International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 6 Issue 9 ǁ Septembre 2017 ǁ PP. 44-51

Efficacy of ethyl acetate fraction of *Cocos nucifera* aqueous extract on the treatment of anemia

Sènou M[1, 2 *], Tchogou AP[1 *], Agbangnan DCP[3], Dougnon TV[1], Ogué P[6], Agossadou A[1], Lalèyè A[4], Loko F[1], Agbonon A[5]& Sèzan A[6].

[1-] *Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 BP 2009 Cotonou, R. Benin*
[2-] *Laboratory of Experimental and Clinic Biology (LaBEC), Faculty of Sciences and Techniques of Dassa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), R. Benin.*
[3-] *Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA); Ecole Polytechnique d'Abomey-Calavi / Université d'Abomey-Calavi (UAC), R. Bénin.*
[4-] *Human Biology Unit, Faculty of Health Science, 01 B.P. 188 Cotonou, R. Benin*
[5-] *Laboratoire de Physiologie/Pharmacologie, Faculté des Sciences, Université de Lomé, Togo.*
[6-] *Laboratory of Biomembranes and Cellular Signaling, University of Abomey-Calavi, Cotonou Benin 01 BP 188 Cotonou Benin.*
[*-] *These authors contributed equally to this work.*
Corresponding author: senouxim@yahoo.fr

Abstract: *Cocos nucifera is a tropical coastal plant whose roots are used in traditional medicine to treat anemia. Its aqueous root extract stimulates erythropoiesis. This work aimed to test "in vivo" the ethyl acetate fraction of the extract.*
Method: *Five groups of five wistar rats each were formed. Four groups were anemic with phenylhydrazine chloridrate at D0. From day 2 to day 15, the anemic groups receive by gavage either distilled water, vitafer (anti-anemic drug), the ethyl acetate fraction of the aqueous root extract of Cocos nucifera at a dose of 40 or 60 mg / Kg of body weight. The non-anemic group served as a control. Their blood was collected on days D0, D2, D7, D10 and D15 for the hemogram and the osmotic resistance of red blood cells.*
Results: *At day 2, phenylhydrazine decreased significant decrease in hemoglobin and in the number of red blood cells, which were corrected at day 10 by the fraction of the extract with a dose-dependent effect. The extract fraction stimulated rapidly a release of immature macrocytes in the first week. The extract did not modify the number of platelets, suggesting some specificity on the red cell line.*
Conclusion: *The ethyl acetate fraction of the aqueous root extract of Cocos nucifera stimulates erythropoiesis more rapidly than the crude extract. The biological activity was dose-dependent and may be related to the flavonoids whose mechanism of action needs to be elucidated.*
Key words: *Cocos nucifera, ethyl acetate, erythropesis.*

Date of Submission: 23-10-2017 Date of acceptance: 07-11-2017

I. Introduction

Anemia is a public health problem that affects people from both developed and developing countries. It is defined as a condition in which the amount of hemoglobin which carries oxygen is low than normal (Bigoniya et al., 2013). Hemoglobin is contained in red blood cells which are the most abundant visible components in the blood system. Their lifespan is about 120 days (Xiong, 2014). Reduced number of erythrocytes can cause anemia and lack of oxygen for body tissues (Libregts, 2011).

There are several types of anemia. Blood loss anemia, aplastic anemia, megoblastic anemia, hemolytic anemia are some examples (Ross and Wilson, 2006; Guyton and Hall, 2007).

Several studies have shown that hemolytic anemia is associated with oxidative stress within erythrocytes. This concept is supported by the fact that hemolytic damage is accompanied by the generation of reactive oxygen species (ROS), glutathione depletion, hemoglobin (Hb) oxidation and Heinz body formation in RBCs. Hemolytic agents have been reported to cause membrane lipid peroxidation and denaturation of cytoskeletal protein (Jollow and al., 2001).

Iron deficiency anemia is the most prevalent type of anemia worldwide and approximately 30% of the world population is affected this anemia (Staubli Asobayire, 2005).

Treatment depends on the type of anemia. It maybe a supply of iron, vitamin B12 orB9orally, treatment withimmunosuppressorsorcorticosteroids,erythropoietininjections,blood transfusion ,or even bone marrow transplantation (Movaffaghi and Hasanpoor, 2006).

Medicinal plants are used all over the world to treat various pathologies (Modak*et al*., 2007). Ethnopharmacoligical information has shown that the use of various herbal plants for the treatment of anaemia is common (Ogwumike, 2002; Akah*et al*., 2010).

The anti-anemic efficacy of some of these plants has even been proven in animal experiments. This is the case, for example, with leaves of *Justicia secunda*Vahl, the foliar sheath of *Sorghum bicolor*, the calyces of *Hibiscus sabdariffa* and the roots of *Cocos nucifera* (Gbénou et al., 2006, Tchogou et al., 2016, Sènou et al., 2016(a) and Sènou et al., 2016(b)).

Although these authors did the phytochemical screening of plant extracts used to determine large groups of chemical compounds, they did not show the groups that correct anemia. It is within this framework that the present work aims to test in vivo the anti-anemic effect of the ethyl acetate fraction of the aqueous root extract of *Cocos nucifera*.

II. Material and methods

Animal Material

Animal material consisted of *Wistar* albino rats of average body weight 145 ± 10 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 (EPAC) of the University of Abomey-Calavi UAC) in Benin Republic. Breeding was done in a well-ventilated room, with a day-night rhythm of 12h. 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

- Identification

Roots of *Cocos nucifera* were collected from Abomey-Calavi in Benin during April 2015. 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.

- Preparation of the aqueous extract

50 g of root powder of *Cocos nucifera* were boiled in 500 ml of distilled water in a 1000 ml flask for 30 minutes. After cooling, the mixture is filtered using the Bushner. This operation is repeated for six times for a total mass of 300 g. The filtrate (the aqueous phase) obtained is recovered and stored in a refrigerator in a jar for liquid-liquid extraction (first fractionation step).

- Fractionation of the extract

Liquid-liquid extraction consists in passing a substance from a solvent, from which it is often difficult to separate, to another (called extraction solvent), from which it will be easily isolable. This operation, usually carried out by stirring, is possible provided that the two solvents are very little or no miscible with one another. But extraction is never 100%, there are always molecules of the compound to be extracted in the solvent in which it is less soluble.

The Liquid-liquid was obtained by successive partitions with solvents of increasing polarity (hexane and ethyl acetate) according to the protocol of Koudoro et al. (2014)**.** In a separatory funnel, was added to the aqueous extract solution the appropriate volume of extraction solvent. After vigorous agitation, the mixture was allowed to settle. After decantation, the two phases were separated by collecting the lower phase (aqueous phase) in a flask and the upper phase (organic phase) in another. The aqueous phase was re-poured into the separating funnel before repeating the following steps. After each extraction step, the organic phases were combined, which constituted the fraction in a jar.

The liquid-liquid extracts obtained was then evaporated using a rotary evaporator at a temperature according to the solvents of polarity. The extractant phase was re-sealed and solidified in an oven at 40°C. The dry residue obtained was reduced to powder and stored in a refrigerator in a brown flask. The yield of the fraction was calculated by the following formula:

$$R = \frac{\text{Mass of fraction}}{\text{Mass of powder}} \times 100$$

In vivo Experimentation

The evaluation of the anti-anemic activity consisted of assessing the impact of *Cocos nucifera* aqueous extract ethyl acetate fraction 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).

- 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 fraction 40 mg / kg of body weight / day or 60 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 40 mg / kg / day of the *Cocos nucifera* ethyl acetate fraction 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 60 mg / kg / day of the *Cocos nucifera* ethyl acetate fraction 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

Hematological 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 use to assess the production of young red blood cells.

Statistical Analysis

Graphs were plotted using Graph pad 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%.

III. Results

Evolution of hemoglobin

The mean hemoglobin ranged from 14 ± 0.4 to 15.7 ± 0.5 g / dl in the different groups of rats at day 0 (Figure 1).

At day 2 (D2), it decreased significantly (P value <0.05) in all phenylhydrazine anemic groups and ranged from 8.1 ± 0.05 to 9.0 ± 0.05 g / dl. It increases very rapidly afterwards in the treated anemic groups and is no longer significantly different from D0 at D7 in the group treated with the ethyl acetate fraction and at D10 in that treated with vitafer (reference drug). At day 15, the mean hemoglobin was 14.7 ± 0.58 g / dl in the vitafer treated group, 14.5 ± 0.74 g / dl in the 40 mg / kg treated group and 15.1 ± 0.63 g / dl in the 60 mg / kg treated group.

In the untreated anemic group, hemoglobin synthesis after D2 is less rapid and the hemoglobin level remained significantly low even at D15 compared to D0.

In the non-anemic (control) group, mean hemoglobin did not significantly vary throughout the experimental period.

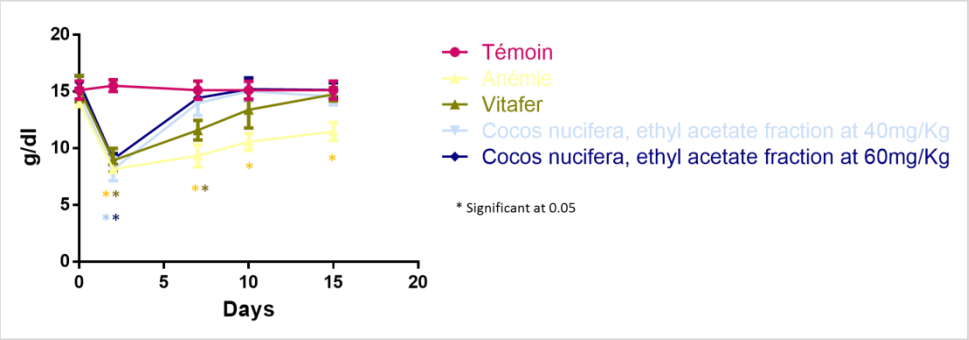

Figure 1. Treatment effect on hemoglobin level

Evolution of the number of red blood cells

The mean number of red blood cells varied from 6.3 ± 0.70 to 7.8 ± 0.72 T / L in the groups at D0 (Figure 2). At D2, it decreased following haemolysis induced by phenylhyrazine and varied from 3.4 ± 0.43 to 3.5 ± 0.32 in anemic groups. Then, it increased very rapidly in the treated groups and was no longer significantly different from D0 at D7 with 60 mg extract fraction / Kg and J10 with 40 mg extract fraction /Kg or vitafer, suggesting a dose-dependent effect of the extract fraction. At day 15, the red blood cell count were 7.1 ± 0.38 T / L for the vitafer treated group, 6.7 ± 1.2 T / L for that treated with 40 mg / kg of extract fraction and 7.1 ± 0.67 T / L for that treated with 60 mg / Kg of extract fraction.

In the untreated group anemic, increasing the number of red blood cells after D2 was lower and even on D14 the mean number of cells (5.2 ± 0.41 T / L) was significantly lower compared to D0. In the non-anemic (control) group, the mean number of red blood cells did not significantly vary throughout the experimental period.

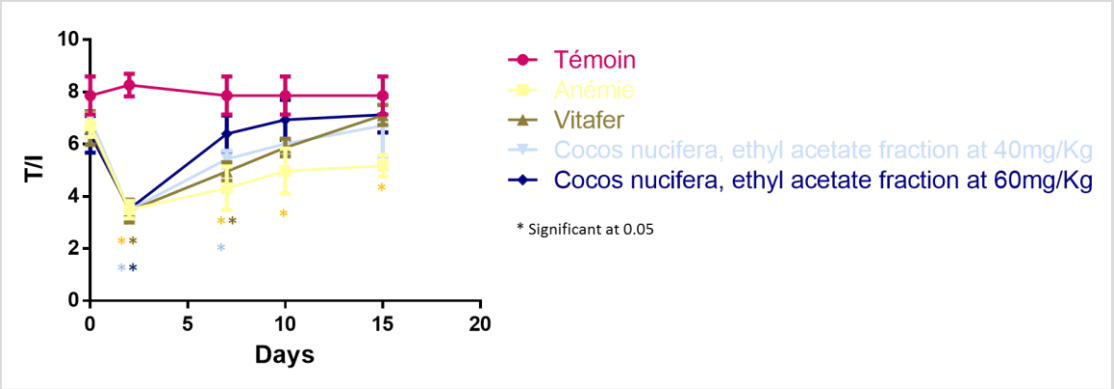

Figure 2. Treatment effect on red blood cells count

Evolution of the Mean Corpuscular Volume (MCV)

At D0, the Mean Corpuscular Volume ranged from 60 ± 1.6 fl to 62 ± 1.3 fl in the different groups of rats (figure 3). It increased very rapidly in all anemic groups and reached its peak (77 ± 2.2 fl to 87 ± 4.3 fl) at D7 in the treated anemic groups, indicating macrocytes release. It then decreased rapidly and on D14 is no longer significantly different from D0 in the groups treated with the extract fraction or vitafer.

In the untreated anemic group, the increase in VGM continued until D14, indicating macrocytes release. In the non-anemic (control) group, VGM did not significantly vary throughout the experimental period.

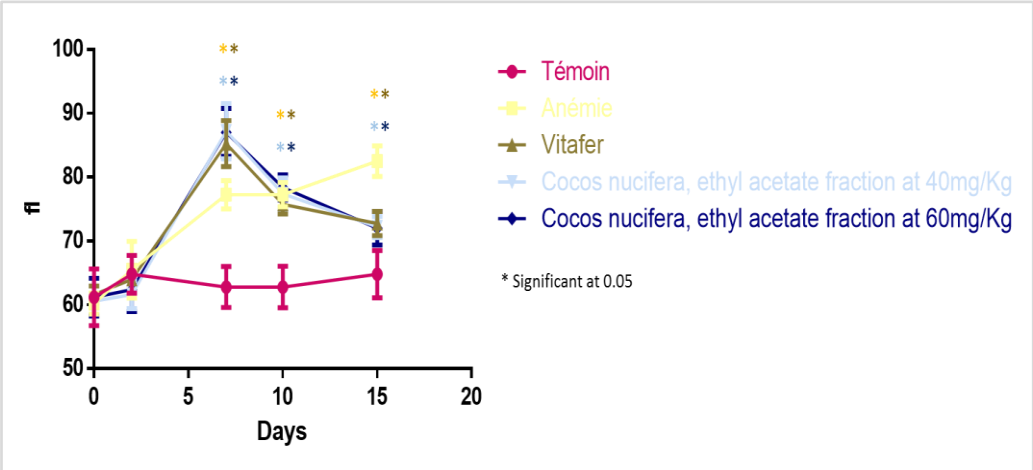

Figure 3. Treatment effect on mean corpuscular volume

Evolution of the mean hemoglobin corpuscular concentration (MHCC)

On Day 0, MHCC ranged from 32 ± 1.2 g / dl to 35 ± 0.7 g / dl in the various groups of rats (figure 4). It increased significantly in all anemic groups at day 2 and ranged from 40 ± 0.6 to 43 ± 1.5 g / dl, indicating saturation of red blood cells present in hemoglobin. Thereafter, a rapid fall was observed in all anemic groups and at D7 was significantly lower than in D0 in the untreated anemic group (31 ± 1.5 g/ dl) and the anemic and low-dose (40 mg / kg) fraction extract treated group (32 ± 1.9 g / dl). This decrease reflects a release of hypochromic erythrocytes (less saturated with hemoglobin) in the circulation. At day 15, the MCC was no longer significantly different from D0 in the anemic groups.

In the non-anemic control group, the MHCC was not significantly varied during the experiment.

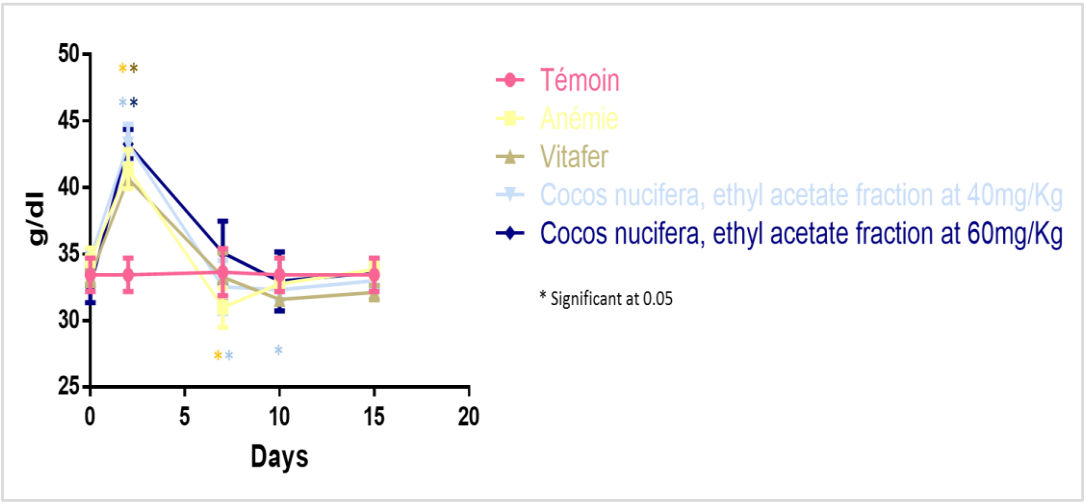

Figure 4. Treatment effect on mean corpuscular hemoglobin concentration

Evolution of the osmotic resistance of erythrocytes

On day 0, the osmotic resistance of the erythrocytes varied from 19 ± 2.5% to 27 ± 9.8% (figure 5). It increased rapidly in anemic groups and reached its peak at D7 (63 ± 6.2% to 71 ± 5.3%), reflecting an increased release of young red cells in the blood circulation. It then decreased in the treated groups and became non-significant at day 15 in vitafer or 40 mg extract fraction / kg groups. In contrast, in the untreated and anemic groups treated with the extract fraction at 60 mg / kg, the osmotic resistance at day 15 increased significantly again compared to day 0 indicating a persistence of liberation of young red cells.

In the non-anemic control group, the osmotic resistance of the erythrocytes was not significantly varied during the experiment.

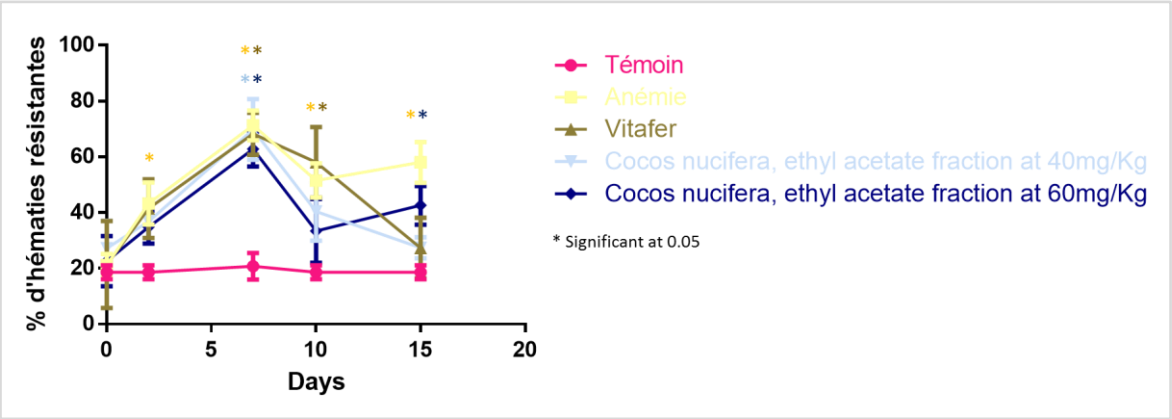

Figure 5. Treatment effect on the red cell osmotic resistance

Changes in the number of blood platelets

The mean number of blood platelets at D0 ranged from 345 ± 135 G / L to 501 ± 90 G / L in the various groups of rats (figure 6).

It increased very significantly with a peak at D2 in all anemic or non-anemic groups (895 ± 107 G / L to 997 ± 157 G / L). It then decreased progressively in all groups until D15 and was no longer significantly different from D0.

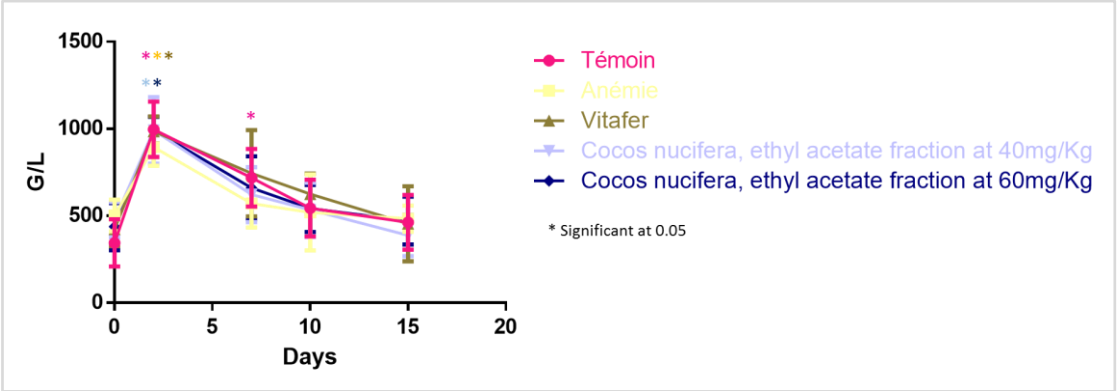

Figure 6. Treatment effect on the number of blood platelets

IV. Discussion

Cocosnucifera is a tropical coastal plant. Its roots are used in Africa to treat anemia. We previously demonstrated in animal experiments the erythropoietic effect of its aqueous root extract (Tchogou et al., 2016). Phytochemical screening of these roots revealed various groups of chemical compounds including tannins, flavonoids, leucoanthocyanins, steroids, quinone derivatives, reducing compounds and mucilage. Since these compounds were also found in other plants with hematopoietic activities such as *Justicia secunda* Vahl and *Sorghum bicolor* for example (Gbébou et al., 2006; Sènou et al., 2016), it would be interesting to investigate the molecular families carrier this biological activity. For this purpose, we tested the fraction of ethyl acetate which isolates mostly flavonoids (Manjusha et al., 2013, Koudoro et al., 2014) on a model of phenylhydrazine anemic rats (BA Naughton et al., 1995; Nakanishi et al., 2003). The effect of this fraction of the extract was compared with that of vitafer, an anti-anemic drug and measured by the evolution of the parameters of the hemogram such as hemoglobin, red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MHCC), blood platelet counts, and osmotic resistance of red blood cells.

Hemoglobin is the indicator of anemia. Its decrease on day 2 by hemolysis induced by phenylhydrazine was very rapidly corrected to 7 by the fraction of extract at 40 or 60 mg / kg. This correction is earlier than that observed with the crude extract (Tchogou et al., 2016) or with the extracts of the leaf sheath of *sorghum bicolor* (Ogwumike, 2002, Godwin et al., 2014) or with the vitafer.

As in the case of hemoglobin, the collapse of the number of red blood cells at D2 following hemolysis induced by phenylhydrazine was very rapidly corrected by the fraction of the extract at 60 mg / Kg from D7 and at 40 mg / Kg from D10. The fraction was more active than the crude root extract, which correction of the number of red blood cells occurred only from D10 at doses of 300 mg / kg or 400 mg / kg (Tchogou et al., 2016). It also stimulated erythropoiesis better than the raw leaf extracts of *Tectona grandis* (Diallo et al., 2008),

*Justicia secunda*Vahl (Gbenou et al., 2006) and the bark of *Mangifera indica* L (Nwinuka et al, 2008). The extract fraction, like vitafer, induced a rapid increase in the mean globular volume with peak at day 7 corresponding to a release of large red cells (macrocytes) into the bloodstream. MCV is returned to normal on day 15, indicating the release of well differentiated red blood cells in the second week in contrast with the crude extract that has continued to release macrocytes in the second experimental week (Tchogou et al., 2016).

In contrast to MCV, the concentration mean corpuscular hemoglobin is low only at D7 in anemic groups untreated or treated with 40 mg / kg of extract fraction. This corresponds to a release macrocytes less saturated hemoglobin (hypochromia) which, however, was corrected later during the second week. This observation also contrasts with that of the crude extract which continued a release of hypochromic erythrocytes even at D15 (Tchogou et al., 2016). A release of hypochromic erythrocytes was also observed by Ogwumike (2002) in his anemic rat model. To confirm that hypochromic macrocytes are young red blood cells, the osmotic resistance of red blood cells has also been determined (Sénou et al., 2016). In all treated anemic groups, the osmotic resistance of red blood cells rapidly increased with a peak at day 7 as observed for MCV, which confirms the hypothesis that these cells are very young and quickly released into the bloodstream to compensate the anemia.

Finally, to check the specificity of action of the extract on the red cell line, we followed the evolution of the number of blood platelets in different groups. It increased with a peak at D2 and then gradually returned to normal at D10 in all anemic or non-anemic groups. The increase reflected a reaction of the body to mobilize the platelets, specialized cells of the hemostasis to stop the haemorrhage linked to the injury of the blood sample collection. The fraction of the extract has no action on the thrombocyte line, indicating that it is not a general stimulator of hematopoiesis and therefore suggests some specificity on erythropoiesis. This finding confirmed the effect of the crude extract of this root and that of the foliar sheath of *Sorghum bicolor* (Tchogou et al., 2016, Sènou et al., 2016).

Since the ethyl acetate fraction largely isolate flavonoids (Manjusha et al., 2013; Koudoro et al., 2014), some molecules of this chemical family would be responsible for the observed erythropoietic activity. Indeed, flavonoids such as orientin and luteolin are powerful antioxidants capable of protecting red blood cells against haemolysis induced by oxidative stress (Fang et al., 2016), and in particular that induced by phenylhydrazine, a powerful oxidant toxic to red blood cells (Nakanishi et al., 2003; Shami et al, 2016.). Moreover, flavonoids have been shown capable of direct stimulation of hematopoiesis and probably via erythropoietin (Abeer et al., 2009; Zhang et al., 2017). Finally, flavonoids are a family of compounds found in several plants with haematopoietic activity. That were the case of the leaves and stem *Manguiferra indica* (Ogbe et al., 2010), the leaves *Telfairia occidentalis* (Alada et al., 2000), the root bark of *Schrebera swietenioides* (Pingali et al., 2015), leaves of *Moringa oleifera* (Nwaehujor et al., 2015) and *Jatropha tanjorensis* (MacDonald et al., 2014).

V. Conclusion

The ethyl acetate fraction of the aqueous root extract of *Cocos nucifera* stimulated hemoglobin synthesis more rapidly than the crude extract. The dose-dependent effect was observed in erythropoiesis. Since ethyl acetate largely isolates flavonoids, the observed effect could be attributed to this family of chemical compounds, which is increasingly also associated with hematopoiesis. However, the study should be continued on a possible synergy action with other families of compounds.

References

[1] Abeer Temraz et al. Treatment of Experimental Hemolytic Anemia by Some Antioxidant Drugs. Journal of Pharmacy Research, 2009; 2(5): 798-802.
[2] Akah PA, Okolo CE, Okoye TC, Offiah NV. 2010. Aqueous extract and methanol fractions of leaves of Brillantaisianitens Lindau. Reverses phenylhydrazine-induced anaemia in rats. Journal of Medicinal Plants Research 4(**3),** 271-277.
[3] Alada, ARA. The hematological effect of *Telfelria occidentalis* diet preparation. African Journal of Biomedical Research, 2000; 3: 185-186.
[4] 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.
[5] Bigoniya P, Singh S, Singh CS, Shukla A. Anti-anemic potential estimation on mice and characterization of flavonoids using high performance thin layer chromatography in *Wrightia tinctoria* bark fraction. J Nat Pharm, 2013; 4: 47-56
[6] Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K, Agbonon A**.** 2008.Effect of Tectona grandis on phenylhydrazine induced anemia in rats. Fitoterapia79**,** 332-6.http://dx.doi.org/10.1016/j.fitote.2008.02.005
[7] Fang An, Shulin Wang, Danhua Yuan, Yuewen Gong and ShuhuaWang. Attenuation of Oxidative Stress of Erythrocytes by Plant-DerivedFlavonoids, Orientin and Luteolin. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine, Volume 2016, Article ID 3401269, 8 pages, http://dx.doi.org/10.1155/2016/3401269.
[8] Ganong, W. F. (2001). *Physiologie Médicale* (1st ed.). *de l'Université Broeck. 828p.*
[9] 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 TradAfr, 14**,** 4
[10] Genetet, B. (1989). Hématologie. *Tech et doc. Lavoisier, éd, Paris* (pp. 3-82).

[11] 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

[12] Guyton and Hall. Text book of Medical Physiology, Saunders, eleventh edition, 2007; 426-427.

[13] Jollow DJ and McMillan DC. Oxidative stress, glucose-6-phosphate dehydrogenase and the red cell. Adv Exp Med Biol., 2001; 500: 595–605.

[14] Koudoro Y. Alain, Dedomè L. S. Oronce, YovoMahudro, Agbangnan D. C. Pascal, TchoboF.Paul, Alitonou G. Alain, Avlessi Félicien, and Sohounhloué D. C. Koko. Chemical characterization, antiradical and antibacterial activities of extracts of the root bark of *Cochlospermum planchoni* of Benin. International Journal of Innovation and Applied Studies ISSN 2028-9324 Vol. 7 No. 4 Aug. 2014, pp. 1582-1594

[15] Libregts S. F.,Guti´errezL., A. M. de Bruin et al., "Chronic IFN-*γ*production in mice induces anemia by reducing erythrocyte lifespan and inhibiting erythropoiesis through an IRF-1/PU.1 axis, "*Blood*, vol. 118, no. 9, pp. 2578–2588, 2011.

[16] Mac Donald Idu et al. Anti-anemic activity of *Jatropha tanjorensis* Ellis and Saroja in Rabbits. Journal of Medicinal Plants Studies, 2014; 2(1): 64-72.

[17] Manjusha Saxena, AbrarHussain Mir, ManikSharm, MohhdYoussufMalla, Sumeerah Qureshi, Mohd IqbalMir and Yogesh Chaturvedi. Phytochemical Screening and in-vitro Antioxidant Activity Isolated Bioactive Compounds from *Tridax procumbens* Linn. Pakistan Journal of Biological Sciences 16 (24) : 1971-1977, 2013

[18] Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A, Devasagayam T. 2007. Indian herbs andherbal 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

[19] Movaffaghi Z, Hasanpoor M (2006). Effect of therapeutic touch on blood hemoglobin and hematocrit level. J Holist Nurs, 24: 41-8. http://dx.doi.org/10.1177/0898010105282527

[20] Nakanishi A, Kinuta K, Abe T, Araki K, Yoshida Y, Liang S, *et al*. Formation of meso, N-diphenylprotoporphyrin IX by an aerobic reaction of phenylhydrazine with oxyhemoglobins. Acta Med Okayama, 2003; 57: 249-56

[21] Naughton, B. A., Moore, E., Bush, M. E., Lapin, D. M., &Domfest, B. S. (1995). Hemostatic alterationsassociated with phenylhydrazine – induced haemolytic anaemia. *Eur J Clin Invest, 25*, 722-7.

[22] Nwaehujor CO et al. Effect of *Moringa oleifera Lam*. Ethanol leaf Extract on Hematology in Phenylhydrazine-induced Anemic Albino Wistar Rats, American Journal of Pharmacological Sciences, 2015; 3(3): 67-73.

[23] Nwinuka, N. M., Monanu, M. O, &Nwiloh, B. I. (2008). Effect of Aqueous Extract of Mangifera indica L. (Mango) Stem Bark on Haematological Parameters of Normal Albino Rats. *Pak J. of Nu, 7*, 663-6. http://dx.doi.org/10.3923/pjn.2008.663.666

[24] Ogbe RJ, Adoga GI, Abu, AH. Antianemic potentials of some plant extracts on phenylhydrazine-induced anemia in rabbits. Journal of Medicinal Plants Research, 2010; 4: 680-684.

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

[26] Pingali PS, Srinivasi P, Reddy MB. Study of Anti- Anemic Effect of *Schrebera swietenioides Roxb*. in rat models. Asian Journal of Pharmaceutical and Clinical Research, 2015; 8(5): 260-263.

[27] Ross and Wilson. Anatomy and Physiology in Health and Illness, Churchill Livingstone Elseiver, Tenth Edition 2006; 70-71.

[28] Sènou M, TchogouAP,Dougnon TV, Agossadou A, F. Assogba F, Kinsiclounon EG, Koudokpon H, Fah L, Fanou B, Akpovi DC, Gbénou J, Lalèyè A, Loko F (a). Efficiency of Sorghum bicolor extract in the treatment of induced anemia on Wistar rats. International Journal of Biosciences 2016; 8 (4): 62-71. http://dx.doi.org/10.12692/ijb/8.4.62-71

[29] Sènou M, Agossadou A, Agbangnan DCP, Tchogou AP, Assogba F, Klotoé JR, Dougnon TV,Gbénou J, Lalèyè A, Sèzan A &Loko F (b). The aqueous extract of *hibiscus sabdariffa* l. calyces effectively corrects induced anemia. *Ijppr.Human, 2016; Vol. 7 (4): 254-265*.

[30] Shami Chaddha and Aman Mittal. Role of flavonoids in the treatment of hemolytic anaemia-A review. European journal of pharmaceutical and medical research,*2016,3(5), 212-216*

[31] Tchogou AP, Sènou M, Dougnon TV, Agossadou A, Assogba F, Kinsiclounon EG, Ewedjè E, Agbangnan DCP, Gbénou J ; Lalèyè A, Loko F. The Aqueous Extract of *Cocos nucifera L.* (Arecaceae) Effectively Treat Induced Anemia. Experimental study on *Wistar* Rats. International Journal of Biology.2016;8: 3; published by Canadian Center of Science and Education.5-54.

[32] Xiong Y. L., *Effect and Mechanism of Exhaustive Exercise Induced Oxidative Stress in Erythrocytes of Rat*, Chongqing University, Chongqing, China, 2014.

[33] Yapi HF et al. Anti-anaemic activity of aqueous and ethanolic extracts of *Entandrophragma angolense* bark on phenylhydrazine-induced anaemic rats. Asian Journal of Biochemical and Pharmaceutical Research, 2015; 3(5): 12-21.

[34] Zhang L., Gong A. G.W., Riaz Kashif, Tsim K. W. K.Protective effect of flavonoids against reactive oxygen species production in sickle cell anemia patients treated with hydroxyurea. FEBS Journal, Volume 7, Issue 3, Pages 318–323, 2017

Sènou M Efficacy of ethyl acetate fraction of Cocos nucifera aqueous extract on the treatment of anemia." International Journal of Pharmaceutical Science Invention(IJPSI), vol. 6, no. 9, 2017, pp. 44-51.