

Niger. J. Physiol. Sci. 38 (December 2023): 247 – 253 www.njps.physiologicalsociety.com

Full length Research Article

Ameliorative Effects of Coconut Water on Hematological and Lipid Profiles of Phenylhydrazine-treated Rats

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Summary: Low amounts of haemoglobin, or red blood cells, are the hallmark of the disorder known as anaemia, which typically affects expectant mothers and small children. The nutrient-rich coconut water is thought to hasten hematopoiesis or make red blood cells. Finding out how coconut water affected the lipid profiles and haematological indices of rats under phenylhydrazine anaesthesia was the aim of the study. Six groups of thirty rats were created: one for phenylhydrazine-untreated groups, one for standard control, one for iron therapy, one for ferrous treatment, and one for coconut water treatment (0.5 ml/kg). Using a haematology analyzer, the amounts of erythrocytes, hematocrit, and haemoglobin were determined. The results showed that compared to the control group, the anaemia produced by phenylhydrazine had significantly lower levels of TG and LDL and greater levels of HDL. After being fed anaemia, rats who received 0.5 millilitres per kilogramme of coconut water had greater levels of HDL and lower levels of TG, LDL, and VLDL. The study discovered that coconut water raised erythrocyte and HB levels in rats with induced anaemia, indicating a beneficial impact on haematological parameters. These results imply that coconut water can help treat anaemia, significantly enhancing blood coagulation and reducing cholesterol. To completely comprehend the mechanisms underlying these effects and to ascertain the most appropriate dosage and course of treatment, more research is necessary. The advantages of coconut water as a potential anaemia therapy alternative are highlighted throughout the entire study.

Keywords: Anaemia, Hematology, Lipid profiles, hematopoiesis, coconut

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Manuscript received- January 2023; Accepted- August 2023

DOI: https://doi.org/10.54548/njps.v38i2.14

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INTRODUCTION

The most prevalent causes of anaemia are hemoglobinopathies, parasite infections, viral diseases like TB, HIV, and malaria, and dietary deficiencies in folate, vitamins B12, and A. Iron deficiency is one of the most common causes of anaemia. In 2019, anaemia affected 36.5% of pregnant women worldwide, 39.8% of children aged 6 to 59 months and 29.6% of non-pregnant women (WHO, 2021). This substantial percentage requires medical care.

To investigate the mechanism of hemolytic anaemia in rats, phenylhydrazine is utilized (Yeshoda 1942; Berger 1985a). Hemolytic anaemia is brought on by phenylhydrazine, which also causes a variety of cellular changes and the oxidative stress-related death of red blood cells. A model for examining the pathogenesis of hemolytic anaemia, the impact of anaemia on other physiological processes, or the onset of related disorders is provided by PHZ-induced toxic anaemia. The majority of currently prescribed anti-anemic drugs have adverse effects, delaying

the development of effective anti-anemic therapies despite significant investments made over the years. Consequently, in recent years, alternative treatments with negligible or no adverse effects have become increasingly popular. When oxidative stress and the predominance of degenerative diseases are present, foods that naturally contain antioxidants can be employed as a method to minimize morbidity and death (Winarsi, 2007; Astuti, 2008). Coconut water is produced from palm plants, typically grown in tropical settings. It is a naturally occurring and highly healthy beverage. Because coconut water contains various nutrients, including vitamins, minerals, antioxidants, amino acids, enzymes, and growth hormones, it offers therapeutic benefits (Bhagya, 2012). Recent research indicates that coconut water is an excellent source of vitamin C and the free-form amino acid L-arginine, which can reduce the risk of lipid peroxidation and heart disease (John Kennedy et al., 2013). The purpose of this study is to look into how coconut water affects the lipid profiles, antioxidant enzymes, and haematological markers of rats that have been exposed to anaemia caused by phenylhydrazine.

MATERIALS AND METHODS

ADAM equipment South Africa) set includes distilled water, formalin, phenyl-hydrazine, a beaker, Micropipettes, a freezer, a timer, and test tubes.

Test Subjects: Wistar rats weighing between 120 and 150 g were used in this study. The animals were purchased from the animal house at Ekiti State University Ado-Ekiti. When the animals arrived, they were randomly assigned to cages. The chambers in which the animals were housed had a temperature of $24\pm2^{\circ}$ C and a relative humidity of 30-70%.

Approach to feeding: Every animal received regular commercial pelleted rat meal and had unrestricted access to water.

Grouping of Animals: The five groups of five animals each were created by randomly assigning the animals to different groups.

Group 1: Normal control (fed a diet that is 100% standard)

Group 2: Positive Control (given an entirely conventional diet and an anaemic environment)

Group 3: Coconut water plus Phenyl Hydrazine

Group 4: Ferrous + Phenylhydrazine

Ferrous+ normal diet is group five.

Phenyl-Hydrazine Administration: The procedure followed Harris and Kugler's (1971) description. All the rats were given subcutaneous injections of 2.5 per cent neutralized phenylhydrazine hydrochloride at a dose of 10 mg/kg body weight in order to induce anaemia, with the exception of the control rats.

Samples Collection: Animals were put to sleep at the conclusion of the experiment (using diethyl ether), and samples of their organs and blood were taken. Through the use of syringe needles to puncture the conspicuous ear vein, three to four millilitres of blood were drawn from each rabbit and placed into bijou bottles, either with or without EDTA (for serum). To prevent coagulation, 1 millilitre of blood was thoroughly mixed with EDTA before being used for haematological testing. When centrifuging the 4 ml of blood for 10 minutes at 1000 rpm, the serum was recovered when the blood had clothed at room temperature. Up until they were utilized in biochemical tests, serum samples were kept at -20°C.

Haematological Test: Full blood count comprising red cell count, Hb, white cell count and differentials, platelets as well as Hb indices were determined from the remaining whole blood that was placed in EDTA test tubes using ABX Micros 60 Haematology Analyzer (Horiba-ABX, Montpellier, France). Thin blood film was prepared and stained using Leishman stain for morphologic assessment of the red blood cells. The stained films were examined under the light microscope using × 40 objectives to select a good area for examination and then a drop of oil placed on the film and examined with the × 100 objective (Adewoyin et al., 2014).

Biochemical Assay

Estimation of Lipid Profiles: The lipid profile parameters were determined using ELITech chemistry reagents kit from ELITech Group Clinical Systems (Paris, France). The cholesterol reagent kit with product code (SL) was used for cholesterol determination, the HDL-C reagent kit with product code (HDL SL 2G) was used for HDL-C determination and LDL-C precipitation and triglycerides reagent kit product code (MONO SL NEW) was used to determine LDL-C and triglycerides. The instrument used was the Mindray B-300 chemistry analyzer manufactured by Shenzhen Mindray Bio-Medical Electronics Company, Limited. The procedures of work and preparation of the working reagents were done as described by the manufacturer (Ge et al., 2015).

Statistics Analysis: All of the data were evaluated as SEM using ONE-WAY ANOVA and n = 5. Using Version 6 of the Graph Pad Prism.

RESULTS

Figure 1a,1b and 1c show the Changes in serum level of lipid profiles in normal control, anemic control, coconut water treated and ferrous treated phenylhydrazine-induced rats. From the results, the rats treated with phenylhydrazine had significantly lower levels of Low density lipoprotein (figure 1a), Triglycerides (figure 1a), Total cholesterol(figure 1b), very low density lipoprotein(figure 1c), and significantly (P < 0.001) increased in levels of High density lipoprotein (figure 1b) after consuming coconut water at a dose of 0.5 ml/kg body weight for 14days.

Additionally, figure 2a 2b and 2c show the Changes in hematological parameters in normal control, anemic control, coconut water treated and ferrous treated phenylhydrazine-induced rats. From the results, it was observed that the percentage of neutrophil (figure 2a), concentration of hemoglobin (figure 2a), percentage of pack cell volume (figure 2b), percentage of lymphocytes(2b), concentration of reb blood cells(2c) and white blood cells (2c) in subjects treated with coconut water, are significantly (p 0.05) increased compared to the anemic untreated group.

DISCUSSION

Anaemia is characterized by a drop in red blood cell count or an increase in haemoglobin concentration within them, both of which are found to be below normal limits, according to MedlinePlus (2021). Medical care is in high demand due to the illness's widespread prevalence (MedlinePlus, 2021). It has been demonstrated that phenylhydrazine causes hemolytic anaemia by oxidative stress and cellular alterations that result in red blood cell loss (Graf and Graf, 2022). Essential insights into the pathophysiology, effects of anaemia on other physiological processes, and associated disorders can be gained from research on PHZ-induced toxic anaemia (Graf and Graf 2022). In anaemic individuals, there is a favourable correlation between serum cholesterol levels and haemoglobin and hematocrit levels (Osman and Ibrahim, 2021). Moreover, iron-induced free radical damage suggests that anaemia patients may be more vulnerable to ischemic heart disease (Spence and Hegele, 2002).

The study discovered that rats treated with phenylhydrazine had significantly lower levels of LDL, VLDL, TC, and TG and higher levels of HDL after consuming coconut water at

a dose of 0.5 ml/kg body weight per day for 14 days. The results of the present investigation are consistent with those of previous studies by Agbafor et al. (2015), Agbafor et al. (2013), Mohammed (Mohammed & Luka 2013), Sandhya et al. (2006), and John Kennedy et al. (2013).

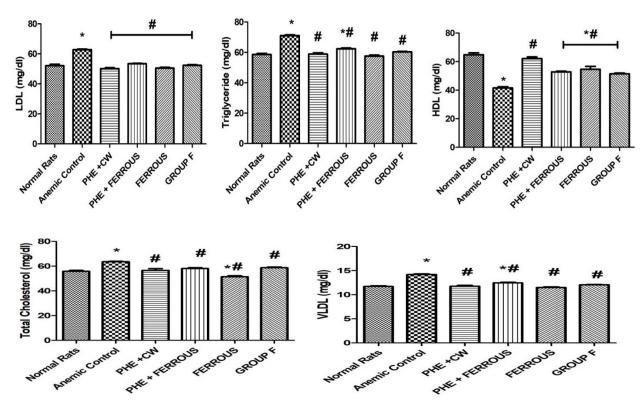


Figure 1: Effects of coconut water on lipid profiles of phenylhydrazine-induced anemia

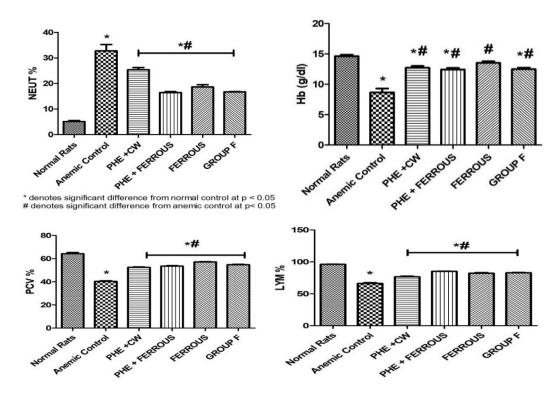


Figure 2a: Effects of coconut water on hematological indices of phenylhydrazine-induced rats

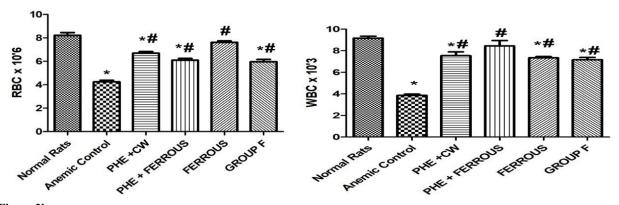


Figure 2b: Effects of coconut water on hematological indices of phenylhydrazine-induced rats

In coconut water, the free amino acids L arginine, ascorbic acid, calcium, magnesium, and potassium all have physiologically active properties and mav chemoprotective effects. Rats fed a diet high in fat and cholesterol showed similar hypolipidemic responses to coconut water and the cholesterol-lowering medication lovastatin (Reddy & Lakshmi 2014). Additionally, vitamin C from coconut water protects HDL cholesterol and helps with the reverse cholesterol transport process, according to McRae's (2008) research. This study discovered that the anaemic rats given coconut water had a considerably lower TG level than the control group. Triglycerides, a kind of fat in the blood, can also increase the risk of heart disease. Given that studies have shown that vitamin C in coconut water boosts intracellular tetrahydrobiopterin (BH4) and NOS activity, this may be related to the vitamin (Sandhya & Rajamohan, 2008).

The findings of this study demonstrate that giving coconut water at a dose of 0.5 ml/200g reduces TG and LDL levels while raising HDL in rats with hydrazine-induced anaemia. Additionally, after receiving coconut water, the experimental groups' haemoglobin values significantly (p 0.05) increased compared to the anaemic untreated group (PHE). PCV, RBC, HB, and WBC all experienced significant increases in values. When rats were given an ethanol leaf extract of yellow mombin, similar outcomes were observed (Asuquo 2013). However, WBC levelscomposed of neutrophils, monocytes, eosinophils, basophils, and lymphocytes—were significantly lower in the anemia-induced group (p 0.05). Indicators of the immune system's response to infection include white blood cells, lymphocytes, and neutrophils; it was discovered that the injection of coconut water significantly decreased these values. Murray (2000) noted that some substances could cause blood cells to synthesize more quickly, indicating that the water may include one or more substances that interact to enhance the production and release of committed stem cells, hematopoietic growth factors, and erythropoietin. However, the finding that the haematological parameter increased in this study is consistent with the findings of Agbafor et al. (2015), which showed that coconut water elevated the haematological parameter in animals. Ascorbic acid, calcium, magnesium, potassium, and the free amino acid L arginine have all been present in coconut water (Sandhya & Rajamohan, 2006; John Kennedy et al., 2013).

These nutrients serve as chemoprotective agents and have a direct impact on the production of bone marrow blood.

In summary, the findings of this investigation suggest that coconut water can prevent anaemia, which could be ascribed to a bioactive component. Furthermore, the coconut water's antioxidant action might be connected to its hematopoietic potential. As a result, the current study backs up the traditional medicine's medicinal use of coconut water to cure anaemia.

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