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# Phyto-Medicine in Gene(s) Targeting Future Direction for Sickle Cell Disease Management

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## Abstract

Sickle cell disease (SCD) is a genetic disease of hemoglobin (Hb) that occurs due to a non- conservative substitution of a polar glutamate (Glu) by non-polar valine (Val) in an invariant region, of hemoglobin β chain-subunit. This change distorts the normal Hb folding resulting in a sticky patch on the surface of the β-chains and associated complications. Nigeria has the largest burden of SCD globally with an estimated 150,000 new born affected annually.

This review is aimed at analyzing the phytomedicines use in Nigeria to ameliorate the crisis associated with SCD and present probable target genetic loci/pathways that this phytomedicines could act upon for effective and enhanced management of SCD in a resource poor environment.

Nucleation is essential in polymerization of Hb and phyto-medicine has been reported to inhibit this pathway. Different loci of genetic variation identified as modulator of SCD phenotype include nucleotide motifs within the β-globin gene cluster and distal genes located on different chromosomes. Fetal hemoglobin (HbF) is equally an important variable and modulator of the clinical features of SCD. Phytomedicine used in the management of SCD ameliorates oxidative stress and modulates cytokines that contributes to SCD complications. Here, we propose the use of phyto-medicine as key component for genetic modification, by twinkling, some transcription effectors/gene modulator such as B-cell lymphoma/leukemia 11A (BCL11A), or an erythroid specific transcription factor (KLF1), which are part of factors mediating HbF gene silencing.

**Keywords:** Sickle cell disease; Fetal haemoglobin; Protein variation; Genetic loci, Point mutation; Phytomedicine

## Background

Sickle cell disease belongs to a family of disease known as hemoglobinopathies. It was first 'discovered' in the United States in 1910, when a physician Dr James Herrick observed sickled shape red cells in the blood of a West Indies patient with pain and anemia. The disease has been known by different names among Africans, particularly, by Nigerian people for many centuries. In Nigeria, records showed that the three main tribes called the SCD by different names. The Yoruba's called SCD sufferers "abiku" [1], the Ibos called it "Ogbanje" [1,2] and the Hausas called it "Sankara-miji" [1,3]. It is the commonest genetic disorder in Nigeria affecting about 4 million Nigerians at prevalence of 2% at birth while over 40 million individuals have sickle cell trait. Nigeria accounts for 75% of infant SCD in Africa [4]. The Table 1 shows the different types of SCD with geographical prevalence and, thalassemia being of the same class of disorder. In this review we discuss plants with phytomedical properties used in Nigeria to ameliorate SCD crisis. Also we propose holistic inclusions of phytomedicines in SCD management.

## Introduction

Biochemically, SCD occurs due to a non- conservative substitution of a polar glutamate (Glu) by non-polar valine (Val) in an invariant region, the sixth position of Hb-β chain-subunit (GAG/GTG; Glu (E) [6] Val (V); rs334) [5-7]. Replacement of this single non-polar amino acid 'valine' results in a biochemical difference that leads to formation of a sticky patch on the surface of the β-chains. The sticky patch is observed on both the oxygenated ("R" Form) and deoxygenated ("T" Form) of HbS. This distort folding and binding pattern of the Hb molecule, due to altered properties. Other known mechanism of polymerization of Hb in SCD involves nucleation, which is due to the aggregation of HbS molecules. At low oxygen level (hypoxia), deoxyhemoglobin S polymerizes inside the red blood cells (RBC). This forms a network of polymers that stiffen and distort cells with rigid, misshape erythrocytes.

Due to oxygen deprivation in the RBC, a critical aggregate of Hb polymer is formed that damages the cellular membrane, promoting aggregation of cellular proteins, stopping the flow of blood in the narrow capillaries and leading to localized oxygen deprivation (anoxia) [5,7,8]. This polymerization is enhanced by the heterogeneous nature of the nucleus, where new polymers are continually formed on pre-existing polymer to form a fourteen inter-wined helical strands of HbS. In each molecule, one of the two β6 Valines of the α2 β2 tetramer is involved in an intermolecular contact with its neighbour in the double strand [7,8]. Formation of deoxy-HbS polymer makes the Hb insoluble, change the biconcave structure of the red blood cells and eventually cause cell lysis, which leads to the various clinical features of SCD [5].

| Hemoglobinopathies | A.A mutation | Origin | Homozygote phenotype |
|---|---|---|---|
| HbS | E>V | Africa, Middle East, India | deoxyHBS [5-7] polymerization, asplenia |
| HbC | E>K | Africa | Mild anemia, [5] splenomegaly |
| HbE | - | South Asia | Unstable, mild [5] hemolysis splenomegaly |
| Thalassemia | | | |
| Types | Origin | Homozygote phenotype [5] | |
| α | South Asia, Mediterranean, Africa | Hydrops fetalis, hemolytic [5] anemia, splenomegaly [5] | |
| β | South Asia, Mediterranean, Africa | Transfusion dependent [5] | |
| Keys: A.A= Amino Acids, E>V= Glutamate to Valine, E>K= Glutamate to Lysine | | | |

**Table 1:** Hemoglobinopathies with disease phenotypes.

It is known that amino acid variations have very large diverse effects affecting protein sequence, structure, stability, interactions, activity, abundance and other properties, such as shape, pKa and pH [7,9,10]. Hence, SCD, demonstrate the importance of amino acid sequence variations in determining structure, functions and, also a good pointer to the fact that, most gene variations usually consist of differences in a single amino acid residue, with variant called alleles. People with SCD are homozygous for the sickle cell allele [11,12] and have red blood cells containing mostly HbS, an abnormal type of Hb. Hemoglobin content of their blood is half the normal value of 15-16 g/100 mL. Sometimes these HbS red blood cells become sickle-shaped with difficulty passing through small blood vessels, such as capillaries (site of gaseous exchange), causing severe pains and interference with normal organ function which is a major crux in early death of people with the SCD phenotype [11].

Sickle cell crisis occur when sickled red blood cells block blood flow to any organ or tissue of the body leading to ischemic and hypoxic injury which manifest as severe and excruciating pain in the affected part of the body [6]. However, individuals with sickle cell allele from one parent are heterozygous thus, they have sickle cell trait. People with sickle cell trait (AS) have both Hb A and S produced in their red blood cells Figure 1, usually more A than S [5]. People with sickle cell trait are generally healthy, as they lead normal life although, their erythrocytes have shorter lifetime than normal.

## SCD Phenotype and Fetal Hemoglobin (HbF)

Genetic variation is a factor in SCD phenotype, as increasing numbers of genetic loci, modulating SCD phenotype have been identified. The variations include nucleotide motifs within the β-globin gene cluster, to distal genes located on different chromosomes [5,13].

Hemoglobin S polymerization is thought to be a major factor and driver of SCD pathophysiology [14], and HbF is an important variable and modulator of the clinical features of SCD. The presence of HbF decreases HbS fiber formation as they act in blocking the assembly of the long polymers of abnormal hemoglobin in a cell via dilution of HbS [5,14]. This dilution delays sickling as HbF do not significantly sickle within 10-20s, being time required to pass from the tissue to the lungs, where oxygenation will take place [5,14]. This suggests that, therapeutic measure targeted towards achieving high concentration of HbF in SCD patients via delayed hemoglobin switching adopted to favor non deletion variant of the hereditary persistence of HbF (HPFH) in cells, will prevent both HbS nucleation and polymerization hence, a worthy experimental option.

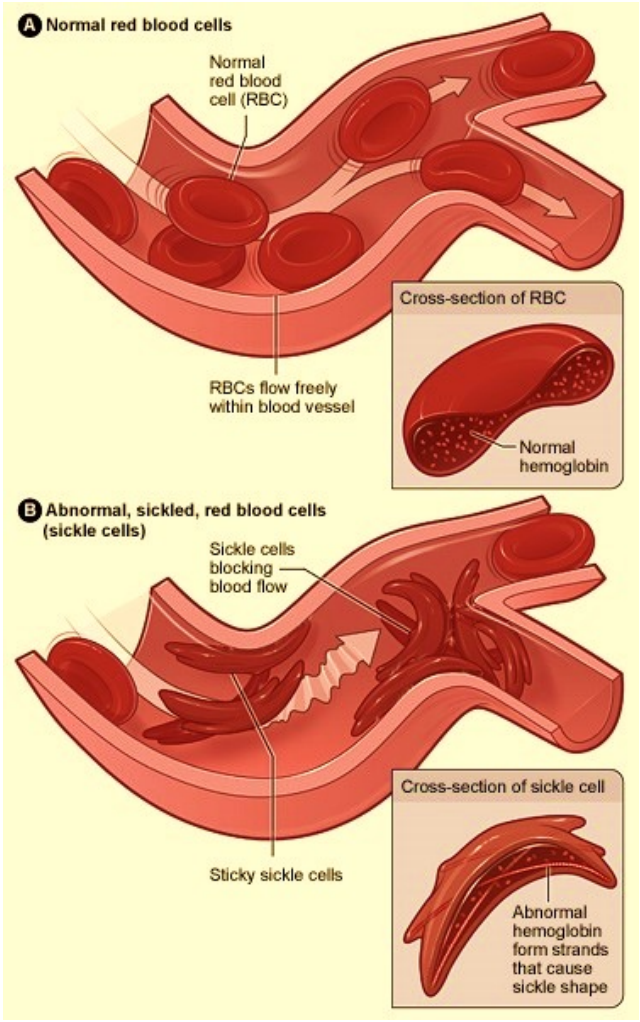


**Figure 1:** A shows normal red blood cells flowing freely in a blood vessel. The inset image shows a cross-section of a normal red blood cell with normal hemoglobin. B shows abnormal, sickled red blood cells blocking blood flow in a blood vessel. The inset image shows a cross-section of a sickle cell with abnormal (sickle) hemoglobin forming abnormal stiff rods. Source: **http://www.nhlbi.nih.gov/health/health-topics/topics/sca**.

Moreover, the relationship of HbF to SCD phenotype and complications had earlier been reported [5]. Studies showed [15,16] existence of 20-fold variation in HbF production amongst normal individuals and those with sickle-cell disease and, this is thought to be influence by factors such as; age, sex, alpha –globin gene number, beta -globin haplotype, and the F-cell production (FCP) locus that has been

mapped to Xp22.2. In studies of HbS subjects from Elsavador, Brazil, Jamaica and France, the FCP locus accounted for 40% of the variation in HbF [17]. HbF differs functionally from HbA because it has a higher oxygen affinity, explained by the low interaction of HbF with 2,3-bisphosphoglycerate (2,3-BPG). This HbF feature is an enabler, as it facilitates the binding of oxygen to hemoglobin, giving fetus easier access to oxygen from the mother's blood stream [16].

There exist three major quantitative trait loci (QTL), for SCD, (Xmn1-HBG2, HBS1L-MYB intergenic region on chromosome 6q23, and BCL11A on chromosome 2p16) and these loci are thought to account for 20–50% of the common variation in HbF levels in patients with SCD. Earlier study suggested independent segregation of HbF with Hb (HBB) containing different HbS mutation in a cluster-like haplotypes [14,15]. Hence, the HBB locus is known to be associated with HbF level. Further, observation of the different haplotypes indicates, the Arabian/Indian (AI) and Senegal haplotypes are associated with milder forms of SCD, whereas the Bantu haplotype is associated with a more severe clinical course [17-19]. SCD patient with AI haplotype have a higher HbF concentration in comparison with other haplotypes [5,17-19]. However, in SCD, HbF does not affect resultant disease phenotypes and complications equally. The failure in, the uniformity of, regulation/modulation, of complications in SCD are attributed to the pathophysiologic events impacting disease complications [17,19]. Also, variants with intermediate effects may be either deleterious or neutral depending on other distal effectors including environment. This had previously been demonstrated, where same variant, in monozygotic twins, causes different phenotype [20].

Due mainly to the complexity of SCD, with roles for both proximal and distal effectors in disease phenotypes, HbF concentrations affects different haplotypes differently [21]. Moreover, not all haplotypes had been studied with same frequency and tenacity [22] and, so upstream and downstream effectors on HbF and its characteristics effect on SCD are not well established. However, from AI haplotype patients, it is shown that increase of HbF keeps HbS more soluble in the deoxygenated state, hence carriers, seems to have mild, albeit no disease symptoms but with a frequent splenomegaly and osteonecrosis [23]. Further, the AI haplotype is characterized by the presence of both Xmn1, Hinc2 restriction endonuclease site and, insertion-deletion polymorphism [19]. Moreover, association between the XmnI polymorphism (rs7482144) in the proximal promoter of the Gα-globin (HBG2) gene and HbF levels is well established in SCD patients [24,25].

In SCD, epidemiological studies, suggested disease complications are mostly linked to sickle vaso-occlusion and blood viscosity and both characteristics, are robustly related to HbF concentration, and whilst, complications associated with the intensity of hemolysis were less HbF dependent [21].

Some other study on the AI haplotype, genetic association protocol [25,26] was used to pinpoint HbF variant (rs4671393) in an intron of the BCLIIA gene (B-cell lymphoma/leukemia IIA), a gene expressed in erythrocyte precursors and implicated in lymphoid malignancies [15,26,27], have the strongest effect bearing, its importance in the control switching of HbF to HbA. Further AI haplotype mapping had long revealed three other single nucleotide polymorphisms (SNPs) (rs28384513, rs9399137 and rs4895441) located in the intergenic region 5' to HBS1L and the MYB (family of transcription factors operational e.g. in neural crest differentiation) gene on chromosome 6q23-q24 [15]. With linkage disequilibrium, these SNPs were found distributed in three blocks referred to as HBS1L-MYB intergenic polymorphism (HMIP) 1, 2 and 3 [14,15]. Common variant within these three blocks had been shown to account for levels of fetal cell (FC) counts. This was mapped to the 6q23 locus, as seen in a European sample study [14,25-27]. However, LD patterns at this genomic region seem different between populations of different race (European and African), origin [26,28-31]. Using such findings, insights can be gained from populations of different race, replicate and refine genetic association signals [25,26,28]. This strategy was instrumental, for the identification of an additional SNP (rs4895441) with good predictive power for pain crisis combined with acute chest syndrome (ACS) rates in SCD [25]. The MYB gene is well studied for its role in hematopoiesis; however, HBS1L is a poorly characterized gene with yet unknown biological functions [26,28,31]. The mechanism on how the various SNPs influences HbF, remain largely unclear [19]. However, to increase HbF in circulation, BCL11A would, be a target, but as a transcriptional factor achieving this goal requires more ingenuity, such that BCL11A expression would only be limited in particular RBC and not in other hematopoietic lineages. Achieving this would require deletion of some specific erythroid enhancer [28]. The use of a zinc finger genome-editing technology that could knock out the erythroid enhancer of BCL11A in hematopoietic stem cell (HSC) of patients affected by SCD was thought of as a way forward (http://investor.sangamo.com/releasedetail.cfm?Release ID=818108).

The success of the human genome project, following genome wide association studies (GWAS), will provide insight to discovery of many more genetic modifiers of SCD. Many studies using GWAS, have mapped disease associated variants and showed for instance, that genetic variation contributes to the heritable risk of glioma [29] and, a novel genes responsible for telomere length regulator gene (TNKS) was identified [30,31], linking diversity with SCD heterogeneity. However, survival in sickle cell anaemia seems to be driven, mostly, by the adverse effects of the disease rather than longevity gene [5]. Such subtle genetic changes (revealed using GWAS), causing substantial difference in protein expression, immune variations with differences to our response mechanism to fighting the infection, form crux of a recent report [26,29]. This sort of study offers a new path to understand genotype-phenotype relationship in a more robust manner; advancing SCD patients care with treatment guided by genomic information.

The handling of immense amount of variation data generated by genome sequencing technologies and relating them to diseases remain a major challenge. Hence, the needs for combinations of genetic probing methods with bioinformatics tools, to predict single nucleotide variations (SNV), for effective rational drug design that would embrace not only synthetic drugs but also naturally occurring anti-sickling products, remain a way forward. In this light, it is heart-warming to welcome a recent consortium (http://www.humanvariomeproject.org/gg2020/index.html#h3-project-leaders) formed to harness developments in human genomics involving the systematic collection and sharing of variation data to fighting haemoglobinopathies especially, thalassemia's and sickle cell disease in low- and middle-income countries. Thus, understanding these corollary between HbF-associated variants on blood cell production and parameters that affects HbF in SCD will be important in shedding light on both SCD molecular mechanisms, etiology of the clinical heterogeneity, its control and defining biological roles for the development of targeted therapies including, phyto-medical therapy [32,33]. Moving forward the incorporation of phytomedicines to target pathways relevant in HbF up regulation and switching might present robust alternatives in the disease management.

## Treatment Options for SCD

Current therapies available for management of SCD are not curative except bone marrow transplantation (Hematopoietic stem cell transplantation).

### Bone marrow transplant

This therapeutic option is neither cheap nor available to most patients due to the lack of a Human Leukocyte Antigen (HLA)-matched bone marrow donor. The procedure was first carried out in 1984 to treat leukemia, accidentally it produced cure for sickle cell disease in the same patient as a side event of the transplantation. The procedure set the precedence for later transplantation efforts directed specifically at SCD. However the transplantation procedure requires a highly compatible family member as a donor and is associated with potentially serious complications. Disease-free survival rate is about 85%, transplant related mortality is 7% while graft failure rate is 9% [34]. The major challenges here is on controlling transgene expression as differentiation is stage restricted, difficult to sustain overtime and moreover, it is erythroid specific.

### Transgene

Using vectors of lenti-virus origin [35] transcriptional effectors with proximal and distal elements permits lineage-specific and elevated -globin expression *in vivo*. This experiment resulted in the production of therapeutic hemoglobin allowing correction of anemia in -thalassemia mice [10,36]. The coming of induced pluripotent stem cells (iPCS) fused with clustered regularly interspaced short palindromic repeats in presence of cas9 endonucleases (CRISPR/Cas9) could provide a veritable tool to engineer a double strand DNA break. Such technology used on wild type - globin gene was shown [10,36], to be suited for treating -thalassemia.

This genome editing technology is a tool that present a promising concept, to extend gene therapy technology by delivering healthy, and removing bad genes. As proof of principle, similar technology was in 2013, used as a correctional measure in patients with Diamond Black fan anemia (DBA) and in Juvenile myelomonocytic leukemia (JMML), which is a form of aggressive myeloproliferative neoplasm found in young children usually initiated by mutations that deregulate cytokine receptor signaling [37]. Patients with DBA on the other hand, develop this condition due to a mutation preventing the bone marrow from producing normal quantities of red blood cells, resulting in severe, sometimes life-threatening anemia [37,38].

In Nigeria, one of the first herbal formulations (Nicosan), produced by the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria, was, tested using transgenic mice with severe hypoxic conditions. Further analysis and efficacy test on SCD patients, using Nicosan showed effective sickling reversal [11,34,39,40].

## Preventive Therapy and Diagnostics

The largest burden of SCD in the world is in Nigeria, with an estimated 150,000 new born affected. A large percentage of the affected do not have access to required blood transfusion but instead rely on traditional phytomedicine to prevent sickling and alleviate painful crisis.

### New born screening

Early diagnosis of SCD in a child is essential in order to prevent complications. Screening programs for newborn involves analysis of blood from heel-prick with a fetal electrophoresis device. Babies found to have SCD are retested after 6 months to validate diagnosis. Children with SCD or sickle cell trait are continuously monitored and followed-up. Parents of affected children are counseled on how to manage the condition.

### Prenatal screening

SCD can also be diagnosed intrauterine using amniotic fluid or placenta tissue. Pre-natal testing could be done as early as 8-10 weeks gestational age, looking at the sickle Hb gene rather than abnormal Hb [31].

### Preventive treatment for SCD

Hydroxyurea became the first (and only) drug proven to prevent complications of SCD in the Multicenter Study of Hydroxyurea in Sickle Cell Anemia, which was completed in 1995 [14]. This treatment increases the amount of HbF in the blood. Increased HbF provides some protection against the effects of HbS, depending on distal variation characteristics. Hydroxyurea treatment reduces the number of vaso-occlusive crises and hospitalizations and also decreases the number of episodes of pain and dactylitis [41-44].

The exert mechanisms by which hydroxyurea induces HbF production are largely unclear, with available study mostly on thalassemia. Here, as one of the mechanism, a cytotoxic effect resulting in stress erythropoiesis, with increased HbF levels occurring as a result, had been proposed [35]. Following this also a more complex effects involving the production of nitric oxide and the soluble guanylyl cyclase and cyclic guanosine monophosphate dependent protein kinase pathway gene, had also been fingered as responsible for hydroxyurea activity [45-47]. In patients with thalassemia, hydroxyurea therapy exerts a 2- to 9-fold increase in mRNA expression, with a more-effective erythropoiesis [48]. Moreover, there seems to be a good correlation between *in vitro* mRNA fold increase and the *in vivo* HbF fold increase [49], although, increases in HbF level do not always correlate with increase in total hemoglobin level in clinical studies. We can only infer hydroxyurea exert same action in SCD patients following similar mechanism as, similar detailed molecular study for SCD seems lacking in the literature.

### Acute transfusion in SCD

Blood transfusion can be used to manage severe anemia that complicates SCD. It could also be beneficial in other SCD complications such as acute stroke, acute chest syndrome, multi-organ failure and surgical procedures on SCD patient.

### Chronic transfusion

Regular or chronic blood transfusion is recommended in a previous stroke to prevent re-occurrence. It is recommended for children with abnormal Trans-cranial Doppler (TCD) ultrasound to reduce the chance of having a first stroke. Chronic blood transfusion may be used in SCD individuals reacting to hydroxyurea. Putting these treatments in context, Table 2 provides a summary of some procedure available in Nigeria for SCD management.

| Category of treatment | Positive effects | Availability/disadvantages |
|---|---|---|
| Clinical | | |
| • Blood transfusion<br>• Bone marrow transplant | | Readily available<br>Not readily available and very expensive for a transition economy like Nigeria |
| Pharmacological | | |
| Hydroxyurea | Increase HbF (HbS antagonist).<br>Increase Nitric oxide inhalation<br>Decreases lipid peroxidation<br>Increase EGR-1 levels | Stimulate pro-inflammation gene. Require close monitoring [47] |
| Hydroxyfusudil | Reduces tissue inflammation | |
| Paludrine and penicillin | Antimalarial and antibacterial drugs, prevent opportunistic infection | |

**Table 2:** Summary of treatment options in SCD management.

## Genetic counseling

Couples who are planning to have children and know that they are at risk of having a child with SCD may want to meet with a genetics counselor. A genetics counselor can answer questions about the risk and explain the choices that are available.

## Medicinal plants used in treatment of sickle cell disease in Nigeria

Phytochemicals found in medicinal plants are known to exhibit pharmacological activity against different ailments and are believed to contain the active ingredients of the medicinal plants. These phytochemicals with varying concentration and distribution from one plant to another include alkaloids, terpenes, phenolic, tannins, saponins etc. Medicinal plants are being used in Nigeria by different communities for treatment of various ailments including SCD [50-53].

The use of medicinal plants for treating various diseases is probably the oldest method that mankind has used to cope with illness [2,54,55]. The effectiveness of medicinal plants as anti-sickling drug was demonstrated decades ago with root extract of *Fagara xanthoxyloides* [2].

Renewed interest in plants as a source of new anti-sickling agent was further stimulated, when Nicosan/Niprisan (a product of extract from four different plants) was reported to possess potent ant-sickling activity [35,36]. Clinical trials have shown that the drug significantly reduces the number of clinical episodes in SCD patients [35,36]. Extract from *Cassus populnea* root (major constituent of Jawaron/Jobelyn – a herbal hematinic used in the management of SCD in South west Nigeria, has been reported [56] to increase Hb, packed cell volume and white blood cell count which are characteristically lower in SCD patients. Other anti-sickling drugs derived from medicinal plants are Ciklavit (produce by Neimeth pharmaceuticals, Lagos), Diascovite and Solamin [56]. Although, synthetic approach to drug discovery has become the standard method of drug development, however, modern pharmacological practice has its origin in medicinal plants [56-58].

In light of the historic success of plants in chemotherapy coupled with the fact that most individuals affected by SCD in Nigeria, live in communities where phytomedicine and alternative medicines are used to fight this disease, and hence most plants with medicinal properties used in SCD management are thought to contain anti-sickling agents [59]. Most of the documented ant-sickling tests using medicinal plants were carried out as *in-vitro* studies; this basically involve collection of blood sample from diagnosed SCD patient and mixing specific quantity of blood with varying concentration/doses of extract prepared from medicinal plants. Anti-sickling effects are analyzed and monitored by observing the ability of the extract to reverse sickled erythrocytes (prevent/reverse polymerization) or prevent deformity of red blood cells [56,57,59]. The treatment of SCD is based on pathophysiology: inhibition of both HbS polymerization and cell dehydration, protection of sickle erythrocyte oxidative induced damage such as hyper-hemolysis, which is the most clinical manifestation of SCD [58-61].

## Some plants with anti-sickling properties used in SCD management in Nigeria

### *Carica papaya*

Dried leaves of *Carica papaya* has been reportedly used [62,63] for the treatment of SCD by local communities in Nigeria. *In vitro* anti-sickling activities of methanol leave extract of *Carica papaya*, carried out by [62-72] showed anti-sickling and membrane-stabilizing activities using p-hydroxybenzoic acid 5 µg/mL and normal saline as positive and negative controls respectively. The anti-sickling activity and osmotic fragility test was carried out on red blood cells obtained from non-crisis state sickle cell patients. The results from this study indicated that the plant extract protected erythrocyte membrane integrity and reduced hemolysis under osmotic stress conditions [70-72]. Pretreatment of SS cell suspensions with *Carica papaya* leaf extract was reported [68] to inhibit formation of sickle cells under severe hypoxia, with only 0–5% sickle cells formed at 40 mins compared with untreated SS cell suspensions which had over 60% sickle cells. The result from these studies is indicative of feasibility of *Carica papaya* as an attractive potential candidate for SCD management/treatment [70-72].

### *Cajanus cajan*

Study on *Cajanus cajan* seed [68] shows that it contains phenylalanine, carjaminose, and hydroxybenzoic acid as active constituents and are thought to be the reason for its anti-sickling effect. The aqueous methanol extract (3:1, v/v) of the seeds of *Cajanus cajan* investigated for anti-sickling properties [43,55,62] showed significant dose-dependent anti-sickling activity. The kinetics of reversal of pre-sickled erythrocyte (HbSS) cells using the extract at 0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL and 2.5 mg/mL showed first-order kinetics with rate constants of $5.833 \times 10^{-3}$ $min^{-1}$, $6.143 \times 10^{-3}$ $min^{-1}$, $5.957 \times 10^{-3}$ $min^{-1}$ $6.00 \times 10^{-3}$ $min^{-1}$ and $6.046 \times 10^{-3}$ $min^{-1}$, respectively [43,55,62].

### *Parquetina nigrescens*

*Parquetina nigrescens* has been reported to exhibit significant anti-sickling activity when administered at low dose, and protects the integrity of the erythrocyte membrane by reducing hemolysis of the HbS erythrocytes [68]. *In-vitro* studies on the anti-sickling activity of *P. nigrescens* extract carried out on blood samples of non-crisis sickle cell patients, showed that pretreatment of HbS cell suspension with the extract inhibited formation of sickle cells under severe hypoxia with only 5% sickle cells at 40 min compared with untreated SS cell suspensions which had over 65% sickle cells compared with the controls [3,39,68,73].

### *Trema orientalis*

Ethanol extract of *T. orientalis* containing mainly anthocyanin's was reported by [3,62-63], to strongly inhibit the sickling of drepanocytes induced by 2% sodium metabisulfite and also restores normal biconcave shape of erythrocytes at a dose of 2.5 mg/mL. The anthocyanin content of *T. orientalis* extracts, probably exert protective effect based on anti-oxidant properties by preventing oxidation and destruction of erythrocyte membrane lipids and Hb. Anthocyanin's are therefore seen as a potential anti-sickling agent for SCD treatment [27,43,44].

## Oxidative stress in SCD patho-biology

Sickle Hb molecules are known to undergo repeated polymerization/depolymerization with formation of greater amounts of reactive oxygen species ($O_2$-), which do lead to a reaction cascade such as blood cell adhesion, vaso-occlusion, ischemia reperfusion injury and hemolysis [74]. Oxidative stress challenges often arise from sources such as radiation, metabolism of xenobiotic, and challenges to the immune system due to abnormal functions [56,75]. From the forgone, SCD are linked to a patho-biological condition with multiple sources of pro-oxidant processes with its attendant consequence and, concomitant chronic and systemic oxidative stress [12,69]. For this reason, phytomedicines/herbal drugs that can target oxidative stress constitute a valuable means for preventing or delaying the development of organ complications [62-65].

As many as 200 human diseases, including sickle cell have association with increased levels of oxidative stress [12]. Oxidative stress, because it is tied to mitochondrial and the generation of the energy necessary to sustain life, occupies a place of central importance, even in SCD [12,76]. Moreover, reactive oxygen species are capable of disrupting nearly any metabolic pathway through their attack on proteins, lipids, and nucleic acids. This is so because, with severe oxidative stress necrosis, causes ATP depletion, preventing controlled apoptotic cell death [12,76].

Thus in an attempt at curbing havoc of free radicals, the use of plants, natural products are beneficial in protecting against oxidative stress-induced damage. They are less toxic compared to synthetic compounds used at their optimum protective dose levels [57,62-65]. Some plant extracts e.g. *Portulaca oleracea*, which we recently reported to treat hyperglycemia and improve glucose tolerance effect in an experimentally induced diabetic rats [69] are thought to exert their anti-oxidative effects, by bolstering insulin secretion via interference with the K+-ATPase channels, membrane depolarization and increase in Ca++ ions influx. Thus, the interests, has always existed in development of potential drug of plant origin, being a good source of potent but non-toxic anti-oxidant [68-69,76]. Antioxidants of plant origin include food supplements such as, vitamin E, C, selenium, phenolic compounds, carotenoids and flavonoids are, all supplements that have found their usefulness in SCD management in Nigeria. Earlier studies in the laboratory indicated that oral administration of carotene and plant extract of spinach, amaranths and linseed to Swiss albino mice protects various tissues against oxidative stress induced by radiation [62-65]. These extracts are thought to participate in biochemical processes and actions that include: activating metabolizing enzymes involved in carcinogenic detoxification; inhibiting reactive oxygen formation; blocking DNA adduct formation and in cell cycle regulation leading to apoptosis [32,53,76,77].

## Biochemical significance of phyto-medical management of SCD

The biochemical effects of plants extracts with medicinal value/properties, may lie in their ability to act early in impairing cell proliferation with a severe arrest and accumulation in G2/M [3]. These events are thought to culminate in the appearance of caspase-3 activation, phosphatidyl serine exposure and nuclear fragmentation, a typical hallmark of apoptotic onset [38,76,77].

Owing to the complexity of the SCD patho-physiology, there are few available drugs of plants origin with anti-oxidative properties on clinical trials; most are mainly in phase III trials, limiting the evaluation of the impact of antioxidant therapies on disease symptoms. However, phytomedicines are presents as supplements and form major components of anti-sickling agents, enabling reduction of oxidative stress that contributes to sickle cell crisis [78]. Moreover, *in vitro* studies, observations in animal models, and studies involving SCD patients have shown interesting results (Tables 3 and 4).

| Supplements | Benefits | Availability |
|---|---|---|
| Zinc | Food supplements, act as antioxidants | In-expensive and readily available in Nigeria [4] |
| Acetyl-L carnitine | | |
| Vit C | Decreases ROS production and increases glutathione concentration | |
| Vit E | Decreases lipid peroxidation and ROS production | |

**Table 3:** Supplements for SCD management.

A most recent study linked catalase deficiency with thymus atrophy due to elevated levels of $H_2O_2$, which is a hallmark of oxidative stress [38,54,56]. However, atrophy rate was diminished with antioxidant, a modulator of metabolism, providing, a clear linkage at the biochemical and mechanistic level between, metabolism, normal immune functions and antioxidants [38,77].

| Phytochemicals | Plant sources | Probable mode of action in management of SCD |
|---|---|---|
| Eugenol, capsaicin, and piperine | *E. caryophyllata* and *Piper guineense*. | Eugenol interacts with vanilloid receptors in order to produce analgesia and other physiochemical effects.<br>Capsaicin excites the nervous system into producing endorphins, which promote a pleasant sense of well-being.<br>Prolonged activation of the TPRV-1 neurons by capsaicin leads to a depletion of presynaptic substance P (a neurotransmitter for pain). Neurons lacking TRPV1 are unaffected by capsaicin [59,60] |
| B-caryophyllene (Cannabinoids) | Constituent of *Piper guineense* | Binds selectively to cannabinoids receptor type-2, which is a major receptor in pain control [3,61-62]. |
| Three isomeric divanilloylquinic acids (burkinabin A, burkinabin B, and burkinabin C)<br>coumarins, vanillic acid, parahydroxybenzoic acid | *Fagara zanthoxyloides* (root) | Increase resistance of erythrocytes to osmotic stress and haemolysis [62-66]. |
| Phenylalanine is thought to be the most active principle in *Cajanus cajan* seed—a component of Ciklavit antisickling phytomedicine, | *Cajanus cajan* (seed) | Antigelling effects of phenyalanine involves decrease gelation of HbS molecules during de-oxygenation [55,65]. |
| Limonoids | *Khaya senegalensis* (stem bark/leaf) | |
| Anthocyanins | *Hymenocardia acida* (leaf) and *Trema orientalis* | Inhibitory effects on aggregation of deoxy-HbS.<br>Inhibitory effects on the sickling of RBCs by scavenging intracellular free radicals [2,3] |
| Allicin | *Garlic* (bulb) | Potent stimulus of TRPV1, a subfamily of transient receptor potential vanilloid (TRPV) group of channels has 6 subfamilies [64,66] |
| 5,7-Dimethoxycoumarin, kempferol, and quercetin | *Carica papaya leaf extract* | Membrane stabilization and protection of membrane integrity under osmotic stress [33-34,39,68] |

**Table 4:** Plants with phytochemical properties for SCD management.

## Future Perspectives and Conclusions

Moving forward, it is safe to argue, that the use of these plants with medicinal value in the management of SCD and other related ailments would continue to be a major part of the health care delivery system in Nigeria and many parts of the world. Hence, the interests in natural products chemistry should be viewed from the growing awareness that many of the secondary metabolites of living things serve important medicinal roles, with many acting majorly as chemical messengers and defensive compounds [78,79]. As such their remains an ample need, for further studies on these plant-extracts as modulators of cytokines being effectors for some SCD complications. This is important in light of recent findings that showed receptor-interacting serine/threonine-protein kinase-3 (RIPK3) enzyme relays signals between the mitochondria "cell's power-houses" and the immune system [80]. It is likely that RIPK3 are involved in natural killer T cells (NKTs) regulation, and these cells play dual roles in autoimmune disease and in cancerous cells destruction [80]. Clearly, this cross talk as observed is important for both lunching of immune responses against tumors and also for regulating inflammatory responses that might come up due to autoimmune diseases. As SCD is propagated via loss of balance in immune response regulation with severe inflammatory disorders as fall out, it may be important to investigate (if any), the presence of "such orphan" molecules with RIPK3-like activity that could be modulated or suppressed using some of the plant extracts and study pathways interference, necessary for inflammatory regulation in SCD.

Several transcription factors regulate the expression of the β globin gene [30]. However, research in this area suggests activation of the globin genes are dependent on the coordination of the locus control region (LCR) in presence of some transcription factors including; GATA binding protein 1 (GATA1), friend of GATA1 (FOG1), BCLIIA, Krueppel-like factor 1 (KLF1) and LIM domain binding protein (LDB1). These transcriptional factors represent the most vital proteins for proper globin gene switching and activation [30,78,79]. The knockdown experiment of BCLIIA in human erythroblast saw increased expression of HbF [30,78,79]. Moreover, induction of HbF via inactivation of analogous gene in mice had earlier been demonstrated [78], implying BCL11A transcription factor when properly modulated could be of significant value in SCD and related hemoglobinopathies management. Thus twinkling of such transcription effectors/gene modulator as BCL11A with for instance phytomedicines e.g. Eugenol, capsaicin, and piperine that have many characteristics attributes (Table 4), may further help in SCD management. Similarly, KLF1 (an erythroid specific transcription factor) associated with globin expression and erythropoiesis with

influence in the switching of HbF to HbA [74,78,79] can be targeted in a holistic manner, encompassing medicinal plants that could be useful in the delay switching process. We think this is an important concept, considering the fact that reduced expression of KLF1 in human erythroblast cell line is linked to viability and differentiation [30], which ameliorates – thalassemia and SCD phenotypic complications [45].

Thus, globin gene expression and its pattern, remain a priority area requiring more investigation. For instance, the dynamics of HbF switching to HbA is an important phenomenon that needs scientific clarity. This singular process provides a model for understanding gene expression and its modulation at the molecular level [45-48]. Dissecting these processes hold the key to the information for developments of holistic approach for sustaining HbF activation in SCD. Most transcription factors already identified to be important in SCD are modulators that are involved in either up-regulation or down regulation of HbF, but as discussed (see above), it is known, HbF does not affects all SCD phenotype equally. Hence, proper study of this switching with harnessing of phytomedicines as a constituent of holistic SCD phenotype management in these regard, can and should be part of the way forward.

A recent review [30], which covers in depth erythropoiesis and iron metabolism in anemia and hypoxia, brings to the forefront the necessary pathways, effectors and modulators, such as; transcription factors, macrophages, protein tyrosine kinases, adhesion molecules, progenitor molecules etc. Under certain conditions, when level of 2,3-BPG is increased due to SCD complications, both erythropoiesis and iron absorption are consequently increased. This is due to the combinations of actions of elevated Epo, activities of duodenal divalent mental transporter 1 (DMmt1), duodenum cytochrome B (DcytB) and ferroportin (Fpn); iron exporter expressed mainly in enterocytes, macrophages and hepatocytes [30]. However, in erythropoiesis and iron metabolism, some epi-genes such as; hypoxia inducible factor-2a (Hif2a), iron receptor protein 1 (Irp1) plays important regulatory role [30]. Previous evidence shows Hif2a to accelerate red blood cells (RBC) response (to conditions that arose due to low oxygen tension leading to reduce oxy-hemoglobin) via increase in Epo synthesis [30]. Managing SCD using multifaceted approaches, information of these natures is valuable in testing the hypothesis that; plants with phytomedical value can further aid RBC against hypoxia. Especially where experiments are designed to accelerate different stages (proerythroblast, basophilic, polychromatic, orthochromatic erythroblasts, reticulocytes and RBC) of erythropoietin differentiation [15,30,54]. When such are used to modulate molecular iron accessibility and its metabolism, particularly if same can also target transferrin receptor 2 (Tfr2) and high ferrous iron (Hfe), bearing in mind that recent study indicates Tfr2 to adjust erythrocyte production according to iron availability and Hfe is implicated in modulating iron homeostasis [15,30]. Such a process could alter levels of other key molecules within this pathway and can potentially slow down SCD complications. This review focused on use of phytomedicines to ameliorate SCD crisis in Nigeria and how probable target genetic loci/pathways could be manipulated for effective and enhanced management of SCD in both resource poor and rich environment. Conclusively, these types of studies, systematically carried out, could form the basis for future direction for the management of persons with SCD and other types of hemoglobinopathies.

## Conflicts of Interests

We declare that there are no conflicts of interests.

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