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

Maximin Sènou1,2, Pascal Atchadé Tchogou1,2, Félicienne Agbogba1,3, Ezechiel Jacques Lokonon1, Espérance Medoatinsa1, Rose Estelle Kanfon4, Victorien Tamégnon Dougnon5, Pascal Cokou Dossa Aghangnan5, Cyrille Boko2, Frédéric Loko2, Anatole Lalèyè5, Amegnona Agbonon6, Lamine Saïd Baba-Moussa7 and Alphonse Sèzan3

1. Experimental and Clinic Biology Laboratory, National School of Applied Biosciences and Biotechnology, National University of Science, Technology, Engineering and Mathematics (UNSTIM), Dassa-Zoumé, R. Benin.
2. Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Cotonou, R. Benin.
3. Laboratory of Biomembrane and Cell Signaling, Faculty of Sciences and Techniques, University of Abomey-Calavi, R Benin.
4. Laboratory of Study and Research in Applied Chemistry, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, R. Benin.
5. Human Biology Unit, Faculty of Health Science, Cotonou, R. Benin.
6. Laboratoire de Physiologie/Pharmacologie, Faculté des Sciences, Université de Lomé, R. Togo.
7. Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, University of Abomey-Calavi R. Benin.

Abstract

Psorospermum febrifugum Spach. (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 phenylhydrazine chloridrate on D0. From day 2 to day 15, some were gavage fed with the ethyl acetate fraction of Psorospermum febrifugum Spach 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 Psorospermum febrifugum root bark aqueous extract stimulated erythropoiesis faster than the
crude extract. Its action seemed specific and dose-dependent. It would probably be related to the flavonoids which action mechanism needs to be explored.

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; Sénou et al, 2017a). Genetic factors and especially endemic sickle cell disease might also be considered to explain this high prevalence (Sénou 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 (Sénou et al, 2016a; 2016b; Tchogou et al, 2016).

Psorospermum febrifugum Spach 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 its 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
Psorospermum febrifugum Spach (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° 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 Koudoro et al. (2014).

In a separatory funnel, was added to the aqueous extract solution the appropriate volume of extraction solvent. After vigorous agitation, the mixture was allowed to settle. After decantation, the two phases were separated by collecting the lower phase (aqueous phase) in a flask and the upper phase (organic phase) in another. The aqueous phase was re-poured into the separating funnel before repeating the following steps. After each extraction step, the organic phases were combined, which constituted the fraction in a jar.

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

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

In vivo Experimentation
The evaluation of the anti-anemic activity consisted of assessing the impact of PsorospermumfebrifugumSpach aqueous extract ethyl acetate fraction on hematological parameters and red blood cells osmotic resistance of anemic female and male Wistar rats.

Induction of anemia
Anemia was induced by 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).

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).
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).

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).
Discussion:

Psorospermum febrifugum Spach 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 hematological 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 Psorospermum febrifugum, 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 al, 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 Psorospermum febrifugum 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 Tectona grandis (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 Psorospermum febrifugum (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.

References:
1. AbeerTemraz and Walid Hamdy El-Tantawi, (2009).Treatment of Experimental Hemolytic Anemia by Some Antioxidant Drugs. Journal of Pharmacy Research, 2 (5): 798-802.
2. AgbogbaFélicienne, Sacramento Téniola Isabelle, TchogouAtchadé Pascal, MedoatinsaEspérance, Kanfon Estelle Rose,Atakpa Eugène, AgbangnanDossa C. Pascal, Loko Frédéric, Lalèyè Anatole, Atègbo Jean-Marc, Sènou Maximin and Sèzan Alphonse, (2019a). The aqueous extract of the root bark of PsorospermumfebrifugumSpach effectively corrects anaemia. Experimental study on Wistar rats. Journal of Applied Biosciences 139: 14137 – 14146. ISSN 1997-5902.
3. Agbogba F, Sènou M, Tchogou AP, Sacramento TI, Medoatinsa E, Kanfon RE, Atakpa E, Dougnon TV, Agbangnan DCP, Loko F, Lalèyè A, BABA- Moussa S.L, Atègbo JM, Sèzan A, (2019b). Study of toxicity of aqueous extract of PsorospermumfebrifugumSpach root bark on Wistar rats. International Journal of BiosciencesVol. 14 (5): 475-482. ISSN: 2220-6655 (Print), 2222-5234 (Online) http://www.innspub.net.
4. Al-Zabedi EM, Kaid FA, Sady H, Al-Adhroey AH, Amran AA, Al-Maktari MT, (2014). Prevalence and risk factors of iron deficiency anaemia. American journal of health research. 2 (5): 119-326.
5. Arbonnier M., (2000). Arbres, arbustes et lianes des zones sèches’Afrique de l’Ouest. CIRAD/MNHN/UICN, pp: 541.
6. Assobayire FS, Adou P, Davidson L, Cook JD, Hurrel R., (2001). Prevalence of iron deficiency withand without concurrent anemia in population group with high prevalences of malaria and other infections:a study in Cote d’Ivoire. American Journal of Clinical Nutrition 74, 776-82.
7. Bhushan P. WHO-CIPIH, (2005). Study nine on traditional medicine, Draft report. 2005, pp. 5-12.
8. Bigoniya P, Singh S, Singh CS, Shukla A., (2013). Anti-anemic potential estimation on mice and characterization of flavonoids using high performance thin layer chromatography in Wrightiatinctoriabark fraction. J Nat Pharm, 4, 47-56.
9. Crawley J., (2004). Reducing the burden of anemia in infants and young children in malaria-endemic countries of Africa: from evidence to action. Am. J. Trop. Med. Hyg. 71 (2), 25-34.
10. De Benoist B, McLean E, Egli I, Cogswell M., (2008). Worldwide prevalence of anaemia 1993-2005- WHO Global Database on Anaemia. WHO-CDC, 48 p.
11. Diallo A, Gbassor M, Vovor A, Eklu-Gadegbeku K, Alikokou K, Agbonon A., (2008). Effect of Tectona grandis on phenylhydrazine induced anemia in rats. Fitoterapia79,332-6. http://dx.doi.org/10.1016/j.fitote.2008.02.005
12. El Hioui M, Aboussaleh Y, Ahami AOT, Farsi M., (2009). Contribution à l’étude de la prévalence de l’anémie chez les enfants préscolaires de la région de Kénitra, Maroc. Antropo 19, 1-5.
13. Fauchet R, Ifrah N, Blanchet O, Dautel M., (1995). Collection BiologieMédicaleHématologie. 2ème édition. Condé-sur-Noireau (France), 437.
14. Ganong, W. F., (2001). Physiologie Médicale (1st ed.). de l'UniversitéBrock. 828p.
15. Genetet, B., (1989). Hématologie. Tech et doc. Lavoisier, éd, Paris, pp. 3-82.
16. Guyton A. and Hall J., (2007). Text book of Medical Physiology, Saunders, eleventh edition, 426-427.
17. Jollow DJ and McMillan DC., (2001). Oxidative stress, glucose-6-phosphate dehydrogenase and the red cell. AdvExp Med Biol., 500: 595–605.
18. Koudoro, Y.A., Dedomè, L.S.O., Yovo, M., Agbangnan-Dossa, C.P., Tchobo, P.F., Alitonou, G.A., Avlessi F., Sohounghoué, D.C.K., (2014). Chemical characterization, antrical and antibacterial activities of extracts of the root bark of Cochlospermumplanchoni of Benin. International Journal of Innovation and Applied Studies 7(4), 1582-1594. http://www.ijias.issr-journals.org/
19. Librengs S. F., Gunirérez L., A. M. de Bruin et al., (2011). “Chronic IFN-y production in mice induces anemia by reducing erythrocyte lifespan and inhibiting erythropoiesis through an IRF-1/PU.1 axis,” Blood, vol. 118(9), pp. 2578–2588.
20. Manjusha S, Abrar H M, Manik S, Mohd Y M, Donerah Q, Mohd Iqbal M and Yogesh C. (2013). Phytochemical Screening and in vitro Antioxidant Activity Isolated Bioactive Compounds from Trigaziprocumbens Linn. Pakistan Journal of Biological Sciences 16 (24), 1971-1977.
21. Movaffaghi Z, Hasanpoor M., (2006). Effect of therapeutic touch on blood hemoglobin and hematocrit level. J Holist Nurs, 24, 41-8. http://dx.doi.org/10.1177/0898010105282527
22. Nakanishi A, Kinnuta K, Abe T, Araki K, Yoshida Y, Liang S, (2003). Formation of meso, N- diphenylprotoporphyrin IX by an aerobic reaction of phenylhydrazine with oxyhemoglobin. Acta Med Okayama, 57: 249-56.
23. Naughton, B. A., Moore, E., Bush, M. E., Lapin, D. M., & Domfest, B. S., (1995). Hemostatic alterations associated with phenylhydrazine – induced haemolyticaemia. Eur J Clin Invest, 25, 722-7.
24. Ogbe RJ, Adoga GI, Abu AH., (2010). Antianaemic potentials of some plant extracts on phenyl hydrazine-induced anaemia in rabbits. J Med Plant Res, 4, 680-684.
25. Ogwumike OO, (2002). Hepatoprotective effect of aqueous extract of the leaf sheath of Sorghum bicolor in albinos rats. African Journal of Biomedical Research 5, 69-71.
26. Ross and Wilson (2006). Anatomy and Physiology in Health and Illness, Churchill Livingstone Elsevier, Tenth Edition, 70-71.
27. Sènou M, Tchogou AP, Dougnon TV, Agossadou A, Assogba F, Kinsiclounon EG, Koudopon H, Fah L, Fanou B, Akpovi DC, Gbénou J, Laléyè A, Loko F., (2016a). Efficiency of Sorghum bicolor extract in the treatment of induced anemia on Wistar rats.International Journal of Biosciences 8 (4), 62-71.
28. Sènou M, Agossadou A, Agbangnan DCP, Tchogou AP, Assogba F, Klotoé JR, Dougnon TV, Gbénou J, Laléyè A, Sézan A &Loko F., (2016b). The Aqueous Extract of Hibiscus sabdariffa L. Calyces Effectively Corrects induced Anemia. International Journal of pharmacaynd pharmaceutical research Vol. 7 (4), 254-265.
29. Sènou M, Agbangnan DCP, ZimeN’Gobi AR, AbissiGY, Agbogba F, Sézan A, Loko F., (2017a). In malaria, acute inflammation level depends on parasitaemia. International Journalof Pharmacy and Biological Sciences 7 (2), 23-30.
30. Sènou M, Lokonon JE, Agbogba F, Agbangnan DCP, Odoulami S, Tchogou AP, Laléyè A, Sézan A, Loko F., (2017b). In vitro study of some characteristics of red blood cells with hemoglobin S. International Journal of Biosciences 10 (1), 311-317.
31. Sènou M, Tchogou AP, Agbangnan DCP, Dougnon TV, Ogué P, Agossadou A, Laléyè A, Loko F, Agbonon A &Sézan A., (2017c). Efficacy of ethyl acetate fraction of Cocos nucifera aqueous extract on the treatment of anemia. International Journal of Pharmaceutical Science Invention 6 (9 l):2017c, 44-51. P-ISSN: 2319 – 670X, E-ISSN, 2319 – 6718. www.ijpsi.org.
32. Tchogou AP, Sènou M, Dougnon TV, Agossadou A, Assogba F, Kinsiclounon EG, Ewedjé E, Agbangnan DCP, Gbénou J, Laléyè A &Loko F.; (2016). The Aqueous ExtractofCocos nucifera L. (Areacaceae) Effectively Treat Induced Anemia. Experimental Study on Wistar Rats. International Journal of Biology 8 (3), 1-9.
33. WHO., (2015). The global prevalence of anaemia in 2011. Report of a scientific group of WHO, Geneva, 43p.
34. Zhang L., GongA. G.W., RiazKashif, TsimK. W. K., (2017). Protective effect of flavonoids against reactive oxygen species production in sickle cell anemia patients treated with hydroxyurea. FEBS Journal, 7 (3), 318–323.