INFLUENCE OF COMBRETTUM NIGRICANS HOT AQUEOUS EXTRACTS ON PHENYLHADRAZINE-INDUCED HEMOLYTIC ANAEMIA IN WISTAR RATS


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Abstract: Anaemia is a major health issue that affects many people, particularly in developing nations. In some cases of anaemia, rural residents in the tropics have to rely on traditional remedies. The goal of this study was to determine Combretum nigricans’s haematogenic capabilities. Using phenylhydrazine (PHZ)-induced anaemia in rats, the aqueous extract (AE) of the bark was tested for haematogenic effects. A total of twenty-eight (28) wistar albino rats of both sexes, weighing between 150 – 250g, were used for the experiment. Rats were randomly divided into seven groups (n = 4/group). Group I, served as the normal control (not infected) received the vehicle (10 ml/kg; Tween 20). Group II, served as positive control (infected but not treated) received no treatment, while groups III, IV, V, VI and VII received 100, 200, 300, 400 and 500 mg/kg of the aqueous extract (AE) respectively daily for two (2) weeks. As markers of anaemia, blood parameters such as red blood cell (RBC) count and hemoglobin concentration (Hb) were measured. The results showed that oral treatment of AE (100–500 mg/kg/day) had a significant (P 0.05) haematogenic effect by reducing the PHZ-induced drop in blood parameters such as Hb, PCV, and RBC. The potency of haematogenicity was shown to be dose-dependent. These findings support the existence of haematogenic components in C. nigricans bark and therefore can be used to maintain physiologically healthy RBC and PVC during anaemic episodes.

Keywords: Combretum nigricans haematogenic, Anaemia, Phenylhydrazine, Hemoglobin, Blood parameters, rats

INTRODUCTION

Phytomedicine has globally been used as alternative to synthetic drugs in the treatment of many diseases such as anaemia, which is a condition characterized by a defined number of red blood cells (RBCs) and haemoglobin in the blood below the normal (Okorie et al., 2020). Despite all recent health-related improvements, anaemia remains an extensive global public health issue affecting the lives of about one-fourth of the world population in a geographically heterogeneous pattern. In this research we aim at exploring the phytomedical potential of as a solution to the prevalence, severity, most common types of anaemia using wistar rat as experimental animals. This is generally accompanied by decreased haemoglobin concentrations and altered RBC shape, leading to reduced oxygen flow to the body’s organs. Prevalence of this diseases is found in underdeveloped and developing countries (Manikanddaselvi et al., 2015). According to a recent research conducted by Moll and Davis (2017), there are other causes of anaemia such as non-access to balance diet, folate and Vitamin B12 deficiencies, chronic inflammation and inherited disorders. Anaemia has been considered to be among the important contributing factors to the global burden of diseases (Gadaga et al., 2009). Although, there are variety of drugs used for the treatment of anaemia, but they are not affordable to numerous poor people in developing countries especially the Sub-Saharan Africa. More so, the rural communities in different parts of the world do not have access to quality drugs for the treatment of anaemia, so rely more on plant products for the treatment of anaemia. Since there is increase in anaemia there should also be a serious desire to establish preventive and curative plans too for the disease. Symptoms may include; weariness, chest tightness or pain, shortness of breath, and elevated heart rate (Schümann & Solomons, 2017; Jin et al., 2019; Dou et al., 2021. According to World Health

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Organization (WHO) report (1968), anaemia is commonly caused by nutritional deficiencies like iron, infectious disease such as HIV, Malaria and other parasitic infections. Anaemia is therefore defined as a drop in the amount of circulating hemoglobin in the blood from normal levels to less than 13 g/dl for males and less than 12 g/dl for females (Akah et al., 2010). In a recent epidemiological research report, the World Health Organization estimated that 2 billion individuals were anaemic globally in 2004, accounting for 30% of the global population (WHO, 1968; Elham et al, 2022). Additionally, over 50% of pregnant women and 40% of infants globally are anaemic, resulting in a high rate of morbidity and mortality, especially in underdeveloped countries (Adusi-Poku et al., 2008; Akah et al., 2010; Okorie et al., 2020). As a result, anaemia has become one of the diseases of global public health concerns.

Approximately 80% of the world human population coming from developing nations rely on traditional medicine or therapeutic herbs for primary health care to cure and manage diseases complementary to conventional medicine (Mukherjee, 2002; Bodeker & Ong, 2005; Bandaranayake, 2006; Al-Ani et al., 2018). Therefore, medicinal herbs still provide excellent health protection to a vast percentage of humanity. The use of herbal remedies has also been widely accepted in many developed countries with complementary and alternative medicine (Calapai, 2008; Braun et al., 2010) thus, represent a substantial proportion of the global drug market (Shukla & Cheryan, 2003; Bodeker & Ong, 2005; World Health Organization, 2005). However, Medicinal herbs are being utilized to cure various forms of anemia in various parts of the world, particularly in the tropics. Today about 50 drugs came from tropical plants (Gurib-Fakim, 2006).

Combretum nigricans is one of tropical medicinal plant with promising therapeutic potential from the Combretaceae family. This plant is characterized by simple broad leaves, smooth bark with an average height up to 4.1 m and self-supporting growth form. This plant is mostly grown in North-Central Nigeria and other parts of the world. Combretum nigricans has been used to cure malaria fever for centuries (Enegide et al., 2021). The seed has been shown to have hypotensive and Central Nervous System (CNS) depressive properties, as well as anti-leishmaniasis properties. Antifungal and cytotoxic effects have also been found for the plant (Enegide et al., 2021; Waterman et al., 2010).

Anemia is defined by a significant drop in red blood cell count, which invariably results in low PCV. Knowing how important red blood cells are to the body’s overall functioning, this is certainly a matter for concern. In view of the foregoing, the goal of this research was to evaluate C. nigricans’ erythropoietic properties as a potential alternative therapy for anemia. To the best of our knowledge as at the time this research was carried out, there was limited information on the promising medicinal potential of C. nigricans as well as its anti-anemic potential. Therefore, it is important to investigate the anti-anemic action of C. nigricans crude extract on anemic induced rats to establish its medicinal potential.

MATERIALS AND METHODS

The bark of C. nigricans was collected from a garden in Mangu Halle of Mangu Local Government Area of Plateau State Northern Nigeria. Fresh Combretum nigricans bark was cleaned with water to remove debris and dirt particles, air dried, and pounded into powder with a pestle and mortar. At room temperature, 200 grams of powdered plant material were macerated in 1000 milliliters of distilled water. To assist extraction, the mixture was left to stand for 48 hours while being agitated intermittently with a glass rod. A muslin cloth with a pore size of 2 mm was used to filter the solution. The filtrate was then filtered again using Whatman filter paper (No. 42) before being decreased in volume using a rotary evaporator set to 40°C. A controlled water bath at 40°C was used to finish the solvent removal and drying process. The resulting crude extract was put in a bottle and stored at 4°C for further and subsequent analysis.

A total of twenty-eight (28) wistar albino rats of both sex, weighing between 150 – 250g, were
used for the experiment. The rats were purchased from the animal house livestock investment Department (LID), National Veterinary and Research Institute (NVRI), Jos, Nigeria. The animals were kept in well-aerated laboratory cages and allowed to acclimatize to the laboratory environment for two weeks before the commencement of the experiment. They were fed, with standard feed (Vital Feeds, Jos, Nigeria) and water was provided ad libitum.

Anaemia was induced in rats via daily oral administration of phenyl hydrazine (PHZ, 60 mg/kg dissolved in Tween 20 as PHZ is sparingly soluble in water) for 7 days while the control group received normal saline. Blood samples were collected from the rat by an ocular puncture on the 7th day of treatment for hematological analysis. Rats that developed anaemia with hemoglobin concentrations lower than 13g/dl were recruited for the study.

Experimental rats were randomly divided into seven groups (n = 4/group). Group I, which was the normal control (Not infected) received the vehicle (10 ml/kg; Tween 20). Group II, which was the positive control (Infected but not treated) received no treatment, while groups III, IV, V, VI and VII received 100, 200, 300, 400 and 500 mg/kg of the aqueous extract (AE) respectively daily for 2 weeks.

RESULTS AND DISCUSSION

Effect of *C. nigricans* on Hb, RBC and PCV in PHZ-induced anaemic animals

Induction of anemia in experimental animals resulted in significant decreases in the levels of most erythropoietic markers when compared to control animals. The red blood cell count (RBC), haemoglobin concentration (Hb), and packed cell volume (PCV) were all reduced significantly (P< 0.05). Treatment of anemic mice with *C. nigricans* at doses of 100, 200, 300, 400, and 500 mg/kg BW dramatically improved RBC, Hb, and PCV levels, with 500 mg/kg BW significantly improving blood parameters to near normal values (Figures 1, 2 and 3).

After two weeks of treatment, aqueous extract (AE) of *C. nigricans* restored PHZ-induced anemia in rats, with blood indices returning to normal. Up to two weeks after treatment, the outcome was dosage dependant. The concentrations of hemoglobin (Hb), red blood cells (RBCs), and packed cell volume (PCV) gradually increased (Figures 1 - 3). At the doses examined, the increment in Hb, RBC, and PCV was significant (P< 0.05) when compared to the control group (Group 1).

![Figure 1. Effects of AE on PCV of PHZ-induced anaemic rats. Data were significant (P < 0.05) vs. control after 2 weeks of treatment](image-url)
Antibiotics are potent drugs that combat infections and, when taken correctly, can save lives. These drug agents function by either preventing bacteria proliferation (bacteriostatic) or killing them (bactericidal), and are often administered for 7 to 10 days or longer. Their clinical application, however, is frequently linked to red blood cell lyses (Garratty, 2012; Giguère et al., 2013; Ighodaro et al., 2020). On the other hand, phenyl hydrazine (PHZ) has been shown to induce hemolytic anemia in rats when supplied parenterally or orally by lowering the concentrations of blood parameters such as Hb, WBC, RBC, and PCV (Jensen et al., 1995; Pandey et al., 2014). This hypothesis is supported by the findings of this study, which found that giving phenylhydrazine (PHZ) to experimental rats at a dose of 60 mg/kg BW resulted in a significant (P<0.05) drop in erythropoietic indices such as red blood cells (RBC), packed cell volume (PCV), and haemoglobin (Hb). However, oral administration of PHZ in rats cause oxidative stress, by generating reactive oxygen species (ROS) in red blood cells thus, leading to cell lyses (Valenzuela et al., 1977). Moreover, haemolysis of RBC by PHZ is said to modify iron metabolism by increasing ferrous transportation express in the spleen, liver and duodenum (Marsh & Koenig, 1982; Augstine OKuna 1982). Giving the significant roles of these molecules in the process of erythropoiesis, the ability of C. nigricans extract to enhance blood levels of main erythropoietic indicators (RBC, PCV, and Hb) in treated-anemic rats is of major pharmacological value. Previous phytochemical screenings of C. nigricans revealed the presence of bioactive phytoconstituents such as saponins, tannins, flavonoids, alkaloids, cardiac glycosides, terpenoids, phenols, and sterols, which may be responsible for the plant's haematopoietic activities (Amaeze et al., 2011; Ayoola et al., 2011). Flavonoids taken orally have been shown to prevent pulmonary bleeding and reduce vascular permeability (Tan et al., 2000). Anti-anemic properties have been documented for saponins and alkaloids. Herbs that contain saponin have been shown to improve blood circulation (Ighodaro et al., 2020; Shi et al., 2004). On this basis, the erythropoietic capabilities of C. nigricans in this study could be attributed to its phytoconstituents, which are well-known haematopoietic agents that regulate the generation of red blood cells directly or indirectly. In response to hemolysis, cellular repair mechanisms are also
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stimulated by the bone marrow through iron metabolism pathways (Marsh & Koenig, 1982; OKuna 1982). According to (Dholi, n.d.), the administration of plants extract stimulates the stem cell line in producing new blood cells through erythropoiesis by countering the activity of PHZ (Naman et al., 2020; Suzanne et al., 2020). However, it can be deduced from this study that; the anti-anemic property of C. nigricans is closely link to its anti-oxidants compounds such as phenol, saponin and flavonoids. Because antioxidants serve as hydrogen or proton donor by stabilizing and delocalizing unpaired electron, chelate with transition metals to terminate the Fenton reaction with prooxidant (Gemede, 2014). Therefore, it can be inferred from this study that; C. nigricans possibly improves erythropoiesis primarily through its potential to improve hemoglobin synthesis or blood availability, based on the level of influence of the plant extract on the several examined markers (Hb).

CONCLUSION
Finally, aqueous extracts of C. nigricans bark corrected anemia caused by phenylhydrazine in rats, suggesting that this could be effective in maintaining physiologically healthy red blood cell counts and packed cell volume (PCV) during any anemic condition.

REFERENCES


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