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RESEARCH ARTICLE

THE ETHYL ACETATE FRACTION OF PSOROSPERMUM FEBRIFUGUM SPACHROOT BARKS AQUEOUS EXTRACT IS A GOOD STIMULATOR OF HEMATOPOIESIS

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Abstract

PsorospermumfebrifugumSpach. (Clusiaceae) is a medicinal plant found in Benin whose root bark was effective in treating anemia. To identify the family of chemical compounds of this organ was responsible for its hematopoietic efficiency, this work aimed to test the ethyl acetate fraction of the aqueous extract of the plant organ on anemic rats.

Methods: Wistar rats were anemic by phenylhydrazinechloridrate on D0. From day 2 to day 15, some were gavage fed with the ethyl acetate fraction of PsorospermumfebrifugumSpach root bark aqueous extract at 40 or 60 mg / kg / day. Others received either vitafer as a reference drug or distilled water (untreated anemic group). Blood samples were collected from these rats and non-anemic control rats at days 0, 2, 7, 10 and 15 for the blood count and osmotic resistance of red blood cells.

Results: At D2, phenylhydrazine significantly decreased hemoglobin and red blood cell number, which were corrected on D7 by the extract fraction with a dose-dependent effect. The extract fraction rapidly stimulated release of macrocytes, immature red blood cells in the first week to compensate the anemia. The extract did not affect blood platelet number, suggesting some specificity of action on the red blood cell line.

Conclusion: The ethyl acetate fraction of Psorospermumfebrifugum root bark aqueous extract stimulated erythropoiesis faster than the

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crude extract. Its action seemed specific and dose-dependent. It would probably be related to the flavonoids which action mechanism needs to be explored.

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Introduction:-

Anemia was a major public health problem in the world and affected mainly underdeveloped countries (De Benoist et al 2008). All ages were concerned but the mostly target were children. According to a recent estimate, about 273 million pre-school children were anemic worldwide, with 62% of cases in Africa (WHO, 2015). The main causes were poverty and lack of hygiene (Ogbe et al, 2010). Parasitic diseases, especially endemic malaria in the tropics, also largely explained this situation (Crawley, 2004; De Benoist et al 2008; Sènou et al, 2017a). Genetic factors and especially endemic sickle cell disease might also to be considered to explain this high prevalence (Sènou et al, 2017b). Iron deficiency was the most common cause of anemia and 30% of the world's population was affected by this anemia (Assobayire et al, 2001; El Hioui et al 2009 ; Al-Zabedi et al, 2014).

Anemia was defined as a decrease in circulating hemoglobin which resulted in a decrease in oxygen supply to the organs (Libregts et al, 2011; Bigoniya et al, 2013). Different types of anemia exist, among which hemorrhagic anemia, megaloblastic anemia, hemolytic anemia, and so on (Ross and Wilson, 2006; Guyton and Hall, 2007). Different studies shown that hemolytic anemia was often due to oxidative stress in the red blood cell characterized by the generation of reactive oxygen species (ROS), glutathione depletion, production of Heinz bodies in the red blood cell, peroxidation membrane lipids and disruption of the globular cytoskeleton (Jollow and McMillan, 2001).

The treatment varied depending on the type of anemia. It could be done by supplementation with iron, vitamin B12, erythropoietin injection, blood transfusion and even bone marrow transplant (Movaffaghi and Hasanpoor, 2006). In underdeveloped countries, the high cost of pharmaceutical drugs forced different populations to use medicinal plants to solve various health problems (Bhushan, 2005). Thus, several plant organs were used in Africa to cure anemia. This is the case of the leaf sheath of Sorghum bicolor, Cocos nucifera roots, Hibiscus sabdariffa calyx (Sènou et al, 2016a; 2016b; Tchogou et al, 2016).

PsorospermumfebrifugumSpach was widely used in traditional medicine in Africa. It grew in savannas and tropical ears and belonged to the family of Hypericaceae (Arbonnier, 2000). Recent studies shown that the aqueous extract of it root bark effectively stimulated erythropoiesis and was not toxic (Agbogba et al, 2019a, 2019b). This work aimed to test in vivo the hematopoietic efficiency of the ethyl acetate fraction of this extract in animal experimentation.

Materials And Methods:-

Animal Material

Animal material consisted of Wistar albino rats of average body weight 173 g approximately, having free access to water and food and acclimated to farming conditions from the pet of the Biomembrane and Cell Signaling Laboratory in Faculty of Sciences and Techniques of Abomey-Calavi University (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. All the rules for animal welfare have been observed.

Identification and Preparation of Plant Material

Identification

PsorospermumfebrifugumSpach (Clusiaceae) roots bark was collected from Atlantic Department in Benin during April 2015. The collected samples were identified and certified at the National Herbarium of Abomey Calavi University under the number AA6625 / HNB. The samples were dried at moderate temperatures (20-25^0 C), protected from moisture for four weeks. They were then crushed into powder and stored in suitable containers at room temperature.

Preparation of the aqueous extract

50 g of root powder of PsorospermumfebrifugumSpach (Clusiaceae) roots bark were boiled in 500 ml of distilled water in a 1000 ml flask for 30 minutes. After cooling, the mixture was filtered using the Bushner. This operation was repeated for six times for a total mass of 300 g. The filtrate (the aqueous phase) obtained was 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 was often difficult to separate, to another (called extraction solvent), from which it would be easily isolable. This operation, usually carried out by stirring, was possible provided that the two solvents were very little or no miscible with one another. But extraction was never 100%, there were always molecules of the compound to be extracted in the solvent in which it was less soluble.

The Liquid-liquid was obtained by successive partitions with solvents of increasing polarity (hexane and ethyl acetate) according to the protocol of Koudoroet 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 PsorospermumfebrifugumSpachaqueous 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 phenylhydrazineChloridrate. Phenylhydrazine was previously dissolved in a DMSO solution diluted to one-tenth in distilled water. It was administered to rats intraperitoneally (IP) at a dose of 40 mg/ kg of body weight / day (Naughton et al, 1995) for two days (D0 and D1).

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:

1. 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.
2. 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.
3. 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.
4. 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 thePsorospermumfebrifugumSpachethyl acetate fraction extract from D2 to D15.
5. 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 PsorospermumfebrifugumSpachethyl 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
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, 1989; Ganong, 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 (Sènou et al, 2016a).

Statistical Analysis
Graphs were plotted using Graphpad software. The results were presented as mean ± 2 SEM (standard error of the mean). 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%.

Results:-

Evolution of hemoglobin
The mean hemoglobin level ranged from 13.6 ± 0.4 to 15.8 ± 0.7 g / dl in the different groups of rats at day 0. It collapsed on D2 following the administration of phenylhydrazine and measured between 9.1 ± 0.2 to 9.3 ± 0.7 g / dl. The hemoglobin level then increased rapidly in the treated groups and was no longer significantly different from its D0 value from D7 in the groups treated with the extract fraction of Psorospermumfebrifugum and from D10 in the group treated with Vitafer®. On day 15, the mean hemoglobin level was significantly higher than on day 0 in the group treated with 60 mg of extract fraction (16.0 ± 0.9 g / dl, P < 0.05). In the anemic and untreated group, the hemoglobin level also increased progressively until J15 (11.7 ± 0.7 g / dl) but its value remained significantly lower compared to J0 (P value < 0.05). In the non-anemic group, the mean hemoglobin level did not significantly change during the experimental period (Figure 1).

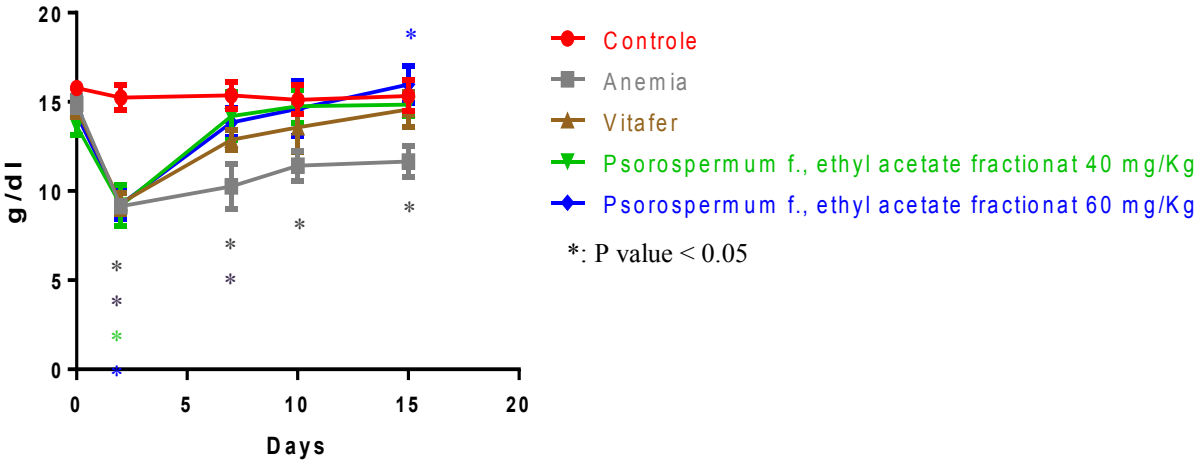

Figure 1:-Treatment effect on hemoglobin

Evolution of the red blood cells number
The mean number of red blood cells ranged from 6.3 ± 0.2 to 7.6 ± 0.6 T / L at day 0 in the groups of rats. It collapsed between 3.1 ± 0.4 and 3.3 ± 0.7 T / L following phenylhydrazine-induced hemolysis. Very rapidly, it increased in the treated groups and was no longer significantly different from D0 at D7 in the groups treated with the extract fraction of Psorospermumfebrifugum and at D10 in that treated with Vitafer®. In the anemic and untreated

group, the number of red blood cells also increased progressively until D15 (4.5 ± 0.7 g / dl) but its value remained significantly low compared to D0 (P value < 0.05). In the non-anemic group, the mean hemoglobin level did not significantly change during the experimental period (Figure 2).

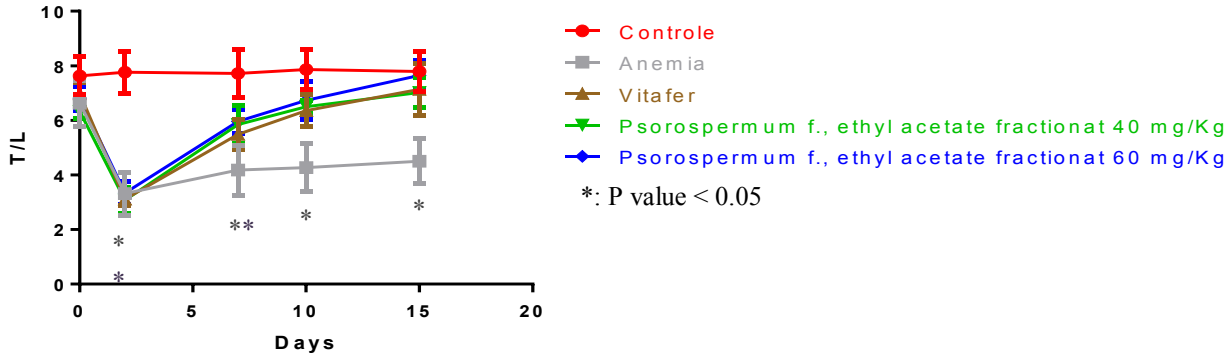

Figure 2:- Treatment effect on red blood cell number.

Evolution of the mean corpuscular volume

Mean corpuscular volume ranged from 60 ± 4 to 63 ± 4 fl at D0 in the groups of rats. It increased and reached its peak on day 7 in the anemic and treated groups (82 ± 6 to 90 ± 5), reflecting a release of macrocytes. It then dropped gradually and was no longer significantly different from D0 in these groups at J15 (P value < 0.05). In the anemic and untreated group, the evolution of the corpuscular volume continued until D15. In the non-anemic group, mean corpuscular volume did not change significantly during the experimental period (Figure 3).

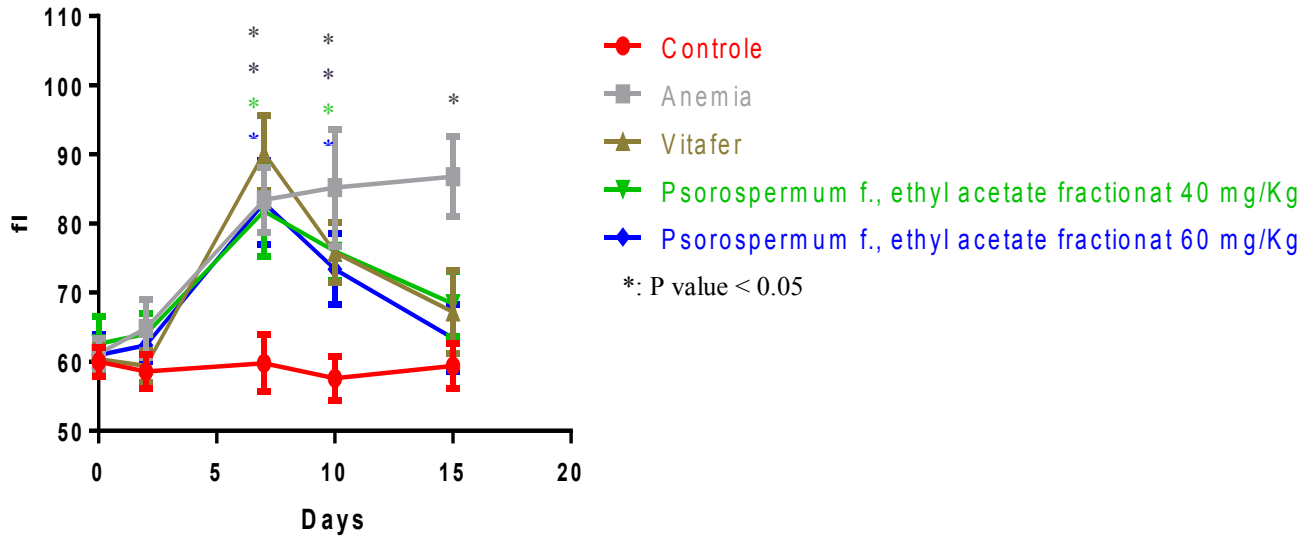

Figure 3:- Treatment effect on mean corpuscular volume.

Evolution of the mean corpuscular hemoglobin concentration

Mean corpuscular hemoglobin concentration ranged from 34.5 ± 0.7 to 35.4 ± 1.2 g / dl at day 0 in the rat groups. It increased significantly on day 2 in the anemic groups (44.3 ± 4 to 50.6 ± 2.3 g / dl, P value < 0.05). It dropped significantly at D7 in all these groups and measured between 28.2 ± 2.2 and 30.6 ± 1.0 g / dl (P value < 0.05). Then it regained to its normal value and was not significantly different from D0 at D10 in the treated groups. In the anemic group and it remained significantly low until J15. Mean corpuscular hemoglobin concentration did not change in the non-anemic group during the experiment (Figure 4).

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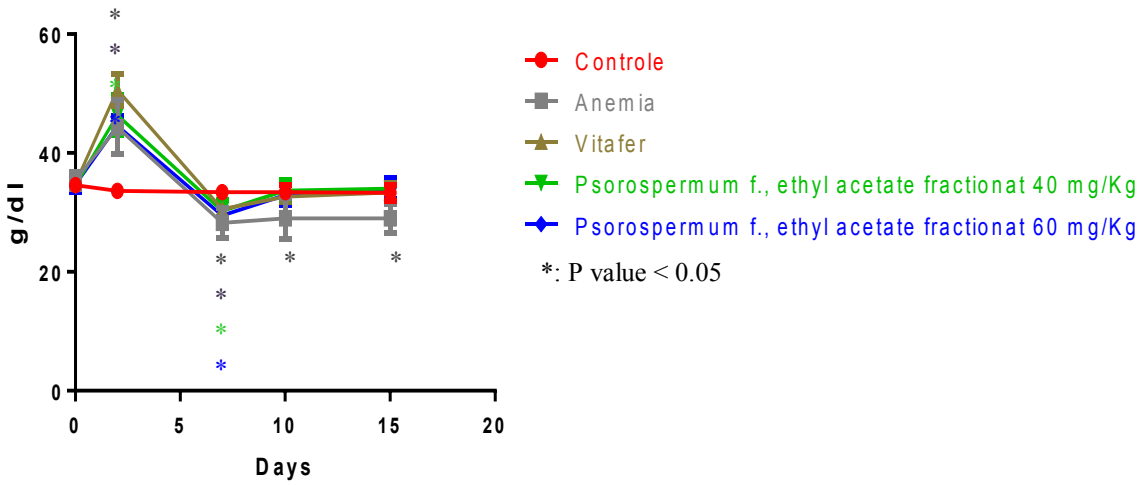

Figure 4:-Treatment effect on mean corpuscular hemoglobin concentration.

Evolution of the osmotic resistance of red blood cells

Osmotic resistance was determined by the percentage of hemolysis-resistant red cells in a hypotonic solution and reflected the proportion of young red blood cells. Osmotic resistance ranged from 0.18 ± 0.03 to 0.24 ± 0.09 at day 0 in the groups of rats. It increased significantly on D2 and peaked in all anemic groups on D7 (0.63 ± 0.1 at 80 ± 0.1,

P value < 0.05), reflecting increased release of young red blood cells. It gradually decreased in all these groups until D15, but the values remained significantly higher than on D0 (Figure 5).

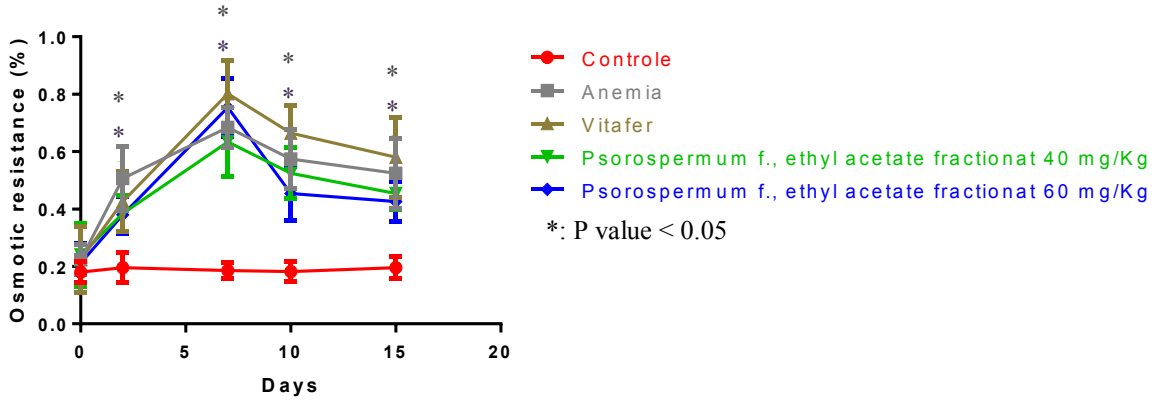

Figure 5:-Treatment effect on blood cell osmotic resistance

Evolution of the blood platelets number

The number of platelets varied from 400 ± 97 to 476 ± 61 G / L in the different groups of rats at day 0. It did not significantly change throughout the experiment, indicating that the treatment did not influence on blood platelets (Figure 6).

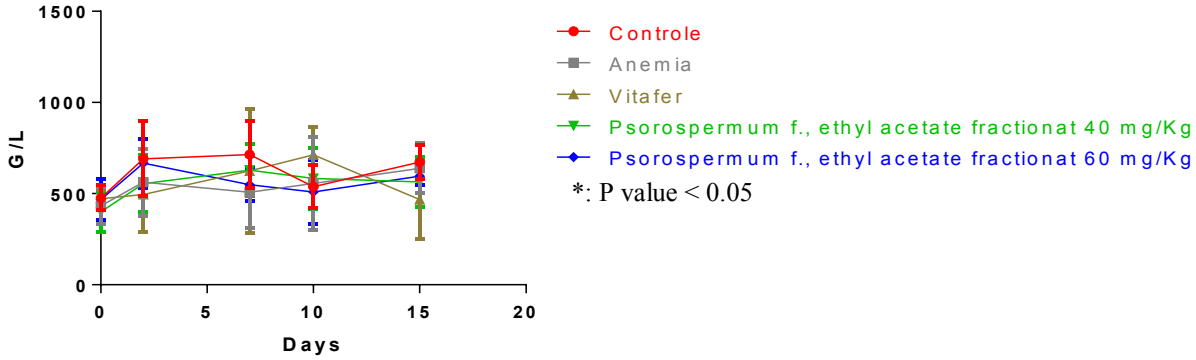

Figure 6:- Treatment effect on Platelets number.

Discussion:-

PsorospermumfebrifugumSpach was a medicinal plant whose root bark aqueous extract effectively healed anemia. Phytochemical screening of the extract revealed a presence of various families of chemical compounds including flavonoids (Agbogba et al, 2019a). This work proposed to search for the family of compounds responsible for hematopoietic activity. For this purpose, a fractionation of the extract was carried out. The ethyl acetate fraction that mainly isolated flavonoids (Manjusha et al, 2013 ;Koudoro et al, 2014) was tested in phenylhydrazine-anemic rats (Naughton et al, 1995; Nakanishi et al, 2003). Changes in haematological parameters such as hemoglobin levels, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin concentration, osmotic resistance of red blood cells and number of blood platelets in rats were monitored for two weeks.

Hemoglobin was the key indicator of anemia. It collapsed on D2 in all groups of rats injected with phenylhydrazine. This decrease was very rapidly corrected on D7 in the groups receiving the extract fraction at doses of 40 or 60 mg / kg / day and at day 10 in the group of rats treated with vitafer®, used as a reference drug. This result was better than that of the crude aqueous extract of Psorospermumfebrifugum, which minimum dose corrected the hemoglobin level only at day 15 (Agbogba et al, 2019a). On the other hand, it confirmed that observed with the ethyl acetate fraction of Cocos nucifera root aqueous extract (Sènou et al, 2017c). At day 15, the hemoglobin level was significantly greater than its D0 value only in the group receiving the 60 mg dose of extract / Kg (high dose) suggesting a dose-dependent effect. Such an observation was also made with the aqueous extract of the leaf sheath of Sorghum bicolor (Sènou et 2016a).

Since hemoglobin synthesis occurred in the red blood cell, we also followed the evolution of these cells. At day 2, the number of cells collapsed by hemolysis in all the groups injected with phenylhydrazine. As for hemoglobin, the decrease of red blood cells number also was corrected on day 7 in the groups treated with the extract fraction at 40 or 60 mg / kg and J10 in the group treated with vitafer®. This result agreed well with that of Psorospermumfebrifugum crude aqueous extract and that of Cocos nucifera roots aqueous extract which effectively stimulated erythropoiesis (Tchogou et al, 2016 ;Agbogba et al, 2019a). Similar observations were made with leaf extracts of Tectonagrandis (Diallo et al, 2008).

Mean cell volume(MCV) increased in all anemic groups with a peak on day 7 in the anemic and treated groups indicating early release into the bloodstream of macrocytes, immature red blood cells to compensate for anemia [31]. The globular volume then decreased progressively in the treated groups and at J15 no longer differed significantly from D0, indicating a well-differentiated red blood cells release at the end of the experimental period. In contrast, in the untreated anemic group, the increase in the blood volume continued until day 15 indicating a continuous release of macrocytes. The observation was similar to that of the crude extract of Psorospermumfebrifugum (Agbogba et al, 2019a).

The mean corpuscular hemoglobin concentration (MCHC) evolved inversely compared to the cell volume. It increased slightly on D2, indicating that red blood cells that resisted hemolysis were the most saturated in hemoglobin. It dropped significantly with a peak on day 7 in the anemic groups, corresponding to the early release into the bloodstream of macrocytes less saturated with hemoglobin to compensate for anemia. The values returned to normal from D10 in anemic treated groups but remained significantly lower compared to D0 in the anemic and untreated group which was consistent with the evolution of the mean corpuscular volume. This result agreed with

those of the crude root bark extract of Psorospermumfebrifugum (Agbogba et al, 2019a), the ethyl acetate fraction of the aqueous root extract of Cocos nucifera (Sènou et al, 2017c) and leaf sheath of Sorghum bicolor (Ogwumike, 2002).

Osmotic resistance was an indicator of the proportion of young red blood cells in the bloodstream (Sènou et al, 2016a). It increased significantly from day 2 with a peak on day 7 in the anemic groups. These observations were consistent with those of MCV and MCHC, which showed increased release of young early immature globules into the bloodstream during the first week of anemia.

In order to show the specificity of the extract fraction action, we followed the evolution of the number of blood platelets which constitute another blood cell line. The number of blood platelets was not influenced by the treatments, suggesting a specificity of the Psorospermumfebrifugum root bark ethyl acetate fraction on the erythrocyte line. This result was similar to that of the crude extract (Agbogba et al, 2019a) and to that of Cocos nucifera root extract ethyl acetate fraction (Sènou et al, 2017c).

Since ethyl acetate mainly isolated flavonoids (Manjusha et al, 2013 ;Koudoro et al, 2014), the observed hematopoietic effect would probably be related to certain members of this family via erythropoietin (Abeer et al, 2009; Zhang et al, 2017)

Conclusion:-

This work demonstrated the efficacy of the ethyl acetate fraction of Psorospermumfebrifugum root bark aqueous extract in the stimulation of erythropoiesis. The action seemed specific and dose-dependent as observed with the crude aqueous extract of the root bark. It could be due to the flavonoids contained in this organ of the plant and mediated by erythropoietin. The mechanism of action was not yet known and worth exploring.

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