding it at a 30–45-degree angle and dragging it across the slide to create a thin smear.
- Allow the smear to air dry before staining.
- Stain the dried smear using a suitable stain like Wright's or Giemsa stain.
- Rinse gently with distilled water and let it air dry.
- Place the slide on the microscope stage and focus on the stained smear using the low-power objective to locate areas with a good distribution of cells.
- Switch to a higher magnification (e.g., 100x oil immersion objective) to count the platelets.
- Use the high-power objective to examine fields of view. Count the number of platelets in multiple high-power fields (HPFs) to get an average count.
- Platelets are typically smaller than erythrocytes and appear as tiny, light-staining fragments.
- Estimate the count based on the number of platelets per field of view and the average number counted.

Statistical Analysis

The data collected from the current study were analyzed using the Statistical Package for Social Sciences (SPSS) software, specifically version 21.0. To determine statistical significance, we employed one-way analysis of variance (ANOVA) followed by a post-hoc multiple comparison test to identify significant differences between groups. A P value of less than 0.05 ($P<0.05$) was used as the threshold to denote statistical significance. All results were reported as mean values with their corresponding standard error of the mean (SEM), providing a measure of the variability around the mean estimates.

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III. RESULTS OF FINDINGS

The results of the study are presented in tables and charts and interpreted accordingly.

Table 1: Effect of administration of aqueous pulp extract of *Raphia hookeri* fruit (APERHF) on Erythrocytes and platelet Parameters in female Wistar rats

Group and Treatment	Erythrocyte ($X10^{12}$/L)	Platelet count ($X10^{9}$/L)
Group 1: Control Group	5.80 ± 0.12	250.00 ± 1.15
Group 2: Low Dose treated (1000mg/kg b.w APERHF)	5.00 ± 0.58	120.00 ± 2.89 [a]
Group 3: Medium Dose treated (2000mg/kg b.w APERHF)	4.40 ± 0.17 [a]	100.00 ± 1.73 [a, b]
Group 4: High Dose treated (3000mg/kg b.w APERHF)	4.80 ± 0.12	94.00 ± 2.31 [a, b]

The erythrocyte (RBC) levels of all treated groups were seen to be reduced when compared to that of the control group but only that of group 3 (treated with medium dose of the APERHF was statistically significant ($p<0.0$).

The platelet count of all different doses of APERHF treated groups of rats were found to be significantly ($p<0.05$) low when compared to that of the control group. There was a progressive reduction in the platelet count with increasing doses of APERHF treatment. The variation of platelet counts across the different doses of APERHF treated groups were statistically significant ($pp<0.05$) when compared to themselves.

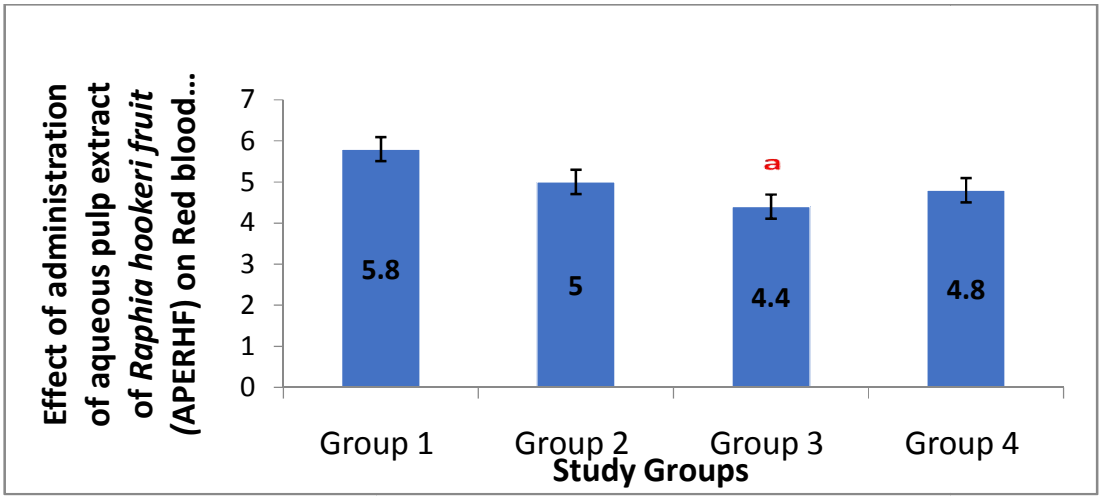

Figure 2: Effect of administration of aqueous pulp extract of *Raphia hookeri* fruit (APERHF)on erythrocyte count in female Wistar rats

The results in Figure 2 show a significant reduction in erythrocyte count with increasing doses of *Raphia hookeri* extract. The control group exhibited the highest erythrocyte count, indicating normal red blood cell production and stability. However, as the dose increased from 1000 mg/kg to 3000 mg/kg, there was a progressive decline in erythrocyte levels, with the medium dose (2000 mg/kg) showed the most pronounced reduction. This suggests that higher doses of APERHF may suppress erythropoiesis or lead to premature breakdown of red blood cells, possibly due to oxidative stress or interference with the bone marrow's erythropoietin activity. These findings imply potential haematological risks associated with prolonged or high-dose consumption of the extract, indicating the need for controlled dosing in therapeutic applications.This reduction indicates a lower concentration of haemoglobin per erythrocyte, suggesting potential disruptions in haemoglobin synthesis or retention. Such changes could impair the oxygen-carrying capacity of red blood cells, potentially leading to hypochromic anaemia. These findings emphasize the need for careful dosing, as excessive intake of APERHF may negatively impact haemoglobin concentration and overall blood health.

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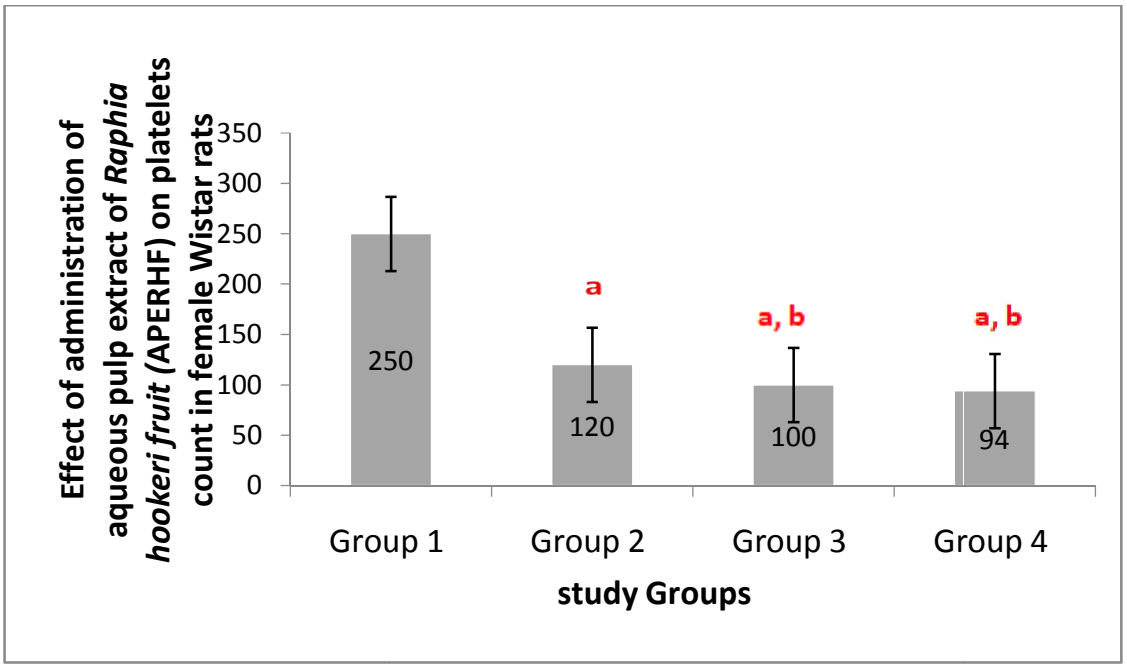

Figure 3: Effect of administration of aqueous pulp extract of *Raphia hookeri* fruit *(*APERHF)on platelets count in female Wistar rats

Figure 2 shows a significant, dose-dependent decrease in platelet count across all treatment groups. The control group exhibited a stable platelet count, indicating normal clotting function. However, as the APERHF dose increased, there was a marked reduction in platelet levels, with the lowest count observed in the high-dose group. This suggests that high doses of APERHF may suppress platelet production or increase platelet destruction, potentially impairing haemostasis. Platelets are essential for blood clotting, and a significant decrease can increase the risk of bleeding. These results highlight the potential risks associated with high-dose APERHF consumption, emphasizing the importance of dose regulation to avoid adverse effects on platelet function.

IV. DISCUSSION

The findings of this study offer valuable insights into the some haematological effects of the aqueous pulp extract of *Raphia hookeri* fruit *(*APERHF) on erythrocyte indices and platelet counts in female Wistar rats. The analysis showed dose-dependent responses in the blood parameters, revealing notable changes across various doses. Specifically, APERHF treatment at higher concentrations resulted in a significant reduction in erythrocyte count and reducing platelet count. These effects suggest potential implications for blood health and highlight the importance of understanding how natural compounds like APERHF interact with erythrocytes and platelets at varying dosages.

The observed reduction in erythrocyte count, particularly in the 2000 mg/kg and 3000 mg/kg treatment groups, suggests a suppressive effect of high-dose APERHF on erythropoiesis or on erythrocyte survival. This outcome is consistent with previous research on high doses of antioxidants, which have been shown to impact erythrocyte membrane integrity and lead to premature breakdown of red blood cells under certain conditions (Sies *et al.,* 2017). Components within APERHF, such as vitamins E and C, are known to combat oxidative stress; however, their excessive presence may disrupt erythrocyte metabolism and contribute to changes in cell size, likely through alterations in cell maturation and membrane stability (Hebbel *et al.,* 1982).This effect could be linked to interactions between APERHF's antioxidant-rich profile and erythrocyte functionality. High antioxidant levels, while protective at moderate doses, might interfere with the reactive oxygen species (ROS) balance and affect cellular haemoglobin retention, as reported in studies on antioxidant impacts on red blood cells (Roberts & Fulwood, 2018). Similar findings have been documented in studies where antioxidant-rich compounds affected cellular iron metabolism, influencing haemoglobin synthesis and storage within erythrocytes (Ganz, 2019).

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The observed reduction in platelet count across the treatment groups, particularly in the 3000 mg/kg dose, suggests that APERHF may impact platelet production or reduce platelet lifespan. Platelets play an essential role in clot formation, and a lower platelet count can compromise haemostasis, potentially leading to an increased risk of bleeding (Harrison & Keeling, 2005). While antioxidants such as vitamins C and E are known to stabilize platelet function by reducing oxidative damage, an excess intake can disrupt the balance and impair thrombopoiesis (Tao *et al.,* 2019). Excessive antioxidants, which reduce ROS levels excessively, may alter the oxidative environment necessary for maintaining platelet production and stability (Oluwaniyi *et al.,* 2014).

The haematological effects observed in this study may be attributed to the high lipid and antioxidant content of APERHF, including vitamins C and E, flavonoids, and phenolic compounds. While these constituents provide significant health benefits, they may also impact blood parameters, particularly when consumed in high quantities. For instance, the lipid content in *Raphia hookeri* could potentially influence cell membrane composition, contributing to changes in membrane stability, as well as the risk of dyslipidaemia if consumed excessively (Pappan & Rehman, 2021). Such dyslipidaemia could lead to oxidative stress in erythrocytes, affecting their survival and morphology. Furthermore, although antioxidants like vitamins C and E are effective in neutralizing ROS, high levels may create an overly reduced cellular environment, impacting cellular processes essential for erythropoiesis and thrombopoiesis (Greco, 2005). Greco's (2005) research illustrated that while moderate doses of vitamins C and E strengthen cellular resilience, high doses can disrupt cellular balance, particularly in blood cells that undergo high oxidative turnover.

V. CONCLUSION

In conclusion, APERHF may offer antioxidant benefits at lower doses; however, high-dose administration could adversely impact haematological health by altering erythrocyte and platelet parameters. These findings emphasize the importance of dose regulation to maximize the therapeutic potential of APERHF while minimizing risks associated with excessive antioxidant intake. This study provides evidence that aqueous extract of *Raphia hookeri* fruit (APERHF) significantly affects erythrocyte indices and platelet counts in female Wistar rats, particularly at high doses. Notably, high-dose APERHF administration reduced erythrocyte count and platelet count. These results indicate that, although APERHF contains beneficial antioxidants, its overuse could have detrimental effects on haematological parameters, specifically erythrocytes and platelets.

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