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Impact of the aqueous extract of Jatropha gossypiifolia leaves in the treatment of anemia induced in Wistar rats

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ABSTRACT*: Introduction: Jatropha gossypiifolia was a medicinal plant generally used by people to treat anemia in the Djougou area in northern Benin. To our knowledge, there were no scientific data available to assess its efficacy with regard to its anti-anaemic effect. This was what justified this research work, which general objective was to evaluate the effectiveness of the aqueous extract of Jatropha gossypiifolia leaves in the treatment of anemia induced in Wistar rats. Methods: Five groups of five Wistar rats were each formed. Four groups of rats were rendered anemic by injection of phenylhydrazine hydrochloride (haemolysis) on the first two days D0 and D1, except for the rats in the negative control group. From the second day D2, the anemic groups were force-fed either with the aqueous extract Jatropha gossypiifolia leaves at the dose of 200 or 300 mg/kg of body weight/day, or with vitafer, the reference drug against 'anemia. The anemic positive control group was treated only with distilled water. Blood samples were taken from all the rats on different days D0, D2, D7, D10 and D15 to assess the impact on the hemogram and the osmotic resistance of the red blood cells. Results: Phytochemical analysis revealed the presence of tannins, flavonoids, anthocyanins, alkaloids, triterpene reducing compound, steroids and mucilages. Both the extract and vitafer completely corrected the anemia within two weeks by stimulating the synthesis of hemoglobin and then the production and early release of immature red blood cells into the bloodstream. Its effect did not seemed to be dose-dependent and but seemed to be quite specific since it did not affect the platelet lineage. Conclusion: Jatropha gossypiifolia leaves showed good therapeutic efficacy and can be considered for transformation into improved traditional medicines (ITM) after studying its biological tolerance and appropriate clinical trials.*

KEYWORDS: *Jatropha gossypiifolia, leaves, phytochemical analysis, anemia, red blood cells, Wistar rats.*

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I. INTRODUCTION

Anemia was a condition in which the number of red blood cells or the level of hemoglobin they contain was lower than normal (WHO, 2020). It caused symptoms such as fatigue, weakness, dizziness and shortness of breath, among others. Anemia was a real global public health problem, particularly in developing countries, which particularly affected young children and pregnant women. During pregnancy, anemia was generally considered a risk factor that could lead to life-threatening complications for the mother and the fetus complications that threaten the life of the mother and the fetus while leading to premature birth and failure of the body at the birth (Sari et al., 2016). The WHO estimated that 1.62 billion people suffer from anemia, i.e. a global prevalence of 24.8% (WHO, 2008). Pregnant women and children under five were the most affected with

a prevalence of 40% and 42% respectively (WHO, 2020). The highest prevalences were recorded in Africa and South-East Asia with 67.7% and 65.5% respectively (WHO, 2008). In Benin, 72% of children aged 6 to 59 months suffered from anemia, of which 3% in its severe form and 41% in its moderate form, and 58% of women aged 15 to 49 suffered from anemia and 26% in its mild form, 30% in its moderate form and 2% in its severe form (INSAE, 2018). The most common causes of anemia were nutritional deficiencies, particularly iron deficiency although folate, vitamin B12 and vitamin A deficiencies are also important causes; haemoglobinopathies; and infectious diseases such as malaria, tuberculosis, HIV infection and parasitoses (WHO, 2020). The most common causes of anemia were nutritional deficiencies, particularly iron deficiency although folate, vitamin B12 and vitamin A deficiencies were also important causes. Hemoglobinopathies; and infectious diseases such as malaria, tuberculosis, HIV infection and parasitosis were also incriminated (WHO, 2020). The treatment varied according to the type of anemia depending on whether it was central anemia due to a defect in bone marrow production, and peripheral anemia due to destruction of red blood cells. It could be a supply of iron, vitamin B12 or B9 by mouth, treatment with immunosuppressants or corticosteroids, injections of erythropoietin, blood transfusion, or even bone marrow transplantation. Bone (Movaffaghi et al., 2006; Seidou, 2013). Given the high cost of treatment, the inaccessibility and adverse effects of conventional synthetic drugs used in developing countries, the search for alternative and/or complementary strategies was necessary for the use of synthetic antianaemics and other conventional drugs.

Herbal medicines were used by the majority of the world's population to cure various diseases. WHO estimated that 80% of rural populations in developing countries depend on traditional medicine (WHO, 2001). They were used for primary health care in rural areas of developing countries. Traditional medicines were derived from medicinal plants, minerals and organic materials; however, herbal medicines were prepared from medicinal plants only (Kar et al., 2008). The use of medicinal plants was growing rapidly in most countries of the world, this use was particularly based on the idea that plants were a natural means of treatment without risk (Akesbi, 2021). Interest in the use of medicinal plants in the treatment of many diseases then increased. Phytotherapy was enjoying new success in more and more countries around the world, particularly in Benin. In addition, a significant number of medicinal plants were now used by traditional medicine to treat many conditions, including anemia and its complications. Africa was full of a multitude of food and therapeutic plants. They improve the nutritional quality of diets due to their chemical composition and medicinal properties (Tchiegang et al., 2004; Dansi et al., 2008). Indeed, of the 300,000 plant species recorded on the planet, more than 200,000 species are found in tropical African countries and have medicinal properties (Souad et al., 2010). Several ethnobotanical surveys carried out in Benin and in several other countries of the sub-region showed that several plants were traditionally used to treat various emerging diseases and many other chronic diseases. However, the therapeutic merits of these plants were only based on purely empirical bases, most often without any scientific proof. In Benin, out of the 30,700 species of plants inventoried in forest ecosystems (Akoegninou et al., 2006), many plants were used by local populations as food plants and others as medicinal plants, including Jatropha gossypiifolia L, commonly called " stomachache shrub", a therapeutic plant used in all Asian countries. A few human and veterinary uses in conventional medication were described for various plant-dependent specificities and arrangements (Pande et al., 2021). Jatropha L. was a member of Crotonoideae subfamily, the Jatropheae clan and to which about 250 species belong. The current family was widely appropriated in tropical and subtropical regions of Asian countries. (Axelsson et al., 2012.). The leaves, seeds, fruits, roots and stems were used to treat various diseases such as leukemia, leprosy, vertigo, gout, intestinal worms, dysphonia, infections of the genital area, ulcers , anemia, dermatitis, abscesses, asthma, diarrhea, fever, fungal skin infections, inflammations and burns. In Africans the seeds were used as a laxative or purgative. Tea, made from the bark, was used in Nigeria to treat intestinal worms. The leaves were boiled and used during a bath for fever and also used as purgatives. The aqueous extract of J. gossypiifolia exhibited anti-malaria properties.

According to the national program of pharmacopoeia and traditional medicine, Jatropha gossypiifolia was classified on the list of medicinal plants widely used by populations. However, in Benin, scientific data on its use as an anti-anaemic were almost non-existent, although it was the subject of several therapeutic uses, particularly in the treatment of infectious diseases. It is therefore essential for scientists to carry out phytochemical and biological studies in order to promote the use of this plant efficiently as an anti-anaemic within communities and to create a synergy between traditional medicine and conventional medicine. It was for this reason that this study was undertaken with the aim of evaluating the impact of the aqueous extract of leaves of Jatropha gossypiifolia in the treatment of anemia induced in Wistar rats.

II. MATERIALS AND METHOD

Animal material

The animal material used in this study consists of albino Wistar strain rats aged 3 to 4 months and with an average body weight of approximately 200 g. These rats were acclimatized to ambient conditions at a constant temperature of 25 ± 1°C in the animal facility of the Experimental and Clinical Biology Unit (UBEC), of the National School of Applied Biosciences and Biotechnologies (ENSBBA) of the National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) in Benin. Breeding was carried out in a well-ventilated room with a cycle of 12 hours of light and 12 hours of darkness. Rats were kept in wire mesh cages with feeders and drinkers, and have free access to drinking water and food. They were supplied with drinking tap water and their daily diet consists of a mixture of granulated feed presented in the form of kibble and marketed by Véto Services (Benin). The breeding enclosure was regularly cleaned to guarantee optimal development of the animals protected from any infection.

Plant material

The plant material was composed of aqueous extracts of leaves of Jatropha gossypiifolia L. The samples studied were fresh leaves harvested in the northern Benin region, specifically in Djougou in the DONGA department and brought back to the laboratory for drying. The samples were spread out in a cold drying room (22°C) for about 14 days, after which time the samples were brittle and were practically anhydrous. Then the dry samples were reduced to powder using an electric grinder. The powders thus obtained were sieved with a sieve with a diameter of 710 µm.

Phytochemical analyzes

Phytochemical analysis was based on the differential reactions (coloration and precipitation) of the main groups of chemical compounds contained in plants according to the classic method of P.J. Houghton and Raman (1998) and which is widely used in the literature with success (Houngbèmè and al., 2014; Ombouma et al., 2021). This analysis includes:

- ✓ Search of alkaloids : It was done through two tests:

- General acid test

5 g of the powder were mixed with 25 mL of 5% dilute hydrochloric acid. The mixture was macerated for 24 hours. 1 mL of the filtrate was collected, to which 5 drops of Mayer's reagent were added. If alkaloids were present, a yellow or cloudy precipitate was observed in the tube.

- Extraction of alkaloids

5 g of powder were placed in 5 mL of 50% ammonia. To this mixture, 25 mL of chloroform ether was added and left to macerate for 24 hours in a stoppered flask. The filtrate was dried over anhydrous sodium sulfate and then extracted with 5 mL of 5% hydrochloric acid twice in succession. To the exhausted filtrate, 5 drops of Mayer's reagent were added. If an alkaloid was present, a precipitate was observed in the tube.

- ✓ Search of polyphenolic compound.

In an Erlenmeyer flask, put 5 g of powder to which 100 mL of boiling water are added. The mixture was left for 15 minutes with continuous stirring, then filtered. This filtrate divided into 6 portions was used for the following research:

- Tannins:

To the first portion of the filtrate, a few drops of 1% ferric chloride were added. The observation of a dark blue, green or black color indicated the presence of tannins.

- Catechin tannins

To 30 mL of the second portion, 15 mL of Stiasny's reagent was added and the mixture was heated in a water bath at 90° C. for 15 minutes. The appearance of a pink precipitate indicated the presence of catechin tannins.

- Galic tannins

After collecting the filtrate, it was saturated with sodium acetate to which a few drops of 1% ferric chloride were added. A blue or black tint indicates the presence of gallic tannins.

- Flavonoids

To 5 mL of the third portion, add 5 mL of hydrochloric alcohol and a pinch of magnesium powder: this was the cyanidin reaction, known as the Shinoda reaction. The appearance of an orange, red or purple color indicated the presence of flavonoids.

- Anthocyanins

A few drops of 5% hydrochloric acid were added to 1 mL of the fourth portion. This mixture was then made alkaline by adding a few drops of 50% ammonia. A red color that increases and turned blue-purple or greenish indicated the presence of anthocyanin.

- Leuco-anthocyanin

To 5 mL of the last portion, add 5 mL of hydrochloric alcohol. The mixture was then heated for 15 minutes in a water bath at 90°C. The observation of a cherry red or purplish color indicated the presence of leuco-anthocyanin.

- ✓ Search of quinone derivatives

In an Erlenmeyer flask, 2 mL of 5% HCl and 2 g of powder were mixed. To this mixture, 20 mL of chloroform were added and we leave stirring continuously for 24 hours. After maceration, 5 mL of ammonia was added to the previous mixture: this was the Born-Trager reaction. A pink or purplish-red color indicated a positive reaction.

- ✓ Search for saponosides

The decoction of 1 g of powder was prepared for 30 minutes in 100 mL of distilled water with moderate boiling. The filtrate cooled then adjusted to 100 mL was distributed in 10 test tubes (height 16 cm×16 mm in diameter) in a geometric series at the rate of 1/10th concentration of the decoction. After adjusting to 10 ml with water and 30 shakings in 15 seconds, the tube was left to stand for 15 minutes. The height of the foam was measured. If it was ≥ 1 cm in one of the tubes, the dilution in this tube was the desired foam index.

- ✓ Search for triterpenoids and steroids

For this research, 10 mL of ethyl alcohol at 70°C was added to 1g of powder. To this mixture, 10 mL of distilled water was added then 2 mL of 10% lead acetate at equal volume V/V. After standing for 15 minutes, 2 ml of 10% aqueous sodium phosphate solution were added to the filtrate. After 15 minutes of rest, the filtrate was collected in a separatory funnel and extracted three times with 5 mL of chloroform (CHCl3). The chloroform solutions were dried over sodium sulphate then divided into two portions and evaporated to dryness (bain-marie).

At the first portion was dissolved by a few drops of acetic acid. To the mixture obtained, 3 mL of a mixture of acetic anhydride-sulfuric acid is added. A purple, blue or green color indicated the presence of triterpenoids.

To the second portion, add 2 drops of an alcoholic solution of dinitrobenzoic acid and 2 drops of 1N sodium hydroxide. The appearance of a purple or wine red color indicated the presence of steroids.

- ✓ Search for cyanogenic derivatives

To 15 mL of distilled water,was added 2 g of the powder then stopper immediately and left to macerate for 1 hour. The neck of the Erlenmeyer flask was covered with paper soaked in picric acid and heated for a few minutes. The appearance of a brown color indicated the release of HCN.

- ✓ Search for mucilages

1 mL of 10% decoction was introduced into a test tube and 5 mL of absolute alcohol was added. The appearance of a fluffy precipitate indicated the presence of mucilage after about ten minutes.

- ✓ Search for coumarins

To 20 mL of ether, 1 g of powder was added then immediately plugged in a small Erlenmeyer flask and left to macerate for 24 hours. The filtrate was adjusted to 20 mL with ether. 5 mL of filtrate was evaporated in a dish in the open air. To the residue obtained, 2 mL of hot water was added and the solution was divided into two test tubes. In one of the tubes, 0.5 mL of 25% ammonia was added. The second tube represented the control. The fluorescence of the two test tubes was observed under UV at 365 nm. Intense fluorescence in the test tube indicated the presence of coumarins.

- ✓ Search for reducing compound

The 10% decoction was obtained by moderate boiling for 3 minutes of a mixture of 50 mL of distilled water and 5 g of powder. After cooling, the filtrate was adjusted to 50 mL with distilled water. 5 mL of filtrate are introduced into a test tube. After heating in a water bath at 90° C. for a few minutes, 1 mL of Fehling's reagent (Fehling's liquor A+Fehling's liquor B in equal volume) was added. The filtrate was heated a few minutes later. The observation of a bright red precipitate indicated the presence of reducing compound.

- ✓ Search of anthracene derivatives

- Free anthracenes

To 1 g of powder, add 10 mL of chloroform and heat carefully for 3 minutes in a water bath. After hot filtration, the mixture was made up to 10 mL with chloroform. 1 mL of the chloroform extract was added with 1 mL of ammonia diluted to 1/2 then stirred. The appearance of a more or less intense red color indicated the presence of free anthracenes.

- Combined anthracenics

• O-heterosides

To part of the residue exhausted with chloroform, 10 mL of distilled water and 1 mL of concentrated hydrochloric acid were added. The test tube kept in a boiling water bath for 15 min was then cooled under a stream of water. The hydrolyzate was obtained after filtration and adjustment to 10 mL. 5 mL of the hydrolyzate was taken and shaken with 5 mL of chloroform. The drawn off organic phase was introduced into a test tube and added with 1 mL of ammonia diluted to 1/2 then stirred (the aqueous phase is kept). The presence of anthracene

was revealed by the more or less intense red coloring. If the reaction was negative or weakly positive, the O-heterosides with reduced genins were sought. To do this, take 5 mL of hydrolyzate and add 3 to 4 drops of 10% FeCl3 (ferric chloride). The mixture, heated in a water bath for 5 min, was then cooled under a stream of water and then stirred with 5 ml of chloroform. To the chloroform phase drawn off and introduced into a test tube, 1 mL of ammonia was added to 1/2 and then stirred. A more or less intense red color indicated the presence of O-heterosides with reduced genins.

- C-heterosides

To the aqueous phase preserved above, 1 ml of 10% FeCl3 is added. The mixture was brought to the boil in a boiling water bath for 30 minutes and then cooled. After stirring with 5 mL of chloroform, the chloroform phase was drawn off and collected in a test tube. Add 1 mL of ammonia diluted to 1/2 and stir. A more or less intense red color indicated the presence of C-heterosidegenins.

- ✓ Cardiotonic glycosides

Prepare an extract from 1 g of drug powder and 10 mL of 60% alcoholic ethanol and 5 mL of a 10% neutral lead acetate solution. Bring to the bain-marie for 10 minutes and filter. Extract with chloroform and divide the organic phase between 3 test tubes, evaporate to dryness and resume with isopropanol. Then enter in:
- tube 1 Baljet's reagent;
- tube 2 Kedde's reagent;
- tube n°3 the Raymond-Marthoud reagent;

Finally, introduce 5 drops of 5% KOH in alcohol into each tube. The presence of cardenolides results in a coloration:
- Orange in tube 1;
- Red-violet in tube n°2;
- fleeting violet in tube n°3.

Preparation of extract

The extraction of the total chemical principles was done using the method of maceration in accordance with the traditional use of plants. Based on the extraction techniques cited in the literature (Houngbeme et al., 2014). 50 g of powder were dissolved in 500 mL of distilled water. The mixture was left stirring continuously for 48 hours. After cooling, the mixture obtained was filtered (3 times in a row) on absorbent cotton and the filtrate was transferred to a 1000 mL flask then subjected to evaporation until dryness at 40° C. Using a rotavapor (HeidolphLaborota 4000 efficient) coupled to a water chiller (Julabo FL 300). The dry residue obtained represented the maceration. Finally, the various dry residues obtained are weighed and the yield was calculated according to the expression:

$$Yield\ (\%) = \frac{Mass\ of\ dry\ extract}{Initial\ mass\ of\ powder} X100$$

Induction of anemia

Anemia was induced in animals according to a standardized protocol from Seidou, 2013. According to this protocol, anemia sets in two days after the end of phenylhydrazine injections (Gbenou et al., 2006). Phenylhydrazine hydrochloride previously diluted in a 10% solution in physiological water, before being administered to the rat by the intraperitoneal route IP, at a dose of 40 mg kg d for two days (D0 and D1). An animal was considered anemic if its hemoglobin level is less than or equal to normal. The control animals (non-anaemia) received only physiological saline.

Evaluation of the anemia-correcting effect of Jatropha gossypiifolia leaf extracts

To evaluate the correction of anemia by extracts of Jatropha gossypiifolia, the rats were divided into five (5) experimental groups at the rate of five (5) animals per group:

Group 1: Negative control, consisting of rats which received 0.9% saline solution only from D2 to D2; and distilled water from D2 to D15;

Group 2: Positive control, consisting of rats which received phenylhydrazine on D0 and D1 and distilled water from D2 to D15;

Group 3: Reference control, consisting of rats which received phenylhydrazine on D0 and D1 and 1 ml/kg/d of body weight of vitafer (Reference drug), from D2 to D15;

Group 4: Test group, consisting of rats which received phenylhydrazine on D0 and Dd1 and 200 mg/kg/d of body weight of Jatropha gossypiifolia leaves extract from D2 to D15;

Group 5: Test group, consisting of rats which will receive phenylhydrazine on D0 and D1 and 300 mg/kg/d of body weight of Jatropha gossypiifolia leaves extract from D2 to D15.

The extract and the reference medicinal product vitafer are administered by force-feeding using a gastric tube.

Hematological analyzes

Blood samples were taken during the manipulation on different days: D0, D2, D7, D10 and D15 for all the groups. Blood was collected by orbital puncture after anesthetizing the rats with chloroform. These samples will make it possible to assess the evolution of hematological parameters in each group (Seidou, 2013). The hemoglobin level, the number of red blood cells, the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), the thrombocyte count and the osmotic resistance of hematic were determined.

These parameters are measured using the PLC SYSTEM KX 21.

Osmotic resistance is the ability of blood cells to resist 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 toto assess the rate 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%.

III. RESULTS

Chemical groups studied in the leaves of Jatropha gossypiifolia

The results of the phytochemical screening carried out on the leaf powders of the species analyzed were summarized in the table below:

Table No.-1: Identified chemical groups:

GROUPES CHIMIQUES	Jatropha gossypiifolia L
Catechic tannins	-
Gallic tannins	+
Flavonoids	+
Leuco-Anthocyanins	-
Anthocyanins	+
Alkaloids	+
Reducing compounds	+
Mucilages	+
Saponosides	-
Cyanogenicderivatives	-
Triterpenes	+
Steroids	+
Coumarins	-
Quinone derivatives	-
Free antracenes	-
C-Geosides	-
O-Heterosides	-
Cardiotonicderivatives	-

(+): positif, (-): négatif

This table showed that the species analyzed contains all the various secondary metabolites. Jatropha gossypiifolia L contains 8 chemical groups such as: gallic tannins, flavonoids, anthocyanins, mucilages, triterpenes, alkaloids, reducing compounds and steroids.

The organ studied did not contain toxic chemical groups, namely cyanogenic and cardiotonic derivatives, which gave it a priori a certain safety as to its use.

Yield of crude extracts

The yield calculated for the prepared extract is recorded in the table below:

Table No.-2: Yield of the extract:

Sample	*Jatropha gossypiifolia L* leaves
Yield ± (%)	10.32 ± 0.13

The yield obtained with the aqueous extract of Jatropha gossypiifolia L was high. The plant was very rich in polar compounds, the extraction was made with water which was a recognized very polar solvent.

Evaluation of the efficacy of extracts of Jatropha gossypiifolia on Wistars rats

- ✓ **Hemoglobin (Hb) level**

Figure 1 showed the evolution of the hemoglobin level in the groups of rats.

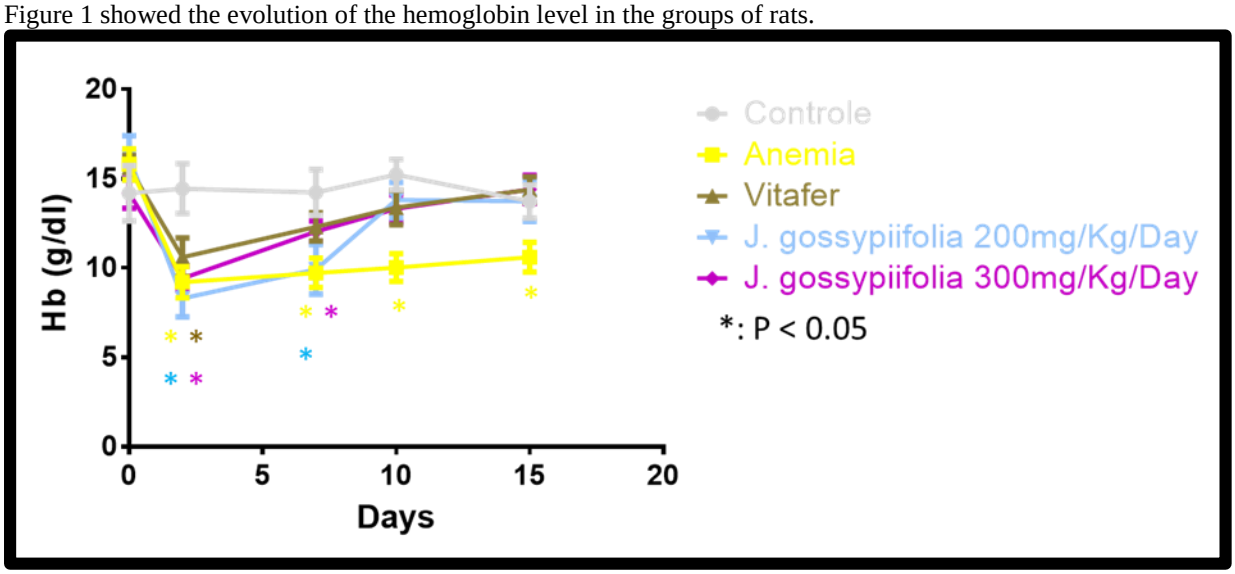

Figure No 1: Evaluation of hemoglobin level

The hemoglobin level varied from 14.2 ± 1.4 to 16.2 ± 1.1 g/dL in the various groups of rats on D0. Phenylhydrazine significantly lowered the hemoglobin level on D2. This drop was completely corrected on D7 by Vitafer® and on D12 by the extract of Jatropha gossypiifolia L at 200 or 300 mg/Kg of body weight. The group of untreated anemic rats did not have such a correction in hemoglobin level.

- ✓ **Red blood cell count**

Figure 2 showed the evolution of the number of red blood cells in the groups of rats.

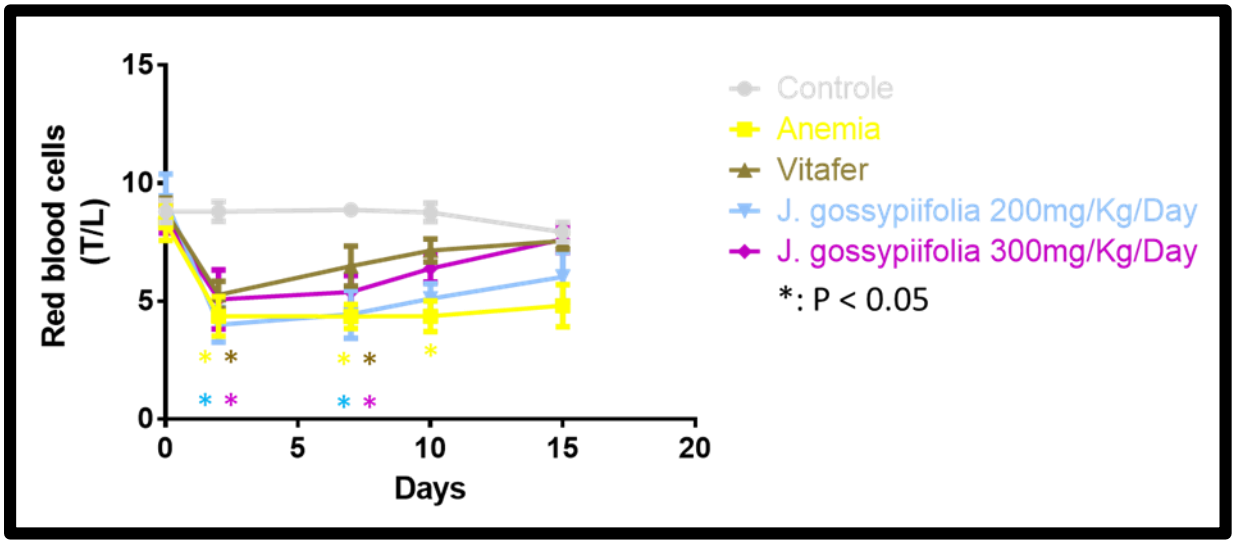

Figure No 2: Evaluation of red blood cells in various groups

The number of red blood cells varied from 8.3 ± 0.7 to 9.2 ± 1.0 T/L in the various groups of rats on D0. Phenylhydrazine significantly lowered the number of red blood cells on D2, reflecting hemolysis of red blood cells. This decrease was corrected on D10 by Vitafer® and by the extract of Jatropha gossypiifolia L at 200 or 300 mg/Kg/D. Only the group of untreated anemic rats did not experience such a correction in the number of red blood cells.

- ✓ **Mean corpuscular volume (MCV)**

Figure 3 showed the evolution of the mean globular volume in the groups of rats.

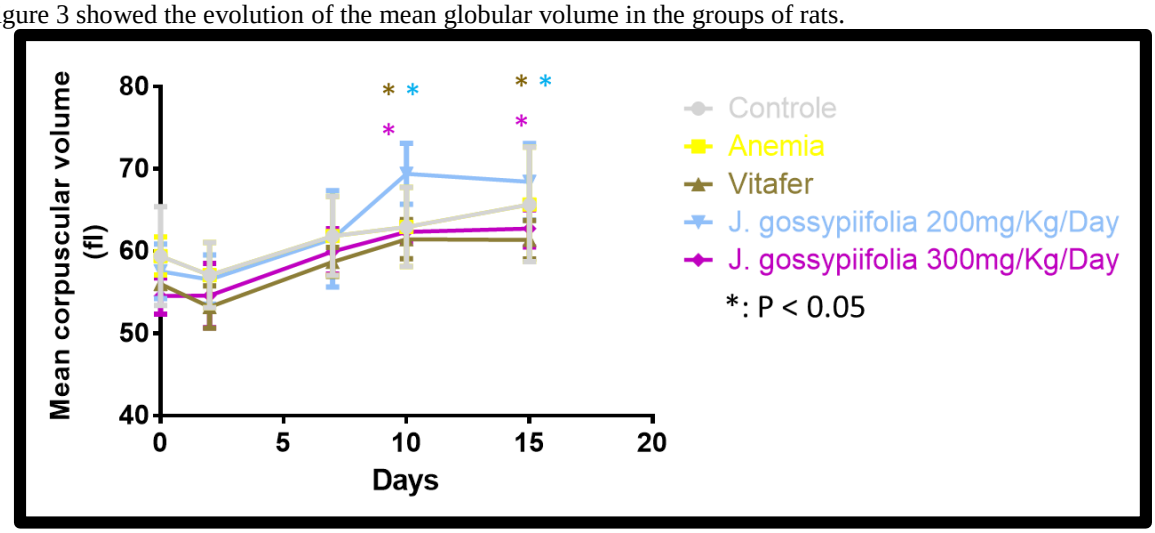

Figure No 3: Assessment of mean corpuscular volume

The Mean Globular Volume varied from 55 ± 4 to 59 ± 5 fl in the various groups at D0. The MCV increased significantly from D10 in the anemic and treated groups, reflecting a release of macrocyte.

- ✓ **Mean Corpuscular Hemoglobin Concentration (MCHC)**

Figure 4 showed the evolution of mean corpuscular hemoglobin concentration in groups of rats.

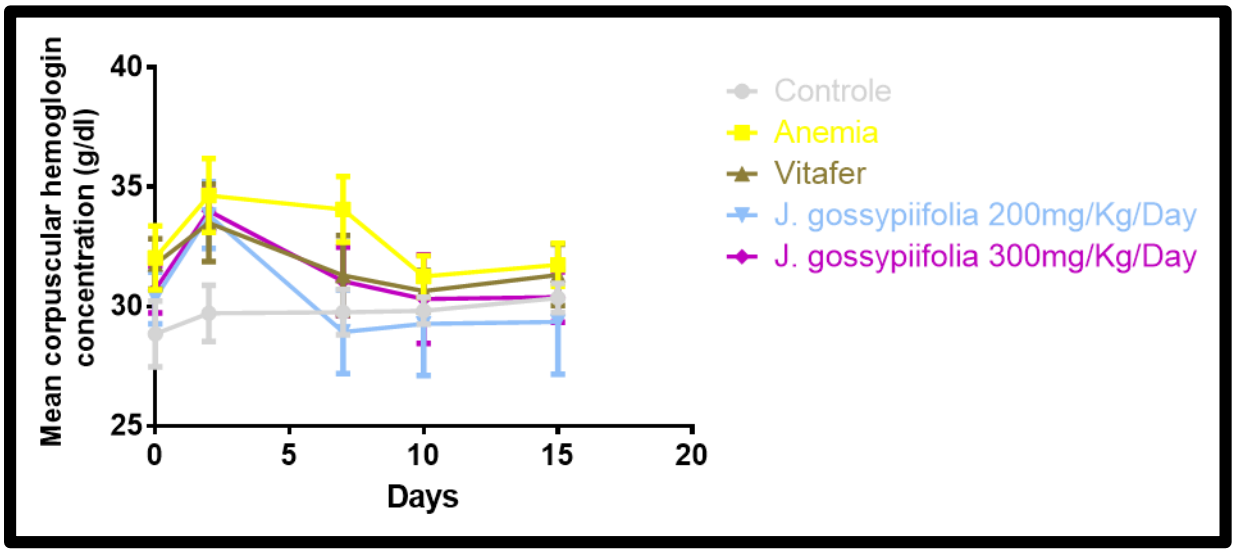

Figure No 4: Evaluation of mean corpuscular hemoglobin concentration

The mean corpuscular hemoglobin concentration varied from 28.9 ± 1.2 to 32 ± 2.7 g/dl in the various groups of rats at D0. It did not change significantly during the experiment.

✓ **Blood platelet count (BPC)**

Figure 5 presented the evolution of the number of blood platelets in the groups of rats.

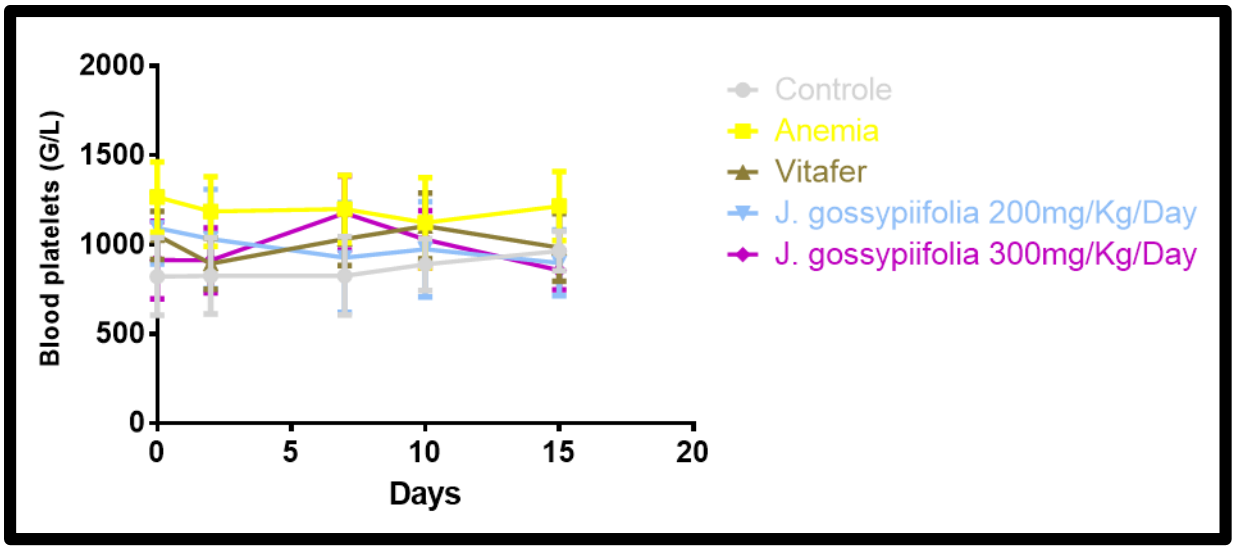

Figure No 5: Evaluation of the number of blood platelets

The number of platelets varied from 822 ± 71 to 1297 ± 218 G/L on D0 in the different groups of rats. It did not change significantly in the groups during the experiment, indicating that the extract did not affect the thrombocyte lineage.

✓ **Osmotic resistance of red blood cells (OR)**

Figure 6 showed the evolution Osmotic resistance of red blood cells in the groups of rats.

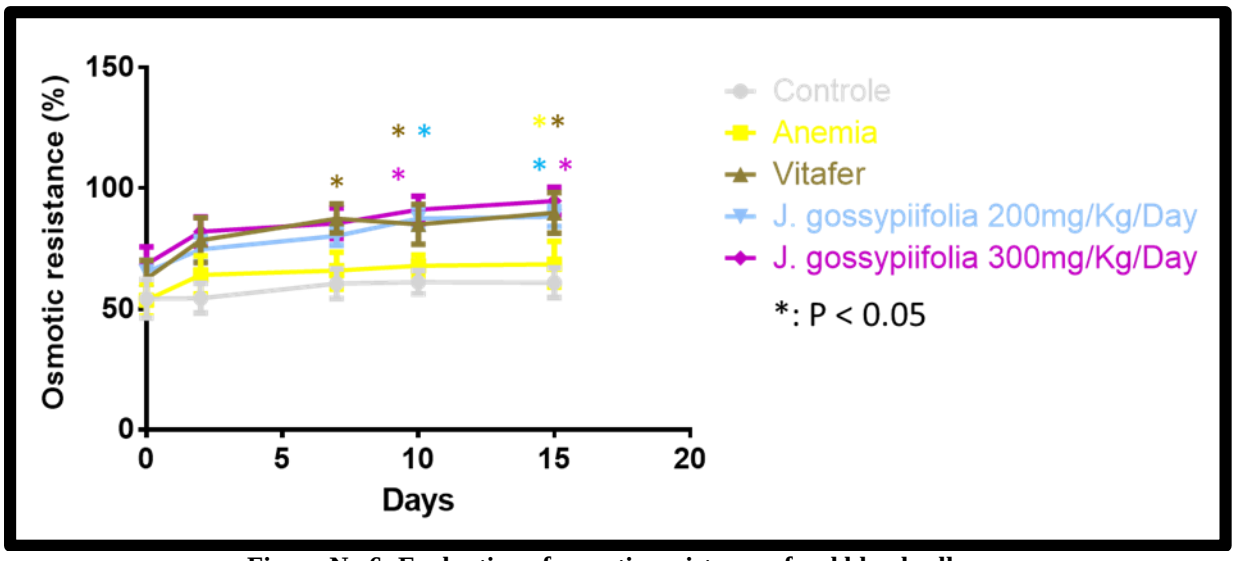

Figure No 6: Evaluation of osmotic resistance of red blood cells

The osmotic resistance of red blood cells varied from 54 ± 6 to 68 ± 14%. It increased significantly in all the anemic groups treated from D10 and in the untreated anemic group at D15, reflecting a massive release of young red blood cells. In the group treated with vitafer (reference), the increase was already significant on D7.

IV. DISCUSSION

The use of Jatropha gossypiifolia L in the treatment of anemia has not been noted documented in the literature to our knowledge. It was to better understand the mechanism of action of this plant and to verify the therapeutic property of the leaves of this plant in the management of anemia that the present study was undertaken, given the high prevalence of the syndrome of anemia. The phytochemical analysis of the leaves of this plant revealed the presence of chemical groups such as flavonoids, mucilages, anthocyanins, alkaloids,

reducing compounds, triterpenes, steroids and tannins. These results differ from those of Kisangau et al. (2007) in Cameroon, Drissa et al in Burkina Faso in 2010, Adjavon et al in Benin in 2012. The absence or presence of certain compounds such as coumarins could be explained by the variability of the harvest period and the difference in environmental media, the method of phytochemical analysis, the stage of maturity of the leaves, etc. However, our results were similar to those of Sènou et al. (2020), Agbogba et al. (2019), from Tchogou et al. (2016) and Gbénou et al. (2006) who detected alkaloids, tannins, flavonoids, anthocyanins in the leaves of Psorospermum febrifugum, in the leaves of Cocos nucifera and in the leaves of JusticiasecundaVahl (Acanthaceae), which are anti-anaemic plants used in Benin .

The analysis of the effectiveness of the aqueous extract of Jatropha gossypiifolia L leaves in the correction of anemia revealed a correction of the hemoglobin level and that of the number of red blood cells on D10 at 200 or 300 mg of extract /Kg of body weight. The effect was less than that of vitafer, the reference drug which corrected the hemoglobin level already on D7. The effect was slower than that of the bark of the roots of Psorospermum febrifugum Spach which corrected the hemoglobin level and the number of red blood cells on D7 at the of 300 mg of extract/Kg of body weight (Agbogba and al, 2019).

The mean globular volume increased significantly from D10, reflecting a release of macrocyte, red blood cells that had not completed their differentiation in the bloodstream to compensate for the anemia. This result differed from that of the aqueous extract of the root bark of Psorospermum febrifugum Spach and that of the leaves of Sorghum bicolor which caused a release of well-differentiated red blood cells from D10 (Sènou et al, 2016; Agbogba et al, 2019).

Mean corpuscular hemoglobin concentration did not significantly increase during experience. This result was better than that of the aqueous extract of Cocosnicifera roots, which led to the release of hypochromic macrocytes on D15 (Tchogou et al, 2016).

The osmotic resistance of the red blood cells increased significantly from D7 in the group treated with vitafer, the reference drug, and from D10 in the groups treated with the extract at 200 or 300 mg/Kg of body weight, reflecting a increased release of young red blood cells into the blood by the bone marrow. This result was similar to that of the aqueous extract of the root bark of Psorospermum febrifugum Spach which showed a high proportion of young red blood cells released into the circulation to compensate for anemia from D15 (Agbogba et al, 2019).

The correction of anemia by stimulation of hemoglobin synthesis and of the number of red blood cells by the aqueous extract of the leaves of Jatropha gossypiifolia L was not different between doses of 200 and 300 mg/Kg of body weight. These results differed from those of Sorghum bicolor leaves and Cocosnicifera roots, the effects of which seem to be dose-dependent (Sènou et al, 2016; Tchogou et al, 2016).

In order to determine the specificity of action of the Jatropha gossypiifolia L leaf extract, we measured the level of blood platelets at the same time as that of red blood cells. The number of platelets did not follow that of red blood cells and did not vary significantly in the different groups during the experiment. This suggested that the extract did not stimulate the thrombocyte lineage. This specificity of action on the erythrocyte lineage has been also observed with the leaves of Sorghum bicolor, Psorospermum febrifugum Spach and the roots of Cocosnicifera (Sènou et al, 2016; Tchogou et al, 2016; Agbogba et al, 2019).

V. CONCLUSION

In order to propose an alternative solution in the management of anemia, we chosed to study Jatropha gossypiifolia L reported in traditional medicine as one of the plants used to treat this syndrome. The aqueous extract from the leaves of this plant corrected anemia by stimulating the production of red blood cells and the synthesis of hemoglobin. This could be explained by the groups of chemical compounds it contained and which were found in other plants with anti-anemic activity. These results would lead to a rationalization of the use of this medicinal plant in the treatment of anemia. It would pave the way not only for its scientific valorization but also for its transformation into Improved Traditional Medicine (ITM) for better accessibility of poor populations to secure anti-anaemic products.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest

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