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Original Research Article

THE SHORT TIME EFFECT OF EXTRACT OF SORGHUM BICOLOR (JOBELYN) ON THE HAEMATOLOGICAL PARAMETERS OF PATIENTS WITH SICKLE CELL ANAEMIA

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Abstract: Sickle cell anaemia in South West Nigeria has a prevalence of 2.4 %. It is characterized by recurrent crisis like bone pain, hyper haemolysis, acute sequestration, red cell aplasia and progressive organ damage. These cause high absenteeism at school and at work with a significant reduction in life expectancy. The phytochemical extract of sorghum bicolor has been shown to have anti-inflammatory and antioxidant effect; and to increase the haemoglobin in experimental rat. The extract is consumed widely in Nigeria by patients with sickle cell anaemia. This study seeks to assess the effect of this extract on haemopoiesis in these patients.

The study population was the patients attending the adult haematology clinic of the Lagos State University Teaching Hospital. It was a randomized open label study with 105 consenting participants. One group was given folic acid 5mg twice daily and paludrine 200mg daily. The other group had in addition, 1gm of extract per day in two divided doses for 4 weeks. The haematological parameters were taken weekly.

After 4 weeks of taking the extract, there were reduction in white blood cells (p= 0.10) and platelet counts (p= 0.03).There were significant reductions in the mean red cell haemoglobin (p=0.0004), mean cell haemoglobin concentration (p=0.0001) while the reduction in mean cell volume and haematocrit changes were minimal (p=0.3and 0.5 respectively).

The reduction in leukocytes and platelets counts suggests an anti-inflammatory effect of the extract which may have a clinically positive effect. The significantly reduced cellular haemoglobin concentration and minimal changes in haematocrit demonstrate that the extract will not unduly increase the red cell haemoglobin concentration which may promote sickling.

Key words: Haematological parameters. Phytochemical. Sickle cell anaemia. Sorghum bicolor

Introduction: The prevalence of sickle cell anemia (haemoglobin SS) in South West Nigeria is about 2.4% and the frequency of heterozygotes who are asymptomatic traits (hemoglobin AS) is stable at 20-25%.[1] Sickle cell anaemia is an autosomal inherited disorder of haemoglobin (Neel and Beet 1947-1949) due to a point mutation in the 6th codon of the beta globin gene (Ingram and Hunt 1956-1958).[2] This mutation results in the substitution of a

hydrophilic amino acid (glutamic acid) by a less hydrophilic amino acid (valine). [2] The variant haemoglobin is therefore less soluble in reduced oxygen tension as seen in the tissue capillaries or rising cytosolic hemoglobin concentration. The precipitation of deoxyhemoglobin makes the red cell more rigid and the membrane expresses increased phosphatidyl serine on their surface thereby making the cell more sticky to the endothelium (Hebbel, Eaton and Steinberg 1980). [2] There is subsequent blockage of the venules with tissue congestion and hypoxia. A vicious cycle of tissue hypoxia and reperfusion becomes established and cytokines that mediate pain and inflammation are released. At reperfusion, reactive oxygen series are released. The rigid cells with the associated membrane lipid peroxidation are prone to increased intravascular and extravascular hemolysis. There is intravascular release of hemoglobin and arginase. Arginase mops the blood of L-arginine, a source of nitric oxide. Polyamines and L-proline are formed from arginine by arginase. These are essential for smooth muscle growth and collagen synthesis.[3] the released hemoglobin reduces the constitutive nitric oxide (Eno). Nitric oxide has vasodilatation effect, it reduces platelet aggregation and endothelial adhesion molecule expression. Reduced nitric oxide will cause increased vascular tone and promote tissue hypoxia with pain and release of inflammatory cytokines. Recurrent hypoxia and reperfusion results in reperfusion tissue injury, chronic anaemia, hyper haemolysis, recurrent bone pain, sequestration of blood in organs like the spleen, the liver, the lungs, the veins of the male erectile organ and progressive organ damage as seen in pulmonary hypertension.[4,5,6]

The phytochemical extract of sorghum bicolor bran has been shown to have anti-inflammatory and anti-oxidant effects due to the presence of phenolic acids, various flavonoids and trace elements.[7, 8] These phytochemicals have been shown to inhibit the gene expression of transcription factor i.e. nuclear factor NF-KB and the activities of tumor necrosis factor, interleukin 1 and COX 2.[9] These are mediators of inflammation and pain. These phytochemicals are also known to have strong anti-oxidant effect. [9] They also increase the production of red blood cells in rat infected with Trypanosoma brucei to cause anaemia.[10]The anti-oxidant effect will reduce the noxious effects of inflammation while the haemopoietic effect should offer some protection against the development of red cell aplasia in sickle cell anaemia which is usually caused by viral infection (Human Parvovirus B19) of erythroid precursors and folate deficiency. It is therefore expected that the extract of sorghum bicolor should give clinical improvement and prevent tissue damage in sickle cell anaemia. This study seeks to assess the effect of the extract on the haematological parameters in sickle cell anaemia.

Methodology: This was an interventional, open label and randomized study to identify the effect of extract of sorghum bicolor on the hematological parameters in patients with sickle cell anaemia. Ethical approval was obtained from the Lagos State University Teaching Hospital health research and ethics committee. The study was registered with Clinical Trials.gov Identifier: NCT01703104.

The patients were recruited from the outpatient department of the Lagos State University Teaching Hospital with explanation. Written informed consents were obtained from the participants or their guardian or parents if below 18 years old. The exclusion criteria were age below 14 years. Presence of co-morbid conditions like tuberculosis, HIV infection, hepatitis infection and patients with severe organ damage like renal failure or cardiomyopathy were also excluded from the study. The participants were initially screened with liver function tests, renal function tests, Mantoux test, HIV, hepatitis screen and electrocardiography. Fingertip prick using rapid kits was used to screen for HIV and hepatitis.

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These test were to exclude participants before randomization.

Participants with haemoglobin SS were randomized using sealed envelopes into 2 groups: one group was placed on the extract capsule 500mg twice daily , folic acid 5mg twice daily and paludrine 200mg daily (group A) while the other group was placed on folic acid and paludrine at the same dosage (group B). After 2 weeks those in group B had the extract added to their treatment due to high missed appointments. The participants were monitored for a total of 4 weeks.

Appointments were given every week to make enquiries on drug adherence and intolerance. Follow up was strengthened by regular text messages and phone calls. Adverse events and abnormal laboratory values were to be reported to the consultant haematologists and the principal investigator immediately.

Questionnaire was used to determine the demography of the participants.

Four milliliters of blood was collected each into EDTA bottles for full blood count and heparinized bottles for chemistry analysis at recruitment. Subsequently blood samples were taken at weekly visits for 4 weeks. The blood collected were analyzed within 2 hours of collection using SYSMEX KX-21N™ automated hematology analyzer by Sysmex Corporation, Kobe, Japan. The chemistry tests were done with VITROS 350 chemistry auto analyzer manufactured by Orth clinical USA.

The recruitment which started in September 2011 took about 10 weeks and therefore the whole study span a period of 14 weeks

All the patients were treated free and appropriately for other clinical presentations during the study period.

Drop Out Rate: The number of participants that signed consent was one hundred and forty five. Twenty-one participants presented for randomization but were excluded from the study because eight had significantly positive Mantoux test, nine were not homozygote haemoglobin S, two were hepatitis positive and two screened positive to HIV. One hundred and forty five participants were randomized. Seventy three started with the extract and seventy two were started on routine drugs.

Eight participants from the group on the extract dropped out. Twenty eight participants dropped out from among those that started on routine drugs

Statistical Analysis: Each parameter was analyzed using the repeated measure ANOVA if the normality test is positive and the Friedman's test if negative. Where there is a significant difference, a linear trend posttest was done. The pre and post extract data were analyzed with the student t test.

Results: The age range was 14 to 45 years with a mean of 24± 8 years. There were forty three males (41%)and sixty two females (59%). All the participants had haemoglobin SS on haemoglobin electrophoresis.

No adverse event of any degree was reported during the study. There was no evidence of deterioration in the health condition of any of the participants.

There was no statistical difference in the means of the baseline values of the haematological parameters in the controls (on routine drugs) and those on Jobelyn.

After 2 weeks of treatment with routine drugs only (control group) and 4 weeks of taking the extract Jobelyn (test group), there were reductions in both groups in the haematocrit, mean cell volume, red cell distribution width, red cell count and white cell count. The reductions were not statistically significant (tables 1 and 2). In both groups, the reduction in haemoglobin concentration, mean cell haemoglobin and mean corpuscular haemoglobin were statistically significant but the reduction in platelet count was only significant in the Jobelyn group (tables 1 and 2).

Discussion: The pathogenesis of the various clinical presentation of sickle cell disease starts with cytosolic precipitation of the mutant deoxy-hemoglobin. This tendency is initiated by a combination of cellular dehydration, tissue hypoxia and the intrinsic property of the mutant hemoglobin to release its oxygen more readily than the normal adult hemoglobin. At a concentration of deoxy hemoglobin that is probably peculiar to the beta globin gene

haplotype and individual hemodynamics, the cell takes on the characteristic sickle cell shape. The variation in the degree of gene expression of alternative genes on the beta globin gene cluster i.e. fetal or gammaglobin genes and on the reduced production of hemoglobin as seen in the beta thalassemia add to the clinical diversity seen in sickle cell disease. Most antisickling agents alter the cellular hemoglobin S concentrate or the proportion of other hemoglobin within the cell. An example is hydroxyurea.[11, 12]Another method of preventing sickling is the prevention of cellular dehydration. Three processes are identified; the Gardos channel inhibition (Calcium activated potassium channel inhibition), magnesium linked potassium- chloride co-transport and chloride permeability pathway.[13, 14]Blockage of cellular loss of potassium through the inhibition of these pathways has been shown to reduce hemolysis and the percentage of dense erythrocytes with a significant amelioration of anemia in sickle cell disease.[15, 16]

This study showed that there was no change in red cell volume but there was a significant decrease in mean cell hemoglobin concentration in patients on the sorghum extract (table 1). A similar event was observed in participants on routine drugs only. This suggests that the sorghum extract does not unduly increase the red cell haemoglobin concentration which will otherwise promote sickling.

The marrow, when challenged, may increase haemopoiesis 6-8 times its normal activity. However infections such as human parvovirus in sickle cell disease may prevent this marrow response thereby causing prolonged red cell aplasia. The phytochemicals in sorghum bicolor have been shown to increase red cell formation in rats whose marrow were damaged by parasitic infection. This was not demonstrated in this study. There was reduction in haemoglobin values in both the participants on Jobelyn and the control group (tables 1 and 2). An explanation may be that the presence of abnormal haemoglobin with lower affinity for oxygen may prevent the hypoxic drive for haemopoiesis. Moreover, the marrow may be at its maximum level of haemopoiesis in the steady state such that there was no potential for further increase in haemopoiesis in both the control and those on Jobelyn. The ability of the extract to protect against aplastic crisis in sickle cell due to parvovirus as seen in the experimental rat infected with trypanosomes can only be demonstrated by a study over a prolonged period, using a larger sample size and including participants in aplastic crisis.[10]

There was reduction in the blood cellular counts in both groups at the end of the study however the values were within normal ranges (tables 1 and 2). This suggests that the extract of sorghum bicolor is not toxic to the marrow and would not increase the red cell mass which may make the blood more viscous (tables1). An increase in blood viscosity will promote blood sequestration in tissues. An increase in red cell mass beyond an optimal level would therefore be a disadvantage in sickle cell anemia.

Once sickled, the cell becomes more rigid, the phospholipid at the surface is altered to favor adhesion of the red cells to the endothelium, platelets and leukocytes. The cells may become activated with increase in their expression of adhesion molecules such as selections and may release agents of inflammation like interleukin 6.[17,18] Agents that block these pathways should reduce the degree of organ damage.

The phytochemicals in sorghum bicolor have very potent anti-inflammatory and antioxidant effects in tissues. [17, 18] They are therefore expected to moderate organ damage, pain, and sequestration crisis in sickle cell anaemia. There were reduction in white cell though not statistically significant but may be clinically important and a significant reduction in platelet counts in this study (table 1).This effect is probably a demonstration of the anti-oxidant and anti-inflammatory effects.[17,18] Extract of sorghum bicolor may therefore contribute to reduction of organ sequestration, painful crisis and progressive organ damage in sickle cell disease.[17]

This study, however, was not designed to determine the in vivo antioxidant and anti-inflammatory effect of sorghum extract in sickle cell anemia.[19, 20.] These could have been

secondary end points. The time interval was too short to make any conclusion on clinical effects.

In conclusion, the reduction in leukocytes and platelets counts suggests an anti-inflammatory effect of the extract which may have a clinically positive effect. The significantly reduced cellular hemoglobin concentration and minimal changes in hematocrit suggests that the extract would not unduly increase the red cell haemoglobin concentration which is a factor that may precipitate sickling. The antioxidant effect may also be beneficial in the reduction of the sickling phenomenon because increased oxidative stress has been shown to contribute to damage to red cell membrane and hence permanent sickling. [19,20.]There is therefore need for randomized, controlled and blinded studies that would include clinical measures like painful crises, jaundice, transfusion requirements, oxidative stress biomarkers, inflammatory markers and weight gain over a longer period.

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Conflict of interest: The investigators are full time college lecturers. The study was funded by Health Forever Product Ltd, the producers of Jobelyn, an extract of sorghum bicolor. Honoraria were provided for the authors, laboratory staff and other staff members that assisted. No other reward was provided nor promised to the authors. A copy of this paper is provided to the sponsors after the completion of analysis and no alteration was made.

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Table 1: Data from participants commenced on extract of sorghum bicolor from the start. Number 65

TESTS	DAY 1 (MEAN +/- SD)	WEEK 2	WEEK3	WEEK 4	P VALUE
PCV IN %	23.383±4.085	23.323±3.984	22.912±3.925	23.156±3.675	0.5455
RBC	2.887±0.689	2.915±0.7036	2.850±0.6807	2.862±0.6561	0.6071
HB	7.198±1.317	7.047±1.256	6.841±1.200	6.829±1.065	0.0013
WBC	11.275±4.329	10.543±3.992	10.480±4.096	10.653±3.972	0.1053
PLATELET	390.41±146.9	400.861±159.51	360.307±137.68	369.646±141.90	0.0344
MCV	82.332±8.567	81.495±495	81.896±8.970	82.415±8.784	0.3534
MCH	25.418±3.543	24.670±3.225	24.527±3.342	24.404±3.199	0.0004
MCHC	30.798±1.847	30.233±1.637	29.887±1.551	29.535±1.170	0.0001
RDW (CV)	24.561±3.947	24.050±3.639	25.398±7.045	24.006±3.734	0.698

PCV- HAEMATOCRIT, RBC – RED BLOOD CELL COUNT X 10^{12}/L,HB – HAEMOGLOBIN CONCENTRATION IN gm/l, WBC- WHITE CELL COUNT X 10^{9}/L, PLATELET X10^{9}/L, MCV MEAN CELL VOLUME IN Femtoliters, MCH – MEAN CELL HAEMOGLOBIN IN PICOGRAM, MCHC – MEAN CELL HAEMOGLOBIN CONCENTRATION IN gm/dl, RDW – RED CELL DISTRIBUTION WIDTH IN PERCENT COEFICIENT OF VARIATION

Table 2: Data From Participants Commenced on Routne Drugs and Change to Include Extract on From Week 3.Number 40

TESTS	DAY 1 (MEAN +/- SD)	WEEK 1	WEEK 2	P VALUE
PCV	25.082±4.848	24.857±4.628	24.922±4.327	0.4789
HB	7.823±1.535	7.753 ±1.486	7.574 ±1.391	0.0037
WBC	10.585±2.375	10.225±2.794	9.8475±2.245	0.2131
PLATELET	395.52±151.5	386.65±132.6	372.35±112.6	0.6398
MCV	81.025±8.073	80.460±8.677	80.110±8.135	0.1335
MCH	25.347±3.414	25.180±3.795	24.377±3.393	<0.0001
MCHC	31.212±1.813	31.182±1.909	30.337±1.695	<0.0001
RDW (CV)	24.215±3.335	23.980±3.100	24.502±2.802	0.4796
RBC	3.146 ±0.774	3.135 ±0.735	3.1655±0.752	0.9773

PCV- HAEMATOCRIT, RBC – RED BLOOD CELL COUNT X 10^{12}/L, HB – HAEMOGLOBIN CONCENTRATION IN gm/l, WBC- WHITE CELL COUNT X 10^{9}/L, PLATELET X10^{9}/L, MCV MEAN CELL VOLUME IN Femtoliters, MCH – MEAN CELL HAEMOGLOBIN IN PICOGRAM, MCHC – MEAN CELL HAEMOGLOBIN CONCENTRATION IN gm/dl, RDW – RED CELL DISTRIBUTION WIDTH IN PERCENT COEFICIENT OF VARIATION

DROP OUT RATE