



Applied nutritional investigation

An open-label, randomized, parallel-group comparative study of the efficacy of sorghum bicolor extract in preoperative anemia



Adetokunbo O. Tayo M.B.B.S.^a, Adedoyin O. Dosunmu M.B.B.S.^b,
Ireti O. Akinola MD^a, Adeniyi Adewunmi M.B.B.S.^a, Olufemi A. Oloyede M.B.B.S.^c,
Akinsegun A. Akinbami M.B.B.S.^b, Bodunrin I. Osikomaiya M.B.B.S.^b,
Samira B.L. Makanjuola Ph.D.^{d,*}

^a Department of Obstetrics and Gynecology, Lagos State University College of Medicine, Lagos, Nigeria

^b Department of Hematology and Blood Transfusion, Lagos State University College of Medicine, Lagos, Nigeria

^c Department of Obstetrics and Gynecology, Olabisi Onabanjo University College of Medicine, Ogun State, Nigeria

^d Department of Pharmacology, Lagos State University College of Medicine, Lagos, Nigeria

ARTICLE INFO

Article history:

Received 22 December 2015

Accepted 9 May 2016

Keywords:

Sorghum bicolor
Preoperative anemia
Erythropoiesis
Myomectomy
Hematinics

ABSTRACT

Objective: Anemia in patients presenting for elective surgery is associated with increased morbidity, allogeneic blood transfusion, and delay of surgery. Extract of sorghum bicolor has been shown to have hemopoietic, immune-stimulating, and antioxidant effects in rats and in patients with HIV. The aim of this study was to determine the effect of the extract in patients with pre-operative anemia booked for myomectomy.

Methods: Consenting patients (N = 66) were randomly assigned to two groups. The test group (n = 34) was given folic acid 5 mg/d, 200 mg iron tablet three times daily, and 500 mg/d of the extract. The control group (n = 32) was given the same doses of folic acid and iron for a period of 3 wk. Blood samples were taken at baseline and weekly for full blood cell count and liver and kidney function tests. Participants were screened for tuberculosis, HIV, hepatitis, and sickle cell anemia.

Results: Increases in red blood cell count, hematocrit, and hemoglobin concentration in participants in the test group were highly significant ($P < 0.0002$, $P < 0.0001$, and $P < 0.0001$, respectively). Participants in the control group had a significant increase in the hemoglobin concentration ($P > 0.04$). The changes in liver enzymes, urea, and creatinine for participants in the test group were within the normal ranges.

Conclusion: The addition of the extract of sorghum bicolor to routine hematinics is superior to the use of routine hematinics alone. Although the difference is not statistically significant, the extract will correct preoperative anemia in an additional 15% of the patients.

© 2016 Elsevier Inc. All rights reserved.

Introduction

Anemia is defined as a condition in which the number of red blood cells (RBCs) with oxygen-carrying capacity known as hemoglobin (Hb) is insufficient to meet the body's physiological needs. The Hb count in nonpregnant women at sea level is 120 g/L,

whereas the cutoff level for severe anemia is 80 g/L and that of mild anemia is 110 g/L [1]. Studies have shown that anemia has a prevalence of about 39.5% in surgical patients and that it is independently associated with increased mortality [2–4]. The Network for the Advancement of Transfusion Alternatives (NATA) therefore recommends the following guidelines in the evaluation and management of preoperative anemia in patients presenting for orthopedic surgery:

1. Screening for anemia should be done ≤ 28 d before surgery.
2. The target for Hb level before elective surgery should be within the normal range (female ≥ 12 g/dL, male ≥ 13 g/dL).

AOT, AOD, and SBLM wrote the manuscript and conducted data analysis. AOT and AOD designed the trial, AOT, AOD, OIA, AA, OAO, AAA, and BIO recruited the participants for the trial. The authors are in full-time employment of the Lagos State University College of Medicine. They do not have any financial interest in the producers or marketers of products referred to in the text.

* Corresponding author. Tel.: +234 809 993 3500; fax: +234 127 06644.

E-mail address: sbatistalobo@hotmail.com (S. B. L. Makanjuola).

according to the World Health Organization's [WHO] criteria).

3. Laboratory testing should be performed to further evaluate anemia due to concurrent multiple micronutrient deficiencies, chronic renal insufficiency, and/or chronic inflammatory disease.
4. Micronutrient deficiencies must be treated and an erythropoietin stimulatory agent should be added to the treatment of anemia [3].

Preoperative anemia, advancing age, female sex, small body size, and comorbidities such as cardiovascular disease are predictive of perioperative allogeneic blood transfusion [3,5]. Blood transfusion is, however, associated with adverse events (AEs) and risk for transfusion-transmissible infections [4,5]. Moreover, perioperative blood transfusion as a result of perioperative anemia, which is worsened by surgical blood loss, release of inflammatory cytokines, and hemodilution during surgery, is associated with increased morbidity and mortality [5,6]. Nonetheless, if allowance is made for risk factors such as the existence of cardiovascular disease, there is no increased mortality due to blood transfusion, provided the Hb level is <7 g/dL [6,7]. As studies have demonstrated, a transfusion trigger of 7 g/dL may present superior outcomes to a more liberal transfusion schedule in critical care patients [8,9]. Therefore, it is more prudent to investigate and treat preoperative anemia >7 g/dL than to give a blood transfusion because blood is a scarce product with huge economic implications.

Recombinant erythropoietin, a glycoprotein normally produced in the cortical interstitial cells of the kidneys and to a minor extent in the liver, has been approved for use because it improves Hb in anemia originating from renal impairment, chronic inflammatory disease, chemotherapy in cancer patients, orthopedic surgery, and preoperative or intraoperative autologous blood transfusion [10–12]. Perioperative use of recombinant erythropoietin has been found to reduce the use of allogeneic blood transfusion and has been associated with increased postoperative Hb levels [12]. The risk–benefit analysis in these conditions is inconclusive due to few cases of AEs like hypertension, thromboembolism, development of anti-erythropoietin antibody, seizures, low potassium levels, and the need for parenteral administration [13–16]. These findings reduced the strength of recommendation of recombinant erythropoietin by NATA in the recommended guidelines for preoperative anemia in orthopedic surgery. Therefore, in most patients with uterine fibroids, where the cause of anemia is essentially due to excessive blood loss, or in proven cases of micronutrient-deficiency anemia, the correction of these factors may be sufficient, particularly in resource-poor countries where the cost of recombinant erythropoietin is deemed high. In such conditions, the combination of phytomedicines with therapeutic routine in different clinical situations may be justified and may represent a significant and viable alternative, particularly in Nigeria where the flora is diverse and plentiful.

The extract is from the leaf sheath of a sorghum bicolor strain grown in South West Nigeria and formulated into a commercial pharmaceutical product under the name Jobelyn®. It contains anthocyanins and anthocyanidins. The most common anthocyanidins found in this variety of sorghum bicolor are the three deoxyanthocyanidins and their derivatives, which include apigenin dimers, apigeninidin-flavene dimers, and luteolinin, and are not commonly found in higher plants [17]. This extract has been proven to have antioxidant, antiangiogenesis, antineoplastic, antiinflammatory, immunomodulatory, and

erythropoietic properties in tissue culture models, experimental rat models, and clinical trials involving patients with HIV [18–23]. It also has been recommended for human consumption since studies on its toxicology profile have provided extensive information about the effects of the extract in different body systems [24]. The purpose of the present study was to assess the erythropoietic activity of sorghum bicolor extract in anemic women being prepared for myomectomy in a tertiary hospital in South West Nigeria.

Methods

This was an interventional, randomized, open-label, positive-controlled, parallel-group clinical study conducted in a single institution, Lagos State University Teaching Hospital (LASUTH). The study was approved by the hospital's ethical review committee and was registered with ClinicalTrials.gov identifier: NCT01670955.

The study was spontaneous and had the external support of Health Forever Products Inc. (Lagos Nigeria) and Hains Herbal Products LLC. (Gaithersburg, MD, USA), which provided the study extract (sorghum bicolor) at no cost. Health Forever Products and Hains Herbal Products had no role in data collection, analysis, and quality control. Data property belongs to the Hematology Clinic, LASUTH. All patients signed a written informed consent to participate in the study.

Over a period of 3 mo, 73 participants were recruited. Inclusion criteria were hematocrit (Hct) $<36\%$ (WHO definition of anemia in nonpregnant women), age ≥ 18 y, presentation ≤ 1 mo before surgery (myomectomy). Exclusion criteria were Hb level <7 g/dL, evidence of renal impairment, evidence of chronic inflammatory disease, sickle cell anemia, and age >55 y.

Intervention

Participants were randomized into two groups using sealed envelopes. The randomization ratio of the treatment arms was 1:1. An investigator at the site managed the randomization process, as well as participant screening and enrollment. The research officer collected and analyzed the blood samples in the laboratory. Figure 1 shows the patient distribution in each of the groups.

Thirty-four participants were randomly allocated to receive the extract of sorghum bicolor and routine hematinics (test group), whereas 32 were allocated to receive routine hematinics alone (control group). The extract sorghum bicolor was harvested, processed, packaged, and supplied by Health Forever Products and Hains Herbal Products.

At recruitment, participants were screened for HIV with determine kit, hepatitis B with rapid kit, tuberculosis with Mantoux test, sickle cell anemia with Hb electrophoresis, and renal impairment with raised serum creatinine. These were to screen for exclusion. Participants with sickle cell traits (6.7%) were included. Participants' baseline tests also were taken.

Thereafter, participants were seen weekly at the hematology clinic (LASUTH) for clinical assessment and 8 mL of venous blood was taken into EDTA bottle (4 mL) and heparin bottle (4 mL). Samples were analyzed within 2 h of collection for full blood count using the SYSMEX KX-21 N™ automated hematology analyzer (Sysmex Corporation, Kobe, Japan). The chemistry tests were done with VITROS 350 chemistry autoanalyzer (Ortho Clinical, Raritan, NJ, USA).

A questionnaire was used to assess the demographic features of the participants.

The primary endpoints were increases in RBC indices over a period of 3 wk. The secondary endpoints were derangement in liver and renal function tests to determine probable toxic effect on the liver and kidneys.

Sample size

The assumptions for the sample size were based on the primary endpoint of increases in RBC indices over a 3-wk period. Effect size, which quantifies the strength of the event was settled based on increases of 3 mg/dL over the 3-wk period for both treatment groups as clinically relevant and specified such an effect to be detected with 80% power (0.80) and a significance level α of 0.05. Data retrieved from patient files of previous years suggest that the data will be approximately normally distributed with an SD of 2.25 mg/dL. The sample size was calculated with 56 patients (28 in each arm). Considering a withdrawal rate of 25%, the number of patients to be enrolled was 70.

Dropout rate

The initial number of participants recruited was 73. Three were HIV positive, two were hepatitis positive, one had high creatinine, and one had a white blood cell count $>12,000/\text{dL}$, which is suggestive of an infective process. Once these

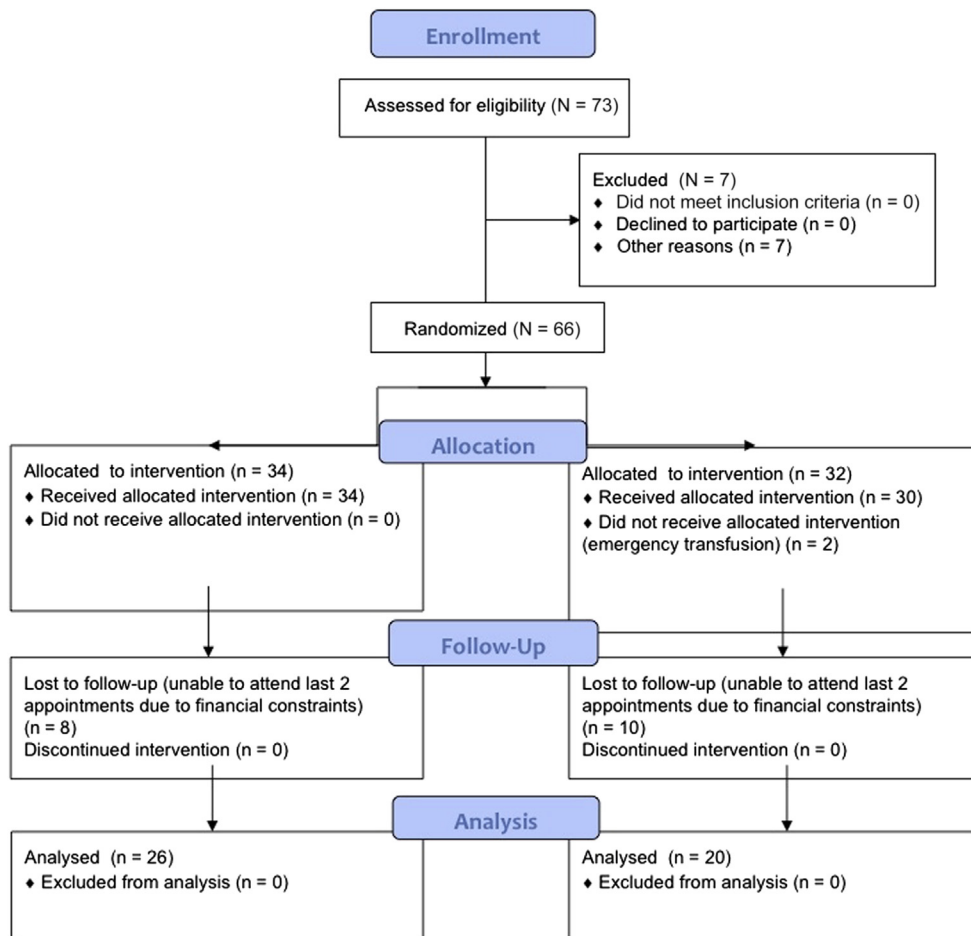


Fig. 1. Participant distribution, intervention, follow-up, and analysis by group.

individuals were excluded, 66 participants were left for inclusion in the intervention. Among the 34 participants (test group) allocated to take sorghum bicolor extract, 8 dropped out. The total number of participants for analysis, therefore, was 26. Among the 32 participants allocated to routine hematinics, 10 dropped out and 2 required transfusion after heavy bleeding. The total number of participants for analysis in the control group was 20. The cause of the high dropout rate was believed to be due to the majority of participants being adults with financial responsibilities that affected their consistency with the last two appointments.

Statistical analysis

The means of the parameters at baseline and after 3 wk were compared using paired test in all participants (Tables 1–3). The differences in appropriate hematologic parameters over the weeks in the test participants were compared

with those of the control group who received routine hematinics (Table 4). The level of significance was $P < 0.05$.

Results

The age range of participants was 19 to 55 y. The mean age was 38 ± 9 y in participants in the test group and 39.8 ± 10.3 y in the control group. There was no significant difference in age between the two groups ($P = 0.96$).

There were no significant differences in the baseline RBC count, Hb concentration, and Hct of the two groups ($P = 0.47$, $P = 0.60$, and $P = 0.31$, respectively).

Table 1
Changes in hematologic parameters in participants receiving routine drugs

Parameter	Baseline 0 wk (N = 26)	After 3 wk (N = 26)	P value
WBC ($\times 10^9$)	6.13 ± 1.91	4.94 ± 0.71	0.06
RBC ($\times 10^{10}$)	3.89 ± 0.68	4.33 ± 0.49	0.0002
Hb (g/dL)	7.916 ± 2.047	9.628 ± 1.469	0.0001
Hct (%)	29.47 ± 5.8	34.98 ± 4.2	<0.0001
MCV (fl)	75.75 ± 0.66	81.52 ± 7.50	<0.0001
MCH (pg)	20.29 ± 3.75	22.32 ± 3.14	0.0002
MCHC (g/L)	26.5 ± 2.4	27.7 ± 1.92	0.0008
Platelet ($\times 10^6$)	292.8 ± 167.0	262.8 ± 122.0	0.26

Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean corpuscular hemoglobin volume; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell

Table 2
Changes in hematologic parameters in participants receiving the sorghum bicolor extract in addition to routine drugs

Parameter	Baseline 0 wk (N = 20)	After 3 wk (N = 20)	P value
WBC ($\times 10^6$)	5.4 ± 2.4	5.5 ± 2.05	>0.05
RBC	3.74 ± 0.7	3.82 ± 1.09	0.129
Hb	7.475 ± 2.096	9.838 ± 2.47	0.04
Hct	27.4 ± 6.8	31.8 ± 6.0	0.267
MCV	72.8 ± 8.8	87.78 ± 21.6	0.0007
MCH	20.4 ± 4.0	33.2 ± 22.9	0.0007
MCHC	27.0 ± 3.7	34.0 ± 1.5	0.0002
Platelet	312 ± 120	342 ± 162	0.2

Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean corpuscular hemoglobin volume; MCV, mean corpuscular volume; RBC, red blood cell; WBC, white blood cell

Table 3

Changes in chemistry parameters in participants receiving the sorghum bicolor extract in addition to routine drugs

Parameter	Baseline 0 wk (N = 26)	After 3 wk (N = 26)	P value
ALT (IU/L)	18.25 ± 2.77	23.45 ± 15.67	0.67
AST (IU/L)	17.4 ± 5.78	24.05 ± 11.83	0.034
Urea (mg/dL)	23.88 ± 12.04	23.19 ± 15.14	0.7
Creatinine (mg/dL)	0.99 ± 0.63	0.74 ± 0.17	0.05

ALT, alanine aminotransaminase; AST aspartate aminotransferase

Participants who were administered the routine drugs alone (Table 1) showed no significant differences in white blood cell, platelet, or RBC counts or in Hct ($P > 0.05$, $P = 0.2$, $P = 0.129$, $P = 0.267$, respectively) during the 3-wk treatment. However, participants in the test group (Table 2), demonstrated significant increases in the RBC count, Hb concentration, and Hct ($P = 0.0002$, $P = 0.0001$, and $P < 0.0001$, respectively). There were also decreases in white blood cell and platelet counts, although these were not statistically significant ($P = 0.06$ and $P = 0.26$, respectively). All participants had significant increases in mean cell volume ($P < 0.0001$ and $P = 0.0007$, respectively), mean cell Hb ($P = 0.0002$ and $P = 0.0007$, respectively) and mean cell Hb concentration ($P = 0.0008$ and $P = 0.0002$, respectively).

The difference in RBC indices after 3 wk (Table 4) were higher in participants in the test group than in control group participants; however, this was not statistically significant ($P \geq 0.3$).

There was a significant increase ($P = 0.034$) in aspartate aminotransferase (AST) from 17.4 to 24.05 IU/L and a significant decrease ($P = 0.05$) in creatinine from 0.99 to 0.74 mg/dL, although the values were still within normal reference ranges after 3 wk of extract use (Table 4).

Discussion

The standard approach to treating patients with mild to moderate anemia is to investigate the cause and treat accordingly [3]. One-third (30%) of preoperative anemia is due to nutritional deficiencies (e.g., iron deficiency, folate utilization, and B₁₂ deficiency) [3,4]. These groups of patients would benefit from simple replacement therapy if the anemia is detected and investigated early at least 28 d before the surgery. Among the participants in the control group, 75% had an increase in Hct levels, whereas only 40% could attain the recommended Hct level of 36% for surgery. Among the participants who had the extract added to their therapy, 80% had an increase in Hct levels and 55% could attain the recommended Hct level of 36%. Additionally, 15% of selected patients, therefore, would benefit from the addition of the sorghum bicolor extract to their hematronics,

thus avoiding blood transfusion, postponement of surgery, or erythropoietin injections.

There were highly significant increases (≤ 0.0001) in Hct, Hb concentration, and RBC count in the test group, whereas only the Hb concentration was significantly increased in the control group. All participants had low baseline mean cell volume and mean corpuscular volume (MCV). When anemia is observed, RBC distribution width (RDW) is used together with MCV to determine the possible causes of anemia. Vitamin B₁₂ or folate deficiencies produce a macrocytic anemia or large cell anemia in which the RDW is elevated in roughly two-thirds of all cases. However, a varied size distribution of RBCs is characteristic of iron-deficiency anemia (IDA) and as such, it shows increased RDW in virtually all cases [25]. In line with this, IDA usually presents with high RDW and low MCV, whereas vitamin B₁₂ and folate deficiencies presents with high RDW and high MCV and mixed deficiency of both iron and vitamin B₁₂ and folate usually presents with high RDW and often MCV is within the normal range [25]. In the present study, preoperative individuals demonstrated low MCV of 72.8 ± 8.8 in the control group and 75.75 ± 0.66 in the test group. This is suggestive of IDA probably due to excessive menstrual bleeding. If so, it is expected that administration of the iron supplement during the 3-wk period would substantially increase the Hb concentration (7.475 ± 2.096 to 9.838 ± 2.47 , $P = 0.04$) and would account for the significant increase in MCV (72.8 ± 8.8 to 87.78 ± 21.6 , $P = 0.0007$); mean cell Hb concentration (27 ± 3.7 to 34 ± 1.5 , $P = 0.0002$); and mean cell Hb (20.4 ± 4 to 33.2 ± 22.9 , $P = 0.0007$) as seen in the control group. However, the addition of the extract increased not only the Hb (7.916 ± 2.047 to 9.628 ± 1.469 , $P = 0.0001$); but also the RBC count (3.89 ± 0.68 to 4.33 ± 0.49 , $P = 0.0002$) and Hct (29.47 ± 3.7 to 34.98 ± 4.2 , $P < 0.0001$). This suggests that the extract supports erythropoiesis by increasing the number of cells in mitosis and RBC mass, whereas the addition of iron increases the total Hb. Therefore, it may be inferred that the participants receiving sorghum bicolor extract would have had a greater increase in RBC indices if they had received more iron supplement because more RBCs would have been produced.

There were no significant differences when the means of the hemogram at baseline and after the third week in the two groups were compared. The increases were higher in participants in the test group, but there were large deviations from the means in both groups, which probably obscured a significant difference. For instance, patients were screened for HIV, hepatitis B, tuberculosis, and sickle cell anemia. However, it would have been meaningful to test for malaria due to its associations to iron status in patients. This is because the malaria parasite requires iron availability for growth and replication, but it appears that the parasite is dependent on a very small pool of iron in the cytoplasm but is sensitive to external iron. Hence, iron supplementation in participants who might have been carrying the malaria virus during the study could have influenced the concentration of iron available to these participants [26]. Other factors obscuring the significance in the hemogram at baseline and after the third week of treatment may include differences in menstrual blood loss associated with the nature and size of the fibroids in this group of participants. Blood loss of >80 mL per menstrual cycle leads to decreased iron levels and increased risk for anemia [27]. Prolonged menstrual blood loss or menorrhagia is a common occurrence in women with fibroids that are located within the uterine wall underneath the endometrium (submucosal) and to a lesser extent in fibroids that are located in the myometrium or muscle of the uterus (intramural), as it may inhibit muscle contracture, thereby preventing normal uterine attempts at hemostasis. In many cases, these fibroids increase

Table 4

Comparison of the differences in RBC indices parameters

Parameter	Mean ± SD test group*	Mean ± SD control group†	P value
Hb	1.86 ± 1.97	1.77 ± 2.3	0.91
RBC	0.44 ± 0.77	0.18 ± 0.9	0.44
Hct	6.57 ± 7.1	3.85 ± 8.0	0.3
MCV	5.92 ± 8.3	5.9 ± 7.1	0.99
MCH	2.0 ± 3.2	3.1 ± 2.6	0.41
MCHC	1.16 ± 2.2	1.08 ± 2.7	0.94

Hb, hemoglobin; Hct, hematocrit; MCH, mean cell hemoglobin; MCHC, mean corpuscular hemoglobin volume; MCV, mean corpuscular volume; RBC, red blood cell

* Test group comprised individuals receiving extract in addition to routine drugs.

† Control group comprised individuals receiving routine drugs only.

the size and volume of the uterus and uterine lining with increased bleeding [28]. As a result, some participants may have experienced substantial decrease in iron levels due to prolonged menstrual blood loss. The size of the fibroid also may have contributed to menstrual blood loss in some participants. However, there are reports that menstrual blood loss does not correlate with fibroid size and subsequent decrease in iron levels [29].

Furthermore, there was a decrease, although not a significant one, in white blood cell and platelet counts in participants in the test group; an increase in these same parameters was observed for participants on routine hematinics alone, but also was not statistically significant. This may reflect the antiinflammatory and antioxidant effects of the extract, which may moderate postoperative wound healing [20,21,23]. A study designed to investigate the postoperative outcome in patients who have been given the extract for preoperative anemia is therefore desirable.

The increase in AST and the decrease in creatinine were within normal range and may not reflect hepatotoxicity effect. Plasma activities of AST and alanine aminotransferase are well-established biomarkers of hepatocellular integrity in vivo [30]. The increase in AST activity may be due to de novo synthesis in the enzyme molecules or adaptation of the liver to the assault from the plant extract leading to higher enzymatic activity than the control [31,32]. On the other hand, decrease in creatinine in participants receiving sorghum bicolor extract and routine therapy represents increased functional capacity of tubular excretion [33]. Like other herbal preparations, sorghum bicolor extract is rich in flavonoids [18], which help increase renal excretion, eliminate excess amounts of creatinine, and increase the blood flow to the kidneys, thus increasing urine filtration [34]. However, it would be prudent to suggest the regular monitoring of liver functions while on long-term extract consumption.

Conclusion

Approximately 15% of patients with preoperative anemia will benefit from the addition of the extract of sorghum bicolor to routine hematinics. However, there is need to screen for chronic inflammatory disease, renal impairment, and other causes of anemia to select patients who will benefit from this simple and low-cost intervention.

Acknowledgment

The authors acknowledge O. Okubena and Health Forever Product Inc., Nigeria, for sponsoring this study. They also acknowledge Gboyega Oyeshoro for administration of the questionnaire and randomization; Rachel Egede for logistics and preanalytical preparation of samples; and M. O. Arogundade and O. Olaosebikan for ensuring the quality of the laboratory tests.

References

- [1] Hemoglobin concentrations for the diagnosis of anemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, Switzerland: World Health Organization; 2011.
- [2] Scott BW, Keyvan K, Wijeyesundera DN, Gordon T. Risk Associated with preoperative anemia in noncardiac surgery: a single-center cohort study. *Anesthesiology* 2009;110:574–81.
- [3] Goodnough LT, Maniatis A, Earnshaw P, Benoni G, Beris P, Bisbe E, et al. Detection, evaluation, and management of preoperative anemia in the elective orthopedic surgical patient: NATA guidelines. *Br J Anaesth* 2011;106:13–22.
- [4] Wu WC, Schiffner TL, Henderson WG, Eaton CB, Poses RM, Sharma SC, et al. Pre-operative hematocrit levels and post-operative outcomes in older patients undergoing non cardiac surgery. *JAMA* 2007;297:2481–8.
- [5] Khanna MP, Hebert PC, Fergusson DF. Review of clinical practice literature on patient characteristics associated with perioperative allogeneic red blood cell transfusion. *Transfus Med Rev* 2003;17:110–9.
- [6] Carson JL, Duff A, Berlin JA, Lawrence VA, Poses RM, Huber EC, et al. Perioperative blood transfusion and postoperative mortality. *JAMA* 1998;279:199–205.
- [7] Foss NB, Kristensen MT, Jensen PS, Palm H, Krshninnikoff M, Kehlet H. The effect of liberal versus restrictive transfusion threshold in ambulation after hip fracture surgery. *Transfusion* 2009;49:227–34.
- [8] Carson JL, Noveck H, Berlin JA, Gould SA. Mortality and morbidity in patients with very low postoperative hemoglobin levels who declined blood transfusion. *Transfusion* 2002;42:812–6.
- [9] Hebert PC, Wells G, Blajehman MA, Marshall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirement in critical care. *N Engl J Med* 1999;340:409–17.
- [10] Singh AK, Szczec L, Tang KL, Bamhart H, Scipp S, Wolfson M, et al. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med* 2006;358:2085–98.
- [11] Bokemeyer C, Aupro MS, Courd A, Fourbet J, Link H, Osterborg A, et al. EORTC Guidelines for the use of erythropoietic proteins in anemic patients with cancer. *Eur J Cancer* 2007;43:258–70.
- [12] Stowell CP, Chandler H, Jove M, Guilfoyle M, Wacholtz MC. An open label, randomized study to compare the safety and efficacy of perioperative epoetin alpha with preoperative autologous blood donation in total joint arthroplasty. *Orthopedics* 1999;22:105–12.
- [13] Cazzola M. How and when to use erythropoietin. *Curr Opin Hematol* 1998;5:103.
- [14] Cassati S, Passerini P, Campise MR, Graziani G, Cesana B, Perisic M, et al. Benefits and risks of protracted treatment with human recombinant erythropoietin in patients having hemodialysis. *Br Med J* 1987;295:1017.
- [15] Eckardt KU, Casadevall N. Pure red cell aplasia due to anti erythropoietin antibodies. *Nephrol Dial Transplant* 2003;18:865.
- [16] Loprinzi C. Epoetin for cancer patients: a boon or a danger? *J Natl Cancer Inst* 2003;95:1820.
- [17] García-Erce JA, Cuenca J, Haman-Alcober S, Martínez AA, Herrera A, Muñoz M. Efficacy of preoperative recombinant human erythropoietin administration for reducing transfusion requirements in patients undergoing surgery for hip fracture repair. An observational cohort study. *Vox Sang* 2009;97:260–7.
- [18] Awika JM, Rooney LW, Waniska RD. Properties of 3 deoxyanthocyanins from sorghum. *J Agric Food Chem* 2004;52:4388–94.
- [19] Ayuba G, Jensen GS, Benson KF, Ademola M, Okubena BA, Okubena O. Clinical efficacy of West African Sorghum bicolor-based traditional herbal preparation Jobelyn shows increased hemoglobin and CD4+ T-lymphocyte counts in HIV+ patients. *J Altern Complement Med* 2013;1:1–4.
- [20] Dykes L, Rooney LW. Sorghum and millet phenols and antioxidants. *J Cereal Sci* 2006;44:236–51.
- [21] Burdette A, Garner PL, Mayer EP, Hargrove JL, Hartle DK, Greenspan P. Anti-inflammatory activity of select sorghum (sorghum bicolor) brans. *J Med Food* 2010;13:879–87.
- [22] Okochi VI, Okpuzor J, Okubena MO, Awoyemi AK. The influence of African formula on the hematological parameters of trypanosome infected rats. *Afr J Biotechnol* 2003;2:312–6.
- [23] Benson KF, Beaman JL, Olu B, Okubena A, Okubena O, Jensen GS. West African Sorghum bicolor leaf sheaths have anti-inflammatory and immune-modulating properties in vitro. *J Med Food* 2013;16:230–8.
- [24] Eniojukan JF, Aina BA. Toxicological profiles of commercial herbal preparation, Jobelyn®. *Int J Health Res* 2009;2:369–74.
- [25] Kjelsberg CR, Perkins SL. Practical diagnosis of hematologic disorders: benign disorders. 5th edition. Chicago, IL: ASCP Press; 2010.
- [26] Scholl PF, Tripathi AK, Sullivan DJ. Bioavailable iron and heme metabolism in *Plasmodium falciparum*. *Curr Top Microbiol Immunol* 2005;295:293–324.
- [27] Hallberg L, Nilsson L. Determination of menstrual blood loss. *Scand J Clin Lab Invest* 1964;16:244–8.
- [28] West CP, Lumsden MA. Fibroids and menorrhagia. *Baillieres Best Pract Res Clin Obstet Gynaecol* 1989;3:357–74.
- [29] Sulaiman S, Khaund A, McMillan N, Moss J, Lumsden MA. Uterine fibroids—do size and location determine menstrual blood loss? *Eur J Obstet Gynecol Reprod Biol* 2004;115:85–9.
- [30] Amacher DE. Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. *Regul Toxicol Pharmacol* 1998;27:119–30.
- [31] Umezawa H, Hooper IR. Aminoglycoside antibiotics. Berlin: Springer-Verlag; 1982.
- [32] Wright PJ, Plummer DT. The use of urinary enzyme measurement to detect renal damage caused by nephrotoxic compounds. *Biochem Pharmacol* 1974;23:65–73.
- [33] Mitchell FL, Veall N, Watts RW. Renal function tests suitable for clinical practice. *Ann Clin Biochem* 1972;9:1–20.
- [34] Francescato HD, Coimbra TM, Costa RS, Biachi Mde L. Protective effect of quercetin on the evolution of cisplatin-induced acute tubular necrosis. *Kidney Blood Press Res* 2004;27:148–58.