*American Journal of Open Research, 2(2), February, 2023, 145-151*
*E-ISSN:* 2834-7668

# INFLUENCE OF *COMBRETUM NIGRICANS* HOT AQUEOUS EXTRACTS ON PHENYLHADRAZINE- INDUCED HEMOLYTIC ANAEMIA IN WISTAR RATS

J. G. Nangbes [1*], M. D. Manje [2], P. C. Igeh [3], B. Jonathan[2], F. O. Okonkwo[2], L. A. Barde [2]

[1]*Department of Chemistry, Plateau State University, Bokkos, P.M.B. 2012 Jos. Plateau State Nigeria*
[2]*Department of Biochemistry, Plateau State University, Bokkos, P.M.B. 2012 Jos. Plateau State Nigeria*
[3]*Department of Microbiology, Plateau State University, Bokkos, P.M.B. 2012 Jos. Plateau State Nigeria*
*e-mail: nangbesjg@gmail.com*

*Corresponding Author

***Abstract:*** *Anaemia is a major health issue that affects many people, particularly in developing nations. In some cases of anemia, rural residents in the tropics have to rely on traditional remedies. The goal of this study was to determine Combretum nigricans's hematinic capabilities. Using phenylhydrazine (PHZ)-induced anemia in rats, the aqueous extract (AE) of the bark was tested for haematinic 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 anemia, 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) haematinic effect by reducing the PHZ-induced drop in blood parameters such as Hb, PCV, and RBC. The potency of haematinicity was shown to be dose-dependent. These findings support the existence of haematinic components in C. nigricans bark and therefore can be used to maintain physiologically healthy RBC and PVC during anaemic episodes.*

***Keywords:*** *Combretum nigricans hematinic, 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, anemia 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 anemia using wistar rat as experimental animals. This is generally accompanied by decreased hemoglobin 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

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).

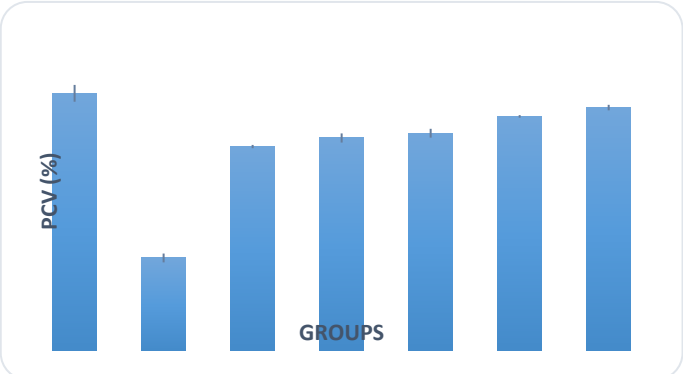


**Figure 1.** Effects of AE on PCV of PHZ - induced anaemic rats. Data were significant ($P < 0.05$) vs. control after 2 weeks of treatment

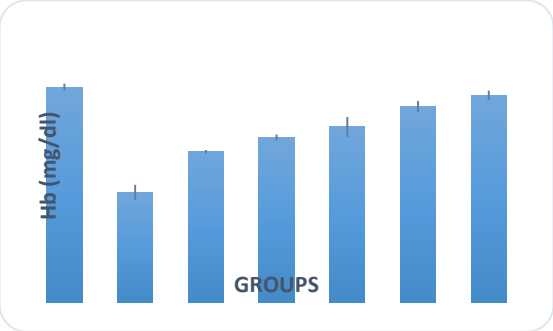


**Figure 2.** Effects of AE on hemoglobin concentrations of PHZ – induced anaemic rats. All values were significant (P < 0.05) vs. control after weeks of treatment.

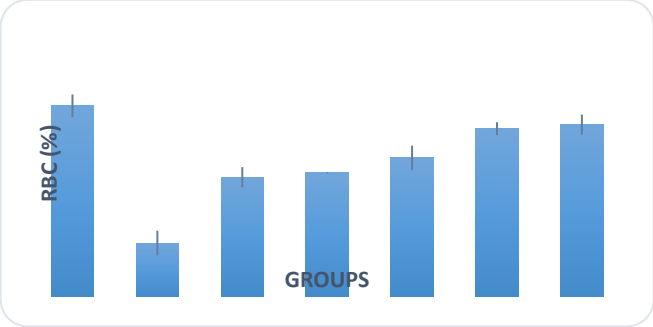


**Figure 3.** Effects of AE on RBC of PHZ – induced anaemic rats. Data were significant (P<0.05) Vs. control from 2weeks of treatment.

Antibiotics are potent drugs that combat infections and, when taken correctly, can save lives. Theses 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 parentally 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 alkaoids. 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

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 erythropoesis 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

Adusi-Poku, Y., Sittie, A., Mensah, M. L., Sarpong, K., Fleischer, T. C., Ankrah, T. C., & Nsiah, D. (2008). Effectiveness and safety assessment of mist tonica, a herbal haematinic. *African Journal of Traditional, Complementary and Alternative Medicines*, **5**(2), 115–119.

Akah, P., Okolo, C., Okoye, T., & Offiah, N. (2010). Aqueous extract and methanol fractions of the leaves of Brillantaisia nitens Lindau reverses phenylhydrazine–induced anaemia in rats. *Journal of Medicinal Plants Research*, **4**(3), 271–277.

Al-Ani, I., Zimmermann, S., Reichling, J., & Wink, M. (2018). Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. *Medicines*, **5**(1), 2.

Amaeze, O., Ayoola, G., Sofidiya, M., Adepoju-Bello, A., Adegoke, A., & Coker, H. (2011). Evaluation of antioxidant activity of Tetracarpidium conophorum (Müll. Arg) Hutch & Dalziel leaves. *Oxidative Medicine and Cellular Longevity*, *2011*.

Bandaranayake, W. M. (2006). Quality control, screening, toxicity, and regulation of herbal drugs. *Modern Phytomedicine: Turning Medicinal Plants into Drugs*, 25–57.

Bodeker, G., & Ong, C.-K. (2005). *WHO global atlas of traditional, complementary and alternative medicine* (Vol. 1). World Health Organization.

Braun, L. A., Tiralongo, E., Wilkinson, J. M., Spitzer, O., Bailey, M., Poole, S., & Dooley, M. (2010). Perceptions, use and attitudes of pharmacy customers on complementary medicines and pharmacy practice. *BMC Complementary and Alternative Medicine*, **10**(1), 1–7.

Calapai, G. (2008). European legislation on herbal medicines. *Drug Safety*, **31**(5), 428–431.

Dholi, S. K. (n.d.). *International Journal of Modern Pharmaceutical Research*.

Dou, L., Gong, X., Wu, Q., & Mou, F. (2021). Therapeutic effects of Sheng Xue Fang in a cyclophosphamide-induced anaemia mouse model. *Pharmaceutical Biology*, **59**(1), 787–796.

Elham, A., Yousef, P., Zahra, M., Ali, M., Leila, D., Farhad, A., Shima, A., Zahra, R., Mohammad, Z., Bahman, C., Hossein, P. and Ali-Akbar, S. (2022). Anemia prevalence, severity, types, and correlates among adult women and men in a multiethnic Iranian population: the Khuzestan Comprehensive Health Study (KCHS); *BMC Public Health* 22:168 1 - 13

Enegide, C., Akah, P., Ofili, C. C., Agatemor, U. M., Ameh, S. F., Dabum, J. L., & Onah, I. A. (2021). Evidence supporting the use of Combretum nigricans as an antimalarial agent in Ethnomedicine. *International Journal of Current Research in Physiology and Pharmacology*, 13–20.

Gadaga TH, Madzina RR, Nembaware N. (2009). Status of micronutrients nutrition in Zimbabwe: A review. *African Journal of Food, Agriculture, Nutrition and Development*; **9**(1):502- 522

Garratty, G. (2012). Immune hemolytic anemia caused by drugs. *Expert Opinion on Drug Safety*, **11**(4), 635–642.

Gemede, H. F. (2014). Potential health benefits and adverse effects associated with phytate in foods: A review. *Glob J Med Res*, *27*, 2224–6088.

Giguère, S., Prescott, J. F., & Dowling, P. M. (2013). *Antimicrobial therapy in veterinary medicine*. John Wiley & Sons.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, **27**(1), 1–93.

Ighodaro, O., Asejeje, F., Adeosun, A., Ujomu, T., Adesina, F., & Bolaji, K. (2020). Erythropoietic potential of Parquetina nigrescens in cephalosporin-induced anaemia model. *Metabolism Open*, *8*, 100064.

Jensen, T. J., Loo, M. A., Pind, S., Williams, D. B., Goldberg, A. L., & Riordan, J. R. (1995). Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. *Cell*, **83**(1), 129–135.

Jin, Y., Meng, X., Liu, S., Yuan, J., Guo, H., Xu, L., & Xu, Q. (2019). Prevalence trend and risk factors for anemia among patients with human immunodeficiency virus infection receiving antiretroviral therapy in rural China. *Journal of Traditional Chinese Medicine= Chung i Tsa Chih Ying Wen Pan*, **39**(1), 111–117.

Manikandaselvi, S., Raj, D. C., Aravind, S., Ravikumar, R., Thinagarbabu, R., Nandhini, S. (2015). Anti-anemic activity of sprouts of Vigna radiata L. in male albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. **7**(11):263-267.

Marsh, W. L., & Koenig, H. M. (1982). The laboratory evaluation of microcytic red blood cells. *CRC Critical Reviews in Clinical Laboratory Sciences*, **16**(3), 195–254.

Moll R, Davis B. (2017). Iron, vitamin B12 and folate. *Medicine*; 45: 198-203.

Mukherjee, P. K. (2002). Problems and prospects for good manufacturing practice for herbal drugs in Indian systems of medicine. *Drug Information Journal: DIJ/Drug Information Association*, **36**(3), 635–644.

Naman, K., Oseni, H., & Enoh, E. (2020). Anti-Anaemic Potential of Methanolic Leaf Extract of *Mucuna Pruriens* on Phenylhydrazine (Phz) Induced Anaemic Albino Wistar Rats. *Fudma Journal of Sciences*, **4**(3), 370–374.

Okorie, A. U., Akuodor, G. C., Aja, D. O. J., Akpan, J. L., Chilaka, J. U., Ezeokpo, B. C., Obiora, E. O. (2020).The effect of Justicia insularis ethanol leaf extract on haematological parameters in Phenylhydrazine-induced anaemic Wistar rats; *Journal of Complementary Medicine Research*, **11(**1) 109 - 116

Pandey, K., Meena, A. K., Jain, A., & Singh, R. (2014). Molecular mechanism of phenylhydrazine induced haematotoxicity: A review. *American Journal of Phytomedicine Clinical Therapeutics*, ***2***(3), 390–394.

Schümann, K., & Solomons, N. W. (2017). Perspective: What makes it so difficult to mitigate worldwide anemia prevalence? *Advances in Nutrition*, **8**(3), 401–408.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from edible legumes: Chemistry, processing, and health benefits. *Journal of Medicinal Food*, **7**(1), 67–78.

Shukla, R., & Cheryan, M. (2003). Stability and performance of ultrafiltration membranes in aqueous ethanol. *Separation Science and Technology*, **38**(7), 1533–1547.

Suzanne, B. B., Adeline, F. Y., Theodora, K. K., Dairou, H., Pradel, K. L., Aristide, K. M., Mathieu, N., Gabriel, A. A., Anne, N. N., & Clerge, T. (2020). Hemopoietic effects of some herbal extracts used in treatment of infantile anemia in Cameroon. *World Journal of Pharmaceutical Research*, **6**(1), 147–155.

Tan, D.-X., Manchester, L. C., Reiter, R. J., Qi, W.-B., Karbownik, M., & Calvo, J. R. (2000). Significance of melatonin in antioxidative defense system: Reactions and products. *Neurosignals*, **9**(3–4), 137–159.

Valenzuela, A., Rios, H., & Neiman, G. (1977). Evidence that superoxide radicals are involved in the hemolytic mechanism of phenylhydrazine. *Experientia*, **33**(7), 962–963.

Waterman, C., Smith, R. A., Pontiggia, L., & DerMarderosian, A. (2010). Anthelmintic screening of Sub-Saharan African plants used in traditional medicine. *Journal of Ethnopharmacology*, **127**(3), 755–759.

World Health Organization. (1968).Nutritional anaemias. Report of a WHO scientific group. Geneva,

World Health Organization, (WHO Technical Report Series, No. 405). Available at http://whqlibdoc.who.int/trs/WHO_TRS_405

World Health Organization. (2005). *Global tuberculosis control: Surveillance, planning, financing: WHO report 2005*. World Health Organization.