



Efficiency of *Sorghum bicolor* extract in the treatment of induced anemia on *Wistar* rats

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Abstract

Sorghum bicolor is a medicinal plant used in Benin to treat anemia. This study aimed to evaluate its therapeutic efficacy in anemia treatment. An aqueous extraction of *Sorghum bicolor* leaves was carried out and analyzed. Five groups of five *Wistar* rats each were formed. The rats of four groups were rendered anemic by injection of phenylhydrazine (hemolysis) in the first two days D0 and D1. From the second to the fifteenth day (D2 to D15), anemic groups were gavaged either by the extract of *Sorghum bicolor* at 200 or 300 mg/kg body weight/day, or by vitafer, a reference drug against anemia. The last anemic group was not treated. The group of non-anemic rats served as a control. Blood samples were collected for all rats on days D0, D2, D7, D10 and D15 to assess blood count and osmotic resistance of red blood cells. The phytochemical analysis revealed the presence of catechol tannins, flavonoids, leucoanthocyanes, steroids, cardenolides, reducing compounds and coumarins. The extract like the vitafer corrected anemia in two weeks by increased stimulation of hemoglobin synthesis, production and the early release of immature red blood cells and its effect was dose dependent. Its action was quite specific and did not affect platelet lineage. *Sorghum bicolor* has an excellent therapeutic efficacy and may be considered for transformation into improved traditional medicines (ITM) after study of its biological tolerance and appropriate clinical trials.

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Introduction

Anemia is a common syndrome observed in various pathological conditions such as genetic defects, infections, etc. (Assobayire *et al.*, 2001). It is defined by a reduction of the normal quantity of blood circulating haemoglobin, less than 13 g/dl for male and less than 12 g/dl for female adults (Okochi *et al.*, 2003). Anaemia affects people of all ages, although the main targets are infants, pregnant women and elderly.

The prevalence of anemia is higher in developing countries. The causes and predisposing circumstances of anemia are multiple. The most important are nutritional deficiencies (Alper *et al.*, 2000; Assobayire *et al.*, 2001; Marti-Carragal *et al.*, 2002), high prevalence of blood parasites such as Plasmodium, Trypanosomes and helminthic infections (Verhoel *et al.*, 2002), as well as pregnancy and breastfeeding (Fricker, 1998; Alper *et al.*, 2000; Scanlon *et al.*, 2000; Assobayire *et al.*, 2001; Carragal-Marti *et al.*, 2002). Iron deficiency is by far the leading cause of anemia in the world with a proportion of about 50% of cases (Alper *et al.*, 2000; Assobayire *et al.*, 2001). More than half of the world's population would experience some form of anaemia in their lifetime (Duff, 2008). Also over 50% of pregnant women and over 40% of infants worldwide are anaemic with a prevailing significant morbidity and mortality rates particularly in the developing world (Holden and Acomb, 2007). Hence, anaemia is one of the leading health disorders posing a great threat to global healthcare.

Treatment varies depending on the type of anemia. It may be a supply of iron, vitamin B12 or B9 orally, treatment with immunosuppressants or corticosteroids, erythropoietin injections, blood transfusion, or even bone marrow transplantation (Movaffaghi and Hasanpoor, 2006).

In holding the high cost of modern drugs in pharmacy, WHO encourages the search for alternatives. The use of plants for therapeutic purposes is a centuries-old practice (Kassel, 2003).

About 60% of the world's population depends on herbal medicine for primary health care (Modak *et al.*, 2007). Ethnopharmacological information has shown that the use of various herbal plants for the treatment of anaemia is common (Akah *et al.*, 2010). *Sorghum bicolor* is a plant used for this purpose in West Africa and Benin (Ogwumike, 2002). This study aimed to investigate the therapeutic efficacy of aqueous extract of this plant on an experimental hemolytic anaemia model.

Material and methods

Animal material

Animal material consisted of *Wistar* albino rats of average body weight 185 g, having free access to water and food and acclimated to farming conditions from the pet of the Research Laboratory in Applied Biology (LARBA) located in the Polytechnic School of the University of Abomey (EPAC) in Benin Republic. Breeding was done in a well ventilated room, with a day-night rhythm of 12 h. The animals were kept in wire mesh cages with metal feeders and drinking troughs. Their daily diet was made from a mixture of food in the form of croquettes and marketed by Vet Services (Benin). The enclosure was regularly cleaned to ensure optimal development of the animals avoid infection.

Identification and preparation of plant materials

Leaves of *Sorghum bicolor* were collected from Tchaourou in Benin during March 2013. The collected samples were identified and authenticated at the National Herbarium of Benin (HNB) at the University of Abomey Calavi. The samples were dried at moderate temperatures (20-25° C), protected from moisture for four weeks. They were then crushed powder and stored in suitable containers at room temperature. 50 g of the powder was boiled in 500 ml of distilled water contained in a 1000 ml flask for 30 minutes. After cooling the filtrate collected is evaporated in a rotary evaporator between 50° C and 60° C. The extract was dried in an oven at 50° C. The dry residue obtained was powdered and kept in the fridge in a black bottle. The yield of the decoction was calculated by the ratio:

$$R = \frac{\text{mass of the powder of the evaporated decoction (g)}}{\text{mass of the vegetable material powder (g)}} \times 100$$

Phytochemical screening of S. bicolor leaves extract

Screening was a qualitative chemical analysis based on differential staining reactions and/or precipitation of the major chemical compounds groups contained in plants. The experimental methodology adopted in this study was that of Houghton *et al.* (1998).

The targeted compound were alkaloids, phenolic compounds, tannins, catechin tannins, gallic tannins, flavonoids, anthocyanins, leuco anthocianin, quinine derivatives, saponosides, triterpenoids, steroids, cardenolides, mucilage, coumarins, reducing compounds and antracene derivatives.

In vivo experimentation

The evaluation of the anti-anemic activity consisted of assessing the impact of *Sorghum bicolor* aqueous extract on hematological parameters and red blood cells osmotic resistance of anemic female and male Wistar rats.

Induction of anemia

Anemia was induced by phenylhydrazine Chloridrate. Phenylhydrazine was previously dissolved in a DMSO solution diluted to one-tenth in distilled water. It was administered to rats intraperitoneally (IP) at a dose of 40 mg / kg of body weight / day (Naughton BA *et al.*, 1995) for two days (D0 and D1).

Experimental protocol

Five groups of five rats each were formed. Group 1 was not anemic and served as control. The rats of other groups were anemic. Groups 3, 4 and 5 were treated with either the vitafer or extract 200 mg / kg of body weight / day or 300 mg / kg of body weight / day from D2 to D15. The extract and vitafer were administered by gavage using a gastric tube. Vitafer is reference drug commonly used to treat anemia. The detail of the protocol is presented as follows:

Group 1: non-anemic control, consisting of rats given the DMSO diluted tenth with distilled water on D0 and D1 and then distilled water only on D2 to D15.

Group 2: anemic control consisting of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and distilled water from D2 to D15.

Group 3: Control reference, made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 1 ml / kg / day of vitafer, from Days 2 to D15.

Group 4: Made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 200 mg / kg / day of the *Sorghum bicolor* aqueous extract from D2 to D15.

Group 5: Made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 300 mg / kg / day of the *Sorghum bicolor* aqueous extract from D2 to D15.

Blood tests

Approximately 2 ml of blood samples were collected in EDTA tube on days: D0, D2, D7, D10 and D15 by orbital puncture after anesthesia rats with chloroform. They were used for the determination of the blood count and osmotic resistance of red blood cells.

Blood count

Haematological parameters such as hemoglobin, the number of red blood cells, mean corpuscular volume and mean corpuscular hemoglobin concentration number of platelets were determined with PLC SYSTEM KX 21 (Genetet B., 1989; Ganong W. E., 2001).

Osmotic resistance of erythrocytes

The test was based on the ability of red cells to resist to hemolysis in a hypotonic solution. Blood was diluted 1/200 in two salt solutions of different concentrations. One was isotonic (0.9% NaCl) and the other hypotonic (0.45% NaCl). Red cells were counted with a Malassez cell. The ratio of the number of red blood cells counted in the hypotonic solution over that of the isotonic solution was the percentage of red blood cells resistant to hemolysis. This test was used to

assess the production of young red blood cells.

Statistical analysis

Graphs were plotted using Graphpad software. In each group, the different means were compared to that of DO using ANOVA one way, Dunnett's Multiple Comparison Test and student t test. The significance level was set at 5%.

Results

Chemical compounds identified in the leaves of Sorghum bicolor

The extraction efficiency is 19.66%. Phytochemical screening of vaporized leaf extract of *Sorghum bicolor* has revealed the presence of catechol tannins, flavonoids, of leucoanthocyanes, steroids, cardenolides, reducing compounds and coumarins (Table 1).

Table 1. Phytochemical screening of *Sorghum bicolor* leaves.

| Compounds | Intensity |
|------------------------------|-----------|
| Alkaloids | - |
| Catechol tannins | + ++ |
| Gallic tannins | - |
| Flavonoids | + |
| Anthocyanins | - |
| Leucoanthocyanins | +++ |
| Quinone derivatives | - |
| Terpenoids | - |
| Steroids | +++ |
| Cardenolides | +++ |
| Saponosides | - |
| Cyanogenic compounds | - |
| Reducing compounds | +++ |
| Mucilages | - |
| Coumarins | + |
| Free anthracenic derivatives | - |
| C-glycoside | - |
| O-glycoside | - |

Legend: + = low intensity; ++ = medium intensity; +++ = high intensity ; - = Negative.

The extract of Sorghum bicolor leaves stimulates hemoglobin synthesis in a dose dependent manner

The mean hemoglobin level was 14.9 to 16.1 g / dl in the different groups of rats on day 0. It decreased significantly in all groups at day 2 after administration of phenylhydrazine (10.0 to 10.8 g / dl; $P < 0.001$). This decrease has been gradually corrected and the hemoglobin level at day 15 was not significantly different from its value of day 0 in the groups treated with vitafer (14.7 g / dl; $p = 0.36$) or 300 mg of extract / kg body weight (15 g / dl; $P = 0.24$). The correction was not over on D15 in rats

treated with low dose (200 mg / Kg) extract (14.8 g / dl; $P = 0.02$), indicating a dose-dependent action of the plant. The hemoglobin level was lower in untreated anemic (12.3 g / dl; $P = 0.01$) (Fig. 1).

The extract stimulates hemoglobin synthesis by activation of erythropoiesis

The mean number of red blood cells is 5.0 to 5.9 T / l on day 0 in the different groups of rats. It fell at day 2 (hemolysis) following administration of phenylhydrazine (2.5 to 3.2 T / l; $P < 0.001$). This decrease was corrected soon at day 7 for treated

groups with vitafer or 300 mg extract / kg. At day 15 correction was complete in all treatment groups and the mean number of red blood cells reached 5.3 T / l (P = 0.1) with the vitafer and 5.6 T / l with the extract at 200 or 300 mg / kg (P = 0.5). Only the untreated

anemic group did not have such a correction (3.5 T / L; P = 0.001) (Fig. 2). A paradoxical slight increase in the mean number of red blood cells was observed at day 2 in the no anemic control group (6.0 T / l; P = 0.06).

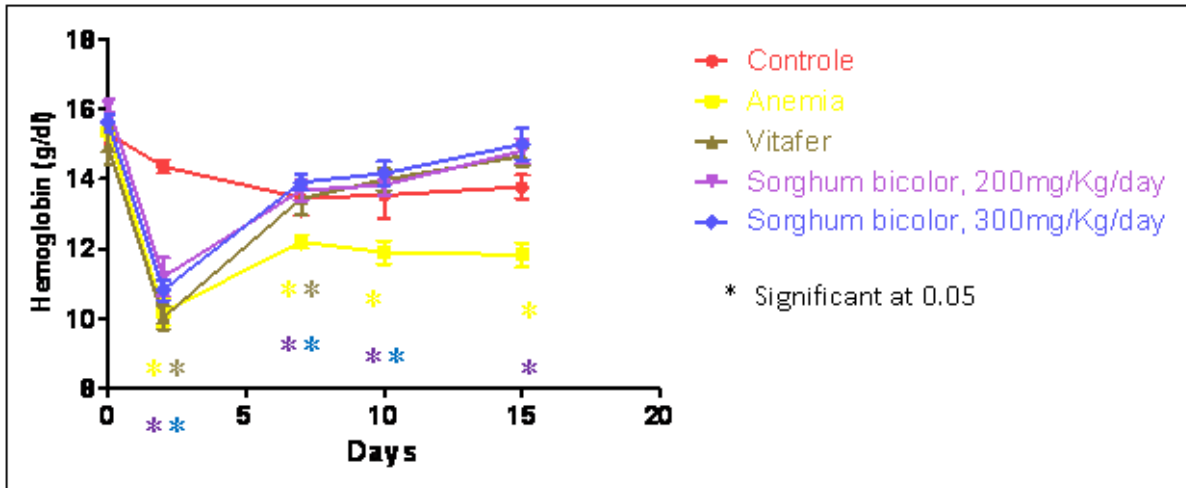


Fig. 1. Treatment effect on hemoglobin level.

Anemia is corrected by a release of immature red cells in the blood

The Mean Corpuscular Volume (MCV) was from 80 to 84 fl in the various groups at Do. It fell at day 2 (64-68 fl, P < 0.04) resulting microcytosis after

administration of phenylhydrazine. The MCV then increase very rapidly and exceeded at D15 its initial value significantly in the group treated with vitafer (90 fl; P < 0.0001) and not significantly in that treated with 300 mg/kg extract (87 fl; P = 0.06).

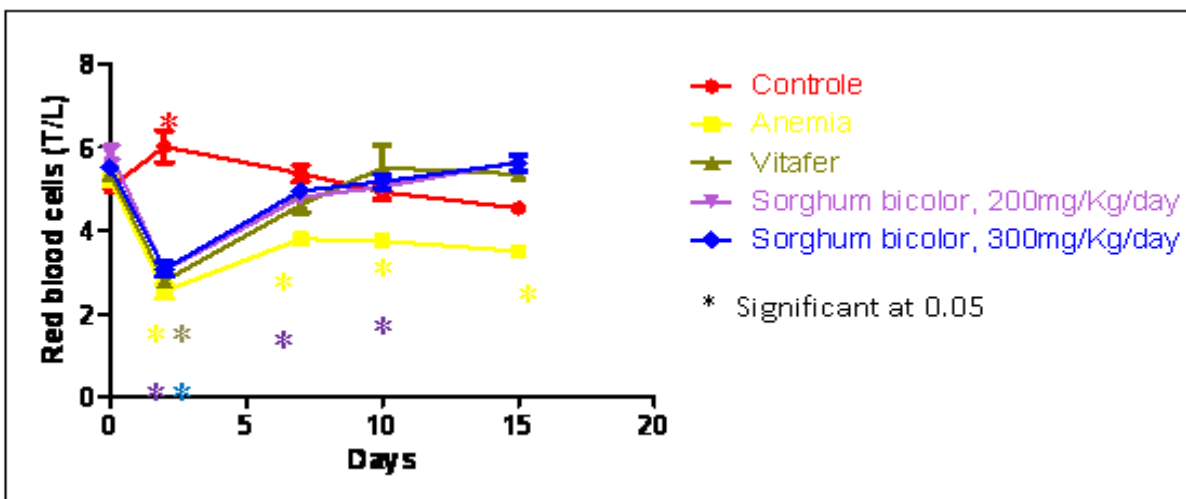


Fig. 2. Treatment effect on red blood cells count.

The increase was more moderate in the group treated with 200 mg / kg of extract and untreated anemic group (83 fl) and started after day 7 in the control no anemic group. The MCV increased reflects a release of immature large red cells (macrocytes) (Fig. 3).

The Mean corpuscular hemoglobin concentration (MCHC) moved inversely compared to the MCV MCHC mean was about 34 to 36 pg in groups at Do. Unlike the MCV, it significantly increased and reached its peak (43-49 pg, P < 0.03) in day 2 in all

anemic groups. It then gradually decreased and became significantly lower at day 15 in the group treated with 300 mg extract / kg (31 pg; $P < 0.01$). This decrease in MCHC reflects a release of red blood cells less saturated hemoglobin (Fig. 4).

Compensation of anemia started with an early release of young red blood cells

The osmotic resistance of red blood cells is 24 to 30%

on day 0 in the different groups. It significantly increased early and peaked on day 7 in the treated groups (70-80%, $P < 0.004$) and day 10 in the untreated anemic group (82%; $P = 0.001$). In the non-anemic group, it first declined at Days 2 and its evolution is more moderate than in the treated groups (Fig. 5). Note that the osmotic resistance is higher when the red cell is young (Gbenou *et al.*, 2006).

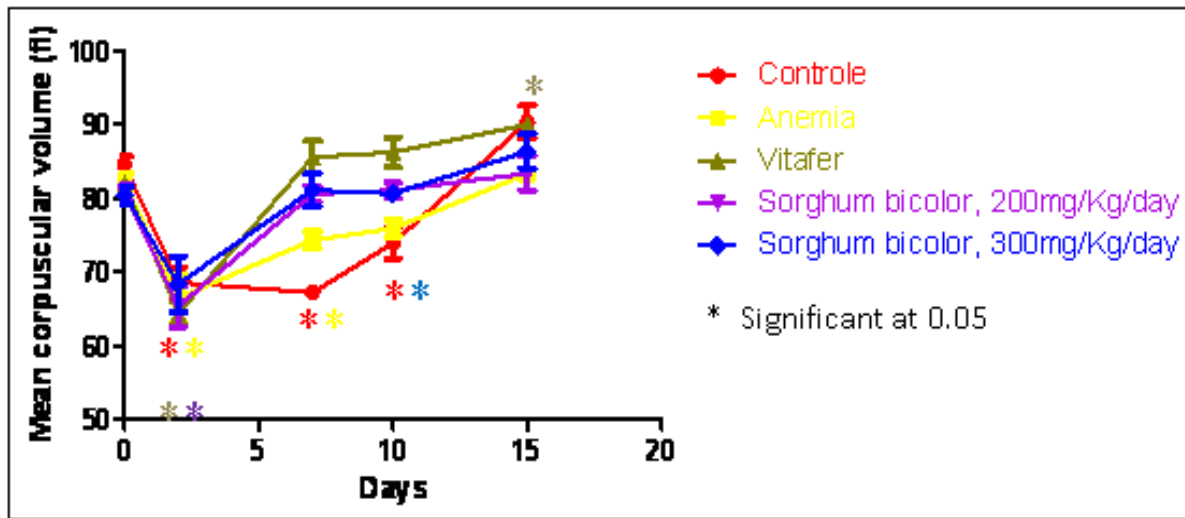


Fig. 3. Treatment effect on mean corpuscular volume.

The extract stimulates erythropoiesis rather specifically

The number of blood platelets on DO was 181 to 325 G / l in all groups of rats (Fig. 6). It has significantly increased and varies between 843 and 991 G/l in all groups anemic or no, in response to injury

(endothelial injury) related to the blood sample puncture. It then decreased very quickly and has regained its original value at day 15 in the different groups, indicating no effect of the extract as well as the vitafer on thrombocyte lineage.

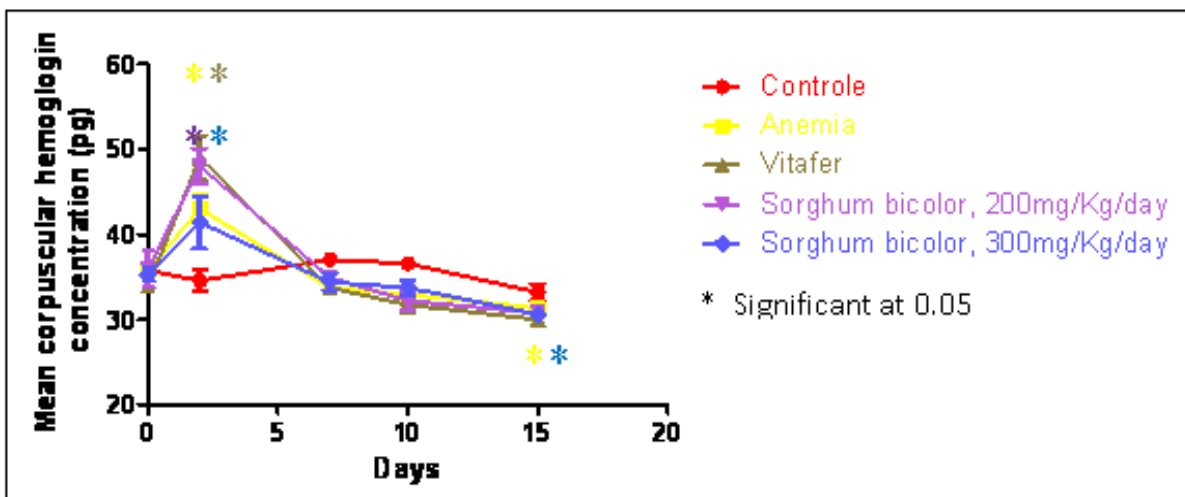


Fig. 4. Treatment effect on mean corpuscular hemoglobin concentration.

Discussion

Sorghum bicolor is a medicinal plant used in Benin to treat anemia. This work showed its therapeutic efficacy on a rat anemic model. For this purpose, a phytochemical analysis of the sprayed aqueous extract of the dried leaves of the plant was first processed. It revealed the presence of tannins, flavonoids, leucoanthocyanes, steroids, cardenolides, reducing compounds and mucilages. These

compounds are consistent with those previously found in the red sorghum (*Sorghum caudatum*) by Agbangnan *et al.* (2012) in Benin. In contrast, Akande *et al.* (2010) in Nigeria did not identify flavonoids and tannins. The absence of these compounds could be explained by the variability of the harvest period, the difference in environmental spheres and detection techniques.

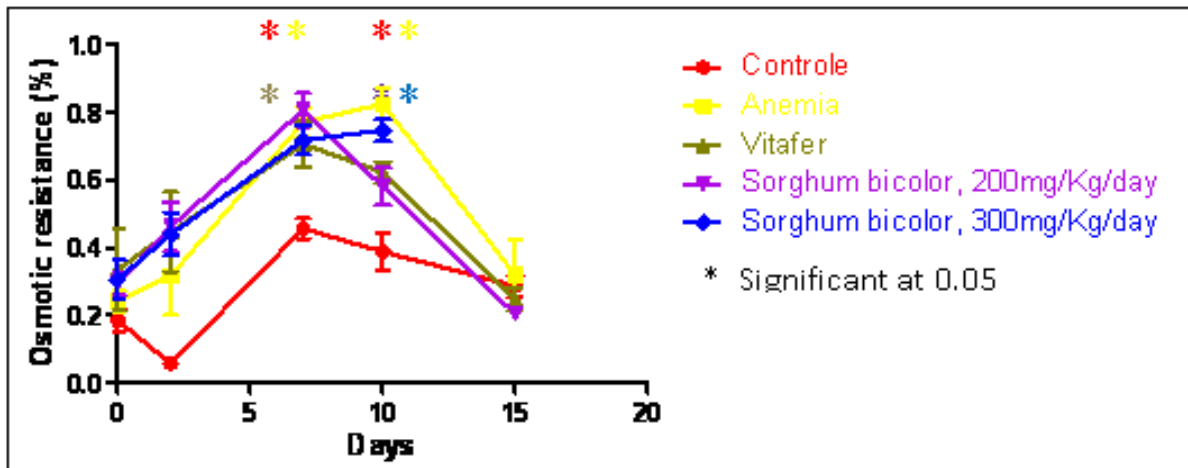


Fig. 5. Treatment effect on the red cell osmotic resistance.

We then induced hemolytic anemia with phenylhydrazine (Naughton *et al.*, 1995) in *Wistar* rats we administered the extract of *Sorghum bicolor* to monitor its effect compared to that of vitafer, a drug commonly used to treat anemia. The effect of treatment was monitored by an evaluation of hematological parameters such as hemoglobin, the number of red blood cells, Mean Corpuscular Volume (MCV), the Mean Corpuscular Hemoglobin Concentration (MCHC) and osmotic resistance of erythrocytes.

Hemoglobin is the key parameter indicator of anemia. The administration of phenylhydrazine created anemia characterized by a significant decrease in hemoglobin at day 2 in the various groups of rats. Anemia was corrected at Day 15 by vitafer and the extract of *Sorghum bicolor* dose-dependent manner. A similar result was obtained by Gbenou *et al.* (2006) who showed a dose-dependent anti-anemia property of *Justicia secunda* Vahl, another medicinal plant used in Benin to treat anemia. However these authors

had used extract doses significantly higher than those of this study. *Sorghum bicolor* based traditional herbal preparation also increased hemoglobin in HIV positive patients (Godwin *et al.*, 2014).

Since hemoglobin is contained in red blood cells, we evaluated the effect of the extract on their production. Changes in the number of red blood cells followed that of hemoglobin. Indeed, the red blood cell count decreased significantly in day 2 in all groups receiving phenylhydrazine (hemolysis). This decline was compensated gradually until day 15 and normal value was found in treated groups by vitafer or *Sorghum bicolor* extract. Such a result was obtained with leaves extracts of *Tectona grandis* in Togo (Diallo *et al.*, 2008) and *Justicia secunda* Vahl in Benin (Gbenou *et al.*, 2006).

In the no-anemic group, a slight increase in the mean number of red blood cells was observed at day 2. It would be a consequence of the organism reaction to compensate for the loss of red blood cells related to

the quantity of blood collected for the various analyzes, by mobilizing and re-injecting into the bloodstream old red blood stagnating in storage organs such as the bone marrow, liver and spleen especially. This assumption is reinforced by the decrease in osmotic resistance of red blood cells we observed in this group at day 2, indicating an increase in the number of old red blood cells in the circulation. The Mean Corpuscular Volume (MCV) and Mean corpuscular hemoglobin concentration (MCHC) are constants for typing anemia. MCV decreased significantly at day 2 after administration of phenylhydrazine indicating microcytosis. This

decrease was offset by the end of the first week in the treated groups and after two weeks in untreated anemic groups of rats. Furthermore, compensation was faster with vitafer than the extract suggesting different mechanisms involved in erythropoiesis in the two cases. This result contrasts that of Ogwumike (2002) in Nigeria which showed a decrease in MCV induced by aqueous extract of *Sorghum bicolor* leaves. However he did not use a model of anemic rats. The increased MCV reflects a release of large immature erythrocytes (macrocytes) (Fauchet *et al.*, 1995).

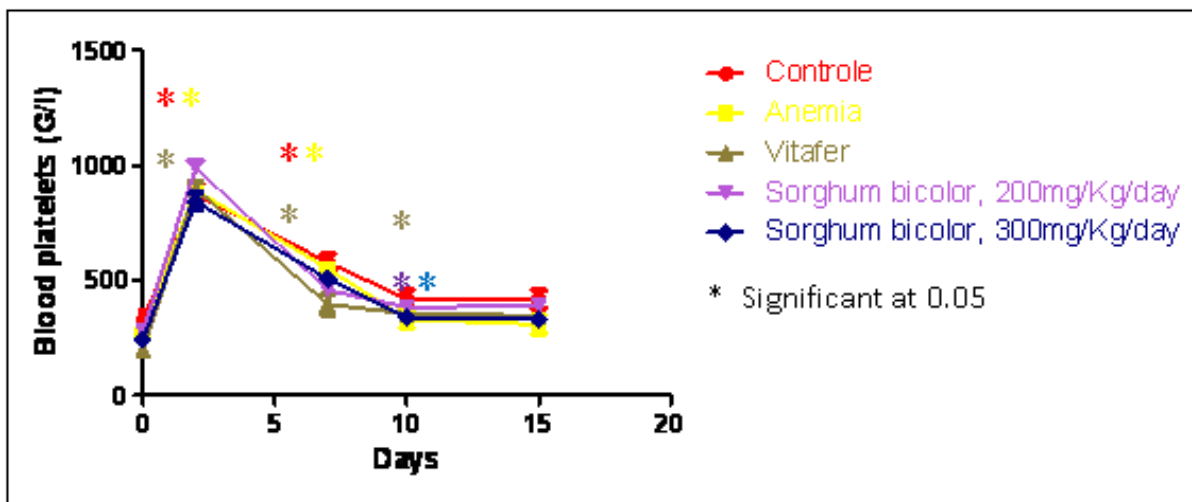


Fig. 6. Treatment effect on the number of blood platelets.

In contrast, the evolution of MCHC was reversed compared to that of the MCV in the experimental groups. A lower MCHC was also observed by Ogwumike (2002) in his model of rats.

This decrease in MCHC reflects a release of red cells less saturated in hemoglobin (hypochromia). It would be a consequence of the release of macrocytes, immature erythrocytes prematurely released into the blood stream to compensate the anemia. To test this hypothesis, we determined the osmotic resistance of red blood cells.

The osmotic resistance increased sharply from Do in all anemic groups, peaked (70-80%) at day 7 in the treated groups and only at day 10 for the untreated anemic group. This indicated that the extracts just as

vitafer increase quickly the proportion of young cells in the blood as a result of a consequent stimulation of hematopoiesis and their premature release into the blood stream before the end of their differentiation. An increased osmotic resistance of red blood cells was also observed with extracts of *Justicia secunda* Vahl in Benin (Gbenou *et al.*, 2006) and *Tectona grandis* in Togo (Diallo *et al.*, 2008).

To investigate the specificity of the extract action, we followed the evolution of blood platelets number during the experiment.

The extract has no obvious effect on thrombocyte lineage, indicating that its action is not extended to all hematopoietic lineages. This specificity of the plant action was one originality of the study.

The mechanism of action of the extract was still unknown. Considering its content in reducing compounds, One could also consider a protection against cell membrane lipid peroxidation (oxidative stress) which could lyse erythrocytes and disadvantage of medullary activity (Awika *et al.*, 2004; Ogbe *et al.*, 2012). The phenylhydrazine-induced hemolysis is due to oxidative stress (Berger, 2007).

Conclusion

The aqueous extract of leaves of *Sorghum bicolor* was efficient against anemia with a dose-dependent effect. It stimulated erythropoiesis rather specifically and favors in anemia compensation early phase, a release of immature red blood cells in the bloodstream. The mechanism of action remains unknown and may involve protection against oxidative stress. Given this finding, this plant can be considered for processing to Improved Traditional Medicine (ITM) after studying its biological tolerance and the appropriate clinical trials.

References

- Agbangnan CDP, Tachon C, Bonin H, Chrostowka A, Fouquet E, Sohounhloue CDK.** 2012. Phytochemical study of a tinctorial plant of Bénin traditional pharmacopoeia: the red sorghum (*Sorghum caudatum*) of Benin. *Scientific Study and Research* **13**, 121-35.
- Akah PA, Okolo CE, Okoye TC, Offiah NV.** 2010. Aqueous extract and methanol fractions of leaves of *Brillantaisia nitens* Lindau. Reverses phenylhydrazine-induced anaemia in rats. *Journal of Medicinal Plants Research* **4(3)**, 271-277.
- Akande IS, Oseni AA, Biobaku OA.** 2010. Effect of aqueous extract of *Sorghum bicolor* on hepatic, histological and haematological indices in rats. *Journal of Cell and Animal Biology* **4**, 137-142.
- Alper BS, Kimber R, Reddy AK.** 2000. Using fainting levels to determine iron deficiency anemia in pregnancy. *Journal of Family Practice* **49**, 829-32.
- Assobayire FS, Adou P, Davidson L, Cook JD, Hurrel R.** 2001. Prevalence of iron deficiency with and without concurrent anemia in population group with high prevalences of malaria and other infections: a study in Cote d'Ivoire. *American Journal of Clinical Nutrition* **74**, 776-82.
- Awika JM, Lloyd W, Rooney, Ralph D, Waniska.** 2004. Anthocyanins from black sorghum and their antioxidant properties. *Food Chemistry*, **90**, 293-301.
- Berger J.** 2007. Phenylhydrazine haematotoxicity. *Journal of Applied Biomedicine* ISSN 1214 0287, **125**, 130.
<http://dx.doi.org/10.1016/j.foodchem.2004.03.058>
- Diallo A, Gbeassor M, Vovor A, Eklugadegbeku K, Aklikokou K, Agbonon A.** 2008. Effect of *Tectona grandis* on phenylhydrazine induced anemia in rats. *Fitoterapia* **79**, 332-6.
<http://dx.doi.org/10.1016/j.fitote.2008.02.005>
- Duff S.** 2008. Types of Anaemia. www.innvista.com, Accessed 15-08-2014.
- Fauchet R, Ifrah N, Blanchet O, Dautel M.** 1995. *Collection Biologie Médicale Hématologie*. 2^e édition. Condé-sur-Noireau (France), 437.
- Fricker J.** 1998. Grossesse et nutrition: l'équilibre de la conception. Hôpital de Bichat, Paris Chole-doc.
- Gbenou JD, Tossou R, Dansou P, Fossou M, Moudachirou M.** 2006. Etude des propriétés antianémiques de *Justicia secunda* Vahl (acanthaceae) chez les rats de souche wistar. *Pharm Med Trad Afr*, **14**, 45-54.
- Godwin I, Ayuba G, Jensen G, Benson K, Okubena A, Okubena O.** 2014. Clinical efficacy of a west african *Sorghum bicolor*-based traditional herbal preparation Jobelyn Shows. Increased Hemoglobin and CD4⁺ T-Lymphocyte Counts in HIV-Positive Patients. *Journal of alternative and*

complementary medicine **20(1)**, 53–56.

<http://dx.doi.org/10.1089/acm.2013.0125>

Holden J, Acomb C. 2007. Anaemia. In: Clinical Pharmacy and Therapeutics, Walker R. and C. Whittlesea (Eds.). Elsevier Churchill Livingstone, New York, 699-701.

Houghton PJ, Raman A. 1998. Laboratory Handbook for the Fractionation of Natural Extracts. London: Chapman and Hall, 22-52.

<http://dx.doi.org/10.1007/978-1-4615-5809-5>

Kassel D. 2003. Des hommes et des plantes, in Histoire de la pharmacie, <http://ordre.pharmacien.fr>, Décembre 2003; 88 p.

Marti-carragal A, Pena-Martí G, Communian G, Munoz S. 2002. Prevalence of anemia during pregnancy result of Valencia (Venezuela) anemia during pregnancy study. *Archivos Latinoamericanos de Nutrición* **52**, 5-11.

Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A, Devasagayam T. 2007. Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition*, **40**, 163–73.

<http://dx.doi.org/10.3164/jcbn.40.163>

Movaffaghi Z, Hasanpoor M. 2006. Effect of therapeutic touch on blood hemoglobin and hematocrit level. *Journal of Holistic Nursing*, **24**, 41-8.

<http://dx.doi.org/10.1177/0898010105282527>

Naughton BA, Moore E, Bush ME, Lapin DM, Domfest BS. 1995. Hemostatic alterations associated with phenylhydrazine – induced haemolytic anaemia. *European Journal Of Clinical Investigation* **25**, 722-7.

Ogbe JR, Adenkola AY, Anefu E. 2012. Aqueous ethanolic extract of *Mangifera indica* stem bark effect on the biochemical and haematological parameters of albinos rats. *Archives of Applied Science Research*, **4(4)**, 1618-22.

Ogwumike OO. 2002. Hemopoietic effect of aqueous extract of the leaf sheath of *Sorghum bicolor* in albinos rats. *African Journal of Biomedical Research* **5**, 69-71.

Okochi YI, Okpuzor J, Alli LA. 2003. Comparison of an African herbal formula with commercially available hematinics. *African Journal of Biotechnology* **2(8)**, 237 – 240.

<http://dx.doi.org/10.5897/AJB2003.000-1048>

Scanlon KS, Yip R, Schieve LM, Cogswell ME. 2000. High and low hemoglobin levels during pregnancy differential risks for paterm birth and small for gestational age. *Obsetrics and gynecology*, 714-8.

Verhoel H, West CE, Kraaighagen R, Nzuko SM, King R, Mbadi M. 2002. Malarial anemia leads to adequately increased erythropoiesis in asymptomatic Kenyan children. *Blood* **100**, 3489-94.

<http://dx.doi.org/10.1182/blood-2001-12-0228>