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The Aqueous Extract of *Cocos nucifera L.* (Arecaceae) Effectively Treat Induced Anemia. Experimental Study on *Wistar* Rats

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Abstract

Anaemia is a serious public health problem especially in developing countries as Benin. *Cocos nucifera* is one of medicinal plants used in Benin to treat anemia. This study aimed to test its therapeutic efficacy in anemia treatment. **Method:** 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 aqueous extract of *Cocos nucifera* 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. **Results:** The phytochemical analysis revealed the presence of tannins, flavonoids, leucoanthocyanes, steroids, quinone derivatives, reducing compounds and mucilage. The extract like the vitafer corrected completely anemia before two weeks by stimulating hemoglobin synthesis, production and early release of immature red cells in the blood stream. Its effect was dose dependent, quite specific and did not affect platelet lineage. **Conclusion:** *Cocos nucifera* has a good therapeutic efficacy and may be considered for transformation into improved traditional medicines (ITM) after study of its biological tolerance and appropriate clinical trials.

Keywords: *Cocos nucifera*, anemia, red cell, *Wistar* rats, phytochemical analysis

1. Introduction

African pharmacopoeia is rich because of the diversity of human groups, languages, customs, and especially the ecological character of areas. The majority of these social groups use this biodiversity of medicinal plants to solve their health problems (Modak et al., 2007; Soetan and Aiyelaagbe, 2009). The use of plants for therapeutic purposes is a centuries-old practice (Kassel, 2003). Among these useful plants, those used to treat anemia caught our attention.

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). It affects people of all ages, but the main targets are infants, pregnant women and elderly (Holden & Acomb, 2007; Duff, 2008).

The prevalence of anemia is higher in developing countries. The main causes are nutritional deficiencies (Alper et al., 2000; Assobayire et al., 2001), parasite infection such as Plasmodium, Trypanosomes and helminthic (Verhoel et al., 2002), pregnancy and breastfeeding (Alper et al., 2000; Scanlon et al., 2000; Marti-Carragal et al., 2002). Iron deficiency is by far the leading cause of anemia in the world and represents about 50% of cases (Alper et al., 2000; Assobayire et al., 2001).

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

In holding the high cost of modern drugs in pharmacy, WHO encourages the search for alternatives (UNESCO, 1998). Ethnopharmacoligical information has shown that the use of various herbal plants for the treatment of anaemia is common (Akah et al., 2010). *Cocos nucifera* is a plant used for this purpose in West Africa and Benin. This study aimed to investigate the therapeutic efficacy of aqueous extract of this plant on an experimental hemolytic anaemia model.

2. Material and methods

2.1 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.

2.2 Identification and Preparation of Plant Materials

Roots of *Cocos nucifera* were collected from Abomey Calavi in Benin during April 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 (20-25^0 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:

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

2.3 Phytochemical Screening of Cocos nucifera Roots Extact

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.

2.4 In vivo Experimentation

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

2.4.1 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).

2.4.2 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 use 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 *Cocos nucifera* 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 *Cocos nucifera* aqueous extract from D2 to D15.

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

2.5 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 and student t test. The significance level was set at 5%.

3. Results

3.1 Chemical Compounds Identified in the Roots of Cocos nucifera

The extraction efficiency is 16%. Phytochemical screening of vaporized leaf extract of *Cocos nucifera* has revealed the presence of catechol tannins, flavonoids, leucoanthocyanes, steroids, quinone derivatives, reducing compounds and mucilage (Table 1).

3.2 The Extract of Sorghum bicolor Leaves Stimulates Hemoglobin Synthesis in a Dose Dependent Manner

The mean hemoglobin level was 14.9 to 16.8 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 10.5 g / dl; $P < 0.001$). This decrease has been gradually corrected and the hemoglobin level at day 10 was not significantly different from its value of day 0 in the groups treated with vitafer (14.7 g / dl), and extract at 200 mg / Kg (13, 7 g / dl) or 300 mg/Kg (14, 6 g / dl). From day 2 to day 15, the increase in hemoglobin was 4.62 g / dl for the treatment vitafer, 3.52 g / dl for the extract at 200 mg / kg and 6.08 g / dl the extract 300 mg / kg, indicating better efficiency of the plant at high doses. The hemoglobin level was lower in untreated anemic (12.3 g / dl; $P = 0.01$) (Figure 1).

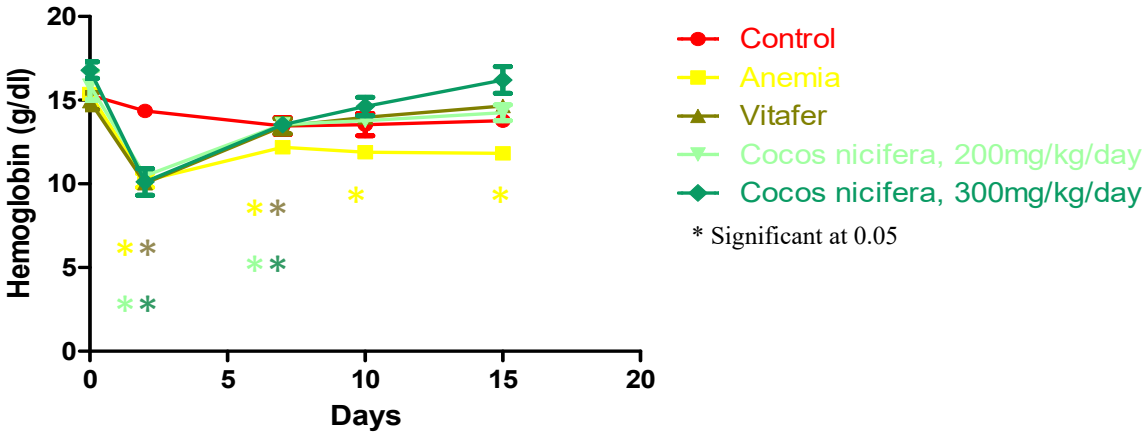

Figure 1. Treatment effect on hemoglobin level

Table 1. Phytochemical screening of *Cocos nucifera* 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.

3.3 The Extract Stimulates Hemoglobin Synthesis by Activation of Erythropoiesis

The mean number of red blood cells is 5.0 to 5.7 T / l on day 0 in the different groups of rats. It fell at day 2 (hemolysis) following administration of phenylhydrazine (2.5 to 3.1 T / l; $P < 0.001$). This decrease was corrected at day 7 for vitafer treated group and at day 10 for the extract treated groups. At day 15, the mean number of red blood cells reached 5.3 T / l with the vitafer and 5.5 T / l with the extract. Only the untreated anemic group did not have such a correction (3.5 T / l; $P = 0.001$) (Figure 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; $P = 0.06$).

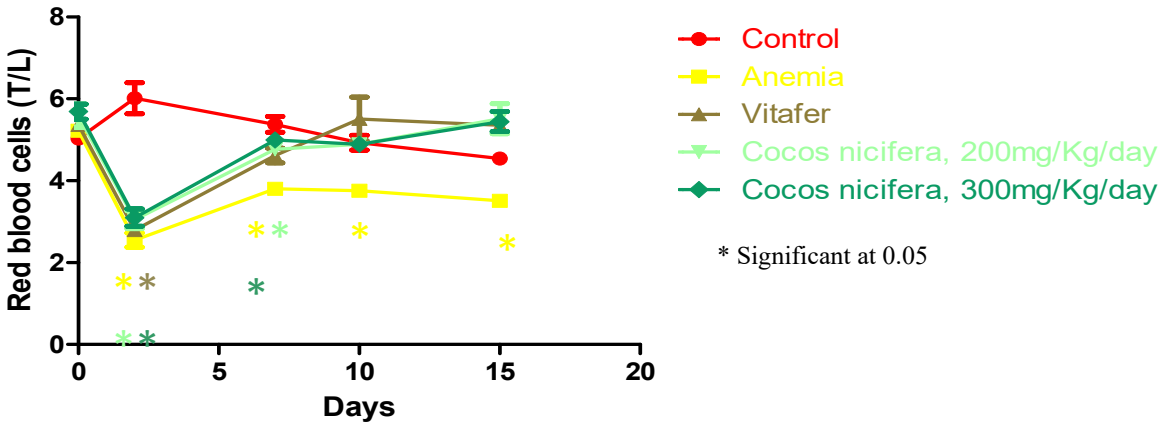

Figure 2. Treatment effect on red blood cells count

3.4 Anemia is Corrected by a Release of Immature Red Cells in the Blood

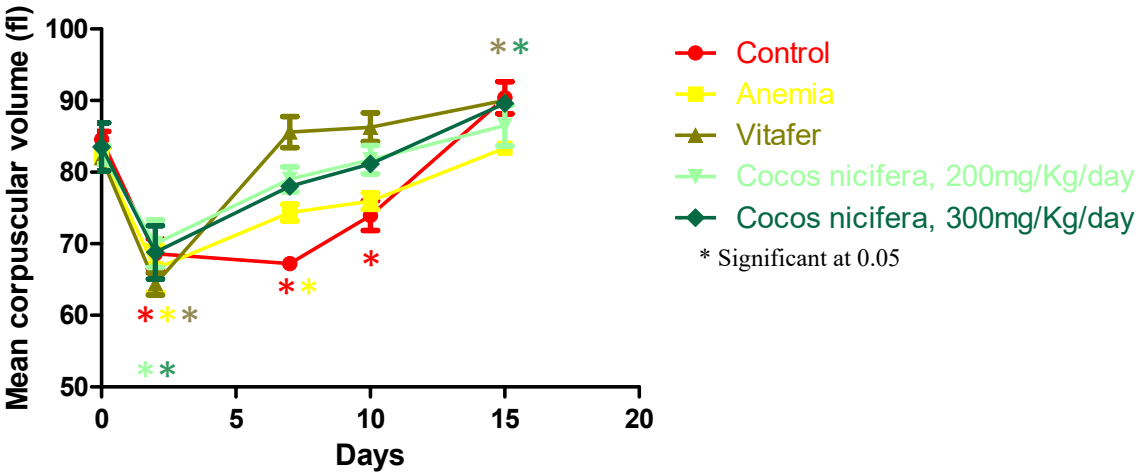

Figure 3. Treatment effect on mean corpuscular volume

The Mean Corpuscular Volume (MCV) was from 82 to 84 fl in the various groups at D0. It fell at day 2 (64-70 fl, $P < 0.05$) resulting microcytosis after administration of phenylhydrazine. The MCV then increase very rapidly in all anemic groups and at D15 significantly exceeded its initial value in the groups treated with vitafer or extract at 300 mg /kg (90 fl; P < 0, 05). The increase was more moderate in the 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) (Figure 3).

3.5 The Mean Corpuscular Hemoglobin Concentration (MCHC) Moved Inversely Compared to the MCV

MCHC mean was about 34 to 36 pg in groups at D0. Unlike the MCV, it significantly increased and reached its peak (43-49 pg, $P < 0.04$) in day 2 in all anemic groups. It then gradually decreased and became significantly lower at day 15 in the groups treated with vitafer (30 pg; $P < 0.01$) or 300 mg extract / kg (29 pg; $P < 0.01$) and anemic untreated group (31 pg; $P < 0.04$). This decrease in MCHC reflects a release of red blood cells less saturated hemoglobin (Figure 4).

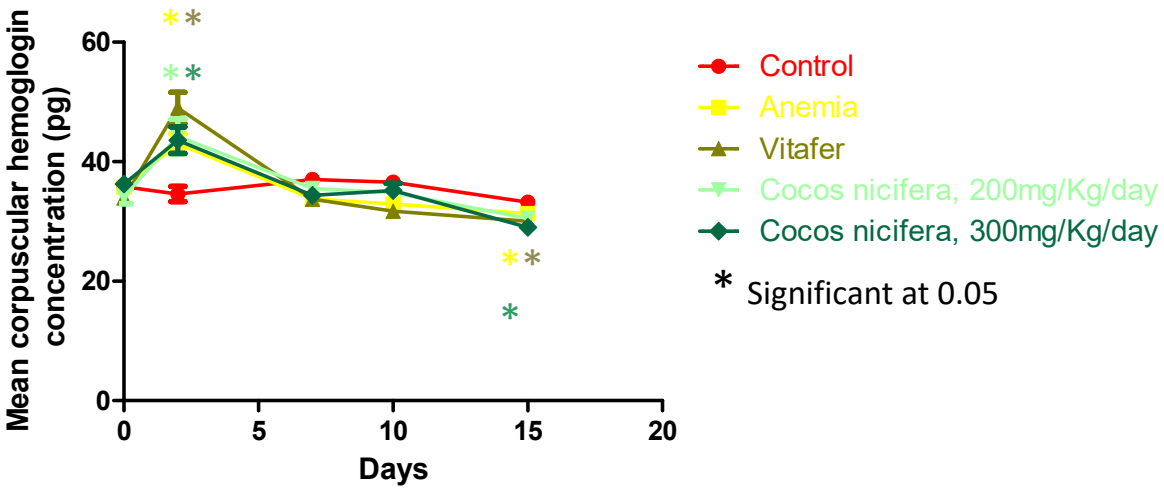

Figure 4. Treatment effect on mean corpuscular hemoglobin concentration

3.6 Compensation of Anemia Started with an Early Release of Young Red Blood Cells

The osmotic resistance of red blood cells is 19 to 34% 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 (Figure 5). Note that the osmotic resistance is higher when the red cell is young (Gbenou et al., 2006).

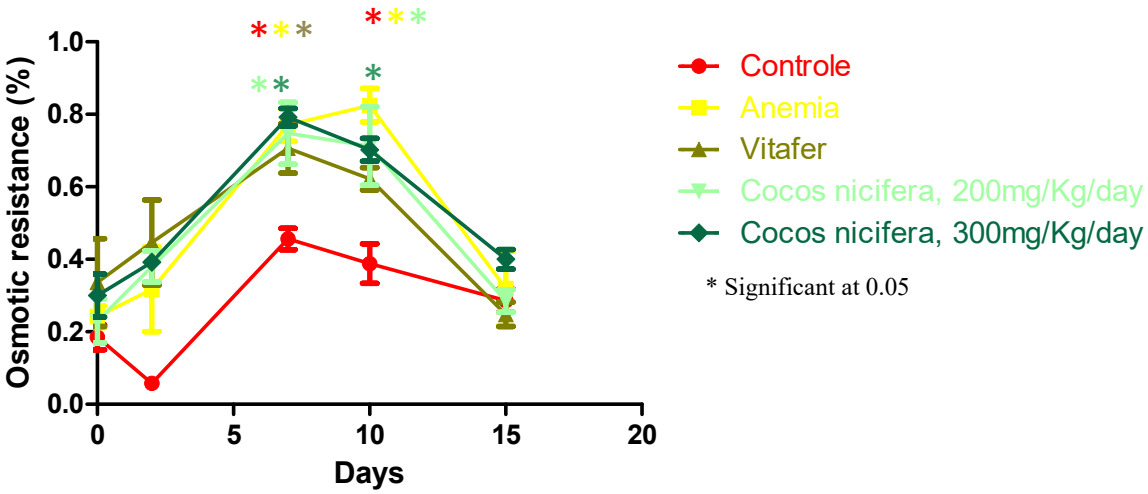

Figure 5. Treatment effect on the red cell osmotic resistance

3.7 The Extract Stimulates Erythropoiesis Rather Specifically

The number of blood platelets on D0 was about 197 to 325 G / l in all groups of rats. It has significantly increased and varies between 842 and 919 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.

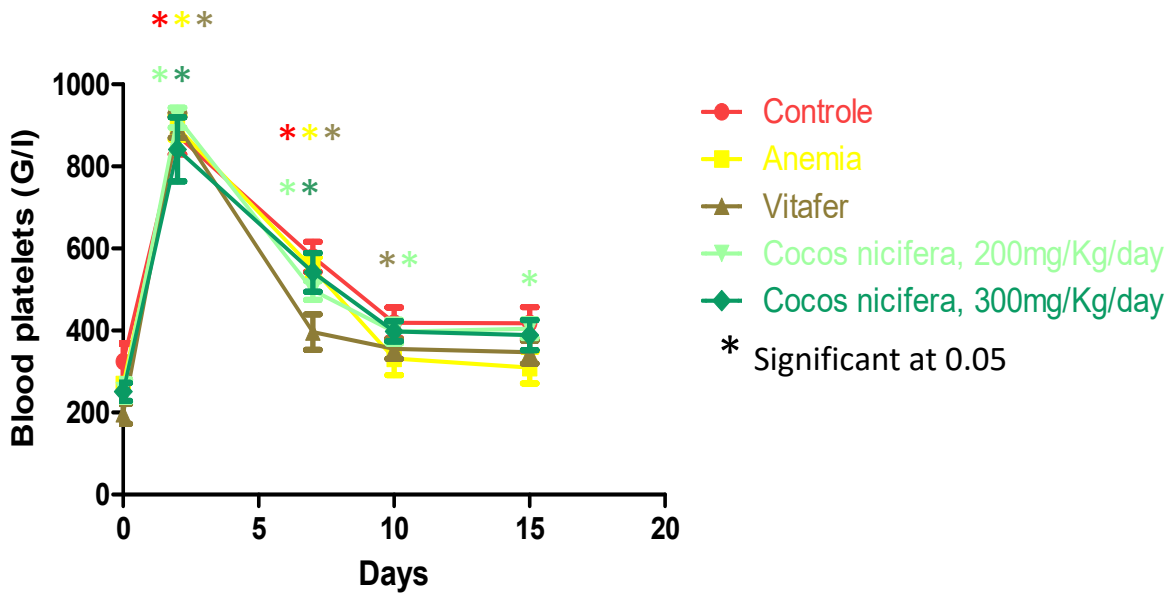

Figure 6. Treatment effect on the number of blood platelets

4. Discussion

Cocos nucifera is a medicinal plant used in Benin to treat anemia. In the present study, its efficacy was demonstrated on experimental model of anemic *Wistar* rats. For this purpose, a phytochemical analysis of the sprayed aqueous extract of the dried roots of the plant was first processed. It revealed the presence of tannins, flavonoids, leucoanthocyanes, steroids, quinone derivatives, reducing compounds and mucilage. The Tannins, flavonoids and leucoanthocyanes were also detected in leave extract of *Justicia secunda Vahl*, another plant use to treat anemia (Gbenou et al., 2006).

We then induced hemolytic anemia with phenylhydrazine (Naughton BA et al., 1995) in *Wistar* rats we administered the extract of *Cocos nucifera* 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 decrease in hemoglobin at day 2 in the various groups of rats. Anemia was corrected from the day 10 by vitafer and the *Cocos nucifera* extract with a dose-dependent effect.

A similar result was obtained by Ogwumike (2002) who showed a dose-dependent anti-anemia property of *Sorghum bicolor*, another medicinal plant used in Benin to treat anemia. *Sorghum bicolor* based traditional herbal preparation also increased hemoglobin in HIV positive patients (Godwin et al., 2014). Furthermore, at the dose 300 mg / Kg, the extract better stimulated the synthesis of hemoglobin that vitafer which is the reference drug. This suggests that the action mechanism of the plant might differ from a simple iron intake.

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 *Cocos nucifera* extract. Such a result was obtained with leaves extracts of *Tectona grandis* in Togo (Diallo et al., 2008), *Justicia secunda* Vahl in Benin (Gbenou et al., 2006) and Stem Bark extract of *Mangifera indica* L. in Nigeria (Nwinuka et al., 2008).

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 at day 7 in the treated groups and at day 10 in the untreated anemic group 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).

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 D0 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).

5. Conclusion

The aqueous extract of roots of *Cocos nucifera* was efficient against anemia in a dose-dependent manner. 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.

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