



# Haematological Assessment of Tender Coconut Water as a Natural Erythropoietic Agent in Wistar Rats

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### **ABSTRACT**

Background and Objective: Coconut water has been traditionally recognized for its numerous health benefits, particularly for hydration and its rich nutrient profile. However, its impact on hematological parameters has not been extensively studied. This study aims to investigate Hematological Assessment of Tender Coconut Water as a Natural Erythropoietic Agent in Wistar Rats. Materials and Methods: The 24 Wistar rats were divided into control and treatment groups, with the latter receiving 10, 20 and 30 mL/kg body weight of Malayan green dwarf hybridized immature coconut water. Hematological parameters, including hematocrit, hemoglobin concentration, RBC count, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC), were assessed. **Results:** The administration of coconut water resulted in significantly higher hematocrit (p = 0.001), hemoglobin (p = 0.002) and RBC counts (p = 0.006) in the treatment groups compared to the control. However, no significant changes were observed in MCV, MCH or MCHC (p>0.05). Additionally, there was no dose-dependent effect on the hematological parameters among the different treatment groups. Malayan green dwarf hybridized immature coconut water significantly improves hematocrit, hemoglobin and RBC counts in Wistar rats without affecting red cell indices. Conclusion: The effects are consistent across different dosages, indicating its potential as a natural supplement for improving hematological health.

## **KEYWORDS**

Coconut water, hematological parameters, erythropoiesis, Wistar rats, haemoglobin, RBC count, natural supplement

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### INTRODUCTION

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Tender coconut water has been traditionally consumed for its numerous health benefits, including its high content of electrolytes, vitamins, minerals and antioxidants. It has been widely used in traditional medicine for hydration and managing certain illnesses, though its impact on erythropoiesis has not been fully examined. Studies have shown that TCW contains bioactive substances such as phytochemicals and phytohormones, which are known to support overall cellular function and metabolism<sup>1</sup>. Coconut water



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### Pharmacologia, 15 (1): 23-28, 2024

has been recognized as a significant option for oral rehydration and has even been employed for intravenous hydration in patients residing in remote areas. It has also been reported to protect against myocardial infarction due to its composition of beneficial polyphenolic compounds, which exhibit antioxidant properties capable of slowing the aging process and preventing cancer and cardiovascular diseases<sup>2,3</sup>.

One of the essential components of the circulatory system is red blood cells (RBCs), which are crucial for oxygen transport. Red blood cells absorb oxygen in the lungs and release it to tissues as they move through the capillary network of the circulatory system<sup>4</sup>. The RBC cytoplasm has a high concentration of hemoglobin, an iron-containing biomolecule that is responsible for oxygen binding and the red colour of blood. Each human red blood cell contains around 270 million hemoglobin molecules and the plasma membrane is made up of proteins and lipids that allow RBCs to deform and stay stable as they transit the body's circulatory pathways<sup>5</sup>. Mature human red blood cells are biconcave in shape and lack a nucleus and organelles, which maximizes their space for hemoglobin, allowing efficient oxygen transport. The production of RBCs, known as erythropoiesis, occurs in the bone marrow at a rate of around 2.4 million cells per second in adults, with each cell circulating for approximately 100-120 days before being recycled by macrophages<sup>6</sup>. Given the crucial role of RBCs in maintaining oxygen levels, interventions that can naturally stimulate erythropoiesis, such as those involving coconut water, are of significant interest. The study aimed to determine whether tender coconut water, a natural-rich bioactive substance, could act as a natural erythropoietic agent in an animal model using Wistar rats.

### **MATERIALS AND METHODS**

**Study area:** The haematocrit value, haemoglobin estimation, red blood cells count and red cell indices were carried out at Niger Delta University Teaching Hospital, Okolobiri. The study was carried out from August, 2022 to January, 2023.

**Study population:** The 24 Wistar rats were bred in animal house of Department of Pharmacology, Niger Delta University, Amassoma, Bayelsa State were used in this study.

**Ethical approval:** College Health Research Ethics Committee, College of Health Sciences, Niger Delta University, Amassosoma, Bayelsa State, provided ethical clearance and approval.

**Experimental design:** This study used 24 Wistar rats (170-200 g) bred in the animal house of the Department of Pharmacology, Niger Delta State, Wilberforce Island, Bayelsa State. Prior to the experiment, the animals were allowed to acclimate in the animal house for approximately 7 days. They were housed in well-ventilated cages that were cleaned and food was replaced on a daily basis at a room temperature of about 37°C. The animals were fed with commercially prepared vital feed *ad libitum* and tap water. They were chosen at random and divided into 4 groups, each with 6 rats. Group A served as the negative control while group B, C and D were given 10, 20 and 30 mL/kg body weight fresh coconut water daily, respectively, via orogastric intubation for 4 weeks.

**Group A:** Vital feed+water (negative control)

**Group B:** Coconut water (10 mL/kg body weight)+vital feed **Group C:** Coconut water (20 mL/kg body weight)+vital feed **Group D:** Coconut water (30 mL/kg body weight)+vital feed

**Collection of blood samples:** After 4 weeks of coconut water treatment, the rats were anesthetized by placing them in a glass chamber containing cotton wool soaked in chloroform and they were then humanely sacrificed one by one. Blood samples (5 mL) were collected from the animals using cardiac puncture and dispense into EDTA bottle for complete blood count. The complete blood counts were analysed 8 hrs after sample collection.

**Method of analysis:** Haematocrit value, haemoglobin estimation, red blood cells count and red cell indices were done by automated hematology analyzer (SYSMEX XP-300)<sup>7</sup>.

**Statistical analysis:** Data analysis was conducted using the Statistical Package for Social Science (SPSS) version 22 windows 10, the result was expressed in Mean $\pm$ SD (standard deviation). Data was obtained from the analysis using paired samples t-test. Values were considered significant at p<0.05 and not significant at p>0.05.

### **RESULTS**

Table 1 compares hematocrit value, haemoglobin value, red blood cell count and red cell indices between control rats and those given 10 mL/kg body weight of Malayan green dwarf hybridized immature coconut water. Wistar rats with coconut water administration had significantly higher hematocrit value, haemoglobin value and red blood cell count than the negative control group (p<0.05) but no significant difference in red cell indices (p>0.05).

**Mean cell haemoglobin concentration (MCHC):** Table 2 shows a comparison of hematocrit value, haemoglobin value, red blood cell count and red cell indices between control rats and those given 20 mL/kg body weight of Malayan green dwarf hybridized immature coconut water. Wistar rats with coconut water administration had significantly higher hematocrit value and haemoglobin values than the negative control group (p<0.05) but no significant difference in red blood cell count and red cell indices (p>0.05).

Table 3 shows a comparison of hematocrit value, hemoglobin value, red blood cell count and red cell indices between control rats and those given 30 mL/kg body weight of Malayan green dwarf hybridized immature coconut water. Wistar rats with coconut water administration show no significant difference in hematocrit value, haemoglobin value, red blood cells count and red cell indices when compared with the negative control group (p>0.05).

Table 4 shows the comparison of 10, 20 and 30 mL/kg Malayan green dwarf coconut water on the treatment group on hematocrit value, haemoglobin value, red blood cell count and red cell indices. The

Table 1: Comparison of hematocrit value, hemoglobin value, red blood cell count and red cell indices between control rats and those given 10 mL/kg of Malayan green dwarf hybrid coconut water

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Hematology parameters	Group A	Group B	t-value	p-value
Hematocrit value (%)	38.33±3.98*	43.00±1.10*	-3.500	0.017
Hemoglobin value (g/dL)	12.72±1.38*	14.20±0.33*	-3.100	0.027
Red blood cells (10 <sup>12</sup> /L)	6.30±0.79*	7.18±0.34*	-3.223	0.023
Mean cell volume (fL)	61.42±3.09	60.10±1.53	0.885	0.417
Mean cell haemoglobin (Pg)	20.35±1.02	19.80±0.44	1.147	0.303
MCHC (g/dL)	33.17±0.34	33.05±0.06	0.833	0.443

Control Wistar rats were compared with those administered 10 mL/kg Malayan green dwarf hybrid coconut water (n = 12), \*Significant differences at p < 0.05 level, Group A: Negative control, Group B: Coconut water administration (10 mL/kg body weight) and  $\pm$ : Mean $\pm$ Standard Deviation (SD)

Table 2: Comparison of hematocrit value, haemoglobin value, red blood cells count and red cell indices between control rats and those given 20 mL/kg of Malayan green dwarf hybrid coconut water

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Hematology parameters	Group A	Group C	t-value	p-value
Hematocrit value (%)	38.33±3.98*	42.67±1.82*	-3.031	0.029
Hemoglobin value (g/dL)	12.72±1.38*	14.20±0.25*	-2.832	0.037
Red blood cells (10 <sup>12</sup> /L)	6.30±0.79	6.63±0.11	-1.008	0.360
Mean cell volume (fL)	61.42±3.09	62.75±1.81	0.978	0.373
Mean cell haemoglobin (Pg)	20.35±1.02	20.50±0.66	-1.327	0.757
MCHC (g/dL)	33.17±0.34	33.00±0.00	1.206	0.282

Control Wistar rats were compared with those administered 20 mL/kg Malayan green dwarf hybrid coconut water (n = 12), \*Significant differences at p < 0.05 level, Group A: Negative control, Group C: Coconut Water administration (20 mL/kg body weight), MCHC: Mean cell hemoglobin concentration and  $\pm$ : Mean $\pm$ Standard Deviation (SD)

Table 3: Comparison of hematocrit value, hemoglobin value, red blood cell count and red cell indices between control rats and those given 30 mL/kg of Malayan green dwarf hybrid coconut water

Hematology parameters	Group A	Group D	t-value	p-value
Hematocrit value (%)	38.33±3.98	42.00±1.10	-1.854	0.123
Hemoglobin value (g/dL)	12.72±1.38	14.00±0.33	-1.933	0.111
Red blood cells (10 <sup>12</sup> /L)	6.30±0.79	7.10±0.27	-2.012	0.100
Mean cell volume (fL)	61.42±3.09	59.40±0.56	1.616	0.167
Mean cell hemoglobin (Pg)	20.35±1.02	19.65±0.38	1.662	0.157
MCHC (g/dL)	33.17±0.34	33.35±0.06	-1.309	0.247

Control Wistar rats were compared with those administered 30 mL/kg Malayan green dwarf hybrid coconut water (n = 12), \*Significant differences at p < 0.05 level, Group A: Negative control, Group D: Coconut water administration (30 mL/kg body weight) MCHC: Mean cell hemoglobin concentration and  $\pm$ : Mean $\pm$ Standard Deviation (SD)

Table 4: Comparison of 10, 20 and 30 mL/kg Malayan green dwarf coconut water on hematocrit value, Hemoglobin value, red blood cells count and red cell indices (n = 12)

Group	N	Hematocrit (%)	Hemoglobin (g/dL)	RBC	MCV	MCH	MCHC
A	6	43±1.095	14.20±0.33	7.18±0.34*	60.10±1.53*	19.80±0.44*	33.05±0.06
В	6	42.67±0.82	14.20±0.25	6.63±0.01*	62.75±1.81*	20.50±0.66*	33.00±0.00
t-value		0.333	0.000	0.550	2.650	0.700	1.050
p-value		0.576	1.000	0.002	0.005	0.030	0.072
Α	6	43±1.095	14.20±0.33	7.18±0.34	60.10±1.53	19.80±0.44	33.05±0.06*
С	6	42±1.10	14.00±0.33	7.10±0.27	59.40±0.55	19.65±0.38	33.35±0.06*
t-value		0.500	0.333	0.153	0.368	0.200	-3.000
p-value		0.271	0.402	0. 565	0.402	0.616	0.000
В	6	42.67±0.82	14.20±0.25	6.63±0.01*	62.75±1.81*	20.50±0.66*	33.00±0.00*
С	6	42±1.10	14.00±0.33	7.10±0.27*	59.40±0.55*	19.65±0.38*	33.35±0.06*
t-value		0.667	-0.571	-2.917	2.913	3.400	-7.000
p-value		0.271	0.271	0.006	0.001	0.011	0.000

\*Significant differences at p < 0.05 level, RBC: Red blood cells (10<sup>12</sup>/L), MCV: Mean cell volume (fL), MCH: Mean cell hemoglobin (Pg) MCHC: Mean cell haemoglobin concentration(g/dL), Group B: 10 mL/kg of body weight Malayan green dwarf hybridized immature coconut water, Group C: 20 mL/kg of body weight Malayan green dwarf hybridized immature coconut water, Group D: 30 mL/kg of body weight Malayan green dwarf hybridized immature coconut water and ±: Mean±Standard Deviation (SD)

effect of doses of Malayan green dwarf hybridized immature coconut water on the treatment groups on hematological parameters showed 20 mL/kg Malayan green dwarf coconut water showed a more significant difference compared with other doses (p>0.05).

# **DISCUSSION**

The administration of coconut water at various dosages revealed a dose-dependent influence on hematocrit, hemoglobin concentration and red blood cell (RBC) count. The results show that administering 10 and 20 mL/kg of coconut water led to a significant increase in hematocrit and hemoglobin levels compared to the control group. Specifically, rats that received 10 mL/kg of coconut water had a higher mean hematocrit value compared to the control group. Similarly, hemoglobin levels were significantly elevated in the treatment group. These increases may be attributed to the high phytochemicals and phytohormones content of coconut water, which has been shown to promote erythropoiesis and enhance oxygen transport in the body<sup>2,8</sup>. Interestingly, no significant increase in hematocrit and haemoglobin levels was observed in rats receiving 30 mL/kg of coconut water. This suggests a threshold beyond which further increases in coconut water consumption may not enhance erythropoietic activity, possibly due to fluid overload or saturation effects<sup>9</sup>.

The RBC count significantly increased in rats administered 10 mL/kg of coconut water compared with the control. This indicates that moderate doses of coconut water may stimulate RBC production, likely through mechanisms involving the hydration of cells and improved cellular function<sup>10</sup>. However, this effect was not observed at higher doses, suggesting that increased coconut water consumption does not proportionally enhance RBC production<sup>11</sup>.

# Pharmacologia, 15 (1): 23-28, 2024

The red cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), showed no significant differences between the control and treated groups across all dosage levels. This indicates that while coconut water improves RBC count and volume, it does not substantially affect the size or hemoglobin content per cell. Such findings suggest that the hematological changes induced by coconut water are more quantitative than qualitative <sup>12</sup>.

The effects of different doses (10, 20 and 30 mL/kg) of coconut water on hematological parameters. The most significant changes in hematocrit and hemoglobin levels occurred at the 20 mL/kg dose, which was more effective than 10 and 30 mL/kg. This suggests that 20 mL/kg may be an optimal dose for enhancing erythropoiesis in Wistar rats, corroborating earlier studies that moderate hydration has a greater impact on hematological parameters than either lower or higher fluid intake<sup>13</sup>. The study suggests that Malayan green dwarf hybridized immature or tender coconut water may serve as a natural erythropoietic agent due to its bioactive components. The significant dose-dependent increase in hematocrit, hemoglobin and red blood cell (RBC) count, particularly at 10 and 20 mL/kg dosages, demonstrates its potential for stimulating erythropoiesis. This finding was relevant in contexts where natural alternatives to synthetic erythropoietic agents are desired, such as in the treatment of anemia or enhancing oxygen transport in the body.

Coconut water could be explored as a natural supplement to boost red blood cell production in clinical and nutritional settings. It could be applied in treating anemia, aiding athletes in recovery by improving oxygen transport or used in areas where access to synthetic erythropoietic drugs is limited. Moreover, coconut water's rich phytochemical and phytohormone content might be valuable in developing functional foods or beverages aimed at improving hematological health.

More studies should be conducted to understand the mechanisms behind the erythropoietic effects of coconut water. Additional research should focus on human trials to validate its safety and efficacy. Given the threshold effect observed beyond 20 mL/kg, future studies should fine-tune the dosage and frequency of coconut water administration to maximize benefits without causing potential fluid overload. Coconut water could be developed as a nutraceutical product specifically designed for individuals with anemia or those seeking to enhance oxygen-carrying capacity. Long-term safety studies should be conducted, particularly at higher doses, to rule out any adverse effects such as fluid overload or electrolyte imbalances.

The study was conducted on Wistar rats and while these results are promising, they may not fully translate to humans. Further research is necessary to confirm the findings in human subjects. The study demonstrates a dose-dependent response, but the lack of significant effects at 30 mL/kg suggests a threshold beyond which coconut water may not be effective, possibly due to fluid overload. This limits the range of beneficial doses and requires careful consideration in dosing regimens. While the study shows quantitative changes (increases in RBC count, hematocrit and hemoglobin), there were no significant improvements in red cell indices such as MCV, MCH and MCHC. This indicates that the effect of coconut water may be limited to stimulating the production of RBCs without enhancing their quality or function in terms of hemoglobin content per cell. Higher doses of coconut water may lead to fluid overload, suggesting that excessive consumption could potentially offset the benefits by causing negative physiological effects.

# CONCLUSION

The administration of Malayan green dwarf hybridized immature coconut water at 10 and 20 mL/kg significantly improves hematocrit, hemoglobin levels and RBC count, particularly at 20 mL/kg. However, no significant effects on red cell indices were observed. These findings suggest that moderate doses of

coconut water could enhance erythropoiesis and could potentially be explored for therapeutic applications in anemia management. Further research is required to clarify the mechanisms involved and to establish the clinical relevance of these findings.

### SIGNIFICANCE STATEMENT

This study demonstrates that tender coconut water, rich in bioactive compounds, significantly enhances erythropoiesis in Wistar rats by increasing hematocrit, hemoglobin concentration and RBC count at specific doses. The optimal dose was found to be 20 mL/kg, which maximized erythropoietic effects without further benefit at higher dosages. These findings suggest that coconut water has the potential to be used as a natural supplement for improving hematological health, particularly in promoting red blood cell production and oxygen transport in the body.

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