Efficacy of the Aqueous Extracts of *Justicia Galeopsis* Leaves on the Improvement of Hematological Parameters in Anemic Rats

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Abstract

Antianemic potential of aqueous of *Justicia galeopsis* leaves was studied using Wistar Albino rats after induction of anemia by phenylhydrazine hydrochloride. Forty rats (20 male and 20 female) subdivided into five groups of eight rats were used. There was a group as control (not anemic) and four other anemic groups which had received by gavage respectively 1 ml/kg of distilled water, 1 ml/kg of body weight of Vitafer (reference drug commonly used to treat anemia), 100 mg/kg of body weight of extract of *J. galeopsis* leaves cooked during 30 min and 30 mg/kg of body weight of extract of *J. galeopsis* leaves cooked during 45 min. Hematological parameters (red blood cells, hemoglobin and hematocrit) were analyzed as indices of anemia and the weights of specific organs (liver, spleen and kidney) were evaluated. The results of this investigation had showed that aqueous extract of *J. galeopsis* leaves cooked improved red blood cells, hemoglobin and hematocrit levels. These extracts were not toxic for liver, spleen and kidney. The administration of 100 mg/kg/day of extract of leaves cooked leaves during 30 min promotes a better recovery rate of the number of red blood cells (94.80 %), hemoglobin level (159.53 %) and hematocrit (117.72 %) than Vitafer and the extract of leaves cooked for 45 min. This is suggestive that aqueous extracts of *Justicia galeopsis* leaves cooked during 30 min may be exploited during 2 weeks in the treatment of anemia.

Keywords: *galeopsis* leaves, water cooking, anemia, hematological parameters

1. Introduction

Anemia is a disease that affects all countries in the world, whether industrialized or not (Ponmozh, & Ramya, 2015). It is also a public health problem that affects almost a quarter of the world's population. This disease affects people of all ages but the most affected are children, pregnant women and the elderly (Senou et al., 2016). There are many causes of anemia (McLean et al., 2007). The most important causes are nutritional deficiencies, then the pregnancy and the high prevalence of blood parasites such as Plasmodium, Trypanosomes (Senou et al., 2016). Among nutritional deficiencies, iron deficiency is the main cause of anemia (Ramakrishnan 2002). There is also a deficiency of folic acid and / or vitamin B 12 which causes megaloblastic anemia (Green, 2017).

Some pharmaceutical drugs are used to treat anemia. These include iron, vitamin B12, folic acid etc. The search for other alternatives to overcome the problem of the high cost of pharmaceutical drugs is encouraged by WHO (Senou et al., 2016). Thus, *Justicia secunda* leaves (Tossou et al., 2008), *Alchormea cordifolia* leaves, *Coco nucifera* milk, *Hibiscus sabdarifa* flowers (Séguénà et al., 2013), and *Jatropha tanjorensis* leaves (Idu et al., 2014) were studied and their anti-anemia properties were discovered. Beyond these trees and shrubs, studies have also shown that the consumption of some leafy vegetables, in particular *Ipomoea batatas* (Osime et al., 2008), *Spinacia oleracea* (Luka et al., 2014), *Moringa olifera* (Madukwe et al., 2013) improve hematological parameters. These plants could therefore be used in place of pharmaceutical drugs (Tossou et al., 2008).

*Justicia galeopsis* is a spontaneous plant whose leaves are eaten as leafy vegetables in Nigeria (Obichi et al., 2015) and Côte d'Ivoire (Kouakou, 2015). In Côte d'Ivoire, the leaves of this plant are very appreciated by populations of Abengourou who like their taste. The cooked dishes of these leaves are offered to distinguished guests or consumed during the festivities (Kouakou, 2015). These leaves contain, in significant quantity, many nutrients in particular iron, vitamin B12, Folic acid, calcium and magnesium (Afolaby et al., 2012; Kouakou,
This plant has advantageous nutritional profile for the health of populations. It could be used like the leaves of *Ipomoea batatas*, *Spinacia oleracea* and *Moringa olifera* in the treatment of anemia. However, it remains little known in the other regions of Côte d'Ivoire. The literature provides information only about their medical uses.

The objective of this study is to test the anti-anemic properties of the aqueous extracts of the leaves of *Justicia galeopsis* on Wistar rats, after induction of anemia by phenylhydrazine hydrochloride.

2. Materials and Methods

2.1 Collection and Authentication of Plant

*Justicia galeopsis* leaves, commonly known in the local language “Agmi” as *Assiaploua* were collected fresh and at maturity from cultivated farmlands located at Abobo. Abobo is one of thirteen communes of the district of Abidjan, Côte d’Ivoire. It is located in Abidjan north between 5°42 north latitude and -4°02 west longitude and at an altitude of 105 meters above sea level (Aka et al., 2013). Plants were identified and authenticated by National Floristic Center (University Felix Houphouët Boigny, Abidjan-Côte d’Ivoire).

2.2 Processing of Plant

The fresh leaves were separated from the stem, washed with distilled water and drained at ambient temperature. Collected leaves were divided into three lots. The first lot is that of the fresh leaves obtained by Agbemafle et al. (2012) method and lyophilized. The other two were cooked at 100 °C respectively during 30 and 45 min by using the method of Randrianatoandro (2010) in the proportion 40 g of leafy vegetables immerged in 200 mL of boiled water. Boiled samples were cooled, crushed with a laboratory crusher (Culatti, France) and lyophilized. All lyophilized samples were ground in fine powder and store in a clean dry air-tight bottle in a refrigerator (4 °C) until required for analyses.

2.3 Preparation of Aqueous Plant Extracts

Fifty grams of powdered plant material was extracted in 1.5 l of distilled water for 24 hours. At the end of extraction time, the mixture was filtered twice through folded cotton and whatman filter grade 1. The filtrate was evaporated at 50 °c for 24 hours in a ventilated oven. The dried powder was then stored in containers at -20 °c until used for bioassay. The powder obtained was dissolved in distilled water (1 ml for 100 g body weight) and administrated by gavage to rats.

The amount of residue administered to a rat is proportional to the amount of leaves consumed by an adult who weighs about 60 kg.

2.4 Animal Handling and Grouping

Eight-week-old male and female *Wistar* albino rats bred in animal house of higher normal school (E.N.S.) at Felix Houphouët Boigny University were used for this study. The rats weighed on average 127.7 g. The animals were housed in locally fabricated cage. They were allowed to acclimatize to the new environment for seven days before the beginning of the experiment. The rats were fed with animal feed (Ivograin) and water *ad libitum*. The experimental protocol and the animal handling procedures were conducted according to the guidelines of the ethics committee of Nangui Abrogoua University (Côte d’Ivoire).

2.5 Experimental Protocol

Forty *Wistar* albino rats were used in this study. The rats were randomly divided into five groups made of eight animals (four male and four female) each. Group 1 was not anemic and served as negative control. The rats of other groups were anemic with group 2 as positive control. Groups 3, 4 and 5 were treated with respectively the Vitafer (1 mg/kg of body weight / day (D)), plant extract cooked during 30 min (100 mg/kg of body weight / day) and plant extract cooked during 45 min (30 mg/ kg of body weight / day) from D2 to D15. The plant 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 1 ml/kg of body weight / day of distilled water on D0 to D15.

Group 2: Anemic control consisting of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 1 ml/kg of body weight / day of 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 D2 to D15.

Group 4: Test 1, made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 100 mg / kg /day of the *Justicia galeopsis* aqueous extract cooked during 30 min from D2 to D15.

Group 5: Test 2, made of rats given the phenylhydrazine at 40 mg / kg / day for two days (D0 and D1) and 30 mg / kg /day of the *Justicia galeopsis* aqueous extract cooked during 45 min from D2 to D15.

The administration of the extracts had begun at D2 (the third day after induction) after ascertainment of the anemia.

2.5 Induction of Anemic Condition

Anemia was induced by phenylhydrazine Chloridrate. Phenylhydrazine was previously dissolved in sterilized normal saline. It was administered to rats intraperitoneally (IP) at a dose of 40 mg/ kg of body weight / day (Naughton al., 1995) for two days (D0 and D1). Rats that were observed to have hemoglobin levels less than 11 g/dL were considered anemic.

2.6 Collection and Preparation of Blood Samples

Blood was collected early in the morning thanks to the technique of amputation of the tail tip (5 mm from the end), previously disinfected with the alcohol 96 °C (Kraus, 1980). Blood was stored in the EDTA (Ethylene-Diamine-Tetra-Acetic acid) tubes for the determination of red blood cell count, hemoglobin and hematocrit. Blood samples were taken on days D0, D2, D7 and D15, the morning after the weighing.

2.7 Evaluation of Hematological Parameters

The hematological parameters included hemoglobin (Hb), red blood cells (RBC) and hematocrit were determined using the automatic analyzer URIT-2900® (Guilin, China).

2.8 Determination of Body Absolute Organ Weights

The body weight of each rat was assessed during the acclimatization period, once before beginning of experience, during the test period (D0, D2, D7, and D15) and on the day of sacrifice. On the day of sacrifice, all the animals were euthanized using diethyl ether in a desiccator. Different organs including the heart, liver, spleen, and kidney were carefully dissected out and weighed.

2.9 Statistical Analysis

Statistical tests of hematological parameters were carried out using software R. The results were expressed in the form of mean ± standard deviation. The statistical significance of the differences between the experimental groups is determined by the Paired t test. Results of the organs are presented as the average ± standard deviation, and the differences among test groups were assessed by one-way analysis of variance followed by Duncan’s New Multiple Range Test using Statistica 7.1 (StatSoft).

3. Results

3.1 Effects of *Justicia Galeopsis* Leaves Aqueous Extract on Red Blood Cells Number

Table 1 shows the effects of oral administration of aqueous extract of *Justicia galeopsis* leaves cooked during 30 and 45 min on red blood cells number. The administration of phenylhydrazine had caused on day D2 a significant reduction (p<0.05) in the red blood cells level in the rats of the four groups (2;3;4 and 5) by about -20.71 to -48.08 %. After treatment with 100 mg/kg of *Justicia galeopsis* aqueous extract cooked during 30 min (extract 1), a gradual recovery was obtained on the following days (+ 10.91 % on D7 and + 94.94 % on D15). On the other hand, in anemic rats that received distilled water, Vitafer and 30 mg/kg of *Justicia galeopsis* aqueous extract cooked during 45 min (extract 2), there was an increase in the number of red blood cells only on day 15 with a recovery rate of respectively 55 %, + 64.69 % and + 60.52 %. The results had showed that extract 1 promotes better recovery than Vitafer, which had a higher recovery rate than extract 2.
The administration of phenylhydrazine had caused on day D2 a significant reduction in the hematocrit rate (p<0.05) in the anemic rats. The results showed that after 15 days of treatment with distilled water, Vitafer, 30 mg/kg of extract 1; 100 mg/kg of extract 2; a gradual recovery was obtained on the following day; +27.56 % on D7 and +117.66 % on D15. After treatment with 100 mg/kg of extract of Justicia Galeopsis leaves cooked during 30 min (extract 1), a gradual recovery was obtained on the following days; +23.75 % on D7 and +108.9 % on D15.

3.2 Effects of Justicia Galeopsis Leaves Aqueous Extract on Hemoglobin Rate

Table 2 shows the effects of oral administration of aqueous extract of Justicia Galeopsis leaves cooked during 30 and 45 min on red blood cells number. The administration of phenylhydrazine had caused on day D2 a significant reduction (p<0.05) in the hemoglobin level in the rats of the four groups (2;3;4 and 5) by about -27.56 % to -58.77 %. After treatment with distilled water, Vitafer, 30 mg/kg of Justicia Galeopsis aqueous extract cooked during 45 min (extract 2) and 100 mg/kg of Justicia Galeopsis aqueous extract cooked during 30 min (extract 1), a gradual recovery was obtained on the following days (D7 and D15). The results had showed that after 15 days of treatment, the reduction in the hemoglobin level was completely corrected in the rats which received Vitafer and those which received the extract of leaves cooked for 30 min, with a recovery rate greater than 100 %. However, the recovery rate of extract 1 is better than that of Vitafer.

3.3 Effects of Justicia Galeopsis Leaves Aqueous Extract on Hematocrit Rate

The effects of oral administration of aqueous extract of Justicia Galeopsis leaves cooked during 30 and 45 min on hematocrit rate are presented in the table 3. The administration of phenylhydrazine had caused on day D2 a significant reduction (p<0.05) in the hematocrit level in the rats of the four groups (2;3;4 and 5) by about -21.33 % to -47.90 %.

After treatment with 100 mg/kg of extract of Justicia Galeopsis leaves cooked during 30 min (extract 1), a gradual recovery was obtained on the following days; +27.53 % on D7 and +117.66 % on D15. In anemic rats that had received distilled water, Vitafer and 30 mg/kg of Justicia Galeopsis aqueous extract cooked during 45 min. The effects of oral administration of aqueous extract of Justicia Galeopsis leaves cooked during 30 and 45 min on hematocrit rate are presented in the table 3. The administration of phenylhydrazine had caused on day D2 a significant reduction (p<0.05) in the hematocrit level in the rats of the four groups (2;3;4 and 5) by about -21.33 % to -47.90 %.

After treatment with 100 mg/kg of extract of Justicia Galeopsis leaves cooked during 30 min (extract 1), a gradual recovery was obtained on the following days; +27.53 % on D7 and +117.66 % on D15. In anemic rats that had received distilled water, Vitafer and 30 mg/kg of Justicia Galeopsis aqueous extract cooked during 45 min.
min (extract 2), there was an increase in the level of hematocrit only on day 15 with a recovery rate of respectively +68.40 %, +81.74 % and +84.64 %.

The reduction in the hematocrit level was completely corrected only in the rats which received the extract of leaves cooked for 30 min, with a recovery rate greater than 100 % (+117.66 %). The recovery rate obtained with the extract is higher than that of Vitafer. The results had showed that extract 1 promotes better recovery than Vitafer. The recovery rate of extract 2 (84.64 %) is higher than that of Vitafer (81.74 %).

Table 3. Effects of Oral Administration of Aqueous Extract of Justicia Galeopsis Leaves Cooked during 30 and 45 Min on Red Blood Cells Number on Hematocrit Rate

| Groups                          | Hematocrit rate (%) |
|---------------------------------|---------------------|
|                                 | D0                  | D2          | D7          | D15         |
| Group 1 (Control)               | 32.50 ± 12.63       | 26.82 ± 14.83| 17.02 ± 9.75| 34.31 ± 6.66|
| Group 2 (Positive control)      | 32.03 ± 9.46        | 20.03 ± 5.76 | 20.02 ± 7.97| 33.73 ± 6.32|
| Variation                       | -37.45 %            | -0.08 %     | +68.36 %    |
| Group 3 (+ 1 ml/kg of Vitafer)  | 34.12 ± 13.97       | 20.48 ± 8.37| 13.16 ± 7.47| 37.22 ± 10.02|
| Variation                       | -39.98 %            | -3574 %     | +117.66 %   |
| Group 4 (+ 100 mg/kg of extract 1) | 29.98 ± 9.41      | 15.62 ± 5.55| 19.92 ± 3.94| 34.00 ± 7.00 |
| Variation                       | -47.90 %            | +27.53 %    | +117.66 %   |
| Group 5 (+ 30 mg/kg of extract 2) | 24.66 ± 9.26      | 19.40 ± 4.86| 16.25 ± 8.89| 35.82 ± 8.89 |
| Variation                       | -21.33 %            | -16.23 %    | +84.64 %    |

*Variation percentage from day D0; *Variation percentage from day D2; *Significant difference (p<0.05); **Highly significant difference (p<0.01); ***Very highly significant difference (p<0.001); extract 1: Justicia galeopsis aqueous extract cooked during 30 min; extract 2: Justicia galeopsis aqueous extract cooked during 45 min

3.4 Effects of Justicia Galeopsis Leaves Aqueous Extract on Liver, Spleen, and Kidney

As depicted in tables 4, oral administration of 100 mg/kg body weight of extract 1 (aqueous extract of Justicia galeopsis leaves cooked during 30 min) and 30 mg/kg body weight of extract 2 (aqueous extract of J. galeopsis leaves cooked during 30 min) in rats at a dose of daily had no significant effect on liver and kidney weights. However, the spleen weight was significantly greater in the anemic rats untreated and treated with Vitafer and the extracts of J. galeopsis leaves when compared to the non-anemic group.

Table 4 Effects of Oral Administration of Aqueous Extract of Justicia Galeopsis Leaves Cooked during 30 and 45 Min on Organ Weights

| Groups                          | Organ weights (g) |
|---------------------------------|------------------|
|                                 | Liver            | Spleen         | Kidney         |
| Group 1 (Control)               | 0.79 ± 0.13      | 0.37 ± 0.14    | 4.11 ± 0.40    |
| Group 2 (Positive control)      | 0.79 ± 0.10      | 0.53 ± 0.19    | 3.97 ± 0.65    |
| Group 3 (+ 1 ml/kg of Vitafer)  | 0.83 ± 0.11      | 0.43 ± 0.10    | 4.05 ± 0.46    |
| Group 4 (+ 100 mg/kg of extract 1) | 0.87 ± 0.15      | 0.55 ± 0.14    | 3.80 ± 0.62    |
| Group 5 (+ 30 mg/kg of extract 2) | 0.86 ± 0.09      | 0.60 ± 0.19    | 4.28 ± 0.70    |

Results are expressed as Mean ± standard deviation (SD) for eight animals per group; means with different superscript letters in the columns are significantly different using the Student’s t-test p < 0.05 was considered statistically significant

4. Discussion

Subacute intoxication of rats with 40 mg per kilogram body weight of Phenylhydrazine (PHZ) for two days resulted in decreasing of red blood cell count, hemoglobin and hematocrit values caused by hemolytic anemia. Administration of PHZ to rats has been reported to result in the production of both aryl and hydroxyl radicals which produce oxidative stress on the red cell membrane resulting in hemolysis by lipid peroxidation Cighetti et al. (1999). According to Ferrali et al. (1997) this lipid peroxidation increase methaemoglobinemia in the blood. These Similar results were reported by Tossou et al. (2008) in rats administered with PHZ to induce anemia.

The group of positive control rats (anemia rats receiving no treatment) had a recovery rate of red blood cells (+55 %), hemoglobin (+ 74.46 %) and hematocrit (+ 68.36 %) lower than that of the other groups which also
received PHZ. Indeed, the body had tried to correct the losses caused by the injection of PHZ but this correction was incomplete until D15. According to Nancy (2011) under normal conditions, the body generates new red blood cells to replace the lost ones but this takes longer; as observed in the untreated anemic rats. So, these rats will need a treatment favoring the increase in hematological parameters following disorders leading to their decrease.

The decrease in red blood cells was almost completely corrected (+ 94.94 %) in the group of rats which had received 100 mg/kg of *Justicia galeopsis* leaves extract cooked during 30 min in contrary to those which had received Vitafer (+ 64.67 %) and 30 mg/kg of extract 2 (+ 60.23 %). The extract of leaves cooked during 30 min allows a better stimulation of the synthesis of red blood cells. These results are better than those of Tossou et al. (2008) who had showed that the administration of 1000 mg/kg of *Justicia secunda* leaf extract increased the number of red blood cells with a recovery of + 98.11 %.

The decrease in hemoglobin induced by phenylhydrazine was completely corrected in the group of rats which received extract 1 and the group of those which received Vitafer. Hemoglobin is the key element in the structure of red blood cells, stimulating their synthesis will also stimulate the production of red blood cells. Therefore, massive production of hemoglobin will cause more red blood cells. Thus, the hemoglobin recovery rate of rats having received the extract of cooked leaves for 30 min being higher (+ 159.53 %) than that of rats having received Vitafer (+ 104.67 %), their red blood cell recovery rate will also be higher than those of rats having received Vitafer and the extract 2 as indicated by le results. The speedy and progressive recovery of anemic rats on treatment with extracts 1 could be due to increased erythropoiesis. According to Koury and Ponka (2004), Folate, vitamin B, and iron are required for optimal erythropoiesis. In fact, according to Loukou et al. (2018), *Justicia galeopsis* uncooked leaves are very rich in vitamin B12 (4173.09 mg/kg) and vitamin C (892.17 mg/kg). Their leaves cooked during 30 min contain more iron (458.98 mg/kg) and vitamin C (4.75±1.60 mg/100g) than the leaves cooked during 45 min which contain 453.93 mg/kg of iron and 3.80 mg/100g of vitamin C (Loukou et al., 2020). This composition of *Justicia galeopsis* leaves extract cooked during 30 min may account for the faster reversal of PHZ induced anemia.

The decrease in hematocrit level observed was completely corrected in rats which received the extract of leaf cooked during 30 min. The hematocrit corresponds to the volume occupied by red blood cells in the blood volume, its value depends on the number of red blood cells in the blood volume. However, this explains the fact that the hematocrit recovery rate of rats having received the extract of the leaves cooked during 30 min is higher than that of the other groups of rats because of its highest recovery rate of red blood cells. This result is better than that observed by Tossou et al. (2008) with the administration of 1000 mg/kg/day of extract of *Justicia secunda* leaves in Benin.

There was no significant difference in the liver and kidney weight for all groups relative to the non-anemic, anemic control and treated with Vitafer and *J. galeopsis* extracts. This indicates that the extracts of *J. galeopsis* leaves were not toxic for those organs at the doses administered. The relative spleen weight was significantly greater in the anemic control and anemic rats untreated and treated when compared to the non-anemic group. This effect is mainly the consequence of the metabolism of phenylhydrazine, as had been suggested earlier. The spleen serves to cleanse the body of damaged old particles transported by the blood (Australasian Society of Clinical Immunology and Allergy 2014). This suggests that an increase in the relative spleen weight might be attributed to the spleen fighting foreign particles due to the anemic condition of the rats. This is also consistent with authors who suggested that the rate of erythropoiesis and 2′5′-A polymerase activity increases after a dose of phenylhydrazine (Orlic et al., 1982).

5. Conclusion

In conclusion, the in vivo study had confirmed the hematinic activity of aqueous extract of *Justicia galeopsis* leaves cooked during 30 and 45 min. The administration of 100 mg / kg / day of extract of leaves cooked during 30 min promotes a better recovery rate of the number of red blood cells (94.80 %), hemoglobin level (159.53 %) and hematocrit (117.72 %) than Vitafer and the extract of leaves cooked for 45 min. The leaves of *Justicia galeopsis* therefore have anti-anemic properties. Therefore, this plant could be used in the management and the treatment of anemia.

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