

## The aqueous extract of the root bark of *Psorospermum febrifugum* Spach effectively corrects anaemia. Experimental study on *Wistar* rats.

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### RESUME

**Objectifs:** *Psorospermum febrifugum* Spach. (Clusiaceae) est une plante médicinale souvent rencontrée en Afrique. L'écorce de ses racines est utilisée au Bénin pour traiter l'anémie. Ce travail a pour but d'étudier l'efficacité thérapeutique de l'extrait de cette écorce de racine dans le traitement de l'anémie.

**Méthodologie et résultats:** Des rats *Wistar* anémiés par injection intra péritonéale de phénylhydrazine chlorhydrate ont été gavés à l'extrait d'écorce de cette racine à 200 ou 300 mg / kg poids / jour, ou au vitafer, un médicament de référence. L'hémogramme et la résistance osmotique des hématies réalisées sur des échantillons de sang des rats à différents jours (J0, J2, J7, J10 et J15) ont montré une correction de l'anémie avec l'extrait au bout de deux semaines. Le criblage de l'extrait a révélé des saponosides, des sucres réducteurs, des polyphénols, des flavonoïdes, des tanins, des protéines, des anthocyanes et des alcaloïdes.

**Conclusion et application des résultats:** L'extrait a complètement corrigé l'anémie en deux semaines par stimulation accrue de la synthèse d'hémoglobine et la libération de jeunes globules rouges dans le sang. Son effet semble dose-dépendant et à la dose de 300 mg / kg, il est encore plus efficace que le vitafer. Cette efficacité remarquable serait certainement liée à sa composition chimique, dont les éléments agiraient de manière isolée ou synergique pour stimuler l'hématopoïèse. Le mécanisme d'action mérite d'être élucidé. De plus, l'extrait n'a aucun effet sur les thrombocytes sanguins suggérant une certaine spécificité d'action sur la lignée érythrocytaire. L'écorce de racine de *Psorospermum febrifugum* Spach. présente une excellente efficacité thérapeutique et pourrait proposée comme un bon candidat pour la

transformation en Médicaments Traditionnels Améliorés (MTA) pour le traitement de l'anémie, après les tests de toxicité aiguë et chronique et des essais cliniques appropriés.

**Mots-clés:** *Psorospermum febrifugum* Spach, anémie, résistance osmotique.

## ABSTRACT

**Objectives:** *Psorospermum febrifugum* Spach. (Clusiaceae) is a medicinal plant found in Africa. Its root bark was used in Benin to treat anaemia. This work aimed to study the therapeutic efficacy of that root bark extract on anaemia treatment.

**Methodology and Results:** Wistar Rats were anaemic by intraperitoneal injection of phenylhydrazine chloridrate. The rats were then fed either with *Psorospermum febrifugum* Spach root bark extract at 200 or 300 mg / kg weight / day, or with vitafer® as the reference drug. Non-anaemic rats served as controls. The blood count and osmotic resistance of red blood cells carried out on blood samples of rats at different days (D<sub>0</sub>, D<sub>2</sub>, D<sub>7</sub>, J10 and D15) showed a correction of anaemia with the extract in two weeks. The extract screening revealed saponosides, reducing sugars, polyphenols, flavonoids, tannins, proteins, anthocyanins and alkaloids.

**Conclusion and application of results:** The extract completely corrected anaemia in two weeks by increased stimulation of hemoglobin synthesis and the production of young red blood cells released into the blood. Its effect seems dose-dependent and at the 300 mg / Kg dose was even more effective than the vitafer. This remarkable efficiency would certainly be linked to its chemical composition, whose elements would act in an isolated or synergistic way to stimulate haematopoiesis.. In addition, the extract had no effect on blood thrombocytes suggesting a certain specificity of action on the erythrocyte line. *Psorospermum febrifugum* Spach root bark had an excellent therapeutic efficacy and could be a good candidate for transformation into improved traditional medicines (ITM) for anaemia treatment, after acute and chronic toxicity tests and appropriate clinical trials.

**Keywords:** *Psorospermum febrifugum* Spach, anaemia, osmotic resistance.

## INTRODUCTION

Anaemia is a common disorder of blood which affects the populations of all ages throughout the world. It constitutes a major public health problem in both rich and developing countries (de Benoist et al., 2008). Anaemia is defined as a lowering in blood hemoglobin level. This disease is of multifactorial nature, mostly affects children and increased their mortality for 3 to 4 times (OMS / UNICEF, 2005). A recent study estimates 273 million (43%) of preschool children affected by anaemia and Africa accounts for most of the cases (62.0%) (WHO, 2015). It increases incidence in developing countries due to poverty and lack of hygiene (Ogbe et al., 2010). Other conditions, such as malaria and haemoglobinopathies are also responsible (Crawley, 2004; de Benoist et al., 2008; Sènou et al., 2017a, 2017b) in Africa. In the tropics, endemicity of malaria, make about 10 to 20% of the population having less than 10 g / dl of haemoglobin (Diallo et al., 2008) against reference

values of 12 g / dl in women and children, 13 g / dl in men (WHO, 1968). Anaemia reduces physical capacity, working aptitude, growth and immune status (Abdullah et al., 2011). Iron deficiency is the leading cause of anaemia and represents the first nutritional deficiency in the world (El Hioui et al., 2009; Al-Zabedi et al., 2014). In women, at their reproductive age anaemia occurs due to menorrhagia and in pregnancy, it is due to excess need of iron (Ramesh et al, 2010; Rajarathinam et al., 2013). Since anaemia is among the most common public health problems, people use medicinal plants to manage it. The main reason is related to the cost of modern medicine that is not affordable to rural populations of poor countries (Bhushan, 2005). Besides, many studies have already confirmed the efficiency of some wild plants and identified the active compound for some diseases (WHO, 2009; Tchogou et al., 2016; Sènou et al., 2016a). *Psorospermum febrifugum* is

widely used in traditional medicine in Africa. It grows in savannahs and tropical areas and belongs to the family of Hypericaceae (Arbonnier, 2000). It is employed in the treatment of varied conditions including fevers and skin problems. Phytochemical screening of the different parts of the plant revealed the presence of various medically active

## MATERIAL AND METHODS

**Animal Material:** Animal material consisted of Wistar albino rats of average body weight  $210 \pm 46$  g, having free access to water and food and acclimated to farming conditions from the pet of Biomembrane and Cell Signalling Laboratory in Faculty of Sciences and Techniques at University of Abomey-Calavi in the 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 Material:** *Psorospermum febrifugum* Spach. (Clusiaceae) roots bark was collected from Atlantic Department in Benin during April 2015. The collected samples were identified and certified at the National Herbarium of the University of Abomey Calavi under the number AA6625 / HNB. The samples were dried at moderate temperatures ( $20-25^{\circ}$  C), protected from moisture for four weeks. They were then crushed into 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  $50^{\circ}$  C and  $60^{\circ}$  C. The extract was dried in an oven at  $50^{\circ}$  C. The dry residue obtained was powdered and kept in the refrigerator in a black bottle.

**Phytochemical Screening of *Psorospermum febrifugum* Spach. (Clusiaceae) roots bark 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 compounds were alkaloids, phenolic compounds, tannins, catechin tannins, gallic acid, flavonoids, anthocyanins, leucoanthocyanin, quinine

compounds (Burkill, 1985). The root bark contained catechuic tannins. Steroids and terpenes are present in the bark and roots (Burkill, 2004). In Benin the root bark is used to manage anaemia. The aim of present study was to test the aqueous extract of the plant roots bark on anaemic Wistar rats.

derivatives, saponosides, triterpenoids, steroids, cardenolides, mucilage, coumarins, reducing compounds and anthracene derivatives.

**In vivo Experimentation:** The evaluation of the anti-anaemic activity consisted of assessing the impact of *Psorospermum febrifugum* Spach. roots bark aqueous extract on haematological parameters and red blood cells osmotic resistance of anaemic female and male Wistar rats.

**Induction of Anaemia:** Anaemia was induced by phenylhydrazine hydrochloride, an oxidant which induces hemolysis (Berger, 2007). 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 et al., 1995) for two days (D0 and D1).

**Experimental Protocol:** Five groups each having five rats were formed. Group 1 was not anaemic and served as control. The rats of other groups were anaemic. Groups 3, 4 and 5 were treated with either the vitafer or extract at 200 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 anaemia (Tchogou et al., 2016). The details of the protocol is presented as follows:

- Group 1: non-anaemic control, consisting of rats given the DMSO diluted one tenth with distilled water on D0 and D1 and then distilled water only on D2 to D15.
- Group 2: anaemic 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 Day 2 to D15.
- Group 4: Rats were given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1)

and 200 mg / kg / day of the *Psorospermum febrifugum* Spach. Roots bark 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 *Psorospermum febrifugum* Spach. Roots bark 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 anaesthesia 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.

## RESULTS

**Main chemical groups identified *Psorospermum febrifugum* Spach. roots bark aqueous extract:** Phytochemical analysis of the root bark of *Psorospermum febrifugum* Spach revealed the major

**Osmotic Resistance of Erythrocytes:** The test was based on the ability of red cells to resist to haemolysis 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 haemolysis. 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 D0 using ANOVA one way, Dunnett's Multiple Comparison Test. The significance level was set at 5%.

families of chemical compounds such as saponosides, reducing sugars, polyphenols, flavonoids, tannins, gallic tannins and catechin tannins, proteins, anthocyanin and alkaloids as indicated in table.

**Table:** Chemical compounds identified in the aqueous extract of *Psorospermum febrifugum* Spach. roots bark.

Compounds	Detection
Saponosides	+
Protein	+
Reducing sugars	+
Polyphenols	+
Flavonoids	+
Tannin	+
Gallic tannin	+
Catechism tannin	+
Anthocyanin	+
Alkaloids	+

Legend: Positive = +

**The extract treats anaemia in a dose-dependent manner:** Anaemia is indicated by the hemoglobin level. At D0, the hemoglobin level is about 14.5 g / dl in the different groups. At D2, it significantly decreased to 8 g / dl following administration of phenylhydrazine ( $p < 0.05$ ). Then, it showed a rapid increase and at day 7, is

no longer significantly different from its D0 value in the group treated with 300 mg / kg *Psorospermum febrifugum* Spach. At day 15, it is no longer significantly low in any group and even significantly exceeds its D0 value in the group treated with 300 mg / kg of the plant extract (15.4 g / dl) (figure 1).

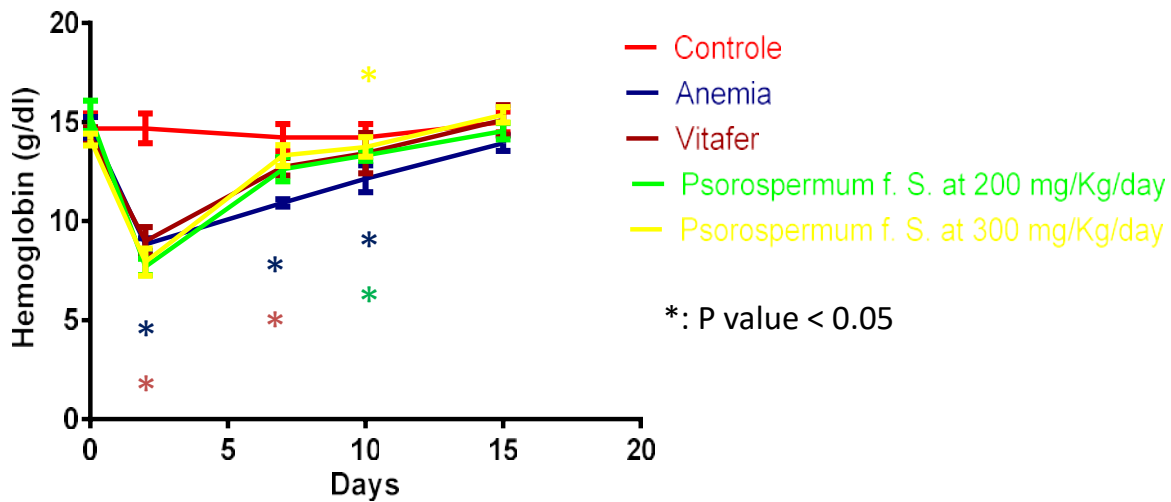


Figure 1. Hemoglobin levels in different groups

**To extract correct anaemia by stimulating erythropoiesis:** Haemoglobin is synthesized by the red blood cell. At D0, the number of red blood cells is about 5 T / l in all groups. It significantly decreased on D2 (about 3 T / L) following the administration of phenylhydrazine. Then, there was a rapid increase in the treated groups and on day 7, no longer significantly different from D0 in the group treated with vitafer or *Psorospermum febrifugum* Spach at 300 mg / kg. At

day 15, it no longer differs from D0 in the group treated with *Psorospermum febrifugum* Spach at 200 mg / kg and even significantly exceeds the value of D0 in that treated with plant at 300 mg / Kg. Note that in the untreated anaemic group, the evolution of the number of red blood cells is more moderate and its value remained significantly low compared to D0 during the entire experimental period (figure 2).

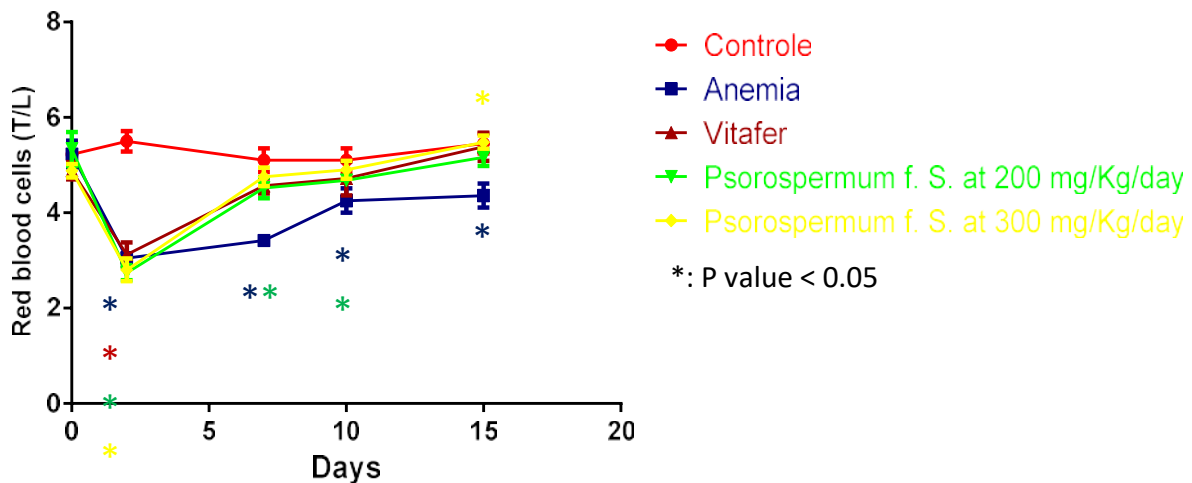


Figure 2. Number of red blood cells in different groups

**The extract and the correct cell volume:** The MCV value at D0 in all groups is about 90 fl. It dropped slightly on D2 in the anaemic groups, then increased significantly and reached its peak on D7 in the vitafer-treated groups (93 fl), the plant at 200 mg / kg (93 fl) or

300 mg / kg (95 fl). Then, the MCV decreased progressively in these groups. In the untreated anaemic group, the MCV significantly increased by Day 10 at the end of the experiment (figure 3).

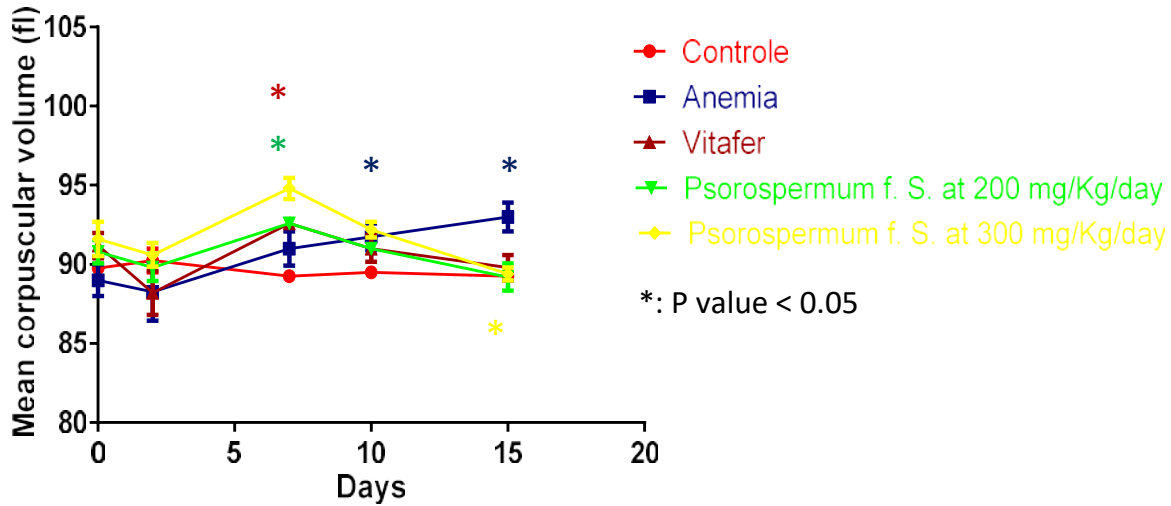


Figure 3. Mean globular volume in the different groups

**The mean corpuscular hemoglobin concentration and the cell volume move inversely:** At D0, the MCHC is at about 31 g/dl in the different groups. On day 7, it was significantly reduced in untreated anaemic groups (27 g / dl) or treated at 300 mg / kg of the plant extract (29 g / dl) and not significant in that treated at

200 mg / kg. After D7, the MCHC then increased in these groups and reached at J15 significantly higher values than D0. In the vitafer-treated group, the MCHC increased on D2 and its increase is significant already on D7 (figure 4).

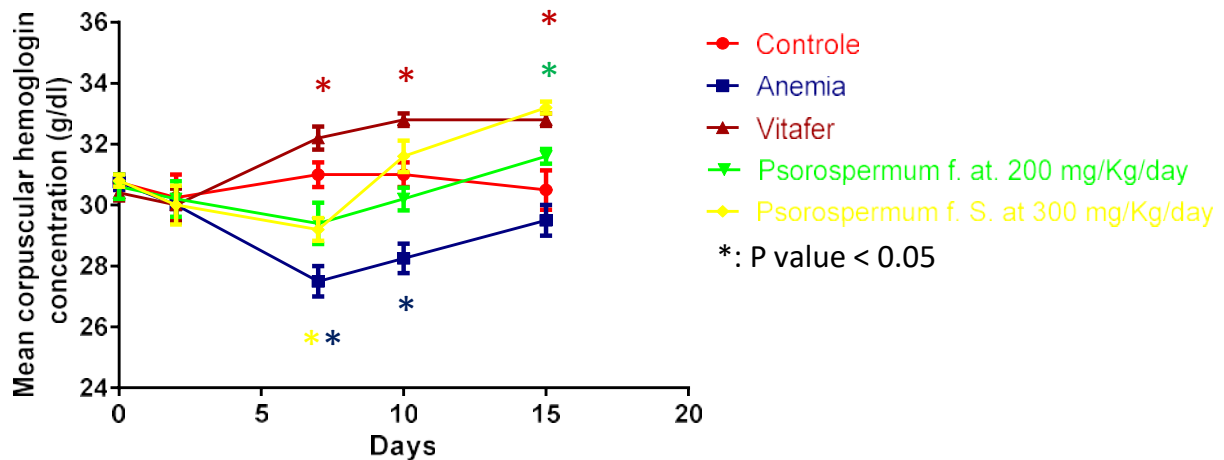


Figure 4. Mean corpuscular hemoglobin concentration in the different groups

**Anaemia is counteracted by an early release of young red cells in the blood:** The osmotic resistance of erythrocytes reflects the proportion of young red cells in the blood. The osmotic resistance of red blood cells

increased significantly in all groups anaemic on day 7. The evolution is more marked later in the group treated with 300 mg / kg of plant extract and less marked in the untreated anaemic group (figure 5).

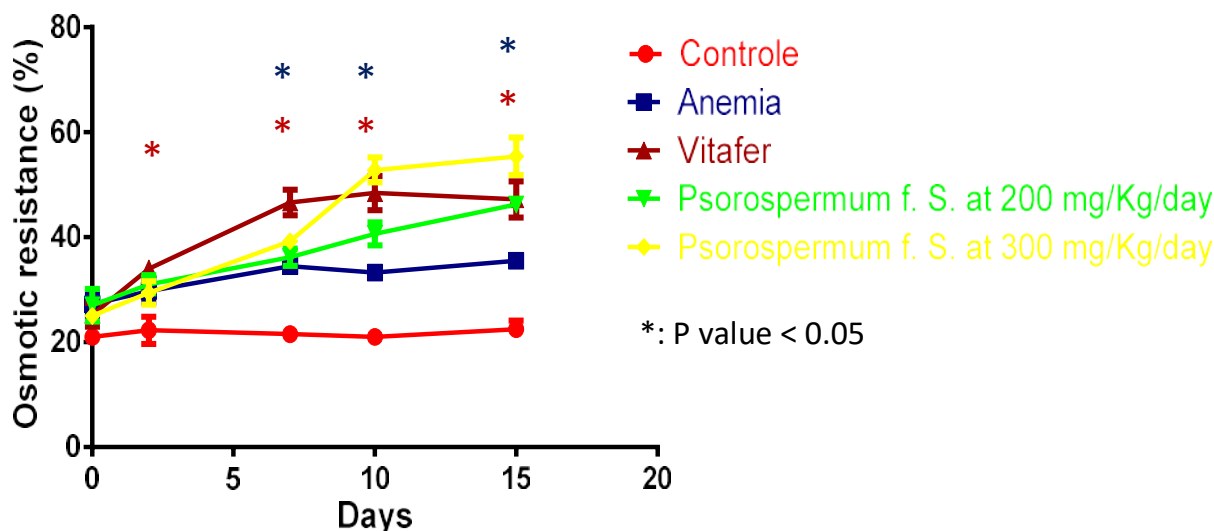


Figure 5. Progression of the red blood cells osmotic resistance in the different groups

**Compensation anaemia does not affect the rate of blood platelets:** The mean rate of blood platelets varies from 508-551 G / l in the various groups of rats at D0. This rate did not display significant change in any

group of rats during the experimental period, indicating that the extract did not stimulate platelets lineage (figure 6).

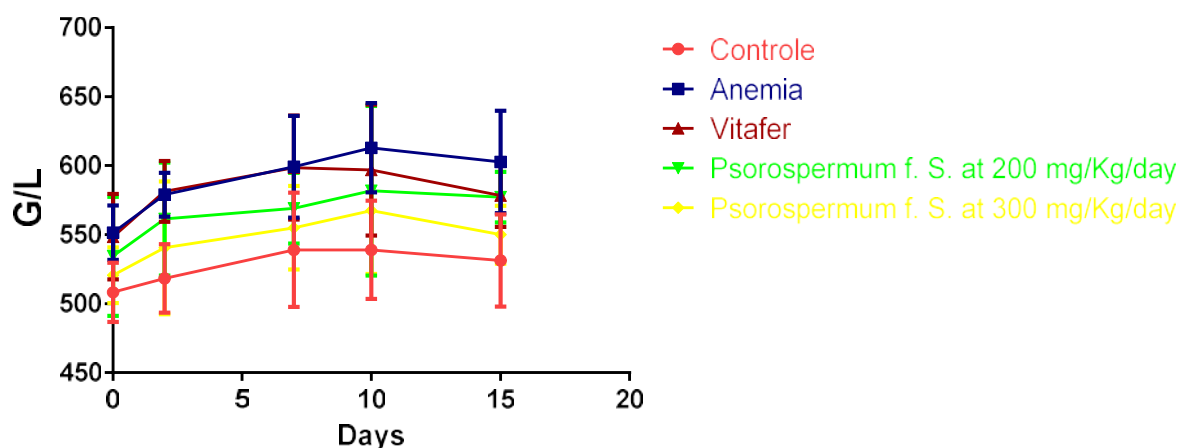


Figure 6. Changes in the number of blood platelets in the different groups

## DISCUSSION

*Psorospermum febrifugum* Spach is a medicinal plant whose root bark is used in Benin to treat anaemia. In this work, the therapeutic efficacy of the aqueous extract of these root barks was tested in vivo on anaemic Wistar rats. For this purpose, a phytochemical screening of the aqueous extract of the root bark of the plant was first carried out to determine the chemical groups. It revealed as saponosides, reducing sugars, polyphenols, flavonoids, tannins, gallic tannins and

catechin tannins, proteins, anthocyanin and alkaloids. These results differ from those of Kisangau et al. (2007) in Cameroon, which detected only phenolic compounds. The absence of these compounds could be explained by the variability of the harvest period and the difference in environmental media. The majority of these compounds were also detected in *Hibiscus sabdariffa* calyces (Obouayeba et al., 2015, Sènou et al., 2016b), foliar sheath of *Sorghum caudatum*

(Agbangnan et al., 2012) and *Cocos nucifera* L roots (Tchogou et al., 2016) which also, showed antianemic properties. We then induce hemolytic anaemia by injecting phenylhydrazine to Wistar rats (Naughton et al., 1995). Anemic rats were treated for two weeks either with distilled water (control group) or with vitafer an anti-anaemia drug (reference group) or with aqueous extract of *Psorospermum febrifugum* Spach root bark at 200 or 300 mg / kg body weight (test groups). The biological effect of the different treatments was measured by the change in hemoglobin, the red blood cell count, erythrocyte constants (mean corpuscular volume, mean corpuscular hemoglobin concentration) and osmotic resistance of red blood cells. The hemoglobin level is the key indicator of anaemia. In all experimental groups, phenylhydrazine administration induced a significant decrease in hemoglobin at day 2. This decrease was compensated as of D7 in rats treated with 300 mg / kg, on day 10 in those treated with vitafer and on D15 in those treated with *Psorospermum febrifugum* Spach at 200 mg / kg. At day 15, the hemoglobin level significantly exceeded its D0 value only in the group treated with 300 mg / kg of extract. Such an observation suggests a dose-dependent effect of the extract. This result is similar to that of Sènou et al. (2016a) who showed a dose dependent antianemic property of *Sorghum bicolor*. They are also similar to that obtained with *Justicia secunda* Vahl (Gbénou et al., 2006). However, in this case the extract doses were much higher than ours. Hemoglobin is a pigment of the Red Blood Cells (RBCs). As hemoglobin, the number of red blood cells collapsed at Day 2 following the haemolysis induced by phenylhydrazine. This decrease was compensated as of D7 in the groups treated with vitafer or with 300 mg / Kg of extract and with D15 for that treated with 200 mg / Kg. Such a result was obtained with leaf extracts of *Tectona grandis* in Togo (Diallo et al., 2008). The osmotic resistance is proportional to the youth of the red cell (Gbénou et al., 2006). It grew strongly from day

2 in all anaemic and treated groups. Its evolution is moderate in untreated anaemic rats. This finding indicates that the extracts as well as the vitafer very rapidly increase the proportion of young cells in the blood as a result of a consequent stimulation of haematopoiesis. Such a finding has also been made with extracts of *Cocos nucifera* root in Benin (Tchogou et al., 2016) and leaves of *Tectona grandis* in Togo (Diallo et al., 2008). The Mean Corpuscular Volume (MCV) significantly increased in the various treatment groups at D7 reflecting microcytosis. This phenomenon indicates that the produced haematia are released early into the blood even before they complete their differentiation. Indeed, the maturation of hematopoietic precursors results in the progressive reduction of their size (Fauchet et al., 1995). The MCV returns to normal at the end of the experiment, suggesting that as the anaemia corrects, the red blood cells complete their differentiation before being released into the blood. The Mean Corpuscular Hemoglobin Concentration (MCHC) has evolved inversely with MCV and confirms the previous suggestion. Indeed, the MCHC first dropped to J7 indicating first a release of hypochromic red cells, so immature before increasing thereafter corresponding to the release of increasingly differentiated red blood cells. These results agree with those obtained on the aqueous extract of the leaf sheath of *Sorghum bicolor* by Sènou et al. (2016a) but differ from those of Ogwumike (2002). However, the latter did not use a model of anaemic rats. Finally, we verified the specificity of the extract action by following the evolution of the blood platelets number. Indeed, the number of platelets was not significantly changed by the treatment, indicating a lack of thrombocyte lineage stimulation. This result suggested that the extract did not stimulate all hematopoietic lineages and therefore showed some specificity of action on erythroid. The same observation was done with aqueous extract of *Hibiscus sabdariffa* calyx or *Cocos nucifera* root (Tchogou et al., 2016; Sènou et al., 2016b).

## CONCLUSION

This work has shown in vivo on a model of anaemic rats, the therapeutic efficacy of root extract of *Psorospermum febrifugum* Spach, a medicinal plant used in Benin to treat anaemia. The extract stimulates the synthesis of hemoglobin and completely corrects the anaemia in two weeks. It stimulates the production

of young red blood cells released into the blood before maturity when the anaemia is severe. The therapeutic efficacy of the extract seems dose dependent and is even superior to that of the vitafer used as a reference drug, suggesting a mechanism of action that deserves to be investigated.



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