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Abstract. The aim of the present study was to investigate the sub-acute toxicological effects of Jobelyn® on pregnant albino rats by employing biochemical, haematological and histopathological methods. A total of 32 pregnant female rats were randomly assigned to four different groups of eight rats each. The control group received distilled water and different doses of Jobelyn®; 250, 500, 1000 mg kg⁻¹ were administered orally once a day for 2 weeks to the other groups. Biochemical analysis revealed a significant decrease (p<0.05) in the levels of alanine aminotransferase, albumin, urea, PCV and Hb in the treatment groups when compared to the control. However, the significant decrease in PCV and Hb was observed solely in the group treated with 1000 mg kg⁻¹ body weight, suggesting that this decrease could be dosedependent. Alkaline phosphatase, total protein, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, eosinophils, basophils, neutrophils, monocytes, lymphocytes, WBC count, revealed no significant difference (p<0.05) when compared to the control. The results show that at an appropriate dosage, the use of Jobelyn® during pregnancy may have no adverse effect on the liver and kidney tissues and may possess hepatoprotective and nephroprotective properties however the histopathological studies revealed that very high levels of Jobelyn may be hepatotoxic.

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 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=0.3 and 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.

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

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 preoperative anemia booked for myomectomy. Methods: Consenting patients (N ¼ 66) were randomly assigned to two groups. The test group (n ¼ 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 ¼ 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.

Toxicological Profiles of Commercial Herbal Preparation, Jobelyn Abstract

PURPOSE: Jobelyn® is a commercial herbal product recommended for the management of anemia related illnesses.
Despite its wide use, there is limited report on its toxicological profile. This study examined the acute and short term chronic toxicity profiles of the product with emphasis on the LD50, gross morphological and histopathological effects. METHODS: Albino mice (mean weight: 16.45±3.14g) were used in this study. For acute toxicity, graded concentrations of Jobelyn® were administered orally and intraperitoneally as single doses to the mice. Intraperitoneal administration of sub-lethal doses daily for 14 days was adopted for the short term chronic toxicity studies. RESULTS: The LD50 following oral and intraperitoneal administration were 215.06 mg/kg (r = 0.916) and 193.37 mg/kg (r = 0.995), respectively. The major behavioral/ morphological effects at high doses were reduction in motor activity, piloerection and sedation. The sub-lethal doses did not significantly modify the normal behavioral repertoire of licking, grooming and sniffing. Histopathological examination also did not indicate severe pathological changes. At the lethal doses, some degree of congestion was noticed in the lung, liver splenic and kidney tissues. Short-term chronic studies did not produce further toxic effects but transient mild sedation and piloerection and histopathological examination revealed only mild congestion in the organs. No death of the animals was recorded during the period of sub chronic toxicity assessment.

CONCLUSION: Jobelyn® is likely to be safe for use in humans when administered at recommended doses. See Link to full Publication

Evaluation of the effects of Jobelyn™ consumption on red blood cell count and quality

Executive Summary

The goals for this clinical study were to examine the effects of Jobelyn™ on the blood count in general, and specifically on red blood cell health in a borderline anemic, otherwise healthy North American population, as a parallel to several studies performed in West Africa, where sickle cell anemia, HIV, malaria, and other microbial diseases affecting red blood cell health, production, and senescence, are prevalent. The outcomes were clear, and included the following: 1) Safety documentation Overall, people consuming Jobelyn™ for 8 weeks had a similar blood count profile as people consuming placebo for 8 weeks. 2) Red blood cell health People consuming Jobelyn™ showed extremely small, but significant changes to red blood cell parameters. However, the changes were not as simple as expected, and point to a complex array of effects in bone marrow and spleen with consumption of Jobelyn™. The surprising reduction in red blood cell counts (mild, but significant), accompanied by an increase in mean cell volume, and changes in other parameters reveals a complex effect of Jobelyn™ on formation of blood cells, suggesting an improved clearance of senescent RBC, accompanied by increased production of new RBC. The changes may also be related to a reduced inflammatory status. Further testing of cytokine profile will help put this data into context. 3) Effects on immune cells Consumption of Jobelyn™ was associated with a rapid increase in the blood levels of monocytes and platelets. Whether this is associated with immune activation as well as bone marrow support is a question for future study. 4) Blood glucose Consumption of Jobelyn™ was in general not
associated with reduced fasting blood glucose in this study population. A few cases showed rapid changes, and based on this data further work may be planned. During the study serum samples were banked from each blood draw. This material is available to pursue further testing without repeating the clinical part of the study. Serum testing may include detailed analysis of pro- and antiinflammatory cytokines, as well as stem cell related growth factors.

Safety

The data presented here helps document basic safety aspects of Jobelyn™ consumption in a North American population. The rapid changes in red blood cell numbers and T cell numbers in West African studies in HIV+ populations could raise the question whether Jobelyn™ consumption is safe to consume for people who have close to normal numbers of such cell types, and whether Jobelyn™ consumption may trigger cellular production in the bone marrow that may be out of control. The data presented in this report clearly documents that Jobelyn™ consumption does not trigger such unhealthy production of cells. This can be seen as an important part of Jobelyn™’s safety data portfolio. The highly specific activation of immune cells, documented in vitro [Benson et al. 2013], could lead to safety related concerns, such as whether Jobelyn™ consumption may trigger overactivation of immune reactions. The current data presented in this report does not suggest such events. Rather, the changes seen were either normalizing or transient, suggesting that Jobelyn™ consumption supports a healthy normalization of many aspects of red and white blood cell production and function.
Sub-acute toxicological effects of Jobelyn® on pregnant albino rats

Abiodun Humphrey Adebayo, Omolara Faith Yakubu, Godwin Eneji Egbung, Olabisi Ibidun Williams, and Olajuwon Okubena

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Sub-acute Toxicological Effects of Jobelyn® on Pregnant Albino Rats

Abiodun Humphrey Adebayo1,a), Omolara Faith Yakubu1, Godwin Eneji Egbung2, Olabisi Ibidun Williams1, Olajuwon Okubena3

1Department of Biochemistry, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria
2Department of Biochemistry, Faculty of Basic Medicine, University of Calabar, Calabar, Cross River State, Nigeria
3Health Forever International, Ikeja, Lagos, Nigeria.

a) Corresponding author: abiodun.adebayo@covenantuniversity.edu.ng

Abstract. The aim of the present study was to investigate the sub-acute toxicological effects of Jobelyn® on pregnant albino rats by employing biochemical, haematological and histopathological methods. A total of 32 pregnant female rats were randomly assigned to four different groups of eight rats each. The control group received distilled water and different doses of Jobelyn®; 250, 500, 1000 mg kg⁻¹ were administered orally once a day for 2 weeks to the other groups. Biochemical analysis revealed a significant decrease (p<0.05) in the levels of alanine aminotransferase, albumin, urea, PCV and Hb in the treatment groups when compared to the control. However, the significant decrease in PCV and Hb was observed solely in the group treated with 1000 mg kg⁻¹ body weight, suggesting that this decrease could be dose-dependent. Alkaline phosphatase, total protein, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, eosinophils, basophils, neutrophils, monocytes, lymphocytes, WBC count, revealed no significant difference (p<0.05) when compared to the control. The results show that at an appropriate dosage, the use of Jobelyn® during pregnancy may have no adverse effect on the liver and kidney tissues and may possess hepatoprotective and nephroprotective properties however the histopathological studies revealed that very high levels of Jobelyn may be hepatotoxic.

INTRODUCTION

Jobelyn® is a multifunctional natural ingredient derived from the leaf sheaths of Sorghum bicolor, a member of the Poaceae family [1]. All parts of the plant, Sorghum (bicolor) have been useful in disease treatment, making the plant a phytomedical. The leaf sheath of Sorghum bicolor is commonly used as a remedy against anaemia by traditional medicine healers [2]. The root is used in treating malaria in Southern Rhodesia; the seed has been employed for the treatment of breast disease and diarrhoea while the stem has been used for tubercular swellings treatment [3]. Recently, focus has been on the leaf sheath of S. bicolor being used as herbal remedy for anaemia and having a boosting effect on blood concentration; possessing hematinic potentials [2, 4]. This powder extract from Sorghum bicolor(Jobelyn®), contains proteins, carbohydrates, dietary fibre as well as bioactive substances known as phytochemicals, which have the potential to significantly affect human health positively [5]. The high concentration of phytochemicals, including proanthocyanidins, anthocyanins, phenolic acids, apigenin, proapigeninidin, apigeninidin, luteolin, naringenins in this wholly, natural, herbal product confers on it, very high antioxidant activity with a total oxygen radical absorbance capacity (ORAC) score of 37,000 μmolTE/g of dry powder[6]. It is capable of inhibiting peroxyl free radicals, hydroxyl free radicals, singlet oxygen and possesses a high capacity for quenching superoxide anions, hence its usefulness in maintaining human health [7]. The inhibition of peroxyl free radicals per gram of jobelyn® is 3,549 μmoleTE/g as compared to 997, 68, 24, 15, 8 and 7 μmoleTE/g of acai berry, cherry tart, blueberry, strawberries, red grape, respectively [8]. This indicates that the antioxidant capacity of Jobelyn® is about 4 times higher than that of acai berry and 50 times than that of tart cherries [8] thus signifying the antiradical properties of Jobelyn® [9]. This herbal formulation is believed to alleviate symptoms of anaemia of diverse origin, among which are malaria, sickle cell disease, leukaemia, stroke, arthritis, pregnancy, enteric fever, helminthiasis, multiple myeloma, and tuberculosis, its use in pregnancy being quite notable [1, 10]. Elevation of packed cell volume (PCV) from 21 percent to 32 per cent has been observed in pregnant women within eight days of ectopic pregnancy operation following the use of Jobelyn® [unpublished]. Despite the wide use of this herbal formulation, there is a dearth of information on its toxicity. This study was therefore conducted to assess the sub-acute toxicological effects of Jobelyn® on pregnant albino rats.
formulation, there is a paucity of studies on its toxicological profile. In relation to its effect on pregnant women, there has been no previous work done to ascertain if it poses health risks on the mother or not. The plethora of previously reported and unreported adverse drug reactions associated with the use of herbal medicines makes it imperative that pre-clinical toxicological studies be carried out on these natural products [11]. Furthermore, the recommendation of the herbal drug as a dietary supplement, establishes the need for toxicological studies on its effects on pregnant women. The primary aim of the present study was to investigate the sub-acute toxicological effect of Jobelyn® by employing the biochemical, haematological, and histological indices in pregnant albino Wistar rats.

MATERIALS AND METHODS

A total of thirty two healthy cyclic female Wistar albino rats weighing 82-190 g were used in this study. Animals were acclimatized for two weeks prior to commencement of treatment housed under standard environmental conditions and fed with a standard rat diet (obtained from Graceline feeds, Ota, Ogun state) with free access to water. During this period of acclimatization, the animals were exposed to a photoperiodicity of 12h light/12h darkness and were periodically assessed for gross morphological/behavioural changes.

Experimental Design

The animals were divided into 4 groups of 8 rats each and treated daily for 2 weeks; beginning from the 5th to 19th day of gestation during the period of foetal organogenesis. Group I served as the control group and received distilled water while groups II, III and IV received 250, 500 and 1000 mg kg⁻¹ of Jobelyn®. All animals were weighed and sacrificed on the 20th day of pregnancy. Their uteri were dissected and blood samples were immediately collected. The liver and kidney was collected, trimmed of excess fat and weighed and a portion was separated for histological studies.

Haematological assays

Blood samples were withdrawn from the heart to measure the levels of haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), WBC differentials (neutrophils, monocytes, lymphocytes, basophils and eosinophil) [12].

Biochemical assays

The commercial test kits for liver function test were purchased from Randox Laboratory, United Kingdom. Standard procedures were used to evaluate the protein concentration [13], aspartate aminotransferase (AST) [14], alkaline phosphatase (ALP) [15], alanine aminotransferase (ALT) [16], albumin [17], direct bilirubin [18], urea [19] and creatinine [20].

Histopathological studies

The organ tissues were fixed in normal saline for a period of 72 h and cut into thin slices 2.1 mm thick. The tissues were dehumidified using liquor. They were thereafter treated with paraffin wax and cast into blocks; tissue sections were then slit into 5μm using microtome and allowed to dry on a slide. The slides were afterwards soiled with haematoxylin-eosin stain, analyzed using a light microscope and photomicrographs recorded [21].

Statistical analysis

Differences between means of biochemical and haematological analysis of all the groups were estimated using a one-way analysis of variance followed by least significant difference test (post hoc) using the SPSS software (SPSS 15 for Windows). The p<0.05 were considered statistically significant. Graph pad prism was used in presentation of charts.

RESULTS
### TABLE 1. Effect of Jobelyn® on Weight of Selected Organs of Pregnant Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER</td>
<td>5.97±0.53</td>
<td>6.03±0.87</td>
<td>6.27±0.45</td>
<td>5.24±0.77</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.47±0.03</td>
<td>0.47±0.04</td>
<td>0.47±0.02</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>HEART</td>
<td>0.45±0.03</td>
<td>0.47±0.04</td>
<td>0.49±0.02</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td>LUNGS</td>
<td>1.13±0.09</td>
<td>1.13±0.13</td>
<td>1.29±0.06</td>
<td>1.15±1.15</td>
</tr>
<tr>
<td>BRAIN</td>
<td>1.41±0.06</td>
<td>1.16±0.18</td>
<td>1.32±0.04</td>
<td>1.35±0.05</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>0.58±0.06</td>
<td>48.50±5.74</td>
<td>0.76±0.05</td>
<td>1.02±0.18</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates. From the Table 1, there was no significant difference p<0.05 in the weight of the kidney, heart, lungs, brain, spleen of treatment groups administered with Jobelyn® when compared with the control group.

### TABLE 2. Effect of Jobelyn® on Liver Homogenate of Pregnant Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/I)</td>
<td>94.09±5.01</td>
<td>90.54±1.42</td>
<td>86.57±1.12</td>
<td>88.16±1.84</td>
</tr>
<tr>
<td>ALP (U/I)</td>
<td>104.42±23.26</td>
<td>237.65±127.25</td>
<td>77.54±14.93</td>
<td>140.66±66.50</td>
</tr>
<tr>
<td>AST (U/I)</td>
<td>118.21±4.10</td>
<td>103.25±17.63</td>
<td>117.02±15.01</td>
<td>136.59±2.57</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>2.08±0.41</td>
<td>2.20±0.43</td>
<td>1.79±0.35</td>
<td>1.55±0.28</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>0.61±0.12</td>
<td>0.65±0.89</td>
<td>0.44±0.08</td>
<td>0.57±0.10</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, significantly different from the control group. ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, TP = Total protein, ALB = Albumin, Dol=Direct Bilirubin. From Table 2, there was no significant difference p<0.05 in the activity of ALT, ALP, AST, TP, Albumin and Direct Bilirubin in the liver homogenate of groups administered Jobelyn® when compared with the control group.

### TABLE 3. Effect of Jobelyn® in Kidney Homogenate of Pregnant Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA (mg/dl)</td>
<td>27.22±1.64</td>
<td>20.98±1.92</td>
<td>10.39±2.63</td>
<td>25.50±9.96</td>
</tr>
<tr>
<td>CREA (mg/dl)</td>
<td>1.83±0.32</td>
<td>1.75±0.32</td>
<td>1.27±0.31</td>
<td>1.55±0.48</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. CREA = Creatinine. From Table 3, there was no significant difference p<0.05 in the levels of urea and creatinine in the kidney homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

### TABLE 4. Effect of Jobelyn® on Selected Lipid Profile Parameters in Plasma of Pregnant Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIGS (mg/dl)</td>
<td>47.02±10.33</td>
<td>41.53±8.05</td>
<td>52.60±8.51</td>
<td>43.48±14.48</td>
</tr>
<tr>
<td>T.CHOL (mg/dl)</td>
<td>66.66±8.19</td>
<td>48.16±1.34</td>
<td>59.26±4.46</td>
<td>53.84±5.90</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. TRIGS = Triglycerides, T. CHOL = Total Cholesterol. From Table 4, there was no significant difference p<0.05 in the levels of Triglycerides and Total cholesterol in the plasma of the treatment groups administered with Jobelyn® when compared with the control group.

### TABLE 5. Effect of Jobelyn® on Selected Lipid Profile Parameters in Liver homogenate of Pregnant Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIGS (mg/dl)</td>
<td>119.52±13.88</td>
<td>102.93±11.85</td>
<td>102.33±9.93</td>
<td>146.34±23.26</td>
</tr>
<tr>
<td>T.CHOL (mg/dl)</td>
<td>25.00±5.01</td>
<td>23.41±3.00</td>
<td>36.95±8.20</td>
<td>32.48±9.29</td>
</tr>
<tr>
<td>HDL CHOL (mg/dl)</td>
<td>206.89±3.73</td>
<td>201.80±0.83</td>
<td>202.77±0.90</td>
<td>207.11±6.76</td>
</tr>
</tbody>
</table>
Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. TRIGS = Triglycerides, TOTAL CHOL = Total Cholesterol, HDL CHOL = High Density Lipoprotein Cholesterol. From Table 5, there was no significant difference p>0.05 in the levels of Triglycerides, Total cholesterol and HDL cholesterol in the liver homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

**TABLE 6.** Effect of Jobelyn® on Selected Lipid Profile Parameters in Kidney of Pregnant Albino Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIGS (mg/dl)</td>
<td>60.24±2.95</td>
<td>60.70±7.55</td>
<td>56.20±3.50</td>
<td>54.52±3.19</td>
</tr>
<tr>
<td>T.CHOL (mg/dl)</td>
<td>48.16±3.32</td>
<td>39.94±3.47</td>
<td>52.46±6.95</td>
<td>42.58±6.31</td>
</tr>
<tr>
<td>HDL CHOL (mg/dl)</td>
<td>21.19±6.11</td>
<td>15.77±3.60</td>
<td>14.74±1.98</td>
<td>14.62±1.31</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. TRIGS = Triglycerides, TOTAL CHOL = Total Cholesterol, HDL CHOL = High Density Lipoprotein Cholesterol. From Table 6, there was no significant difference p<0.05 in the levels of Triglycerides, Total cholesterol and HDL cholesterol in the kidney homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

**TABLE 7.** Effect of Jobelyn® on Haematological Parameters in Plasma of Pregnant Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>250 mg kg⁻¹</th>
<th>500 mg kg⁻¹</th>
<th>1000 mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOSINO (x 10¹²/L)</td>
<td>41.00±3.49</td>
<td>34.80±4.76</td>
<td>38.11±1.88</td>
<td>40.29±4.14</td>
</tr>
<tr>
<td>NEUTRO (x10¹²/L)</td>
<td>46.50±1.55</td>
<td>56.80±5.32</td>
<td>52.67±1.70</td>
<td>54.50±2.81</td>
</tr>
<tr>
<td>BASO (x 10¹²/L)</td>
<td>4.0±1.73</td>
<td>3.4±1.03</td>
<td>2.25±0.37</td>
<td>1.86±0.55*</td>
</tr>
<tr>
<td>LYMPH (%)</td>
<td>4.25±0.63</td>
<td>5.00±1.30</td>
<td>5.22±0.97</td>
<td>3.83±1.08</td>
</tr>
<tr>
<td>MONO (%)</td>
<td>1.50±0.29</td>
<td>1.0±0.0</td>
<td>1.57±0.30</td>
<td>1.40±0.24</td>
</tr>
<tr>
<td>PCV (x 10¹²/L)</td>
<td>55.5±6.51</td>
<td>48.50±5.74</td>
<td>51.38±1.46</td>
<td>41.50±1.74*</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>17.90±2.10</td>
<td>15.65±1.85</td>
<td>16.57±0.47</td>
<td>13.39±0.56*</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates. *p<0.05: significantly different from the control group. WBC COUNT = White Blood Cell Count, NEUTRO = Neutrophils, EOSINO = Eosinophils, BASO = Basophils, LYMPH = Lymphocytes, MONO = Monocytes, PCV = Packed Cell Volume, Hb = Haemoglobin. From Table 7, there was no significant difference p>0.05 in the levels of neutrophils, eosinophils, basophils, lymphocytes, monocytes, white blood cell count, whereas, there was a significant difference p<0.05 in levels of haemoglobin and packed cell volume in the plasma of the treatment groups administered with Jobelyn® when compared with the control group.

**Histopathological examination**

In the histopathological examination, hepatocytes in control group revealed normal nuclei membrane, rough endoplasmic reticulum and mitochondria (Fig. 3A). But in the 250 mg kg⁻¹ group, mild to moderate portal inflammation was observed (Fig. 3B). The 500 mg kg⁻¹ group (Figure 3C) revealed moderate inflammation, while moderate to severe portal inflammation was observed in the 1000 mg kg⁻¹ (Fig. 3D).

**DISCUSSION**

Toxicological assessment of drugs, herbs and extracts are necessary to establish the safety limit of these substances in animals. These are then commonly used to assess the possible health risk in humans [22]. The liver is a metabolically active organ responsible for many vital life functions, some of such primary functions include; bile production and excretion; excretion of bilirubin, cholesterol, hormones, and drugs; metabolism of fats, proteins, and carbohydrates; storage of glycogen, vitamins, and minerals; synthesis of plasma proteins, such as albumin, and clotting factors, as well as blood detoxification and purification [23]. Due to these important activities, the liver is one of the body's organs most subject to injury. It is the central organ in the metabolism and detoxification of drugs and toxins. Consequently, drugs affect the liver more frequently than any other organ and place the liver at great risk for toxic damage [24]. The cells in the liver contain proteins called enzymes that drive these chemical reactions. When liver cells are damaged or destroyed, the enzymes in the cells leak out into the blood. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) are specific markers of
hepatic injury and hepatocellular necrosis [25]. A significant decrease (P<0.05) in the activity of alanine aminotransferase (ALT) was observed in groups treated with 500 and 1000 mg/kg body weight of Jobelyn® when compared with the control (Fig 1). Hence, suggesting that Jobelyn® may possess hepatoprotective properties. ALT is localized in the cytosol of the hepatocytes and is a more sensitive marker of hepatocellular damage as opposed to AST which can also be produced from other tissues like heart, kidney and pancreas other than the liver [26]. However, AST and ALP levels were not significantly different (P<0.05) in the treated groups when compared with the control groups. A significant decrease (P<0.05) in albumin levels in the group administered 500 mg/kg body weight of Jobelyn® was observed (ig 1). A similar study conducted by Salawu et al. [27] and Mancini-Filho[28] revealed similar results. The possible hepatoprotective effects of Jobelyn® could be attributed to the presence of significant levels of flavonoids such as tannins and anthocyanidins which help to clear up free radicals. It has been established that free radicals play a significant role in various pathogenesis, inflammatory diseases and can result in necrosis of the liver [29, 30]. Flavonoids are known for their ability to exhibit antioxidant potential, and their effects on human health and nutrition are considerable. The mechanisms of action of flavonoids are through chelating or scavenging processes [31]. Chemically, the remarkable antioxidant properties of flavonoids is due to the hydrogen donating substituents (hydroxyl groups), which are attached to the aromatic ring structures of flavonoids, enabling the flavonoids to undergo a redox reaction and thus helping them to rapidly scavenge free radicals easily. Flavonoids also mediate their antioxidant effect by a stable delocalization system, which consist of heterocyclic and aromatic rings as well as multiple unsaturated bonds, and helps to delocalize the resulting free radicals [32]. Finally, the presence of certain structural moieties which are capable of forming transition metal chelating complexes that can regulate the production of reactive oxygen species such as O₂⁻ and OH is also a mechanism through which flavonoids mediate their antioxidant effect [33]. Sorghum bicolor, the plant extract of Jobelyn® is known to possess high concentration of flavonoids which include tannins and anthocyanins, significantly possessing high concentrations of dimeric 3-deoxy-anthocyanidins [34, 35]. Phenolic compounds, which have also been found to exist in high concentrations in Sorghum bicolor [36] are considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living system, further establishing the antiradical properties of Jobelyn®[36].Epidemiological studies have suggested association between the consumption of phenolic acid - rich foods or beverages and the prevention of many diseases [37]. The potential hepatoprotective properties of this wholly herbal drug is further supported by the significant decrease (P<0.05) in albumin levels in the group administered 500 mg/kg body weight of Jobelyn®. This could have been pregnancy-induced, as albumin is often decreased in normal pregnancy as a consequence of haem dilution. It is noteworthy though, that a low albumin level is often temporary, and is not a reliable way to diagnose liver disease. In contrast, the concentrations of the transaminases (alanine and aspartate) and γ-glutamyltransferase normally increase during pregnancy. Thus, the decreased levels of ALT observed in pregnant rats treated with Jobelyn® is a further proof of the possible hepatoprotective effect of Jobelyn®. The histological findings revealed mild to moderate portal inflammation in the liver of animals treated with 250 and 500 mg/kg bodyweight of Jobelyn®, while the liver of rat treated with 1000 mg/kg bodyweight of Jobelyn® showed moderate to severe portal inflammation (Fig 3). This is possibly as a result of the dose of Jobelyn® administered to the animals as the severity of liver abnormality increased with increasing dosage. Bilirubin levels were not significantly different (p<0.05) when compared with the control. Bilirubin is a waste product from the breakdown of red blood cells. The liver processes bilirubin so it can be excreted in stool. Bilirubin flows through the liver's bile ducts, dissolved in bile. Bilirubin blood levels may be elevated in people with impaired bile flow. This can occur in severe liver disease, gallbladder disease, or other bile system conditions. Very high bilirubin levels cause jaundice, in which the skin and whites of the eyes turn yellow [38]. Bilirubin can be a useful liver function test in people with a known bile flow problem. Higher than normal levels of direct or indirect bilirubin may indicate different types of liver problems. Occasionally, higher bilirubin levels may indicate an increased rate of destruction of red blood cells. Hence, the stable level of bilirubin in animals treated with Jobelyn® further supports the safety and possible hepatoprotective role of Jobelyn®. A significant decrease (p<0.05) in Urea concentration was observed, suggesting that Jobelyn® may have protective effects on renal function. Salawu et al. [27] also observed similar effects when Sorghum bicolor aqueous extract was administered to rats fed with low and high iron diet. This implies that the administration of Jobelyn® has no toxic effect on the kidney and may thereby preserve the integrity of the kidney. Urea is the major end product of protein metabolism in humans and other mammals. Factors which tend to increase urea excretion include increases in glomerular filtration rate, increased dietary protein intake, protein catabolic conditions, and water diuretic states. Factors which reduce urea excretion include low protein intake and conditions which result in low urine output such as dehydration), although low urea levels are seen during normal pregnancy. Biochemical analysis revealed significant decrease (p<0.05) in the levels of Hb and PCV in only the group administered 1000mg/kg which could be due to the high-dose (Table 7). The PCV (packed cell volume) determines the percentage of red blood cells in the plasma. During Pregnancy, however, decreased hematocrit levels are observed, especially in the last trimester as plasma volume increases. Haemodilution occurs physiologically in pregnancy [39]. This may result in lower haemoglobin concentrations than in the non-pregnant state. Sorghum contains such hard-to-find nutrients as iron, calcium and potassium. In the past, doctors prescribed sorghum as a daily supplement for those low in these nutrients. No significant difference was observed in
the levels of triglyceride, total cholesterol and high density cholesterol. Implying that Jobelyn® poses no threat on the body as relates to diseases caused by increased cholesterol such as atherosclerosis. In addition to the high content of anti-inflammatory phenolic compounds, sorghum contains several groups of bioactive compounds with the capacity to induce pro-inflammatory immune responses. Water-soluble beta-glucans are found in sorghum that showed biologically active beta-glucans capable of initiating macrophage activation.

**FIGURE 1.** Effects of Jobelyn on plasma of pregnant albino rats. ALT = Alanine aminotransferase, ALP = Alkaline phosphatise, AST = Aspartate transaminase, TP = Total protein, ALB = Albumin. Values are presented as mean ± standard error of mean (SEM) of 8 replicates; *significant at p < 0.05 as compared with control

**FIGURE 2.** Effects of Jobelyn on Kidney functions of pregnant albino rats. Values are presented as mean ± standard error of mean (SEM) of 8 replicates; *significant at p < 0.05 as compared with control.

**RECOMMENDATION**

Jobelyn® should be subjected to further analysis to clearly ascertain the mechanisms involved in its suggested haematocrit boosting property, as well as its proposed hepatoprotective and renal protective property, with emphasis on pregnant women. This would help further confirm the safety of the drug for consumption by pregnant women.
FIGURE 3. Photomicrograph of liver tissues of rat (H&E x 400) (A) with normal features in the control (B) treated with 250 mg/kg bodyweight of Jobelyn® Showing mild to moderate portal inflammation, H&E x 400 (C) treated 500 mg/kg bodyweight of Jobelyn® (D) treated with 1000 mg/kg bodyweight of Jobelyn®.

CONCLUSION

In conclusion, Jobelyn may enhance liver and kidney functions in pregnant women when taken at an appropriate dose. It may also boost haematocrit level, and thus could be ideal for intake by pregnant women.

REFERENCES

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

Dosunmu A. O1, Akinbami A. A1, Onakoya J. A. A2, Yemitan O. K.3, Adebola4 Arogundade M.O.1

1Department of haematology and blood transfusion. Lagos State University College of Medicine
2Department of chemical pathology. Lagos State University College of Medicine, IKEJA.
3Department of Pharmacology. Lagos State University College of Medicine. IKEJA.
4Department of Medicine, Lagos State University College of Medicine, IKEJA.

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%. Sickle cell anemia 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). This mutation results in the substitution of a
hydrophilic amino acid (glutamic acid) by a less hydrophilic amino acid (valine). 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).

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. 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.

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. 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. These are mediators of inflammation and pain. These phytochemicals are also known to have strong anti-oxidant effect. 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.
These tests 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 Symsex 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. 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. 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.

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 a 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. 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 (table1). 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. 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. 

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.  

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.

Acknowledgement: This study was made possible by grant from health forever products ltd. The extract was also provided by health forever products ltd. We acknowledge the assistance of the staff of the LASUTH/IHVN laboratory in sample analysis and the pharmacy department of the hospital for providing the drugs. We wish to thank Miss Ife Agboola, Miss. Egede Rachel and Mr. Gboyega Oyesoro for their excellent work in providing the logistic for patients, collection of samples and data input respectively.

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.

References:
12. Katherine C W, Neil Granger D. Sickle cell disease: Role of reactive oxygen and nitrogen


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

<table>
<thead>
<tr>
<th>TESTS</th>
<th>DAY 1 (MEAN +/- SD)</th>
<th>WEEK 2</th>
<th>WEEK 3</th>
<th>WEEK 4</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV IN %</td>
<td>23.383±4.085</td>
<td>23.323±3.984</td>
<td>22.912±3.925</td>
<td>23.156±3.675</td>
<td>0.5455</td>
</tr>
<tr>
<td>RBC</td>
<td>2.887±0.689</td>
<td>2.915±0.7036</td>
<td>2.850±0.6807</td>
<td>2.862±0.6561</td>
<td>0.6071</td>
</tr>
<tr>
<td>HB</td>
<td>7.198±1.317</td>
<td>7.047±1.256</td>
<td>6.841±1.200</td>
<td>6.829±1.065</td>
<td>0.0013</td>
</tr>
<tr>
<td>PLATELET</td>
<td>390.41±146.9</td>
<td>400.861±159.5</td>
<td>360.307±137.68</td>
<td>369.646±141.90</td>
<td>0.0344</td>
</tr>
<tr>
<td>MCV</td>
<td>82.332±8.567</td>
<td>81.495±495</td>
<td>81.896±8.970</td>
<td>82.415±8.784</td>
<td>0.3534</td>
</tr>
<tr>
<td>MCHC</td>
<td>30.798±1.847</td>
<td>30.233±1.637</td>
<td>29.887±1.551</td>
<td>29.535±1.170</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

PCV- HAEMATOCRIT, RBC – RED BLOOD CELL COUNT X 10¹²/L, HB – HAEMOGLOBIN CONCENTRATION IN gm/l, WBC- WHITE CELL COUNT X 10⁹/L, PLATELET X10⁹/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 COEFFICIENT OF VARIATION
Table 2: Data From Participants Commenced on Routine Drugs and Change to Include Extract on From Week 3. Number 40

<table>
<thead>
<tr>
<th>TESTS</th>
<th>DAY 1 (MEAN +/- SD)</th>
<th>WEEK 1</th>
<th>WEEK 2</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
<td>25.082±4.848</td>
<td>24.857±4.628</td>
<td>24.922±4.327</td>
<td>0.4789</td>
</tr>
<tr>
<td>HB</td>
<td>7.823±1.535</td>
<td>7.753±1.486</td>
<td>7.574±1.391</td>
<td>0.0037</td>
</tr>
<tr>
<td>WBC</td>
<td>10.585±2.375</td>
<td>10.225±2.794</td>
<td>9.8475±2.245</td>
<td>0.2131</td>
</tr>
<tr>
<td>PLATELET</td>
<td>395.52±151.5</td>
<td>386.65±132.6</td>
<td>372.35±112.6</td>
<td>0.6398</td>
</tr>
<tr>
<td>MCV</td>
<td>81.025±8.073</td>
<td>80.460±8.677</td>
<td>80.110±8.135</td>
<td>0.1335</td>
</tr>
<tr>
<td>MCH</td>
<td>25.347±3.414</td>
<td>25.180±3.795</td>
<td>24.377±3.393</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MCHC</td>
<td>31.212±1.813</td>
<td>31.182±1.909</td>
<td>30.337±1.695</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RDW (CV)</td>
<td>24.215±3.335</td>
<td>23.980±3.100</td>
<td>24.502±2.802</td>
<td>0.4796</td>
</tr>
<tr>
<td>RBC</td>
<td>3.146±0.774</td>
<td>3.135±0.735</td>
<td>3.165±0.752</td>
<td>0.9773</td>
</tr>
</tbody>
</table>

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 COEFFICIENT OF VARIATION

DROP OUT RATE

166 DID INITIAL TESTS FOR EXCLUSION

21 EXCLUDED (8 MANTOUX POSITIVE, 9 NOT HOMOZYGOTE, 2 HEPATITIS, 2 HIV)

145 RANDOMISED

145 RANDOMISED

73 ON EXTRACT

72 ON ROUTINE DRUGS

8 DROP OUT

28 DROP OUT IN FIRST WEEK

4 HAD POOR FOLLOW UP FOR SAMPLING

65

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

\textsuperscript{a}Department of Obstetrics and Gynecology, Lagos State University College of Medicine, Lagos, Nigeria  
\textsuperscript{b}Department of Hematology and Blood Transfusion, Lagos State University College of Medicine, Lagos, Nigeria  
\textsuperscript{c}Department of Obstetrics and Gynecology, Olabisi Onabanjo University College of Medicine, Ogun State, Nigeria  
\textsuperscript{d}Department of Pharmacology, Lagos State University College of Medicine, Lagos, Nigeria

\textbf{Abstract}

\textbf{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 preoperative anemia booked for myomectomy.

\textbf{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.

\textbf{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.

\textbf{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.

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 \( \leq 28 \) d before surgery.  
2. The target for Hb level before elective surgery should be within the normal range (female \( \geq 12 \) g/dL, male \( \geq 13 \) g/dL)
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 anthrocyanidins. The most common anthocyanidins found in this variety of sorghum bicolor are the three deoxyanthrocyanidins and their derivatives, which include apigenin dimers, apigenin–flavone 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 hematoctrit (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 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/dL, which is suggestive of an infective process. Once these
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 \( 38 \pm 9 \) y in participants in the test group and \( 39.8 \pm 10.3 \) 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline 0 wk (N = 26)</th>
<th>After 3 wk (N = 26)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC ( (\times 10^9) )</td>
<td>( 6.13 \pm 1.91 )</td>
<td>( 4.94 \pm 0.71 )</td>
<td>0.06</td>
</tr>
<tr>
<td>RBC ( (\times 10^10) )</td>
<td>( 3.89 \pm 0.68 )</td>
<td>( 4.33 \pm 0.49 )</td>
<td>0.0002</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>( 7.916 \pm 2.047 )</td>
<td>( 9.628 \pm 1.469 )</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>( 29.47 \pm 5.8 )</td>
<td>( 34.98 \pm 4.2 )</td>
<td>0.0001</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>( 75.75 \pm 6.6 )</td>
<td>( 81.52 \pm 7.50 )</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>( 20.29 \pm 3.75 )</td>
<td>( 22.32 \pm 3.14 )</td>
<td>0.0002</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>( 26.5 \pm 2.4 )</td>
<td>( 27.7 \pm 1.92 )</td>
<td>0.0008</td>
</tr>
<tr>
<td>Platelet ( (\times 10^6) )</td>
<td>( 292.8 \pm 167.0 )</td>
<td>( 262.8 \pm 122.0 )</td>
<td>0.26</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline 0 wk (N = 20)</th>
<th>After 3 wk (N = 20)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC ( (\times 10^9) )</td>
<td>( 5.4 \pm 2.4 )</td>
<td>( 5.5 \pm 2.05 )</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RBC</td>
<td>( 3.74 \pm 0.7 )</td>
<td>( 3.82 \pm 1.09 )</td>
<td>0.129</td>
</tr>
<tr>
<td>Hb</td>
<td>( 7.475 \pm 2.096 )</td>
<td>( 9.838 \pm 2.47 )</td>
<td>0.04</td>
</tr>
<tr>
<td>Hct</td>
<td>( 27.4 \pm 6.8 )</td>
<td>( 31.8 \pm 6.0 )</td>
<td>0.267</td>
</tr>
<tr>
<td>MCV</td>
<td>( 72.8 \pm 8.8 )</td>
<td>( 87.78 \pm 21.6 )</td>
<td>0.0007</td>
</tr>
<tr>
<td>MCH</td>
<td>( 20.4 \pm 4.0 )</td>
<td>( 33.2 \pm 22.9 )</td>
<td>0.0007</td>
</tr>
<tr>
<td>MCHC</td>
<td>( 27.0 \pm 3.7 )</td>
<td>( 34.0 \pm 1.5 )</td>
<td>0.0002</td>
</tr>
<tr>
<td>Platelet ( (\times 10^6) )</td>
<td>( 312 \pm 120 )</td>
<td>( 342 \pm 162 )</td>
<td>0.2</td>
</tr>
</tbody>
</table>

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
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 ($P = 0.0002$ 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 ($P = 0.3$).

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 hematins, 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 present 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 patients 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 of 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 3
Changes in chemistry parameters in participants receiving the sorghum bicolor extract in addition to routine drugs

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

ALT, alanine aminotransaminase; AST, aspartate aminotransferase

### Table 4
Comparison of the differences in RBC indices parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD test group*</th>
<th>Mean ± SD control group †</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>1.86 ± 1.97</td>
<td>1.77 ± 2.3</td>
<td>0.91</td>
</tr>
<tr>
<td>RBC</td>
<td>0.44 ± 0.77</td>
<td>0.18 ± 0.9</td>
<td>0.44</td>
</tr>
<tr>
<td>Hct</td>
<td>6.57 ± 7.1</td>
<td>3.85 ± 8.0</td>
<td>0.2</td>
</tr>
<tr>
<td>MCV</td>
<td>5.92 ± 8.3</td>
<td>5.9 ± 7.1</td>
<td>0.99</td>
</tr>
<tr>
<td>MCH</td>
<td>2.0 ± 3.2</td>
<td>3.1 ± 2.6</td>
<td>0.41</td>
</tr>
<tr>
<td>MCHC</td>
<td>1.16 ± 2.2</td>
<td>1.08 ± 2.7</td>
<td>0.94</td>
</tr>
</tbody>
</table>

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 activity may be due to de novo synthesis in the test group; an increase in these same parameters was observed for preoperative 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 administrative support; and A. O. Tayo et al. / Nutrition 33 (2017) 113–117

References


Conclusion

Approximately 15% of patients with preoperative anemia will benefit from the addition of the extract of sorghum bicolore 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.

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References

Sub-acute Toxicological Effects of Jobelyn® on Pregnant Albino Rats

Abiodun Humphrey Adebayo¹,a), Omolara Faith Yakubu¹, Godwin Eneji Egbung², Olabisi Ibidun Williams¹, Olajuwon Okubena³

¹Department of Biochemistry, College of Science and Technology, Covenant University, PMB 1023, Canaan land, Ota, Ogun State, Nigeria
²Department of Biochemistry, Faculty of Basic Medicine, University of Calabar, Calabar, Cross River State, Nigeria
³Health Forever International, Ikeja, Lagos, Nigeria.

a) Corresponding author: abiodun.adebayo@covenantuniversity.edu.ng

Abstract. The aim of the present study was to investigate the sub-acute toxicological effects of Jobelyn® on pregnant albino rats by employing biochemical, haematological and histopathological methods. A total of 32 pregnant female rats were randomly assigned to four different groups of eight rats each. The control group received distilled water and different doses of Jobelyn®; 250, 500, 1000 mg kg⁻¹ were administered orally once a day for 2 weeks to the other groups. Biochemical analysis revealed a significant decrease (p<0.05) in the levels of alanine aminotransferase, albumin, urea, PCV and Hb in the treatment groups when compared to the control. However, the significant decrease in PCV and Hb was observed solely in the group treated with 1000 mg kg⁻¹ body weight, suggesting that this decrease could be dose-dependent. Alkaline phosphatase, total protein, triglycerides, cholesterol, HDL cholesterol, LDL cholesterol, eosinophils, basophils, neutrophils, monocytes, lymphocytes, WBC count, revealed no significant difference (p<0.05) when compared to the control. The results show that at an appropriate dosage, the use of Jobelyn® during pregnancy may have no adverse effect on the liver and kidney tissues and may possess hepatoprotective and nephroprotective properties however the histopathological studies revealed that very high levels of Jobelyn may be hepatotoxic.

INTRODUCTION

Jobelyn® is a multifunctional natural ingredient derived from the leaf sheaths of Sorghum bicolor, a member of the Poaceae family [1]. All parts of the plant, Sorghum (bicolor) have been useful in disease treatment, making the plant a phytoceutical. The leaf sheath of Sorghum bicolor is commonly used as a remedy against anaemia by traditional medicine healers [2]. The root is used in treating malaria in Southern Rhodesia; the seed has been employed for the treatment of breast disease and diarrhoea while the stem has been used for tubercular swellings treatment [3]. Recently, focus has been on the leaf sheath of S. bicolor being used as herbal remedy for anaemia and having a boosting effect on blood concentration; possessing hematinic potentials [2, 4]. This powder extract from Sorghum bicolor(Jobelyn®), contains proteins, carbohydrates, dietary fibre as well as bioactive substances known as phytochemicals, which have the potential to significantly affect human health positively [5]. The high concentration of phytochemicals, including proanthocyanidins, anthocyanins, phenolic acids, apigenin, proapigeninidin, apigeninin, luteolin, naringenins in this wholly, natural, herbal product confers on it, very high antioxidant activity with a total oxygen radical absorbance capacity (ORAC) score of 37,000 μmolTE/g of dry powder[6]. It is capable of inhibiting peroxyl free radicals, hydroxyl free radicals, singlet oxygen and possesses a high capacity for quenching superoxide anions, hence its usefulness in maintaining human health [7]. The inhibition of peroxyl free radicals per gram of jobelyn® is 3,549 μmoleTE/g as compared to 997, 68, 24, 15, 8 and 7 μmoleTE/g of acai berry, cherry tart, blueberry, strawberries, red grape, respectively [8]. This indicates that the antioxidant capacity of Jobelyn® is about 4 times higher than that of acai berry and 50 times than that of tart cherries [8] thussignifying the antiradical properties of Jobelyn® [9]. This herbal formulation is believed to alleviate symptoms of anaemia of diverse origin, among which are malaria, sickle cell disease, leukaemia, stroke, arthritis, pregnancy, enteric fever, helminthisis, multiple myeloma, and tuberculosis, its use in pregnancy being quite notable [1, 10]. Elevation of packed cell volume (PCV) from 21 percent to 32 per cent has been observed in pregnant women within eight days of ectopic pregnancy operation following the use of Jobelyn® [unpublished]. Despite the wide use of this herbal
formulation, there is a paucity of studies on its toxicological profile. In relation to its effect on pregnant women, there has been no previous work done to ascertain if it poses health risks on the mother or not. The plethora of previously reported and unreported adverse drug reactions associated with the use of herbal medicines makes it imperative that pre-clinical toxicological studies be carried out on these natural products [11]. Furthermore, the recommendation of the herbal drug as a dietary supplement, establishes the need for toxicological studies on its effects on pregnant women. The primary aim of the present study was to investigate the sub-acute toxicological effect of Jobelyn® by employing the biochemical, haematological, and histological indices in pregnant albino Wistar rats.

MATERIALS AND METHODS

A total of thirty two healthy cyclic female Wistar albino rats weighing 82-190 g were used in this study. Animals were acclimatized for two weeks prior to commencement of treatment housed under standard environmental conditions and fed with a standard rat diet (obtained from Graceline feeds, Ota, Ogun state) with free access to water. During this period of acclimatization, the animals were exposed to a photoperiodicity of 12h light/12h darkness and were periodically assessed for gross morphological/behavioural changes.

Experimental Design

The animals were divided into 4 groups of 8 rats each and treated daily for 2 weeks; beginning from the 5th to 19th day of gestation during the period of foetal organogenesis. Group I served as the control group and received distilled water while groups II, III and IV received 250, 500 and 1000 mg kg⁻¹ of Jobelyn®. All animals were weighed and sacrificed on the 20th day of pregnancy. Their uteri were dissected and blood samples were immediately collected. The liver and kidney was collected, trimmed of excess fat and weighed and a portion was separated for histological studies.

Haematological assays

Blood samples were withdrawn from the heart to measure the levels of haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), WBC differentials (neutrophils, monocytes, lymphocytes, basophils and eosinophil) [12]

Biochemical assays

The commercial test kits for liver function test were purchased from Randox Laboratory, United Kingdom. Standard procedures were used to evaluate the protein concentration [13], aspartate aminotransferase (AST) [14], alkaline phosphatase (ALP) [15], alanine aminotransferase (ALT)[16], albumin[17], direct bilirubin [18], urea[19] and creatinine[20].

Histopathological studies

The organ tissues were fixed in normal saline for a period of 72 h and cut into thin slices 2.1 mm thick. The tissues were dehumidified using liquor. They were thereafter treated with paraffin wax and cast into blocks; tissue sections were then slit into 5μm using microtome and allowed to dry on a slide. The slides were afterwards soiled with haematoxylin-eosin stain, analyzed using a light microscope and photomicrographs recorded [21].

Statistical analysis

Differences between means of biochemical and haematological analysis of all the groups were estimated using a one-way analysis of variance followed by least significant difference test (post hoc) using the SPSS software (SPSS 15 for Windows). The p<0.05 were considered statistically significant. Graph pad prism was used in presentation of charts.

RESULTS
### TABLE 1. Effect of Jobelyn® on Weight of Selected Organs of Pregnant Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER</td>
<td>5.97±0.53</td>
<td>6.03±0.87</td>
<td>6.27±0.45</td>
<td>5.24±0.77</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.47±0.03</td>
<td>0.47±0.04</td>
<td>0.47±0.02</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>HEART</td>
<td>0.45±0.03</td>
<td>0.47±0.04</td>
<td>0.49±0.02</td>
<td>0.49±0.04</td>
</tr>
<tr>
<td>LUNGS</td>
<td>1.13±0.09</td>
<td>1.13±0.13</td>
<td>1.29±0.06</td>
<td>1.15±1.15</td>
</tr>
<tr>
<td>BRAIN</td>
<td>1.41±0.06</td>
<td>1.16±0.18</td>
<td>1.32±0.04</td>
<td>1.35±0.05</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>0.58±0.06</td>
<td>48.5±5.74</td>
<td>0.76±0.05</td>
<td>1.02±0.18</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates. From the Table 1, there was no significant difference p<0.05 in the weight of the kidney, heart, lungs, brain, spleen of treatment groups administered with Jobelyn® when compared with the control group.

### TABLE 2. Effect of Jobelyn® on Liver Homogenate of Pregnant Albino Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/I)</td>
<td>94.09±5.01</td>
<td>90.54±1.92</td>
<td>86.57±1.12</td>
<td>88.16±1.84</td>
</tr>
<tr>
<td>ALP (U/I)</td>
<td>104.42±23.26</td>
<td>237.65±127.25</td>
<td>77.54±14.93</td>
<td>140.66±66.50</td>
</tr>
<tr>
<td>AST (U/I)</td>
<td>118.21±4.10</td>
<td>103.25±17.63</td>
<td>117.02±15.01</td>
<td>136.59±2.57</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>2.08±0.41</td>
<td>2.20±0.43</td>
<td>1.79±0.35</td>
<td>1.55±0.28</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>0.61±0.12</td>
<td>0.65±0.89</td>
<td>0.44±0.08</td>
<td>0.57±0.10</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, significantly different from the control group. ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, TP = Total protein, ALB = Albumin, DBIL = Direct Bilirubin. From Table 2, there was no significant difference p<0.05 in the activity of ALT, ALP, AST, TP, Albumin and Direct Bilirubin in the liver homogenate of groups administered Jobelyn® when compared with the control group.

### TABLE 3. Effect of Jobelyn® in Kidney Homogenate of Pregnant Albino Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA (mg/dl)</td>
<td>27.22±1.64</td>
<td>20.98±1.92</td>
<td>10.39±2.63</td>
<td>25.50±9.96</td>
</tr>
<tr>
<td>CREA (mg/dl)</td>
<td>1.83±0.32</td>
<td>1.75±0.32</td>
<td>1.27±0.31</td>
<td>1.55±0.48</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. CREA = Creatinine. From Table 3, there was no significant difference p<0.05 in the levels of urea and creatinine in the kidney homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

### TABLE 4. Effect of Jobelyn® on Selected Lipid Profile Parameters in Plasma of Pregnant Albino Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIGS (mg/dl)</td>
<td>47.02±10.33</td>
<td>41.53±8.05</td>
<td>52.60±8.51</td>
<td>43.48±14.48</td>
</tr>
<tr>
<td>T.CHOL (mg/dl)</td>
<td>66.66±8.19</td>
<td>48.16±1.34</td>
<td>59.26±4.46</td>
<td>53.84±5.90</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. TRIGS = Triglycerides, T. CHOL = Total Cholesterol. From Table 4, there was no significant difference p<0.05 in the levels of Triglycerides and Total cholesterol in the plasma of the treatment groups administered with Jobelyn® when compared with the control group.

### TABLE 5. Effect of Jobelyn® on Selected Lipid Profile Parameters in Liver homogenate of Pregnant Albino Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIGS (mg/dl)</td>
<td>119.52±13.88</td>
<td>102.93±11.85</td>
<td>102.33±9.93</td>
<td>146.34±23.26</td>
</tr>
<tr>
<td>T.CHOL (mg/dl)</td>
<td>25.00±5.01</td>
<td>23.41±3.00</td>
<td>36.95±8.20</td>
<td>32.48±9.29</td>
</tr>
<tr>
<td>HDL CHOL (mg/dl)</td>
<td>206.89±3.73</td>
<td>201.80±0.83</td>
<td>202.77±0.90</td>
<td>207.11±6.76</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. HDL CHOL = High density lipoprotein cholesterol.
Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. TRIGS = Triglycerides, TOTAL CHOL = Total Cholesterol, HDL CHOL = High Density Lipoprotein Cholesterol. From Table 5, there was no significant difference p>0.05 in the levels of Triglycerides, Total cholesterol and HDL cholesterol in the liver homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

**TABLE 6.** Effect of Jobelyn® on Selected Lipid Profile Parameters in Kidney of Pregnant Albino Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (A)</th>
<th>250 mg kg⁻¹ (B)</th>
<th>500 mg kg⁻¹ (C)</th>
<th>1000 mg kg⁻¹ (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIGS (mg/dl)</td>
<td>60.24±2.95</td>
<td>60.70±7.55</td>
<td>56.20±3.50</td>
<td>54.52±3.19</td>
</tr>
<tr>
<td>T.CHOL (mg/dl)</td>
<td>48.16±3.32</td>
<td>39.94±3.47</td>
<td>52.46±6.95</td>
<td>42.58±6.31</td>
</tr>
<tr>
<td>HDL CHOL (mg/dl)</td>
<td>21.19±6.11</td>
<td>15.77±3.60</td>
<td>14.74±1.98</td>
<td>14.62±1.31</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates p<0.05, not significantly different from the control group. TRIGS = Triglycerides, TOT CHOL = Total Cholesterol, HDL CHOL = High Density Lipoprotein Cholesterol. From Table 6, there was no significant difference p<0.05 in the levels of Triglycerides, Total cholesterol and HDL cholesterol in the kidney homogenate of the treatment groups administered with Jobelyn® when compared with the control group.

**TABLE 7.** Effect of Jobelyn® on Haematological Parameters in Plasma of Pregnant Rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>250 mg kg⁻¹</th>
<th>500 mg kg⁻¹</th>
<th>1000 mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOSINO (x 10¹²/L)</td>
<td>41.00±3.49</td>
<td>34.80±4.76</td>
<td>38.11±1.88</td>
<td>40.29±4.14</td>
</tr>
<tr>
<td>NEUTRO (x10¹²/L)</td>
<td>46.50±1.55</td>
<td>56.80±5.32</td>
<td>52.67±1.70</td>
<td>54.50±2.81</td>
</tr>
<tr>
<td>BASO (x 10¹²/L)</td>
<td>4.0±1.73</td>
<td>3.4±1.03</td>
<td>2.25±0.37</td>
<td>1.86±0.55*</td>
</tr>
<tr>
<td>LYMPH (%)</td>
<td>4.25±0.63</td>
<td>5.00±1.30</td>
<td>5.22±0.97</td>
<td>3.83±1.08</td>
</tr>
<tr>
<td>MONO (%)</td>
<td>1.50±0.29</td>
<td>1.0±0.0</td>
<td>1.57±0.30</td>
<td>1.40±0.24</td>
</tr>
<tr>
<td>PCV (x 10¹²/L)</td>
<td>55.5±6.51</td>
<td>48.50±5.74</td>
<td>51.38±1.46</td>
<td>41.50±1.74*</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>17.90±2.10</td>
<td>15.65±1.85</td>
<td>16.57±0.47</td>
<td>13.39±0.56*</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of 8 replicates. *p<0.05: significantly different from the control group. WBC COUNT = White Blood Cell Count, NEUTRO = Neutrophils, EOSINO: =Eosinophils, BASO = Basophils, LYMPH = Lymphocytes, MONO = Monocytes, PCV: =Packed Cell Volume, Hb = Haemoglobin. From Table 7, there was no significant difference p<0.05 in the levels of neutrophils, eosinophils, basophils, lymphocytes, monocytes, white blood cell count, whereas, there was a significant difference p<0.05 in levels of haemoglobin and packed cell volume in the plasma of the treatment groups administered with Jobelyn® when compared with the control group.

**Histopathological examination**

In the histopathological examination, hepatoytes in control group revealed normal nuclei membrane, rough endoplasmic reticulum and mitochondria (Fig. 3A). But in the 250mg kg⁻¹ group, mild to moderate portal inflammation was observed (Fig. 3B). The 500 mg kg⁻¹ group (Figure 3C) revealed moderate inflammation, while moderate to severe portal inflammation was observed in the 1000 mg kg⁻¹ (Fig. 3D).

**DISCUSSION**

Toxicological assessment of drugs, herbs and extracts are necessary to establish the safety limit of these substances in animals. These are then commonly used to assess the possible health risk in humans [22]. The liver is a metabolically active organ responsible for many vital life functions, some of such primary functions include; bile production and excretion; excretion of bilirubin, cholesterol, hormones, and drugs; metabolism of fats, proteins, and carbohydrates; storage of glycogen, vitamins, and minerals; synthesis of plasma proteins, such as albumin, and clotting factors, as well as blood detoxification and purification [23]. Due to these important activities, the liver is one of the body's organs most subject to injury. It is the central organ in the metabolism and detoxification of drugs and toxins. Consequently, drugs affect the liver more frequently than any other organ and place the liver at great risk for toxic damage [24]. The cells in the liver contain proteins called enzymes that drive these chemical reactions. When liver cells are damaged or destroyed, the enzymes in the cells leak out into the blood. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) are specific markers of
hepatic injury and hepatocellular necrosis [25]. A significant decrease (P<0.05) in the activity of alanine aminotransferase (ALT) was observed in groups treated with 500 and 1000 mg/kg body weight of Jobelyn® when compared with the control (Fig 1). Hence, suggesting that Jobelyn® may possess hepatoprotective properties. ALT is localized in the cytosol of the hepatocytes and is a more sensitive marker of hepatocellular damage as opposed to AST which can also be produced from other tissues like heart, kidney and pancreas other than the liver [26]. However, AST and ALP levels were not significantly different (P<0.05) in the treated groups when compared with the control groups. A significant decrease (P<0.05) in albumin levels in the group administered 500 mg/kg body weight of Jobelyn® was observed (ig 1). A similar study conducted by Salawu et al. [27] and Mancini-Filho [28] revealed similar results. The possible hepatoprotective effects of Jobelyn® could be attributed to the presence of significant levels of flavonoids such as tannins and anthocyanidins which help to clear up free radicals. It has been established that free radicals play a significant role in various pathogenesis, inflammatory diseases and can result in necrosis of the liver [29, 30]. Flavonoids are known for their ability to exhibit antioxidant potential, and their effects on human health and nutrition are considerable. The mechanisms of action of flavonoids are through chelating or scavenging processes [31]. Chemically, the remarkable antioxidant properties of flavonoids is due to the hydrogen donating substituents (hydroxyl groups), which are attached to the aromatic ring structures of flavonoids, enabling the flavonoids to undergo a redox reaction and thus helping them to rapidly scavenge free radicals easily. Flavonoids also mediate their antioxidant effect by a stable delocalization system, which consist of heterocyclic and aromatic rings as well as multiple unsaturated bonds, and helps to delocalize the resulting free radicals [32]. Finally, the presence of certain structural moieties which are capable of forming transition metal chelating complexes that can regulate the production of reactive oxygen species such as O2- and OH is also a mechanism through which flavonoids mediate their antioxidant effect [33]. Sorghum bicolor, the plant extract of Jobelyn® is known to possess high concentration of flavonoids which include tannins and anthocyanins, significantly possessing high concentrations of dimeric 3-deoxy-anthocyanidins [34, 35]. Phenolic compounds, which have also been found to exist in high concentrations in Sorghum bicolor [36] are considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living system, further establishing the antiradical properties of Jobelyn® [36]. Epidemiological studies have suggested association between the consumption of phenolic acid - rich foods or beverages and the prevention of many diseases [37]. The potential hepatoprotective properties of this wholly herbal drug is further supported by the significant decrease (P<0.05) in albumin levels in the group administered 500 mg/kg body weight of Jobelyn®. This could have been pregnancy-induced, as albumin is often decreased in normal pregnancy as a consequence of haem dilution. It is noteworthy though, that a low albumin level is often temporary, and is not a reliable way to diagnose liver disease. In contrast, the concentrations of the transaminases (alanine and aspartate) and γ-glutamyltransferase normally increase during pregnancy. Thus, the decreased levels of ALT observed in pregnant rats treated with Jobelyn® is a further proof of the possible hepatoprotective effect of Jobelyn®. The histological findings revealed mild to moderate portal inflammation in the liver of animals treated with 250 and 500 mg/kg bodyweight of Jobelyn®, while the liver of rat treated with 1000 mg/kg bodyweight of Jobelyn® showed moderate to severe portal inflammation (Fig 3). This is possibly as a result of the dose of Jobelyn® administered to the animals as the severity of liver abnormality increased with increasing dosage. Bilirubin levels were not significantly different (p<0.05) when compared with the control. Bilirubin is a waste product from the breakdown of red blood cells. The liver processes bilirubin so it can be excreted in stool. Bilirubin flows through the liver's bile ducts, dissolved in bile. Bilirubin blood levels may be elevated in people with impaired bile flow. This can occur in severe liver disease, gallbladder disease, or other bile system conditions. Very high bilirubin levels cause jaundice, in which the skin and whites of the eyes turn yellow [38]. Bilirubin can be a useful liver function test in people with a known bile flow problem. Higher than normal levels of direct or indirect bilirubin may indicate different types of liver problems. Occasionally, higher bilirubin levels may indicate an increased rate of destruction of red blood cells. Hence, the stable level of bilirubin in animals treated with Jobelyn® further supports the safety and possible hepatoprotective role of Jobelyn®. A significant decrease (p<0.05) in Urea concentration was observed, suggesting that Jobelyn® may have protective effects on renal function. Salawu et al. [27] also observed similar effects when Sorghum bicolor aqueous extract was administered to rats fed with low and high iron diet. This implies that the administration of Jobelyn® has no toxic effect on the kidney and may thereby preserve the integrity of the kidney. Urea is the major end product of protein metabolism in humans and other mammals. Factors which tend to increase urea excretion include increases in glomerular filtration rate, increased dietary protein intake, protein catabolic conditions, and water diuretic states. Factors which reduce urea excretion include low protein intake and conditions which result in low urine output such as dehydration, although low urea levels are seen during normal pregnancy. Biochemical analysis revealed significant decrease (p<0.05) in the levels of Hb and PCV in only the group administered 1000mg/kg which could be due to the high-dose (Table 7). The PCV (packed cell volume) determines the percentage of red blood cells in the plasma. During Pregnancy, however, decreased hematocrit levels are observed, especially in the last trimester as plasma volume increases. Haemodilution occurs physiologically in pregnancy [39]. This may result in lower haemoglobin concentrations than in the non-pregnant state. Sorghum contains such hard-to-find nutrients as iron, calcium and potassium. In the past, doctors prescribed sorghum as a daily supplement for those low in these nutrients. No significant difference was observed in
the levels of triglyceride, total cholesterol and high density cholesterol. Implying that Jobelyn® poses no threat on the body as relates to diseases caused by increased cholesterol such as atherosclerosis. In addition to the high content of anti-inflammatory phenolic compounds, sorghum contains several groups of bioactive compounds with the capacity to induce pro-inflammatory immune responses. Water-soluble beta-glucans are found in sorghum that showed biologically active beta-glucans capable of initiating macrophage activation.

![Graphs of plasma and kidney functions](image)

**FIGURE 1.** Effects of Jobelyn on plasma of pregnant albino rats. ALT = Alanine aminotransferase, ALP = Alkaline phosphatise, AST = Aspartate transaminase, TP = Total protein, ALB = Albumin. Values are presented as mean ± standard error of mean (SEM) of 8 replicates; *significant at p < 0.05 as compared with control.

**FIGURE 2.** Effects of Jobelyn on Kidney functions of pregnant albino rats. Values are presented as mean ± standard error of mean (SEM) of 8 replicates; *significant at p < 0.05 as compared with control.

**RECOMMENDATION**

Jobelyn® should be subjected to further analysis to clearly ascertain the mechanisms involved in its suggested haematocrit boosting property, as well as its proposed hepatoprotective and renal protective property, with emphasis on pregnant women. This would help further confirm the safety of the drug for consumption by pregnant women.
FIGURE 3. Photomicrograph of liver tissues of rat (H&E x 400)(A) with normal features in the control (B) treated with 250mg/kg bodyweight of Jobelyn® Showing mild to moderate portal inflammation, H&E x 400 (C) treated 500 mg/kg bodyweight of Jobelyn® (D) treated with 1000 mg/kg bodyweight of Jobelyn®.

CONCLUSION

In conclusion, Jobelyn may enhance liver and kidney functions in pregnant women when taken at an appropriate dose. It may also boost haematocrit level, and thus could be ideal for intake by pregnant women.

REFERENCES

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Original Research Article

Toxicological Profiles of Commercial Herbal Preparation, Jobelyn®

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Abstract

PURPOSE: Jobelyn® is a commercial herbal product recommended for the management of anemia related illnesses. Despite its wide use, there is limited report on its toxicological profile. This study examined the acute and short-term chronic toxicity profiles of the product with emphasis on the LD$_{50}$, gross morphological and histopathological effects.

METHODS: Albino mice (mean weight: 16.45±3.14g) were used in this study. For acute toxicity, graded concentrations of Jobelyn® were administered orally and intraperitoneally as single doses to the mice. Intraperitoneal administration of sub-lethal doses daily for 14 days was adopted for the short-term chronic toxicity studies.

RESULTS: The LD$_{50}$ following oral and intraperitoneal administration were 215.06 mg/kg ($r = 0.916$) and 193.37 mg/kg ($r = 0.995$), respectively. The major behavioral/morphological effects at high doses were reduction in motor activity, piloerection and sedation. The sub-lethal doses did not significantly modify the normal behavioral repertoire of licking, grooming and sniffing. Histopathological examination also did not indicate severe pathological changes. At the lethal doses, some degree of congestion was noticed in the lung, liver splenic and kidney tissues. Short-term chronic studies did not produce further toxic effects but transient mild sedation and piloerection and histopathological examination revealed only mild congestion in the organs. No death of the animals was recorded during the period of sub-chronic toxicity assessment.

CONCLUSION: Jobelyn® is likely to be safe for use in humans when administered at recommended doses.

Keywords: Jobelyn, safety profile, LD50, toxicity

Joshua F Eniojukan$^1$

Bolajoko A Aina$^2$

$^1$Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

$^2$Department of Clinical Pharmacy & Biopharmacy, Faculty of Pharmacy, University of Lagos, Idiaraba, Lagos, Nigeria.

For Correspondence:

Tel: +234-805-4104203

Email: jofeniojukan@gmail.com
Introduction

The Alma Ata declaration of 1978 encouraged the use of all available resources for primary healthcare and recommended that government should give high priority to using traditional health practices and incorporate proven traditional remedies into National Drug Policy and Regulations [1]. As much as 80% of people in developing world are said to depend on traditional medicines for primary healthcare [2]. Over the years, there has been worldwide recognition of the vital role of herbal medicines in healthcare [3, 4]. Unfortunately, most of the herbal medicines are poorly regulated and controlled in many countries. Nevertheless, there is a dearth of scientific proofs of effectiveness and safety of herbal preparations, which is required for marketing authorization [5, 6].

Jobelyn®, manufactured by Health Forever Products Ltd., Lagos, Nigeria, is a commercial herbal preparation from Sorghum bicolor leaf-sheaths which contains carbohydrates, protein, tannins, saponins and iron. It is claimed to stimulate rapid production of red blood cells and maintains the integrity of white blood cells even with the presence of viral or bacterial infections. Jobelyn® is said to strengthen the immune system and thereby enhances body’s defensive mechanisms. Unpublished studies revealed the anti-trypanosomal activities of the product and haematinic effects in laboratory animals. The manufacturers recommend it as remedy for anemia in sickle cell anemia, cancer, HIV/AIDS, malaria, typhoid fever, aplastic anemia and pregnancy. Preliminary pilot preclinical studies in mice showed that Jobelyn® does not create significant adverse effects but it significantly lowered serum creatinine and cholesterol levels. However, unpublished toxicological evaluation of the product in mice has shown its lethal effects at high doses. Although Jobelyn® is currently used by people in many countries, the clinical efficacy and safety have not been scientifically reported [7]. The main objective of this work was to evaluate its toxicity profiles after acute and short-term chronic administration.

Materials and Methods

Animals

Healthy albino mice (58 males and 50 females; average weight, 16.45 ±3.14 g; weight range 13.75 – 19.48 g) were obtained from the Animal House of the College of Medicine, University of Lagos, Nigeria. The animals were kept in clean cages (10 mice/cage) in well-ventilated room and allowed unrestricted access to livestock feeds (from Ladokun feeds, Ibadan) and fresh water. They were also allowed to acclimatize to the environment for one week before each experiment. During this period of acclimatization, the animals were periodically assessed for gross morphological/behavioral changes. The animal cages were cleaned out of waste alternate days.

Acute toxicity testing

This was carried out to determine dose-response effects, sub-lethal and lethal doses, and to calculate the LD₅₀ using both the oral and intraperitoneal routes

Oral Route: 42 mice were divided into 7 equal groups (A – G). Mice in groups A to F were administered 6, 12, 18, 24, 30 and 42 ml/kg of 20% solution of JOBELYN® (8.2 mg/ml) orally. The animals in group G that served as control, were given 0.3 ml of de-ionized water orally.

Intra-peritoneal (IP) route: 36 mice were also divided into 6 equal groups (A – F). Mice in groups A to E were given 6, 12, 18, 24, 30 and 60 ml/kg of the stock solution of Jobelyn® (8.2 mg/ml) intra-peritoneally whilst animals in group F, serving as control, received 0.3 ml of de-ionized water orally.

For each of the above routes, all the animals were monitored for gross morphological and
behavioral changes (including changes in locomotor activity, pilo-erection, normal behavioral repertoire [grooming/licking/biting], sedation, aggressiveness, catalepsy, appetite, urination, defecation, vomiting, sneezing/wheezing) over 72 hr and the LD$_{50}$ was determined using probit analysis within 95% confidence limit. The animals were then sacrificed and essential organs (preserved in 10% formaldehyde solution for 4 weeks before processing) subjected to histological examination for pathological changes. The various organs were processed using the automatic tissue processor. This technique involved dehydrating the well-fixed 3 mm-sized tissues placed in tissue baskets with their respective labels by passing them through graded alcohol. They were then moved into xylene solution baths and then placed in molten wax for impregnation. The solidified blocks were trimmed and sectioned using the Rotary microtome at 5 µ thickness. Sections were then floated on water bath at 50 °C and picked up using albuminized microscopic slides. The cut sections were dried on hot plates at 60 °C and then stained by haematoxytocin and eosin to demonstrate tissue structures.

Sub-acute toxicity testing/short-term chronic toxicity

Thirty (30) mice were divided into three equal groups (A – C). Those in groups A and B were given 0.1 and 0.2 ml of 20% solution of JOBELYN® (8.2 mg/ml) IP daily for 2 weeks while those in group C (control) were given 0.3 ml de-ionized water intraperitoneally daily for the same period. The animals were monitored for morbidity and mortality (including changes in locomotor activity, piloerection, normal behavioral repertoire [grooming/licking/biting], sedation, aggressiveness, catalepsy, appetite, urination, defecation, vomiting, sneezing/wheezing), sacrificed after 15 days and essential organs (preserved in 10% formaldehyde solution for 4 weeks before processing) were examined histologically for pathological changes as described earlier.

Results

Acute toxicity testing

Following oral treatment of the animals, the observed changes in behavior of the animals is presented in Table 1. The mortality rates and probit analysis report are recorded in Table 2. Reduced mobility accompanied the administration of Jobelyn® from the 0.3 ml dose level within 1 hr. However, the animals were alert, except at high doses where a slight degree of sedation was noted. There was also some degree of pilo-erection and the surviving animals recovered motility soon after 48 hr. The normal behavioral repertoire was maintained. There was no noticeable reduction in appetite or changes in urine output. The LD$_{50}$ values for oral route was found to be 215.06 (147.30 – 313.99) mg/kg. The summary of observed behavioral changes following intraperitoneal administration of Jobelyn®, mortality rates and probit analysis are presented in Tables 3 and 4, respectively.

Short-Term Chronic Toxicity Testing

The observed behavioral changes following the administration of 0.1ml and 0.2 ml of the stock solution of Jobelyn to 2 groups of ten mice each daily for 2 weeks are presented in Table 5. Histopathological examination revealed no marked pathological changes in the heart and kidneys but moderate congestion in the lungs and liver and slight congestion in the spleen at lethal doses.

Discussion

Most of the toxicological studies report that toxic effects due to the use of herbal medicine are associated with hepatotoxicity. Other toxic effects on the kidneys, nervous system, blood, and cardiovascular system,
### Table 1: Behavioral changes following acute oral doses of Jobelyn®

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Dose (mg/kg)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>Normal behavioral repertoire; no sedation; no piloerection over the 72 hr of observation.</td>
</tr>
<tr>
<td>0.1</td>
<td>49.85</td>
<td>Mobile, alert, grooming and licking; slight piloerection. Very active over the 72 hr of observation.</td>
</tr>
<tr>
<td>0.2</td>
<td>99.70</td>
<td>Slight reduction in motility; normal repertoire, slight piloerection. Active over the 72 hr of observation. No mouse died.</td>
</tr>
<tr>
<td>0.3</td>
<td>149.54</td>
<td>Reduced mobility, slight sedation and piloerection over 24 hr. Recovered motility, active and feed normally over the next 48 hr. 1 mouse died after 24 hr.</td>
</tr>
<tr>
<td>0.4</td>
<td>199.39</td>
<td>Reduced motility, sedation and piloerection over 24 hr. 2 mice died after 24 hr. Recovered motility after 36 hr although not very active. Fully recovered after 48 hr.</td>
</tr>
<tr>
<td>0.5</td>
<td>249.24</td>
<td>Marked reduction in motility. 3 mice died within 24 hr. Recovered motility after 72 hr.</td>
</tr>
<tr>
<td>0.7</td>
<td>348.95</td>
<td>Marked reduction in motility. 5 mice died within 24 hr.</td>
</tr>
</tbody>
</table>

### Table 2: Mortality rates and probit analysis result after acute oral administration of Jobelyn®

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Dose (mg)</th>
<th>Dose (mg/kg)</th>
<th># mice that died</th>
<th>Mortality</th>
<th>% Mortality</th>
<th>Probit +5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>6</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.82</td>
<td>49.85</td>
<td>6</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2</td>
<td>1.64</td>
<td>99.70</td>
<td>6</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.3</td>
<td>2.46</td>
<td>149.54</td>
<td>6</td>
<td>1/6</td>
<td>17</td>
<td>4.0458</td>
</tr>
<tr>
<td>0.4</td>
<td>3.28</td>
<td>199.39</td>
<td>6</td>
<td>2/6</td>
<td>33</td>
<td>4.5601</td>
</tr>
<tr>
<td>0.5</td>
<td>4.10</td>
<td>249.24</td>
<td>6</td>
<td>3/6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>5.74</td>
<td>348.95</td>
<td>6</td>
<td>5/6</td>
<td>83</td>
<td>5.9542</td>
</tr>
</tbody>
</table>

LD<sub>50</sub> value = 215.06 mg/kg; r = 0.916; confidence limit = 147.30 – 313.99 mg/kg

### Table 3: Observed behavioral changes after acute IP administration of Jobelyn®

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal behavioral repertoire. Alert.</td>
</tr>
<tr>
<td>0.2</td>
<td>Normal behavioral repertoire. Slight piloerection. 1 mouse died after 24 hrs. Full recovery after 48 hrs.</td>
</tr>
<tr>
<td>0.3</td>
<td>Slight reduction in motility, mild sedation over 24 hrs. 2 mice died within 24 hrs. Recovers fully after 48 hrs.</td>
</tr>
<tr>
<td>0.4</td>
<td>Reduced motility, sedation, piloerection over 48 hrs. 3 mice died after 24 hrs.</td>
</tr>
<tr>
<td>0.5</td>
<td>Reduced motility, marked sedation over 48 hrs. 4 mice died after 24 hrs.</td>
</tr>
<tr>
<td>1.0</td>
<td>Reduced motility, marked sedation over 12 hrs. All died within 24 hrs.</td>
</tr>
</tbody>
</table>
Table 4: Mortality rates and probit analysis result after acute IP administration of Jobelyn®

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Dose (mg)</th>
<th>Dose (mg/kg)</th>
<th># mice</th>
<th># mice that died</th>
<th>Mortality</th>
<th>% Mortality</th>
<th>Probit +5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>6</td>
<td>0</td>
<td>0/6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.2</td>
<td>1.64</td>
<td>99.70</td>
<td>6</td>
<td>1</td>
<td>1/6</td>
<td>17</td>
<td>4.0458</td>
</tr>
<tr>
<td>0.3</td>
<td>2.46</td>
<td>149.54</td>
<td>6</td>
<td>2</td>
<td>2/6</td>
<td>33</td>
<td>4.5601</td>
</tr>
<tr>
<td>0.4</td>
<td>3.28</td>
<td>199.39</td>
<td>6</td>
<td>3</td>
<td>3/6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>4.10</td>
<td>249.24</td>
<td>6</td>
<td>4</td>
<td>4/6</td>
<td>67</td>
<td>5.4399</td>
</tr>
<tr>
<td>1.0</td>
<td>8.20</td>
<td>498.48</td>
<td>6</td>
<td>6</td>
<td>6/6</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

LD₅₀ value = 193.37 mg/kg; r = 0.995; confidence limit = 131.54 – 284.25 mg/kg

Table 5: Observed behavioral changes after subchronic IP administration

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal behavioral repertoire throughout the 14 days of observation. No mice died.</td>
</tr>
<tr>
<td>0.1</td>
<td>Normal behavioral repertoire. Slight piloerection. No mice died. Slight reduction in appetite</td>
</tr>
<tr>
<td>0.2</td>
<td>Reduced motility, piloerection. Slight sedation. No mice died. Slight reduction in appetite</td>
</tr>
</tbody>
</table>

as well as medicinal herbs' mutagenicity and carcinogenicity have also been published in medical journals [5]. The true incidence of hepatic damage caused by herbal medications is unknown. In the case of Chinese herbal remedies, the incidence of hepatotoxicity has been estimated at between 0.2% and 1% [6]. No accurate estimate of the prevalence of herbal remedies in Africa has been reported.

The manufacturers of Jobelyn recommend as much as 500 mg per dose (2 capsules) and 1.5 g per day (6 capsules) for humans. For any average adult weighing 70 kg, the recommended doses translate to a dosage of about 7.14 mg/kg per dose and 21.42 mg/kg/day, which are much smaller than the LD₅₀ in this study. Even though the extrapolation of data from animals to humans is anticipated and not definitive, the recommended dosage regimen in man can be said to be comparatively very safe. For the oral route, the product has a tolerance limit of 99.70mg/kg. This gives a large room for dosage manipulation, which may be applicable to man. In this study, Jobelyn® has been found to produce only toxic effects at high doses in the animals suggesting that Jobelyn is relatively safe when used at usual doses for a long time in the animals. Lethal consequences of the product may be expected in sufficiently high dosages.

Some behavioral changes in the animals (reduced motility and sedation) may not be mutually exclusive; i.e. one could be responsible for the other. These effects may also have been responsible for the seeming loss of appetite that was observed at high doses in this study. The eye irritation observed in the animals when the product was administrated through the oral route is attributable to direct contact of the product with the eye during administration.

**Conclusion**

There are indications that Jobelyn could be safe in humans when used at at doses recommended by the manufacturers. However, further toxicological screening in
humans is required to ascertain the safety profile.

Acknowledgements

This work was supported by a grant obtained from Health Products Forever Ltd., Lagos. We appreciate also the assistance of Dr. Ojo of the Morbid Anatomy Department, College of Medicine/LUTH, Idiaraba, for carrying out the histological evaluations. We appreciate the efforts of Messrs. Ogunyakin and David, the Principal Technologist and Laboratory Assistant, respectively, in the Department of Clinical Pharmacy and Biopharmacy, CMUL, Idi-Araba, Lagos, for their cooperation and technical assistance during this project.

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November 8, 2013.

Report for:
Olajuwon Okubena
Managing Director
Health Forever Products
No. 7 Dipeolu Street
Ikeja, Lagos, Nigeria
100001
Phone: +13015150139
234-1-4704093; 4970845-6
Info@health-forever.com
www.health-forever.com

Report 14: Evaluation of the effects of Jobelyn™ consumption on red blood cell count and quality.

Study Coordinator
Michelle Lenninger, M.A.

Research Director
Gitte Jensen, PhD.

Analyst
Kathleen F. Benson, PhD.

Executive Summary

The goals for this clinical study were to examine the effects of Jobelyn™ on the blood count in general, and specifically on red blood cell health in a borderline anemic, otherwise healthy North American population, as a parallel to several studies performed in West Africa, where sickle cell anemia, HIV, malaria, and other microbial diseases affecting red blood cell health, production, and senescence, are prevalent.

The outcomes were clear, and included the following:

1) Safety documentation

Overall, people consuming Jobelyn™ for 8 weeks had a similar blood count profile as people consuming placebo for 8 weeks.

2) Red blood cell health

People consuming Jobelyn™ showed extremely small, but significant changes to red blood cell parameters. However, the changes were not as simple as expected, and point to a complex array of effects in bone marrow and spleen with consumption of Jobelyn™. The surprising reduction in red blood cell counts (mild, but significant), accompanied by an increase in mean cell volume, and changes in other parameters reveals a complex effect of Jobelyn™ on formation of blood cells, suggesting an improved clearance of senescent RBC, accompanied by increased production of new RBC. The changes may also be related to a reduced inflammatory status. Further testing of cytokine profile will help put this data into context.
3) Effects on immune cells

Consumption of Jobelyn™ was associated with a rapid increase in the blood levels of monocytes and platelets. Whether this is associated with immune activation as well as bone marrow support is a question for future study.

4) Blood glucose

Consumption of Jobelyn™ was in general not associated with reduced fasting blood glucose in this study population. A few cases showed rapid changes, and based on this data further work may be planned.

During the study serum samples were banked from each blood draw. This material is available to pursue further testing without repeating the clinical part of the study. Serum testing may include detailed analysis of pro- and anti-inflammatory cytokines, as well as stem cell related growth factors.
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Report

Purpose

The purpose of the study was to evaluate the timing and magnitude of improvements to red blood cell health with consumption of Jobelyn™, to incorporate data into existing marketing efforts and to publish data in the medical literature as a peer-reviewed credible publication.

Introduction

Health Forever Products has case studies in Nigeria, as well as testimonials from all over the world, of seeing a robust increase in hemoglobin within days and within a few weeks, in people with serious cases of anemia present under either disease conditions like sickle cell, Malaria, HIV, or cancer. In parallel, improvements in red blood cell health were also seen in many healthy people with general low blood counts due to undetermined factors.

A clinical study on anemia is currently ongoing in Nigeria. The study population is focused on women and aims at evaluating whether Jobelyn™ consumption can help increase hemoglobin and thus reduce risk factors associated with gynecological surgery.

http://clinicaltrials.gov/ct2/show/NCT01670955

As a parallel to the ongoing study in Nigeria, this study protocol helped to systematically examine the effects of Jobelyn™ on anemic conditions in an otherwise healthy population, and helped document the speed and magnitude of improvements in a population without concomitant infections or sickle cell anemia.

Study design

Twenty-five human subjects of both genders were enrolled, and twenty-three completed the study with testing over the period of 8 weeks. Both genders were enrolled in the study, but we expected more women to be eligible, due to effects of menses and prolonged consumption of birth control pills.

Recruiting of study participants happened via NIS Labs. The study location was Klamath Falls, Oregon, which is located in the high desert of central Oregon, where people live and work at an altitude of 4,000-5,000 feet above sea level.
Subjects were monitored at baseline, and after 3 days, 7 days, and 2, 4, and 8 weeks.

A blood sample was taken at each visit. The primary purpose of the blood draws was to perform a complete blood count (CBC) with differential count. Each CBC was performed in triplicate at NIS Labs, using an AC-T 5-diff Coulter counter (Beckman-Coulter).

Fasting blood glucose was performed using a glucometer which was calibrated on each morning of every clinic visit for this study.

The blood draws also allowed for serum banking. This provides the option to add testing later without having to repeat a clinical study. Such testing may include antioxidant status and a number of test types for collecting data on inflammatory status. The baseline, 7 day, and 4, and 8 week samples align with the planned blood draws from the proposed chronic pain study, and this will allow synchronized testing at a later time, and a combined evaluation of inflammatory and other metabolic markers across both studies. This will allow data collection of antioxidant capacity and anti-inflammatory effects of Jobelyn™ across several studies, for a more robust sample size.
Study population

We recruited a total of 25 healthy subjects of both genders. Upon written informed consent, they went through a screening process to verify anemia or borderline anemic conditions. They were randomized to receive either Jobelyn™ or placebo.

Inclusion criteria

- 18-65 year old people of both genders
- Borderline anemic (This is compensated for 4,000-5,000 feet altitude of study location):
  - Males: Hemoglobin 13.5 g/dL or lower
  - Females: Hemoglobin 11.5 g/dL or lower

Exclusion criteria

- Known diagnosis with pernicious, aplastic, or sickle cell anemia, thalassemias;
- Splenectomy;
- Serious active illness within past 12 months;
- Active cancer and/or chemotherapy within the last 12 months;
- Major surgery during past 8 weeks;
- Received blood transfusion past 8 weeks;
- Having donated blood for 6 weeks prior to study, or planning to donate blood during the 8 week study;
- Consuming high doses of vitamin B12;
- Significant active uncontrolled disease (such as lymphoma, cirrhosis, nephritis, uncompensated heart failure);
- Use of multiple medications, indicating that the person’s self-reported state of ‘good health’ is questionable;
- History of drug abuse past 2 years;
- Record of non-compliance in previous studies;
- Display of cognitive impairment or mental instability during pre-screening and screening;
- Poor compliance during screening visits;
- Any other condition or observation that the study coordinator judges may adversely affect the person’s ability to complete the study;
- Currently experiencing intense stressful events/ life changes that would negatively affect compliance;
- Pregnant, nursing, or trying to become pregnant;
- Women not using effective contraception;
- Food allergies related to ingredients in test product.
Prescreening

The pre-screening involved an interview to document gender, age, estimated BMI, medical/surgical history, diet/lifestyle, current health issues, medication, and supplement use.

Screening

The screening visit involved signing a consent form, asking specific questions, verifying current BMI, and taking a blood draw to determine baseline hemoglobin levels.

Demographics of the study population

We recruited a total of 25 healthy subjects of both genders. Upon written informed consent, they went through a screening process to verify anemia or borderline anemic conditions. They were randomized to receive either Jobelyn™ or placebo.

Table 1. Demographics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Jobelyn™</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females:</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Age average*</td>
<td>44.1 ± 16.2</td>
<td>55.6 ± 7.5</td>
</tr>
<tr>
<td>Age range</td>
<td>20.9 - 65.3</td>
<td>44.4 - 64.6</td>
</tr>
<tr>
<td>BMI average*</td>
<td>26.3 ± 4.3</td>
<td>25.5 ± 5.7</td>
</tr>
<tr>
<td>BMI range</td>
<td>22.7 - 34.4</td>
<td>18.2 - 32.9</td>
</tr>
<tr>
<td>Males:</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Age average*</td>
<td>56.7 ± 5.9</td>
<td>40.2 ± 18.8</td>
</tr>
<tr>
<td>Age range</td>
<td>49.4 - 63</td>
<td>22.3 - 64.1</td>
</tr>
<tr>
<td>BMI average*</td>
<td>31.3 ± 4.9</td>
<td>27 ± 4.6</td>
</tr>
<tr>
<td>BMI range</td>
<td>26 - 38.8</td>
<td>20.5 - 31.1</td>
</tr>
</tbody>
</table>

* The average ± standard deviation is shown.
Consumables

Jobelyn™ was provided by Health Forever Products. A placebo powder was produced by NIS Labs. Rice flour was used, as it represents another grain, and of a type that very few people have allergies to. Other presumed inert substances can cause gastrointestinal upset, or have their own biological effects. Food color was added to the rice flour until a desired color was reached, and then was crushed into a powder that resembled Jobelyn™. The product and placebo were both encapsulated in identical, clear capsules.

Each study participant consumed 2 capsules per day during the study. This translated to the consumption of 500mg of Jobelyn™ for the people in the Jobelyn™ group.
Recruitment

Figure 2. Consort flow chart of the study participants.
Randomization

For a study of this nature, it is ideal to randomize study participants of each gender separately, so there is less risk of having more people of one gender in the placebo group and of the other gender in the active product group. Therefore, the randomization was performed as follows: A coin was tossed to determine the group assignment of the first female study participant. The first male study participant was then allocated to the other group. For subsequent volunteers, the group assignment was assigned alternately within each gender. The exception was family members or very close friends, who would be assigned to the same group to eliminate the risk of an accidental switch of bottles during the study within one household.

Compliance

Compliance during a study involves adhering to the study guidelines and consuming the allocated test product on a daily basis, as well as avoiding making major life style or diet changes during the study.

Compliance pertaining to consumptions was tracked by counting the remaining capsules in the returned bottles. Compliance to other requirements was tacked during interviews at each visit.

Table 2. Compliance based on capsule count.

<table>
<thead>
<tr>
<th>Compliance during</th>
<th>Average compliance (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>first 2 weeks</td>
<td>96.59%</td>
</tr>
<tr>
<td>second two weeks</td>
<td>94.37%</td>
</tr>
<tr>
<td>last 4 weeks</td>
<td>96.36%</td>
</tr>
<tr>
<td>entire study</td>
<td>97.12%</td>
</tr>
</tbody>
</table>

* These % include all study participants, except drop-outs.
Statistical analysis

Two kinds of statistical analyses were performed. The independent 2-tailed t-test was used to examine differences between the Jobelyn™ and placebo group for each time point. The unpaired t-test was performed both on raw data and on the percent changes seen for each parameter.

In addition, the paired t-test was used to compare each group’s scores before and after the intervention. This type of statistical analysis is ideal for comparing repeat measurements of the same subject over time.
Results

The complete blood count (CBC) data is presented in the following order:

- White blood cell count (WBC)
- Red blood cell count (RBC)
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Red cell distribution width (RDW)
- Platelet (PLT)
- Mean platelet volume (MPV)
- Neutrophil percentages (NE%)
- Lymphocyte percentages (LY%)
- Monocyte percentages (MO%)
- Eosinophil percentages (EO%)
- Basophil percentages (BA%)
- Neutrophil number (NE#)
- Lymphocyte number (LY#)
- Monocyte number (MO#)
- Eosinophil number (EO#)
- Basophil number (BA#)

On the following pages, data graphs are shown for each CBC parameter. For each parameter, there are two graphs, reflecting two different ways of analyzing the data.

- **Top graphs:**
  - Data are shown in a graph format that allows ‘between-group’ analysis, using the independent 2-tailed t-test.
  - For most data sets, there are no significant differences at any time point between the two groups.

- **Bottom graphs:**
  - The exact same data are shown in a format that allows a closer look at any minor fluctuations within each group, and allows indication of statistical significant changes within each group, using the ‘within-subject’ paired 2-tailed t-test.
  - Many graphs show one of the following:
    - Some significant changes within the Jobelyn™ group, without a matching change in the placebo group.
    - Or no change in Jobelyn™ group, despite a change in the Placebo group, such as may be caused by seasonal changes including allergies.
Figure 3. White blood cell (WBC) counts are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average WBC counts in the placebo group and the Jobelyn™ group.

The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, minor fluctuations in WBC counts were seen. A general mild reduction in WBC counts were seen for the placebo group over the 8-week study period. During the same time, no change was seen in the group consuming Jobelyn™, except a drop at Day 28 (borderline significant (*) when compared to baseline), which returned to the original level at 8 weeks. The data suggest that seasonal or other environmental changes occurring in the general population during that time were not affecting people as much if they were consuming Jobelyn™.
Figure 4. Red blood cell (RBC) counts are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average RBC counts in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, minor fluctuations in RBC counts were seen over time in the Jobelyn™ group ($p<0.01$, **). During the same time, no change was seen in the group consuming Placebo. The data suggest that Jobelyn™’s support of macrophage function may lead to an improved clearance of senescent RBC in the spleen, which if the study had been longer (3-4 months), and had allowed for a complete replenishment of the RBC pool, should be expected to return to normal.
Figure 5. Hemoglobin levels (HGB) (g/dL) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average hemoglobin levels in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, minor fluctuations in hemoglobin were seen. A mild reduction in hemoglobin was seen for the group consuming Jobelyn™ at Day 56 (p<0.001, *** when compared to baseline for the Jobelyn™ group). This follows the data for the red blood cell (RBC) count.
Figure 6. Hematocrit (HCT) (% volume) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average hematocrit in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, minor fluctuations in hematocrit were seen. A very mild reduction in hematocrit was seen for the group consuming Jobelyn™ at Day 56 (p<0.01, **) when compared to baseline for the Jobelyn™ group). This follows the data for the red blood cell (RBC) count.
Figure 7. Mean corpuscular volume (MCV) is shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average MCV in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when 'within-subject' analysis was performed for each group separately, minor fluctuations in MCV were seen. A very mild increase in MCV was seen for the group consuming Jobelyn™ at Day 56 (p<0.05, *) when compared to baseline for the Jobelyn™ group. Interestingly this change seen in the context of the previous red blood cell associated parameters may suggest that over time, with daily consumption of Jobelyn™, fewer but larger red blood cells are produced.
Figure 8. Mean corpuscular hemoglobin (MCH) is shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average MCH in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, minor fluctuations in MCH were seen in both groups. A very mild decrease in MCH was seen for the group consuming Jobelyn™ at Day 56 (p<0.01, **) when compared to baseline for the Jobelyn™ group. This result may reflect that hemoglobin production (per cell) remained constant while the cell size slightly increased (MCV).
Figure 9. Mean corpuscular hemoglobin concentration is shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average MCHC in the placebo group and the Jobelyn™ group.

The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, very minor decreases in MCHC were seen in both groups. This decrease was significant for the placebo group (p<0.05, *), and highly significant for the Jobelyn™ group (p<0.01, **) at Day 56 when compared to each respective baseline. This result may reflect that hemoglobin production (per cell) remained constant while the cell size slightly increased (MCV).
Figure 10. Red cell distribution width (RDW) is shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average MCHC in the placebo group and the Jobelyn™ group. The bottom graph serves to examine the data for any minor fluctuations. No significant changes were seen for either the placebo or the Jobelyn™ group, neither by between-group or within-subject analysis.
Figure 11. Platelet numbers (PLT) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to examine for differences between the average MCHC in the placebo group and the Jobelyn™ group at each time point. Interestingly, at Day 3, a significant difference was seen between the two groups, where a mild decrease in the placebo group, and a mild increase in the Jobelyn™ group resulted in significance between the Day 3 data (p<0.05, *).

The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, minor decreases in PLT were seen in both groups. This decrease was significant for the placebo group (p<0.05, *) at Day 3, and highly significant (p<0.01, **) at Day 56 when compared to baseline.
Figure 12. Mean platelet volume (MPV) is shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average MCHC in the placebo group and the Jobelyn™ group, using between-group statistical analysis. The bottom graph serves to examine the data for any minor fluctuations. No significant changes were seen for either the placebo or the Jobelyn™ group, using within-subject analysis.
Figure 13. Neutrophil percentages (NE%) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average NE% in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, very minor decreases in NE% were seen in both groups. This decrease was highly significant for the placebo group (p<0.01, **) at Day 56, and significant for the Jobelyn™ group (p<0.05, *) at Day 3 when compared to each respective baseline. The slow reduction in the placebo group may reflect seasonal changes. The apparent rapid change in the Jobelyn™ group needs to be evaluated in context of neutrophil numbers (NE#), see Figure 18, below. The combined data suggest that the rapid drop in NE%, while NE# remain constant, suggests a rapid increase of another white blood cell type.
Figure 14. Lymphocyte percentages (LY%) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average LY% in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, very minor decreases in LY% were seen in both groups. This decrease was highly significant for the placebo group (p<0.01, **) at Day 56 when compared to baseline.
Figure 15. Monocyte percentages (MO%) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to examine for significant differences at each time point between the average MO% in the placebo group and the Jobelyn™ group. At Day 14, a significant difference was seen between the placebo and Jobelyn™ groups, where the Jobelyn™ group showed a transient decrease in MO%, compared to the placebo group at the same time point (p<0.05, *). The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, two changes were noteworthy in the Jobelyn™ group. In the Jobelyn™ group, a rapid increase in MO% was seen at Day 3 (p<0.05, *), followed by the reduction at Day 14 when compared to baseline. This is parallel to decreases in MO#, see Figure 20, below.
Figure 16. Eosinophil percentages (EO%) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average EO% in the placebo group and the Jobelyn™ group.

The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, there were no significant changes seen in either group. Interestingly, there was a rapid decrease in the Jobelyn™ group, already after three days of consumption.
Figure 17. Basophil percentages (BA\%) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average EO\% in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, there were only very minor fluctuations in the BA\% over the 8 weeks.

*When evaluating this data, keep in mind the very low frequency of basophil cells. The normal range for BA\% is 0-2\%, so all data were minor fluctuations well within this normal range.*
Figure 18. Neutrophil number (NE#) % are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average NE# in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, a minor decrease in NE# was seen in the placebo group. This decrease was trending towards significance (p<0.1, (*)) at Day 56 when compared to baseline. The slow reduction in the placebo group may reflect seasonal changes. The NE# remained more constant in the Jobelyn™ group over the 8-week period.
Figure 19. Lymphocyte number (LY#) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average LY# in the placebo group and the Jobelyn™ group. At Day 28, the mild reduction in the Jobelyn™ group was borderline significant (p<0.1, (*) when compared to the lack of changes in the placebo group over the same time period. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, the only noticeable change was the mild reduction in LY# in the Jobelyn™ group at Day 28 (p<0.01, **).

*Keeping in mind that the normal range for LY# in 1-4 x 10³/µL for males and 0.9 – 3.6 10³/µL for females, the fluctuations seen are very minor, and well within the normal range.*
Figure 20. Monocyte number (MO#) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to examine for significant differences at each time point between the average MO# in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, a rapid increase in MO# was seen at Day 3, followed by the reduction at Day 14 when compared to baseline (p<0.05, *). The levels of MO# returned to baseline at Day 56. This is parallel to decreases in MO%, see Figure 15, above.

The fluctuations seen during the first 2 weeks of the study may reflect effects on Jobelyn™ consumption on monocyte numbers as part of increased immune surveillance, returning to pre-study levels after 8 weeks.
Figure 21. Eosinophil number (EO#) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average EO# in the placebo group and the Jobelyn™ group.

The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, there were no significant changes seen in either group.
Figure 22. Basophil number (BA#) are shown as the group averages ± SEM for each visit/blood draw. The top graph serves to illustrate that there were no significant differences at any time points between the average BA# in the placebo group and the Jobelyn™ group. The bottom graph serves to illustrate that when ‘within-subject’ analysis was performed for each group separately, there were only very minor fluctuations in the BA# over the 8 weeks.

When evaluating this data, keep in mind the very low frequency of basophil cells. The normal range for BA# is 0-0.1 x 10³/µL %, so all data were minor fluctuations well within this normal range.
Figure 22. Fasting blood glucose levels are shown as the group averages ± SEM for each visit/blood draw. The baseline levels of fasting blood glucose were similar in the placebo and the Jobelyn™ groups, and showed that the study population overall was pre-diabetic with an average fasting blood glucose level above 110 mg/dL. There were no significant differences at any time points between the average fasting glucose levels in the placebo group and the Jobelyn™ group. Even though there were no statistically significant differences, it is interesting that the group consuming Jobelyn™ showed lower fasting glucose levels than the placebo group from Day 14 and during the rest of the 8-week study.
Conclusions

Safety

The data presented here helps document basic safety aspects of Jobelyn™ consumption in a North American population. The rapid changes in red blood cell numbers and T cell numbers in West African studies in HIV+ populations could raise the question whether Jobelyn™ consumption is safe to consume for people who have close to normal numbers of such cell types, and whether Jobelyn™ consumption may trigger cellular production in the bone marrow that may be out of control. The data presented in this report clearly documents that Jobelyn™ consumption does not trigger such unhealthy production of cells. This can be seen as an important part of Jobelyn™’s safety data portfolio.

The highly specific activation of immune cells, documented in vitro [Benson et al. 2013], could lead to safety related concerns, such as whether Jobelyn™ consumption may trigger over-activation of immune reactions. The current data presented in this report does not suggest such events. Rather, the changes seen were either normalizing or transient, suggesting that Jobelyn™ consumption supports a healthy normalization of many aspects of red and white blood cell production and function.

Red blood cell health

Previous and ongoing studies in West Africa have seen very rapid improvement in RBC status, including improvements in hemoglobin in an HIV+ population. The data from these studies were performed in study populations where some common health challenges affecting RBC health include sickle cell anemia and parasitic infections such as malaria, i.e. conditions associated with accelerated RBC senescence and clearance.

In the study performed at NIS Labs, quite different results were obtained. Several factors may help explain this data in context of the West African studies. We suggest that the very mild, but highly significant changes seen in all parameters of RBC health, associated with consumption of Jobelyn™, may be associated with the following:

- Jobelyn™ activation of RBC production, leading to increased production of new RBC that are slightly larger than senescent RBC, thereby affecting RBC mean cell volume;
Jobelyn™ activation of macrophage function, leading to a better clearance of senescent RBC over time;

These mild changes in relative cell numbers seen associated with Jobelyn™ consumption should be interpreted with an open mind, since there are several unknown factors. The data should be considered in context of overall effect on bone marrow function and production of many different cell types.

An alternative suggested explanation of the reduced RBC numbers may be if Jobelyn™ has negative effects on iron absorption – however, this goes against the West African data where a much more challenged population saw huge benefits. Alternative explanations include production of healthier RBC with a proper senescence process, and healthier clearance of senescent RBC by spleen macrophages.

It is also possible that an ‘overworked’ bone marrow at high altitude and chronic low-grade inflammation may have been the status of the recruited subjects, where Jobelyn™ consumption allowed the marrow to produce a slightly lower amount of red blood cells of a higher quality, and redirect bone marrow efforts to an increased production of immune cells as needed (such as the increase in platelets and monocytes by Day 3).

Whether this also allowed the bone marrow to increase the production of stem cells was not answered by this study, buy could be addressed in future studies.

Interestingly, the population was pre-diabetic, as almost the entire population started the study with fasting glucose levels at 100 mg/dL or above. The association between circulating glucose levels and anemia is illustrated by the prevalence of anemia in a large proportion of diabetic patients.

**Immune cells**

Several observations are of interest here.

A rapid increase in monocytes was seen at Day 3, followed by a slight decrease at Day 14, after which time the monocyte percentage and numbers returned to baseline levels. This suggests that the initial consumption of Jobelyn™ initiated an immune response to latent or potential pathogens, which a slightly compromised immune system had been unresponsive to. (The elevated fasting glucose levels and borderline anemic conditions are suggestive of a possible association with mild systemic stressors, including low grade chronic inflammation).
Changes were seen in the platelet numbers in the placebo group, where a slight reduction happened across the 8-week study period. In contrast, the platelet levels in the Jobelyn™ group showed a mild increase at Day 3. This increase became statistically significant with the removal of one outlying data set, such that at Day 3 there was a significant difference in platelet numbers between the placebo and the Jobelyn™ group. This supports the suggestion of a rapid effect of Jobelyn™ on bone marrow production of various cell types. Interestingly, the progenitor stem cells that differentiate into red blood cells are shared with the progenitor path that leads to production of platelets and monocytes.

**Fasting blood glucose**

The study population was almost entirely comprised of pre-diabetic people with a fasting glucose level of 100 mg/dL or higher at study start. This was not associated with obesity, as many study participants had low-normal range BMI.

The measure of fasting glucose levels during this study aimed at collecting pilot data on whether Jobelyn™ consumption would be associated with regulation of fasting blood glucose. No significant changes were seen. There were a few cases where a drop in fasting glucose levels was seen at day 3 compared to baseline. Further study of the effects of Jobelyn™ consumption on fasting blood glucose is warranted, also in overweight and obese people.

**Recommendations for future studies**

The following options for further work may help increase our understanding of Jobelyn™ on regenerative functions, including red blood cell production and immune status:

1) Testing of comprehensive cytokine profile on the banked serum samples from this study may point to mechanisms that could further explain the data presented in this report; this may for example include the increased production of anti-inflammatory, immune regulatory, and stem cell supportive cytokines;

2) A study of longer duration, allowing us to follow RBC levels and quality for at least the 120 day typical life span of RBC:
   a. Study would examine markers for RBC senescence;
   b. Study would drill into more detail regarding immune cells:
i. Even though lymphocyte numbers seemed constant in both groups, it is possible that subsets of T and B lymphocytes were changed in the Jobelyn™ group, compared to the placebo group;

ii. This may include important changes within the lymphocyte population, for example involving T regulatory cells and natural killer cells, as well as antigen-presenting monocyte/macrophage and dendritic cell types.

3) Study may address other cell types, such as circulating stem cells.

References