Scientific Research Publishing

Journal of Biosciences and Medicines, 2024, 12, 295-306
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Aqueous Extract of *Ceiba pentandra* Stimulates the Production of Fetal Hemoglobin in Sickle Cell Patients

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How to cite this paper: Nsimba, M.M., Mihatano, M.B., Mbamu, B.M., Lubongo, A.D., Kolombo, T.N., Mukengeshaie, J.M. and Lami, J.N. (2024) Aqueous Extract of *Ceiba pentandra* Stimulates the Production of Fetal Hemoglobin in Sickle Cell Patients. *Journal of Biosciences and Medicines*, **12**, 295-306.
https://doi.org/10.4236/jbm.2024.128023

Received: July 18, 2024
Accepted: August 20, 2024
Published: August 23, 2024

Abstract

Subsequent studies have demonstrated the reversed activity of the aqueous extract of *Ceiba pentandra* on the deformity of sickled red blood cells in hypoxia conditions. The observation which related to an *in vitro* study had given rise to hopes as to the management of sickle cell disease (SCD) by the use of this plant species. In this paper, the authors aimed to investigate the effect of the aqueous extract of *C. pentandra* on the production of fetal hemoglobin in SCD patients. The work carried out hemoglobin electrophoresis, for a period of six months, on blood samples from SCD patients who voluntarily undergone routine treatment, based on the medicinal recipe prepared from the bark of the trunk and branches of *C. pentandra*, in a hospital center of herbal medicines located in Kinshasa. The medicinal recipe called *BEAT-SS* is a patented product of the hospital center named *Centre de Phytothérapie Moderne NIECA*. Blood samples from patients under treatment were taken to evaluate the behavior of different forms of hemoglobin (hemoglobin S, hemoglobin F and hemoglobin A_2). Agarose gel electrophoresis with integrated reading was used for the separation of the different forms of hemoglobin, as well as their dosage on each sample of sickle blood. A reduction in the proportion of hemoglobin S and an increase in the proportion of fetal hemoglobin were found in all sickle cell patients during the treatment period. This observation could affirm that the management of sickle cell patients using the recipe prepared from the aqueous extract of *C. pentandra* could increase the level of fetal hemoglobin in these patients.

Keywords

Sickle Cell Disease, Hemoglobin S, Fetal Hemoglobin, *Ceiba pentandra*

1. Introduction

Sickle cell disease (SCD) is a genetic worldwide spread pathology with chronic hemolytic anemia, infectious condition, vaso-occlusive crises and organs failure, due to the presence of an abnormal hemoglobin S molecule (HbS). The disease is most particularly encountered in Sub-Saharan Africa where there are 400,000 SCD newborn children each year for a total of 500,000 in the world. This situation constitutes a real public health problem [1]-[3]. Democratic Republic of Congo (DR Congo) is the second African country most affected by the disease after Nigeria with a prevalence of 2% children among newborns, estimated at 50,000 SCD children born each year [4] [5].

Apart from bone marrow transplantation and gene therapy which, moreover, remains a therapy not allocated to all patients due to the non-availability of donors, high cost as well as therapeutic failure [6]-[8], SCD is currently not medically curable; hence conventional treatment remains generally preventive and symptomatic. The management of modern medicines to treat symptoms and prevent infections is necessary and is a simple measure that prevents the onset of vaso-occlusive crises. This can positively improve the quality of life of affected patients or increase their life expectancy. However, follow-up that must be continuous is still limited or lacking in sub-Saharan African countries; not all patients have easy access to it. That is why many patients cannot accommodate with. Most children with the severe form of the disease die before the age of five, often from infectious causes or severe anemia; in DR Congo for example, more than 50 percent of the affected children at this age, are concerned [9] [10].

Along with efforts in referral hospitals for patients with SCD, researchers in many African countries hope to find better treatment alternatives for the management of the disease [10] [11]. Thus, regarding this concern, the use of medicinal plants is a much extended practice. Some scientists devote their studies to research on medicinal plants which are already identified and being used by the population or healers [12]-[15]. Since ancient times, plants have always been components of medicinal preparations and have been exploited by populations for their therapeutic virtues, to this day the practice has not diminished especially in lower income areas [16]-[18]. In DR Congo, a center located in Kinshasa called *Centre de Phytotherapie Moderme NIECA* (*CPMN*) has been using traditional medicine, which they named *BEAT-SS*, for the management of the disease for several years already. The drug is prepared from the aqueous extract of the bark of the trunk and branches of *Ceiba pentandra*. According to the herbalists, clinical improvement has been observed in patients undergoing treatment with the prepared recipe. The center claimed that a significant reduction in sickle cell

symptoms, or even a complete cure is observed in some patients who regularly take the recipe, without resorting to blood transfusion or other modern drugs. They also believe that *BEAT-SS* would have an inhibiting effect on the production of abnormal HbS because patients who are treated for a long time, no longer show sickle cell symptoms at the end of treatment.

The intracellular polymerization of HbS molecules in hypoxia conditions leads to the sickling of red blood cells (RBCs) and this is the main phenomenon responsible for the multitude of pathophysiological consequences observed in patients with SCD [19]. Many researches have been directed towards inhibiting sickling of RBCs, others have focused on the increased HbF level which militates in favor of SCD patients who see their episodes of vasoocclusive crises reduced. Recent insights into HbF regulation in sickle cell patients have prompted medication to induce HbF expression [20]-[23]. For the latter reason, hydroxyurea has been used for more than twenty years in sickle cell patients [24]-[26].

To test the possibility that HbF expression may occur when sickle cell patients are treated with the recipe from the aqueous extract of *C. pentandra*, we proceeded to the electrophoretic profile of hemoglobin molecules of SCD patients who are monitored and treated with *BEAT SS* at *CPMN* over a period of six months. Level and electrophoretic migrations of hemoglobin molecules (HbS, HbF and HbA2) at alkaline pH (8.5) were performed. Hence, we hypothesized that the recipe prepared from *C. pentandra* for the management of SCD at *CPMN* would exhibit antisickling activity by the enhancing the production of HbF.

2. Material and Methods

2.1. Plant Material

Medication for sickle cell patients is administered within the *CPMN* which, according to information received by its staff, uses a decoction. The bark of trunk and branches of *C. pentandra* were dried at room temperature and then ground. 100 gr of the obtained powder was introduced into a container and boiled with 5 liters of water for 5 minutes while stirring. Then the container was sealed tightly and its contents were left to cool at room temperature for 24 hours. The mixture was filtered through absorbent cotton. The filtrate extract ready for use was packaged in appropriate bottles. The partitioned extracts were administered to sickle cells patients as a crude drug. Patients were taking medication freely with the agreements they established with the phytotherapy center.

2.2. Blood Samples

CPMN staff selected 15 sickle cell patients to conduct this study which was scheduled for six months. For the criteria selection, the patients should have been those who were visiting the center for the first time, with the aim of starting the treatment with the medicinal recipe from *C. pentandra*; no patient should receive any blood transfusion one month before and during the six-month trial

period; and patients should follow treatment continuously without interruption. Only 5 patients, whose ages varied between nine months and five years, remained at the end of the study among the 15 retained due to the constraints imposed above. The patients included two girls (3 years and 4 years) and three boys (9 months, 4 years and 5 years). Three blood samples provided by *CPMN* were taken from each patient: the first at the beginning of the experiment and the other two each time after three months. 3 ml of blood collected from different patients, was introduced each, in a plastic tube containing 0.05 ml of 10 % ethylenediamine-tetraacetic acid (EDTA) as anticoagulant. The samples were placed in an icebox for commuting to the laboratory for the experiment. Blood samples were provided under the authorization of the Congolese ethics committee (N˚d'approbation ESP/CE/200/2023).

2.3. Hemoglobin's Separation and Material

1 ml of blood sample was centrifuged for 5 minutes at 5000 rpm. Then, the plasma was removed, and the red blood cell's pellet was washed twice with 10 volumes of saline which were removed each time from the surface. 130 µl of hemolyzing solution was added to 10 µl of red blood cell's pellet and shaken in a vortex for 10 seconds, then incubated for 5 minutes at room temperature. After that, 10 µl of hemolyzed sample was introduced in each well of the applicator. The applicator was placed in the humid chamber, teeth facing up; and leaved to diffuse 5 minutes after depositing the last sample.

Hemoglobin separation was performed by electrophoresis using the *Hydrasys system* which is an automatic apparatus that ensures electrophoretic migration, drying, staining, distaining, and final drying, after application of samples. This semi-automated system allows the separation of normal hemoglobin, the detection of the main abnormal hemoglobin, as well as their staining. Reading at 570 nm by densitometer gives a relative estimation of each individualized zone and makes it possible to define the relative concentrations (percentages) of each fraction of hemoglobin. The preparation of samples for analysis and their application are the only manual steps in the process. The software allows to control the operation of the automaton (migration, coloring) via a touch screen. The automaton is then connected to a computer equipped with software that allows the acquisition, elaboration, processing and conversion of images from the reader. Electrophoretic material was provided by *Sebia Diagnostics* [27].

3. Results

The quantification in percentage of the different fractions of hemoglobin molecule for a sickle blood patient after electrophoresis is illustrated in **Figure 1**. It is noticed that the S-Hemoglobin fraction migrates in a central position between fractions A and A_2; hemoglobin F on the other hand is located between A and S. The diagram displayed the peak of hemoglobins and their percentages on the lower part. Since it concerns a sickle cell patient and therefore homozygous SS, there were no traces of hemoglobin A on the diagram.

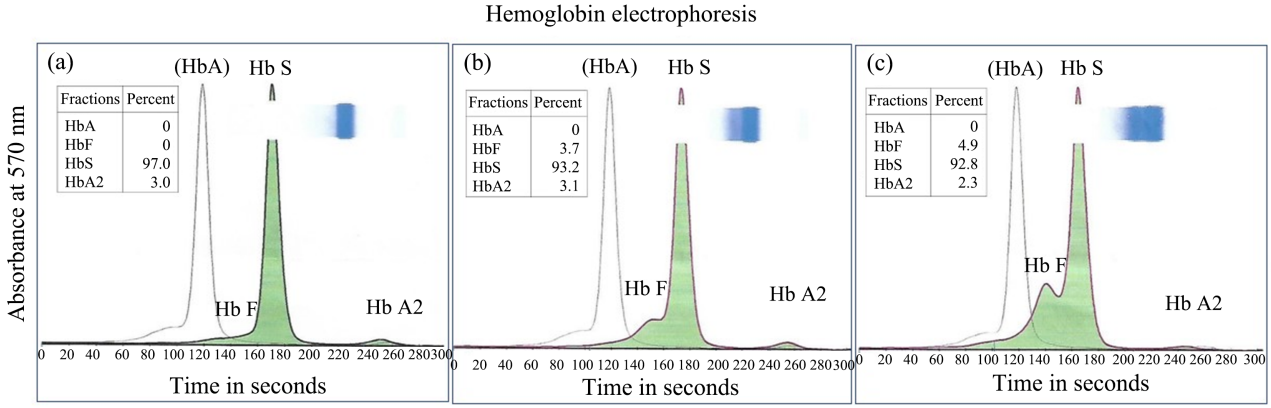

Figure 1. Illustration of the location and the quantification as percentage distribution of different fractions of hemoglobin's (Hb-F, Hb-S and Hb-A2) of a sickle blood sample after electrophoresis migration, as converted from the computer software coupled to the densitometer. (a) at the beginning of treatment with the medicinal recipe, (b) after 3 months of treatment and (c) after 6 months of treatment.

The percentage distribution of the different fractions throughout the study can be seen on Table 1. It was noticed that all the five patients had over 95% (96.8 ± 1.3) of Hemoglobin-S fraction at the beginning. After the patients have been subject for treatment by the extract for 3 months, the percentage of Hemoglobin-S fraction decreased to 93.8 ± 2.4 and then continued to decrease to 90.3 ± 3.9 after 6 months as displayed as average percentage on Table 2 and illustrated on Figure 2. It has been clearly observed that the response to this reduction was not the same for each patient.

Table 1. Percentage distribution of the different fractions of hemoglobin's (hemoglobin S, hemoglobin A2 and hemoglobin F) of the five sickle patients after treatment with *C. pentandra* extract for a period of 6 months.

	Start			3 Months			6 Months		
	Hb S	Hb A_2	Hb F	HbS	Hb A_2	HbF	HbS	Hb A_2	HbF
Patient 1	97	3	0	93.2	3.1	3.7	92.8	2.3	4.9
Patient 2	98	2	0	94.3	3.4	2.3	91.9	3	5.1
Patient 3	96.1	3.9	0	96.3	3.7	0	92.5	3.3	4.2
Patient 4	95	2.2	2.3	90	4.7	5.3	83.5	2.7	13.5
Patient 5	98	2	0	95	2.7	2.1	91	4.6	4.4

Table 2. Mean percentage of Hemoglobin's S, F and A2 for the five patients from baseline, 3 months and 6 months after being subjected to aqueous extract of *C. pentandra*.

Treatment Period	HbS % Mean ± SD	HbF % Mean ± SD	HbA2 % Mean ± SD
Start	96.8 ± 1.3	0.5 ± 1.0	2.6 ± 0.8
After 3 months	93.8 ± 2.4	2.7 ± 1.9	3.5 ± 0.7
After 6 months	90.3 ± 3.9	6.4 ± 3.9	3.4 ± 0.7

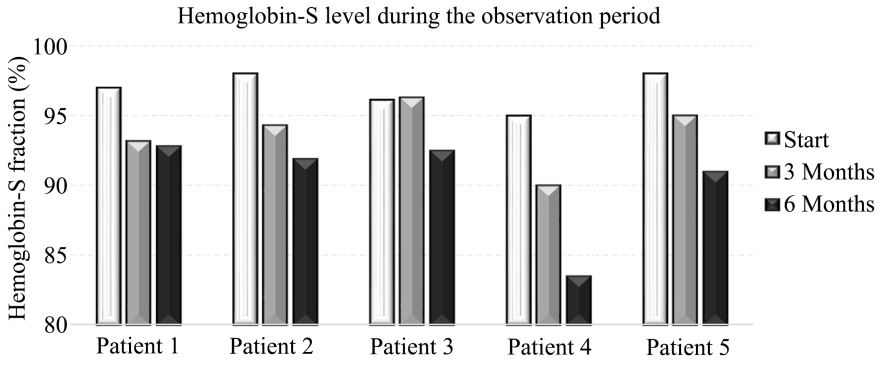

Figure 2. Evolution of the proportion of Hemoglobin-S fraction from the start to 6 months of each sickle cell patient (patient 1 to patient 5) subjected to the aqueous extract of *C. pentandra*.

On the other hand, it was noticed that the rate of fetal hemoglobin increased from the beginning (0.5% ± 1.0%) up to 6.4% ± 3.9% after 6 months of treatment, for the average of all patients, the response to this increase was not observed in the same way for each patient (**Table 2**). Patient 4 especially, recorded an increase of more than 10% (2.3 to 13.5) and he was the only one with some level of fetal hemoglobin, although low, while all four others had shown no trace of fetal hemoglobin at the beginning of treatment (**Figure 3**). The observation was also noticed for the same patient with regard to the decrease in the level of hemoglobin S (95% to 83.5%) as it can be seen in **Table 1**. For hemoglobin A_2, no significant change was noticed after 6 months for all patients.

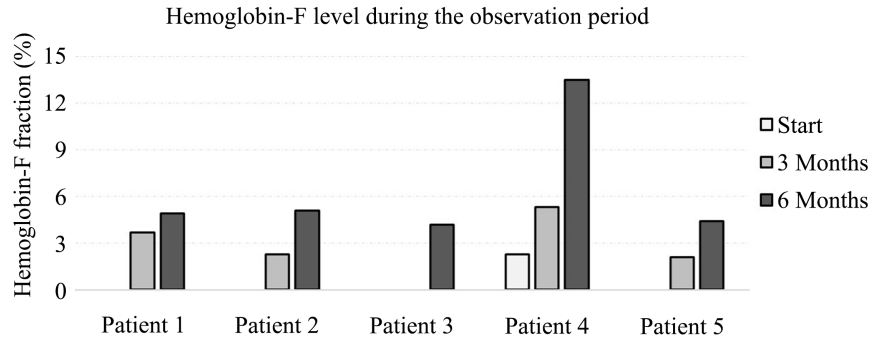

Figure 3. Evolution of the proportion of Hemoglobin-F fraction from the start to 6 months of each sickle cell patient (patient 1 to patient 5) subjected to the aqueous extract of *C. pentandra*.

4. Discussion

The sickling of RBCs, which is initiated by intracellular polymerization of hemoglobin S, is the first phenomenon that initiates the multitude of pathophysiological consequences observed in patients with SCD. The sickling of RBCs causes vascular complications and ischemia of various organs such as the liver, spleen, kidneys or lungs, by sequestration of sickled blood cells; the phenomenon can cause infarction of these organs [28] [29]. In addition, certain infections such as

malaria are incriminated in SCD patients because they tend to aggravate morbidity and mortality in this population group. Plasmodium falciparum can aggravate anemic conditions by destroying already damaged RBCs and increase painful crisis [30]-[32]; hence the interest in combating polymerization to prevent damage. Finding solutions to combat the polymerization has always been a concern of many researchers who strive to find strategies that will bring a glimmer of hope in the treatment of SCD, this is an option that has always been mentioned in several studies [33]-[35]. Over the years, researchers have proven that the production of fetal hemoglobin has a beneficial effect by inhibiting the polymerization of hemoglobin S, then avoiding the sickling of RBCs [33]. The increase in the rate of fetal hemoglobin can, in fact, attenuate the clinical manifestations in patients and lead to the choice of a curative treatment [36]-[38].

Many studies have oriented research on synthetic products or chemical compounds that stimulate the production of fetal hemoglobin such as decitabine [39] [40], butyrate [41] and hydroxurea [33] [42]-[44]. The authors have mentioned the decrease in the incidence of vasooclusive events, infections, transfusions and death. Besides this, medicinal plants also are concerned by these researches [45]-[47].

The aqueous extracts of *C. pentandra*, used in the management of SCD in a traditional medicine hospital center in the Democratic Republic of Congo, have already demonstrated efficacy in reversing the sickling of RBC as well as their aggregation [48] and; exhibited also significant antithrombin activity and prolonged clotting time by Activated Partial Thrombin Time [49]. The present study observed a decrease in hemoglobin S level coupled with an increase in fetal hemoglobin in all five sickle cell patients who were followed. Hemoglobin A_2, on the other hand, did not undergo any change. Hemoglobin A_2 can be found at a level of less than 3 percent in adults. Patients in the present study were all hemoglobin S homozygous; it is noticed that in some cases of double heterozygous S-thalassemia, an increase in hemoglobin A_2 may be noted which, could be beneficial in preventing polymerization in these patients [50]-[53], but this was not the case in this study. One of the patients, in particular, showed a strong significant increase compared to others and it was the one who had shown a slight level of fetal hemoglobin when the others had none at the start.

It has been known that fetal hemoglobin is a modulator of the clinical manifestations of SCD, an effect mediated by its exclusion from the sickle cell hemoglobin polymer [23]. This statement could argue that the aqueous extract of *C. pentandra* which has been observed to present a positive response to the regeneration of fetal hemoglobin, could attenuate the pathophysiology, the clinical evolution and offer prospects for curative treatment of SCD. However, the level of fetal hemoglobin and its distribution among sickle erythrocytes is highly variable [37]. The response on the expression of fetal hemoglobin that we noticed in the study might be similar to that evoked by other authors concerning hydroxyurea, which denote that fetal hemoglobin responses to hydroxyurea vary

among patients with sickle cell disease and are, at least in part, genetically regulated [54].

We noticed in all ways that the patient who presented a certain level of fetal hemoglobin's fraction at the start, responded better to the desired action of regeneration than the other patients who had not presented anything in terms of fetal hemoglobin rate. However, we did not mention the genetic aspect of each patient from the start to discuss the variation in the expression of fetal hemoglobin of each individual. The genetic aspect had not been mentioned in this study, we consider that future studies will focus on it and be able to reveal more about the aspect.

Taking into account other researches already carried out on the recipe based on the aqueous extract of *C. pentandra*, which has demonstrated very promising results, we can also affirm that the recipe has therefore, properties to reduce the proportion of hemoglobin S in sickle cell patients and acts on erythropoiesis for the stimulation of fetal hemoglobin production. It could be said with conviction that this phytomedicine might be useful for improving the management of sickle cell patients.

5. Conclusion

The phytomedicine for the management of sickle cell disease which was developed by the Phytotherapy Center NIECA prepared by using the aqueous extract of trunk and branches of *C. pentandra* and named *BEAT-SS* is used for several years and subjected for several studies. In the present work, the authors stated the reduction of the proportions of hemoglobin S coupled with the increasing of fetal hemoglobin fraction. Generation of fetal hemoglobin is associated with the reduction of the pathophysiologic effects associated with sickling and complications in SCD. Therefore, the result of the present study suggested that phytomedicine would significantly contribute to improving the management of sickle cell patients.

Acknowledgements

The authors would like to thank MPOY LOUMAN, the General Administrator of the *BIAMBA MARIE MUTOMBO HOSPITAL*, for allowing us to use the Hospital medical laboratory for free analysis and providing the equipment for electrophoresis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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