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Full Length Research Paper

Anti-anaemic effect of methanol seed extract of *Sphenostylis stenocarpa* (African yam bean) in Wistar albino rats

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The African yam bean, *Sphenostylis stenocarpa* (Hoschst ex. A. Rich) Harms, Fabaceae, is one of the edible legumes cultivated in Africa for human and animal nutrition. The acute toxicity (LD_{50}) test and haematological parameters of anaemic rats treated with the methanol extract of the plant seeds were investigated for days 0, 4 and 8. The extract was found not to be toxic up to 5000 mg/kg, indicating the safety of the plant for both human and animal consumption. The effects of the oral administration of the extract in anaemic rats at 100, 200 and 400 mg/kg body weight showed a significant increase ($p < 0.05$) in the packed cell volume (PCV), red blood cells (RBC) and haemoglobin concentrations of rats after the fourth day of treatment with the extract. The increase was comparable with a standard drug, Ranferon, used in the treatment of anaemia. There were no significant ($p > 0.05$) difference in the packed cell volume (PCV), red blood cells (RBC) and haemoglobin concentrations of the rats after the 8th day of treatment. The results revealed that the extract administration in rats restored the anaemic conditions in the treatment groups thus giving credence to the use of the plant in the management of anaemia.

Key words: Toxicity, *Sphenostylis stenocarpa*, aanaemia, methanol seed extract.

INTRODUCTION

Medicinal plants have served through the ages as the mainstay in the treatment of diseases and preservation of human and animal health (Yakubu et al., 2009). Plants have provided a good source of anti-infective agents and phyto-medicines that have shown great promise in the treatment and management of numerous kinds of infections and diseases (Iwu et al., 1999). Most people in rural areas utilize these plants to bring about cure and relief to disease conditions with little or no knowledge about the safety and toxicity of such plants.

Anaemia is a worldwide problem that is highly prevalent in developing regions of the world (Talata et al., 1998). It is a condition in which the oxygen carrying capacity of the blood is reduced, indicating a sign of an underlying disease (Ochei and Kolhatkar, 2008). It has been a major public-health problem affecting a greater percentage of the world's population (Allen, 2000; Haidar, 2010). Anaemia can result from non-nutritional factors, such as haemorrhage, infections, chronic disease states, or drug toxicity, and from nutritional factors, including deficiencies of iron, certain vitamins, copper and protein (Zhang et al., 2003; Haidar, 2010). Anaemia could be hemolytic or haemorrhagic depending on its etiology. The causes of haemolytic and haemorrhagic anaemia are found in the erythrocytes themselves or in the peripheral blood rather than in the bone marrow (Dancie and Lewis, 1991). This indicates that the are regenerative due to a compensatory increase in erythropoiesis in the bone marrow

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(McGrath, 1993). African yam bean (*Sphenostylis stenocarpa* Hoschst ex. A. Rich, Harms, Fabaceae) is an herbaceous leguminous plant occurring throughout tropical Africa and some parts of the world (Porter and Doyle, 1992). It is an underutilized legume and its seeds are potential sources of supplement in human and ruminant livestock diet (Ajayi et al., 2009). The whole seed is rich in potassium and phosphorus (Oshodi et al., 1995). The seeds are also rich in magnesium, calcium, iron and zinc but low in sodium and copper (Edem et al., 1990). The seed has high protein content, which is used in supplementing the protein requirements of families (Onyeike et al., 2002). The amino acid profile of the seed showed that the seed contain lysine and methionine, which are some of the limiting amino acids in most vegetable seed proteins (Evans and Heismer, 1979). The seeds have been shown to have anti-sickling effect on sickled haemoglobin due to its high content of hydrophobic amino acids (Nwaoguikpe, 2008).

In many developing countries, herbal medicines are assuming greater importance in primary health care, and their international trade has increased (Oladiji et al., 2007). The seeds of “Ijiriji” or “Azama” as are popularly called within many localities in South Eastern Nigeria, are claimed by a reasonable number of the inhabitants to cure anaemia. This study was carried out to give scientific evidence to the claim that the plant is used in the treatment of aneamia in folklore medicine.

MATERIALS AND METHODS

Experimental animals

Forty eight male albino Wistar rats (*Rattus novergicus*) weighing between 180 to 220 g were obtained from the Animal House of the Faculty of Biological Sciences, University of Nigeria, Nsukka and were used in the study. The animals were housed individually in steel metabolic cages of dimensions 33 × 20.5 × 19 cm, with cleaning of the cages done once daily. They were acclimatized for one week before the commencement of the study. All through the study, they were fed *ad libitum* on standard pellet feed (Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and freely provided drinking water. The rats were divided into six groups (n = 8). Group 1 received 1 ml of normal saline without induction of anaemia and was designated as normal anaemic control (NAC), group 2 received 20 mg/kg of Ranferon with induction of anaemia, designated as Ranferon (treated with a standard drug, Ranferon); group 3 received 1 ml of normal saline with induction of anaemia and was regarded as the anaemic control (AC); group 4 received 100 mg/kg of methanol extract (ME 100); group 5 received 200 mg/kg of methanol extract (ME 200); and group 6 received 400 mg/kg of methanol extract (ME 400).

Plant

Dried seeds of *S. stenocarpa* were purchased from Orba market in Nsukka Local Government Area, Enugu State. The seeds were authenticated by Mr Alfred Ozioko at the Biodiversity Centre, Nsukka, Enugu State.

Table 1. Phase 1 and II of the acute toxicity test of methanol seed extract of *Sphenostylis stenocarpa.*

Parameter	Dosage (mg/kg) body weight	Mortality
Phase I		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/1
Group 2	2900	0/1
Group 3	5000	0/1

The acute toxicity test of the seeds of *S. stenocarpa* showed no death up to 5000 mg/kg body weight. The LD_{50} was found to be above 5000 mg/kg body weight

Preparation of methanol seed extract of *S. stenocarpa*

The seeds were pulverized to powder with a hand Mill. About 500 g of the pulverized seeds was macerated in 1.5 L of methanol with thorough shaking at regular intervals for 24 h at room temperature (26 to 28°C). The resulting solution was then filtered using Whatman No. 1 filter paper. A constant weight of the extract was obtained by evaporating the solvent at 60°C in an oven. The extractive weight of the extract is 18 g.

Acute toxicity (LD_{50}) test

The method of Lorke (1983) was used for the acute toxicity test. Thirteen (13) albino mice were utilized in this study. The test involved two stages. In stage one; the animals were grouped into three (3) different groups of three rats each. They were administered 10, 100 and 1000 mg/kg body weight, respectively and in the second stage, 1600, 2900 and 5000 mg/kg body weight of the extract were administered to the animals. The administration of the extract was done orally. The methanol extract of *S. stenocarpa* seeds were found not to be toxic up to 5000 mg/kg (Table 1).

Induction of anaemia

The baseline haematological parameters were assayed on the rats before the induction of anaemia. Anaemia was induced according to the method described by Stone (1954). About 1 ml of blood was removed from each rat daily for about 5 days until the rats became anaemic. Anaemia was induced in all the groups except Group 1 (Normal Anaemic Control) and their haematological parameters determined after the induction of anaemia to ascertain the level of anaemia in the rats. The following haematological parameters-packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and differential white blood cell count were assayed after 4 and 8 days of treatment with the extract using the methods described by Ochei and Kolhatkar (2008).

Statistical analysis

Data were mean of 3 replicates ± standard deviation (SD). Statistical analysis was carried out using statistical package for Social

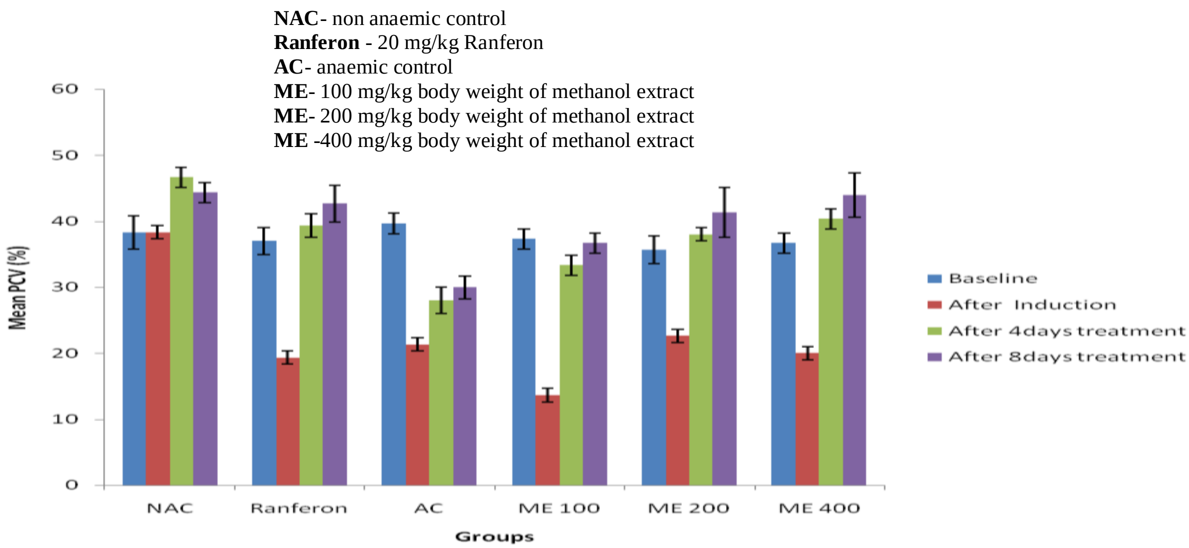

Figure 1. Effect of extract on packed cell volume (PCV). The figure shows the mean PCV values of rats administered different doses of methanol extract of *S. stenocarpa* seeds. A significant increase ($p < 0.05$) was observed in the mean PCV values of groups 3, 4 and 5 after 4 days of treatment with the plant extract. There was no significant difference ($p > 0.05$) after the 8th day of treatment.

Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean ± standard deviation. P < 0.05 was considered significant.

RESULTS

Effect of extract on packed cell volume (PCV)

The methanol seed extract at 100, 200 and 400 mg/kg body weight (ME 100, 200 and 400) significantly ($p < 0.05$) increased the PCV of the experimental (treated) anaemic rats. The increase was non-dose related but was more pronounced after the initial four days of treatment (Figure 1). The PCV of the animals in the non-anaemic control (NAC) group was modestly increased. Further treatment beyond day 4 did not show significant ($p >0.05$) increase in the PCV of all the treatment groups (Figure 1). The increase in the PCV was not significant after the 8th day of treatment.

Effect of extract on cell counts

Administration of the plant seed extract showed a significant ($p < 0.05$) increase in the RBC counts of the treated anaemic rats (Figure 2).The increase in the RBC count of the experimental groups treated with 100, 200 and 400 mg/kg body weight of methanol extract (ME 100, 200 and 400) were found to be in a dose-dependent manner during the first four (4) days of treatment. This increase continued after the 8th day of treatment. The RBC counts of the animals in the experimental groups after the 8th day of treatment did not significantly ($p > 0.05$) increase compared with those observed after the initial 4 days of treatment. The RBC counts of the anaemic control (AC) group was found to be increased also, but this increase in the RBC counts in the AC group showed a lesser effect compared to those of the experimental (ME 100, 200 and 400) and the Ranferon groups (Figure 2).

The WBC counts of the animals in the groups ME 100, 200 and 400 showed no significant ($p > 0.05$) increase during the first four (4) days of treatment (Figure 4). A decrease in the WBC counts of the animals was observed after the 8th day of treatment. The decrease was also not significant ($p > 0.05$). These patterns were seen in both the experimental groups and the AC group. However, a significant ($p < 0.05$) increase was observed in the Ranferon group, the group treated with a standard drug, during the first four (4) days of treatment which was observed to be constant after the 8th day of treatment.

The plant extract produced significant alterations in the neutrophil and lymphocyte counts (Figures 5 and 6). The neutrophils significantly ($p < 0.05$) increased during the first four days of administration with the extract with the exception of ME 200 (treated with 200 mg/kg body weight of methanol extract) group which showed no significant ($p < 0.05$) decrease in the neutrophil counts. Extract administration for eight days at 100 mg/kg body weight (ME 100) resulted in a significant ($p < 0.05$) increase in the neutrophil counts (Figure 5). The effect of the extract

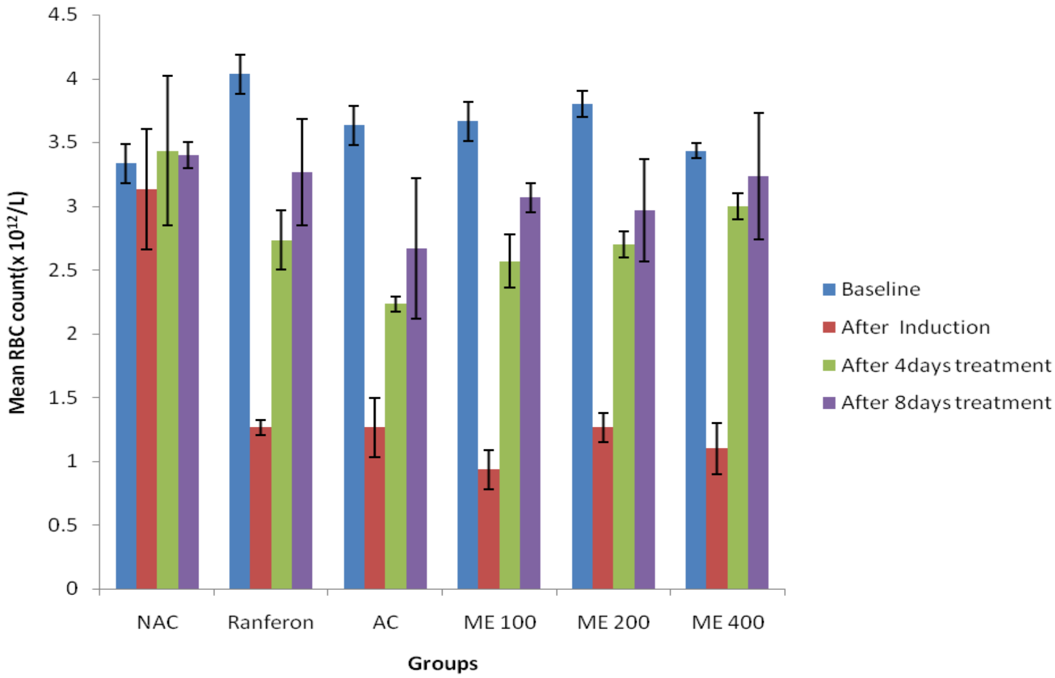

Figure 2. Effect of extract on red blood cell counts. The figure shows the mean RBC counts of rats administered different doses of methanol seed extract of *S. stenocarpa.* There was a corresponding significant increase ($p < 0.05$) in the mean RBC levels of all the test groups after treatment with the seed extract after 4 days. The continued treatments after the first four days of treatment did not show significant ($p > 0.05$) increase in the RBC counts.

administration for 4 days on the lymphocytes showed no significant ($p > 0.05$) decrease in the lymphocytes with the exception of ME 100 group that showed a significant ($p < 0.05$) decrease in the lymphocyte counts. A further reduction was also observed after 8 days of treatment.

Effect of extract on haemoglobin

The administration of extract at various doses 100, 200 and 400 mg/kg body weight (ME 100, 200 and 400, respectively) showed significant ($p < 0.05$) increases in the haemoglobin concentrations of the treated animals during the first 4 days of treatment (Figure 3). The increase was found to be in a dose-dependent manner and the increase in the experimental (ME 100, 200 and 400) groups compared favourably with the Ranferon (treated with a standard drug) group. Further administration of the extract for 8 days produced an increase in the haemoglobin concentration which was found not to be significant (Figure 3).

DISCUSSION

Anaemia is a widespread public health problem affecting about one third population, especially the poor class of the socioeconomic group in third world countries (Karine and Jennifer, 2007). It is associated with an increased risk of morbidity and mortality, especially in pregnant women and young children. It is a disease with multiple causes, both nutritional (vitamin and mineral deficiencies) and non-nutritional (infection) that frequently co-occur (McLean et al., 2007). Therefore, the availability of local plants capable of ameliorating the condition is indispensable. It is therefore necessary to use plants and its products in the management and treatment of anaemia in Africa.

Assessment of haematological parameters can be used to determine the extent of deleterious or beneficial effects of foreign compounds including plant extract on blood (Yakubu et al., 2009). The routine indices used in the diagnosis of anaemia are packed cell volume, haemo-globin and red blood cell counts. The present study was aimed to investigate the levels of PCV, haemoglobin and red blood cell counts on anaemic rats treated with methanol extract of *S. stenocarpa* seeds. The results from this study showed that the methanol seed extract of *S. stenocarpa* increased the PCV, haemoglobin and red blood cell counts of the animals within the first four days of treatment. These increases were further observed with continued administration of the extract. The significant

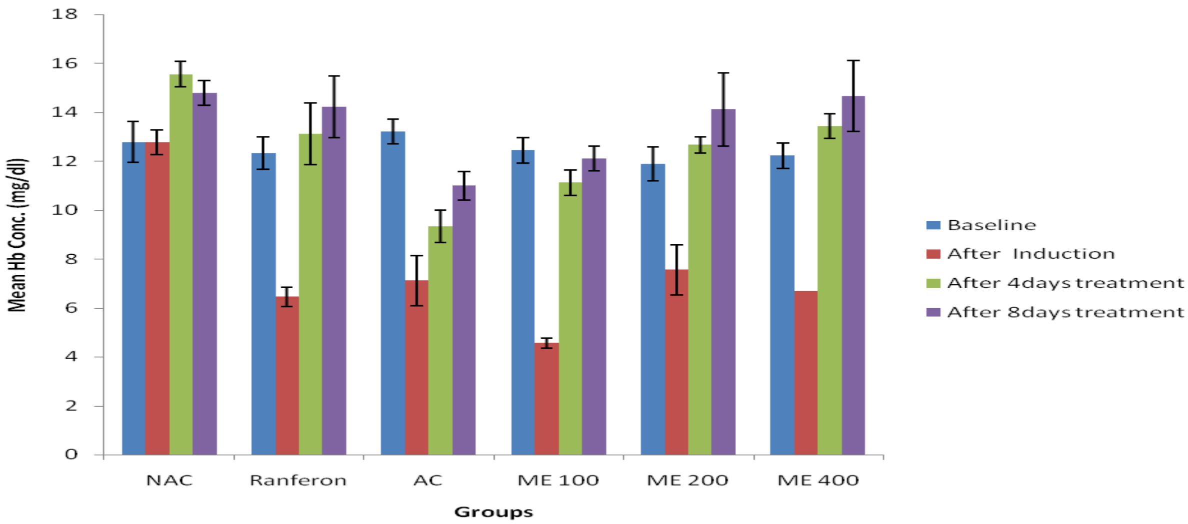

Figure 3. Effect of extract on haemoglobin concentration. The haemoglobin concentrations of rats in groups 4, 5 and 6 increased significantly ($p < 0.05$) after four days of treatment with the plant extract. Treatments after four days did not show significant ($p > 0.05$) increase in the haemoglobin concentrations.

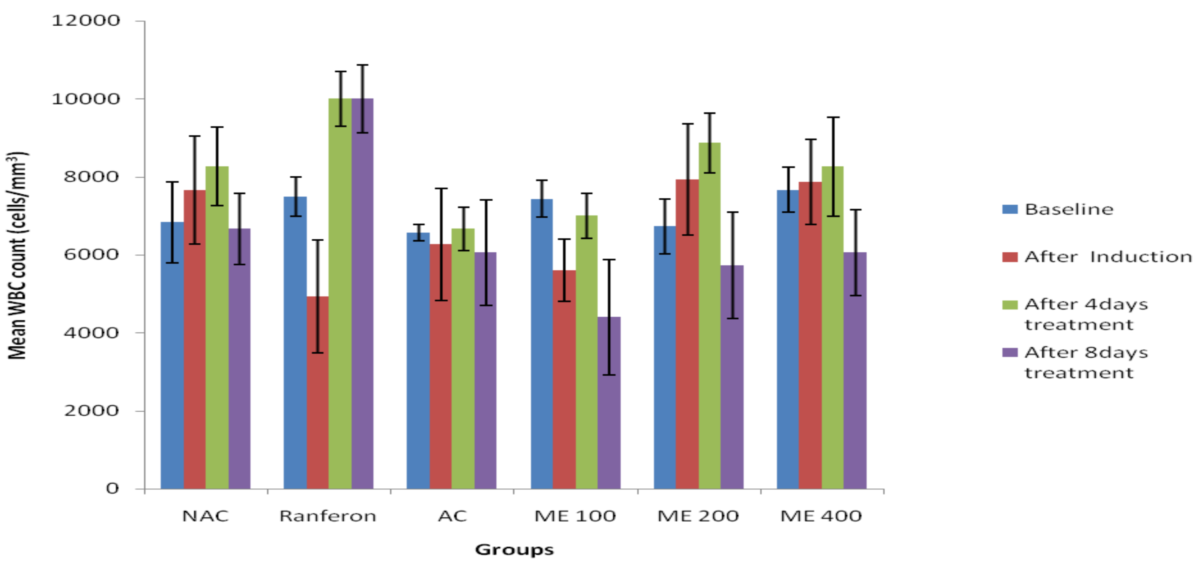

Figure 4. Effect of extract on white blood cell count. There were no significant differences ($p > 0.05$) in the white blood cell counts of the test groups relative to those of controls after four days. But relative to the controls, a significant increase ($p < 0.05$) was observed in the white blood cell counts of group 4.

are indications that the animals recovered from anaemia. Hence, it may be said that the plant extract increased the haemaglobin and red blood cell counts of anaemic rats. These effects may be attributed to the induction of the haemtopoietic pathway by the plant extract. It is reported that African yam bean seeds contain high quantities of iron (33 mg/kg) (Ajayi et al., 2009) and calcium (61 mg/100 g) (NRC, 2007). Iron is the oxygen-carrying component of haemoglobin, where it functions in the delivering of oxygen to cells and tissues (De-Gruchy, 1976). Administration of the plant extract resulted in significant increases in the haemoglobin levels in all the dose groups. The plant has been reported to contain high amounts of proteins with essential amino acids such as lysine and leucine (Onyenekwe et al., 2000). These nutrients could have been harnessed by the anaemic rats

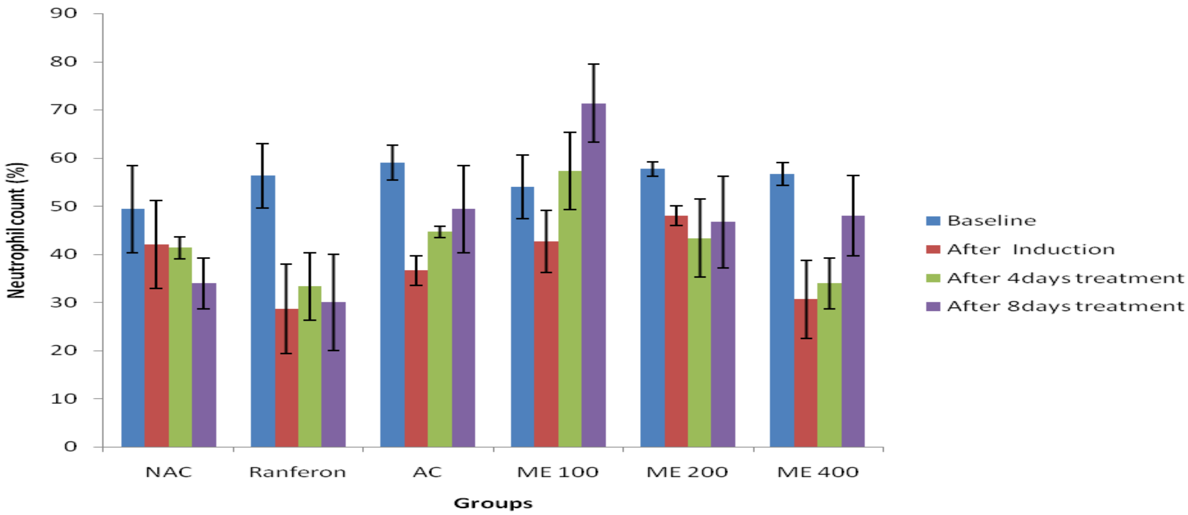

Figure 5. Effect of extract on neutrophil count. The figure above shows the mean Neutrophil counts of rats administered different doses of methanol seed extract of *S. stenocarpa.* The neutrophil levels increased after treatment with the plant extract. In all the experimental groups except in group 1, the control.

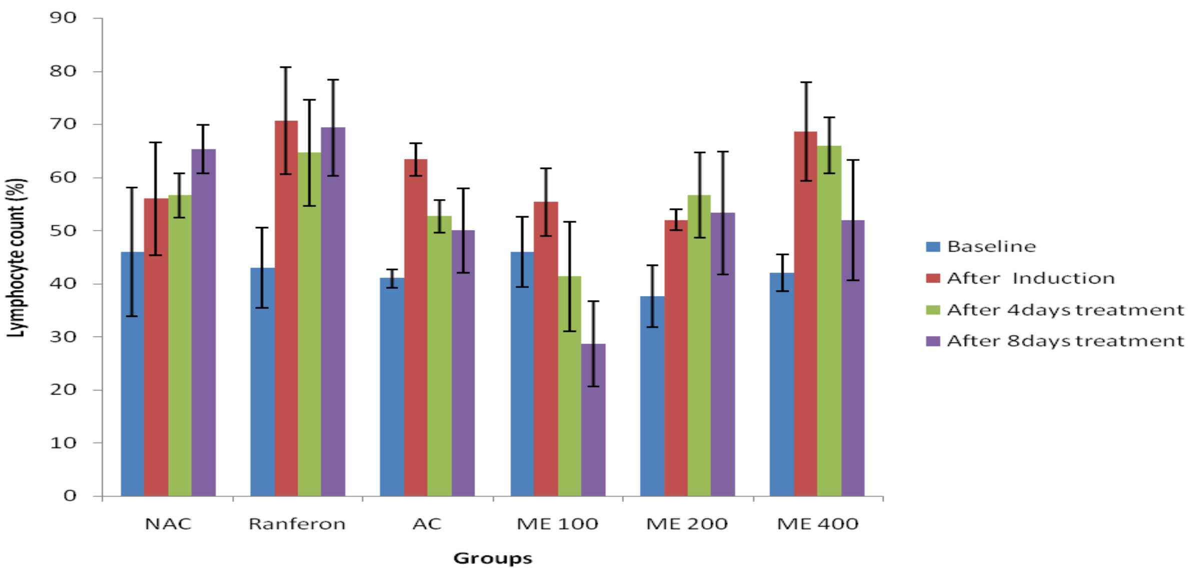

Figure 6. Effect of extract on lymphocyte count. The figure above shows the mean Lymphocyte counts of rats administered different doses of methanol seed extract of *S. stenocarpa*. The extract showed no significant (p >0.05) decrease in the lyphocyte counts with the exception of ME 100. Further reduction was observed after 8 days of extract adinistration.

in the building and restoration of blood cells. The RBC counts of the treated animals increased significantly and the extract can further be said to have some potential to stimulate erythropoietin that is released by the kidney (Polenakovic and Sikole, 1996; Sanchez-Elsner et al., 2004).

The WBC count of the animals increased after the first four days of treatment but further treatment beyond day four showed a decrease in the WBC counts of the animals. The initial increase in the WBC count of the animals could be as a result of the anaemia that was induced in the animals. The animal's system may have assumed the cause of the anaemia to be a result of infection or a disease condition. The system may therefore have concentrated on the production of white blood cells to combat the assumed infections. A similar pattern was observed in the lymphocyte counts. White blood cells and lymphocytes have been known to increase rapidly following foreign attack by pathogens and the systems normal physiologic response will be to boost the body's defense mechanisms (Eyong et al., 2004). The loss of blood may have been noticed by the animals system as a result of an underlying disease or infection and thus produce more of the white blood cells to combat the suspected infection or diseased condition. The WBC counts were observed to decrease after the 8th day of treatment. This could possibly be attributed to the fact that the animals system may have recognized that the actual cause of anaemia may not have been infection or disease.

Conclusion

This study has lent credence to the use of the plant seeds in the treatment and management of anaemia. This may have been possible due to the presence of some blood boosting components in the plant. Although further studies are required to identify the actual component that is responsible for the haemoglobin build up. The plant has been used as protein supplement, where many of its nutritional properties have been harnessed. Since anaemia has been implicated in the etiology of many diseases, the consumption of this plant for its nutritional and medicinal properties is therefore strongly recommended.

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REFERENCES

Allen LH (2000). Anaemia and iron deficiency: effects on pregnancy outcome. Am. J. Clin. Nutr. 71**:**1280-1284.

Ajayi FT, Akande SR, Adegbite AA, Idowu B (2009). Assessment of seven under-utilized grain legume foliages as feed resources for ruminants. Livestock Res. Rural Dev. 21:9.

Dancie JV, Lewis SM (1991). Blood-cell morphology in health and disease. In: Dancie, JV, Lewis, SM (eds), Practical Haematology, 7th ed. Churchill Livingstone, Edinburgh, pp.87-114.

De-Gruchy GC (1979). Clinical haematology in Medical Practice. Blackwell Scientific Publication. Oxford, pp. 33-57.

Edem DO, Amugo CI, Eka OU (1990). Chemical composition of yam bean (*Sphenostylis sternocarpa*). J. Trop. Sci. 30: 59-63.

Evans ID, Haismer DR (1979). Rheology of gelatinized starch suspension. J. Texture Stud. 10:347–370.

Eyong EU, Umoh IB, Ebong PE, Eteng MU, Antai AB, Akpa AO (2004). Haematoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. Niger. J. Physiol. Sci. 19(1-2):1-6.

Haidar J (2010). Prevalence of anaemia, deficiencies of iron and folic acid and their determinants in Ethiopian women. J. Health Popul. Nutr. 28(4):359-368.

Iwu MM, Duncan, AR, Okunji CO (1999). New Antimicrobials of plant origin. In: Janick, J (ed), Perspectives in New crops and New uses. ASHS Press, Alexandria. pp. 457-462.

Karine T, Jennifer F (2007). An update on Anemia in less developed countries. Am. J. Trop. Med. 77(1):44-51.

Lorke D (1983). Determination of Acute Toxicity. Arch. Toxicol. 53:275-279.

McGrath JP (1993). Assessment of hemolytic and hemorrhagic anemia in preclinical safety assessment studies. Toxicol. Pathol. 21(2):158-163.

McLean E, Cogswell M, Egli I, Wojdyla D, Benoist B (2007). Worldwide Prevalence of Anemia in Preschool Aged Children, Pregnant Women and Non-Pregnant Women of Reproductive Age. In: Badham, J, Zimmermann, MB, Kraemer, K (eds) The Guidebook of Nutritional Anemia. Sight and Life Press, Basel, p. 11.

National Research Council (NRC) (2007). Lost Crops of Africa Vol II: Vegetables and Yam bean. Development, Security and Cooperation Policy and Global Affairs (DSC) Washington, D. C., pp 322- 344.

Nwaoguikpe RN (2008). Effects of the extracts of African yam beans (*Sphenostylis stenocarpa*), Hyacinth beans (*Lablab purpureus*) and Bambara groundnut (*Voandzeia subterrenea*) on the polymerization and the Fe^{2+}/Fe^{3+} ratio of sickle cell blood. Niger. J. Biochem. Mol. Biol. 23(2):46-50.

Ochei J, Kolhatkar A (2008). Medical Laboratory Sciences: Theory and Practice. Tata McGraw Hill, New York, pp 663-665.

Oladiji AT, Jacob TO, Yakubu MT (2007). Anti-anaemic potentials of aqueous extract of *Sorghum bicolor* (L.) moench stem bark in rats. J. Ethnopharmacol. 111:651-656.

Onyeike EN, Omubo-Dede TT (2002). Effect of heat treatment on the proximate composition, energy values and levels of some toxicants in African yam bean (*Sphenostylis stenocarpa*) seed varieties. Plant Foods Hum. Nutr. 57:223-231.

Onyenekwe PC, Njoku GC, Ameh DA (2000). Effect of cowpea processing methods on flatus causing oligosaccharides. Nutr. Res. 20:349-358.

Oshodi AA, Ipinmoroti KO, Adeyeye EI, Hall GM (1995). *In vitro* multienzyme digestibility of protein of six varieties of African yam bean flours. J. Sci. Food Agric. 69:373-377.

Polenakovic M, Sikole A (1996). Is erythropoietin a survival factor for red blood cells? J. Am. Soc. Nephrol. 7(8):1178-1182.

Porter D, Doyle JJ (1992). Origins of the African yam bean *(Sphenostylis stenocarpa* Leguminosae*)*; Evidence from Morphology, Isozymes and Chloroplast DNA. Econ. Bot. 46:276-292.

Sanchez-Elsner T, Ramirez JR, Rodriguez-Sanz F, Varela E, Bernabew C, Botella LM (2004). A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and Smads. J. Mol. B. 36**(**1):9-24.

Stone SH (1954). Method of obtaining venous blood from the orbital sinus of rats or mouse. Science 119:100-102.

Talata S, Svanberg U, Mduma B (1998). Low dietary iron availability is a major cause of anemia: a nutrition survey in the Lindi District of Tanzania. Am. J. Clin. Nutr. 68:171–178.

Yakubu MT, Oladiji AT, Akanji MA (2009). Mode of cellular toxicity of aqueous extract of *Fadogia agresti* (Schweinf. Ex Hiern) stem in male rat liver and kidney. Human Exp. Toxicol. 28(8):469-478.

Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD (2003). Plasma folate, vitamin B_6, vitamin B_{12}, homocysteine and risk of breast cancer. J. Nat. Cancer Inst. 95:373-80.