

RESEARCH ETHICAL REVIEW APPLICATION

TITLE OF RESEARCH WORK: The Emerging Role of Aloe Vera Extracts in The Management of Sickle Cell Disease (SCD): A study of Patients in Government hospitals in Abuja-FCT, Nigeria.

INTRODUCTION/BACKGROUND & LITERATURE REVIEW:

INTRODUCTION/BACKGROUND

Sickle cell disease (SCD) is a quadrumvirate of anemia and its sequelae, pain syndromes, organ damage including infection, and comorbid conditions (Embury *et al.*, 1994; Serjeant, 2001; Ballas, 1998; Benjamin, 2001).

Sickle cell anemia is prevalent in Africa, the Middle East, and parts of India. It is common in geographical areas where malaria is widespread. Hemoglobin in most individuals is present in soluble form. However, in sickle cell disease, hemoglobin precipitates as insoluble crystals, which lead to an abnormal shape and size of RBCs with subsequent phagocytosis of the effected corpuscles (Porter, 2018; Badawy *et al.*, 2018). In sickle cell disease, the combination of ongoing haemolysis (rupture of rbc), blood vessel blockage, frequent infections, and the body's attempt to deal with these conditions (via the immune system's inflammatory response) all combine to create an internal environment, which is toxic.

The choice of natural products for management of different conditions is increasing worldwide, due to the effectiveness, affordability and fewer side effects of such treatments. Aloe vera is one of the most popular medicinal plants, widely used for the prevention or

treatment of skin diseases, metabolic diseases, cardiovascular diseases and cancers throughout the world.

It has been used in traditional and folk medicines for thousands of years to treat and cure a variety of diseases. Although the plant is native to northern parts of Africa, it has rapidly spread across the world because its cultivation is easy. The plant has been used by Egyptians, Assyrians, and Mediterranean civilizations, as well as in Biblical times. A variety of aloe species are still used in folk medicines of Africa and Asia. Hunters in the Congo reportedly rub their bodies in the clear mucilaginous gel to reduce perspiration; some African tribes apply the gel for chronic conjunctivitis; the gel is used in India for the treatment of asthma (Morton, 1961).

We propose to examine the role of the aloe vera leaf extract – acemannan – in improving the quality of life and reducing complications of sickle cell disease patients, with a view to establishing it as an adjunct in the management of sickle cell disease.

LITERATURE REVIEW

SCD is a chronic haemolytic disorder that is marked by tendency of haemoglobin molecules within red cells to polymerise and deform the red cell into a sickle (or crescent) shape resulting in characteristic vasoocclusive events and accelerated haemolysis. It is inherited in an autosomal recessive fashion in either the homozygous state or double heterozygous state. When inherited in the homozygous state, it is termed sickle cell anaemia (SCA). Other known SCD genotypes include haemoglobin SC disease, sickle beta plus thalassaemia, and sickle beta zero thalassaemia (which has similar severity with sickle cell anaemia), haemoglobin SD Punjab disease, haemoglobin SO Arab disease, and others (Adewoyin, 2015).

About 5–7% of the global population carries an abnormal haemoglobin gene (Modell and Darlison, 2008). The most predominant form of haemoglobinopathy worldwide is sickle cell disease. The greatest burden of the disease lies in sub-Saharan Africa and Asia (Piel *et al.*, 2013). The prevalence of sickle cell trait ranges between 10 and 45% in various parts of sub-Saharan Africa (Serjeant and Serjeant, 2001). In Nigeria, carrier prevalence is about 20 to 30% (Fleming *et al.*, 1979). SCD affects about 2 to 3% of the Nigerian population of more than 160 million. Recent estimate from a large retrospective study by Nwogoh *et al* (2012) in Benin City, South-South Nigeria revealed an SCD prevalence of 2.39% and a carrier rate of about 23%.

Pathophysiology and Complications of SCD

Sickle-cell anemia is the result of a mutation of the β -globin gene. Sickle β -globin chains differ from the normal in that valine is substituted for glutamic acid at the number six position. This structural alteration, which involves only one of the 146 amino acids that form the β -globin chain, allows the polymerization and gelation of HbS on deoxygenation. Formation of long fibers of deoxygenated S is responsible for the distortion of the normally discoid red cell into a sickle cell, and the sickling of erythrocytes is, in turn, responsible for the myriad clinical manifestations of sickle-cell anemia (Mentzer and Wang, 1980).

In recent years, two major phenotypes of SCD have been recognized: recurrent vaso-occlusion on one end and hemolysis on the other. While the different models of gelation and rheological abnormalities would explain the former, they do not adequately explain the chronic vasculopathy that is the hallmark of the disorder. It is now recognized that SCD is a chronic inflammatory state with extensive oxidative stress involving several enzyme systems and ischemia/reperfusion injury, with nitric oxide (NO) dysregulation playing a

central role. Another model of SCD pathophysiology has therefore been proposed that involves the interaction of hemolysis and the endothelium (Kato *et al.*, 2009)

Although oxygenated blood from patients with sickle-cell disease has normal viscosity, deoxygenation, with resultant sickling, leads to a large increase in whole-blood viscosity, because of the increased rigidity of the sickled red cell. Such rigidity also impairs passage of individual sickled red cells through the microcirculation. The ensuing reduction in blood flow may lead to regional infarction - the so-called vaso-occlusive crisis. Although the presence of sickled red cells is regarded to be of paramount importance in the initiation of a vaso-occlusive crisis, abnormal interactions between such cells and the vascular endothelium or the coagulation system have recently been suggested as additional contributing factors. The increased mechanical fragility of sickled red cells is thought to be responsible for the other major clinical manifestation of sickle-cell anemia - chronic hemolytic anemia. Sickling is usually reversible on reoxygenation of sickled erythrocytes. However, after repeated cycles of sickling, some cells may become irreversibly sickled and fail to regain their normal shape, even after oxygenation. Irreversibly sickled cells make up 0-40 percent of the circulating red cells in patients with sickle-cell anemia and are the sickle cells noted on the air-dried peripheral blood smear. These cells have an unusually short survival and contribute to the hemolytic anemia of sickle-cell disease. Their possible role in the initiation of painful crises has been suspected but never defined (Mentzer and Wang, 1980).

While Hb is safely packaged within the plasma membrane of the RBC, its release, following hemolysis, sets off an intense inflammatory response with eventual NO depletion. NO is a critical endogenous vasodilator synthesized by endothelial cells from its

obligate substrate L-arginine, which is converted to citrulline by NO synthases. SCD is characterized by a state of NO resistance, inactivation and impaired availability with consequent vasoconstriction, platelet activation, up-regulation of adhesion molecules, thrombin generation and endothelial intima proliferation culminating in arterial stenosis and eventual occlusion, with dire consequences (Morris, 2011). The release of arginase from the lysed RBCs reduces available arginine by redirecting its metabolism to ornithine (instead of citrulline and NO), with the formation of polyamines and proline, which promote smooth muscle proliferation and collagen synthesis (Durante *et al.*, 2007)

The subphenotypes of SCD which are directly attributable to hemolysis and endothelial dysfunction include pulmonary hypertension, stroke, priapism and leg ulcers. The biomarkers of this process include free plasma hemoglobin, arginase, reticulocyte count, serum lactate dehydrogenase and bilirubin. The risk of pulmonary hypertension, i.e. a tricuspid regurgitant velocity of ≥ 2.5 m/s, is currently the strongest predictor of early death in adult SCD patients (Gladwin *et al.*, 2004).

Aloe Vera

Aloe barbadensis Miller, commonly referred to as Aloe vera, is one of more than 400 species of Aloe belonging to family Liliaceae that originated in South Africa, but have been indigenous to dry subtropical and tropical climates, including the southern United States of America (USA) (Reynolds and Dweck, 1999). Aloe vera is a succulent plant with thick, fleshy, serrated, lanceolate-shaped leaves of green-greyish color. Aloe vera inner gel is obtained from the lower leaves of the plant by slicing the leaf open. Numerous studies have reported that aloe leaf possesses numerous functions, which are attributed to the presence

of polysaccharides, such as immunomodulation, antimicrobial, antiviral, anti-cancer, anti-inflammatory properties (Liu *et al.*, 2019).

The phytochemical and anti-sickling properties of the leaf and gel extracts of the aloe vera plant were studied. The phytochemical composition of the gel and leaf extracts revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols at various concentrations. The determination of anti-sickling effects of these extracts was directed towards the inhibition of sickle cell polymerization and improvement of Fe²⁺/Fe³⁺ ratio of Sickle hemoglobin (HbS) in the presence of the extracts (Nwaoguikpe *et al.*, 2010). A study done by Ejele and Alinnor (2008) showed that various metabolites of aloe vera inhibited sickling invitro, thus increasing the number of unsickled erythrocytes, thus suggesting that the reversal of the sickling phenomenon could be achieved.

As stated by Surjushe *et al.*, (2008), aloe vera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids, important for performing all its actions. Many of these actions include antioxidant functions (with vitamins A, C and E, minerals like magnesium, hormones like gibberellins), anti-inflammatory functions (with bradykinase), analgesic and antiseptic functions, and immune modulation (with the glucomannans; notable example is acemannan).

Acemannan: Acemannan, a β -(1,4)-acetylated soluble polymannose, is the major bioactive polysaccharide of Aloe vera, from which gel and skin it is extracted (Sierra-Garcia *et al.*, 2014). Acemannan is a kind of storage polysaccharide, an acetylated glucomannan, which is located in the protoplasts of parenchyma cells that contain many polysaccharides in the cell wall matrix (Femenia *et al.*, 1999).

Of the immune functions of aloe vera, acemannan appears to play a big role, due to its activation of immunomodulation. A large number of in vitro and in vivo studies have confirmed the immunomodulatory activity of acemannan on splenic lymphocyte, macrophage and dendritic cells.

Acemannan, an important immunoenhancer, can enhance the lymphocyte response to alloantigen. In addition, the mechanism may be related to the release of IL-1 from the ordered nuclear cells under the protection of alloantigen (Christaki and Florou-Paneri, 2010). The spleen, which combines the adaptive immune system and the innate immune system, is the largest secondary immune organ of the body (Cesta, 2006).

Acemannan could upregulate the cytokines like TNF- α and IL-1 and improve hematopoiesis, such as peripheral lymphocytes counts, spleen cellularity and spleen index. What's more, acemannan has a greater stimulatory activity for white blood cell (WBC) counts and spleen cellularity as well as on the absolute numbers of lymphocytes, neutrophils, monocytes in irradiation-induced myelosuppression mice, as stated by Egger *et al.*, (1996). Macrophages are an important part of the monocyte macrophage system and have a variety of functions in the immune response. Dendritic cells (DCs) initiate and regulate highly pathogenic and specific adaptive immune responses, which are the core of the development of immune memory and tolerance. Studies found that acemannan promoted nonspecific immunity, cellular immunity and humoral immunity (Liu *et al.*, 2019). Lots of in vitro and in vivo experiments have indicated that acemannan has scavenging effects on free radicals (Bozzi *et al.*, 2007). The chelating activity and reduction ability of iron ion were verified by further studies. The cellular studies have showed that

the polysaccharides could inhibit the production of reactive oxygen (ROS), thus reducing the damage caused by oxidative stress (Salah *et al.*, 2017).

It was found that acemannan could stimulate macrophage cytokine production, nitric oxide release, surface molecule expression, and cell morphologic changes. The production of the cytokines IL-6 and TNF-alpha were dependent on the dose of acemannan provided. Nitric oxide production, cell morphologic changes and surface antigen expression were increased in response to stimulation by a mixture of acemannan and IFN-gamma (Zhang and Tizard, 1996).

These results suggest that acemannan may function, at least in part, through macrophage activation, regulation of hematopoiesis, regulation of reactive oxygen species and nitric oxide production, which are needed in the management of sickle cell disease.

STATEMENT OF PROBLEM: 50-70% of all children born with sickle cell disease in Nigeria will not live to see their fifth birthday because of death from complications of the disease. Current drugs available (blood tonics, hydroxyurea, pain relievers, antibiotics and antimalarials) alone are not efficacious enough, and doctors are thus limited in their treatment of the disease. Moreover, people in remote areas where there is little to no access to good health care tend to have more crises and complications. What is needed is a safe approach to treating sickle cell disease patients in remote as well as in urban areas. We are therefore looking at administration of the active ingredient of aloe vera, called acemannan as a possible, safe and cost-effective method for the treatment of sickle cell disease in Nigeria and other African countries.

PURPOSE OF RESEARCH

We are building on the existing knowledge of acemannan to examine its effect in children with sickle cell disease, who typically have ischemia, immune deficiency and reduced bone density. We wish to explore the possibility of using this very safe aloe vera extract as a possible adjunct in the treatment of this dreaded condition.

AIMS/OBJECTIVES OF RESEARCH

Aim: To examine the role of the aloe vera leaf extract – acemannan – in improving the quality of life and reducing complications of sickle cell disease patients.

Objectives: To

1. Determine specific doses of Acemannan required to improve FBC and other parameters in patients with sickle cell disease.
2. Determine doses of Acemannan required to reduce frequency and severity of crises in children aged 6 months - 12 years by at least 50%.
3. Compare the perceived quality of life between sickle cell disease patients on current drugs available (blood tonics, hydroxyurea, pain relievers, antibiotics and antimalarials), to sickle cell disease patients on both the current drugs available and Acemannan supplements.

METHODOLOGY OF THE STUDY

Study Design: This is a randomized trial of sickle cell disease patients on regular medication for the disease. 300 patients attending three specific government owned clinics, 100 from each, during the period of recruitment, with a genotype test result indicating HBSS/HBSC, and consent to participate, will be included in the study.

Study population: The study population will consist of male and female children aged 6 months to 12 years, who are sickle cell disease patients (with the HBSS/HBSC genotype).

Exclusion Criteria: Participants must not be less than 6 months or 12 years of age and must not have complications like leg ulcers, strokes, and avascular necrosis of the head of the femur.

Sampling Technique: Participants will be selected from all cases that meet the minimum criteria for patients with sickle cell disease, having moderate to severe crises, until a 100 patients with SCD are recruited. This will be done in the three government hospitals.

Participants: Consenting patients with sickle cell disease will be recruited from the outpatient clinics of these hospitals after the study has been explained to the potential participants. The principal investigator and coinvestigators will confirm all diagnosis using information obtained from patient registration and intake forms, along with clinical history, existing case notes, laboratory tests and interviews with the patients.

Study Tools: They include:

1. An intake form that will record several parameters like: crises frequency, duration and severity; presence of pains in select parts of the body like chest, abdomen, joints and general bone pain; full blood count; weight, height and BMI; body temperature; pulse rate; pallor; oxygen saturation; and abdominal examination (for organ enlargement).
2. Follow-up forms, to record the same parameters as stated above and the intervention administered.

Intervention: The polysaccharide-based multinutrient formula to be used in the study is an aloe polymannose multinutrient complex (APMC) called acemannan, with trade name “Immun,” which will be administered orally. A nutritional supplement has been sold by several commercial entities for over 20 years, and has been used on sickle cell disease

beneficiaries in the foundation before, with no observable adverse effect. According to the company's literature (www.alovea.com), the APMC contains:

1. 200-milligram of certified organic aloe vera inner leaf gel powder (Alovéa AE™)
2. Stabilized rice bran, which is an extremely powerful source of vitamins and minerals, essential amino acids, omega-3 fats, CoQ10, complete protein and fiber, and contains more antioxidants
3. Sunflower lecithin, that is rich in choline and other essential fatty acids such as phosphatidylinositol, and
4. Vegetable Capsule

The final product is a powder in capsules, packaged in 34-gram containers with 56 capsules inside, which dissolves readily in any liquid.

50% of the participants will consume the routine medication alone, while the other 50% will consume one capsule of the APMC orally, two times per day – along with the APMC.

We will show the primary caregiver how to administer the APMC at baseline assessment and the participant will take the first dose at the facilities to ensure compliance with the method, and to monitor for any complications or adverse effects.

PROCEDURE FOR DATA COLLECTION

We will collect sociodemographic and clinical data from subjects using a case record form (patient intake form). The case record form will include age, sex, and family history, weight, full blood count (FBC) containing the packed cell volume (PCV), last crisis, crises frequency and severity, level of pain experienced during each crisis. Data will also be collected on subsequent visits to the hospital using sickle cell follow-up forms. The forms will include any change in crises frequency and severity, weight and recent full blood

count. One research assistant will extract sociodemographic and clinical data in each hospital into a research spreadsheet for analysis. The principal investigator will train the assistants, and coinvestigators will interview the participants.

QUALITY ASSURANCE (PROCEDURE TO ENSURE THE QUALITY OF DATA)

Qualified personnel will administer the therapies and collect blood samples for FBC. The information gotten will be confidentially kept, with no unauthorized personnel having access to it. We will also engage in repeat questioning for reliability testing and data entry will be done using Microsoft Excel.

Descriptive statistics such as mean and standard deviation will be used to summarize quantitative variables such as weight, packed cell volume, while frequencies and proportions will be used for qualitative variables.

METHOD OF OBTAINING INFORMED CONSENT WHERE RELEVANT

Research participants will at all times be fully informed about the research process and purposes, and must give assent to their participation in the research before being allowed to participate in the research.

SUMMARY OF ETHICAL ISSUES INVOLVED IN THE RESEARCH

Voluntary participation in research: The participants may withdraw from the research at any time.

Informed consent: Research participants' parents or guardians will at all times be fully informed about the research process and purposes, and must give consent to their participation in the research before patients are allowed to participate in the research.

Safety in participation: The subjects will not be placed at a risk or harm of any kind.

Confidentiality: Confidentiality and anonymity of subjects will be protected at all times.

Trust: Participants will not be subjected to any acts of deception or betrayal in the research process or its published outcomes.

CONSEQUENCES OF THE STUDY FOR LOCAL COMMUNITY, ENVIRONMENT AND PARTICIPANTS

The results of this study will provide some insights on sickle cell disease management that can be applied in Nigeria to design successful health care financing strategies, planning, drawing up policy on the care of sickle cell patients in Nigeria.

RESULTS OF EARLIER RELATED STUDIES

Immunomodulation Studies: Zhang *et al.* (1996) demonstrated that acemannan, in combination with interferon gamma, has an effect on the release of nitric oxide, interleukin-6 and tumor necrosis factor- α by macrophages.

Antiviral and Antimicrobial Activity: Azghani *et al.*, in 1995 measured the adherence of several different strains of *Pseudomonas aeruginosa* to human lung epithelial cells and compared the effects in the presence and absence of acemannan. Addition of acemannan to the mixture of radiolabelled bacteria and cells inhibited bacterial binding to the lung cells, but the experiments indicate that acemannan is a relatively non-specific inhibitor of adherence and the effect is dissimilar to the mannose-containing receptor on bacteria or host cells.

Peritoneal macrophages, when treated with acemannan *in vitro*, increased the expression of major histocompatibility complex II (MHC II) and enhanced endocytosis, phagocytosis, nitric oxide production, tumor necrosis factor- α (TNF- α) secretion (Liu *et al.*, 2006).

Toxicology Studies:

Review Expert Panel found that acemannan has no significant toxicity in mice, rats or dogs at maximum dose levels of 200, 50, and 50 mg/kg, respectively, administered intraperitoneally and intravenously at 4 day intervals over 30 days. In the diet of dog, doses of 1500 mg/kg a day, and doses up to 2000 mg/kg a day in mice had no observable toxic effect (Sierra-Garcia *et al.*, 2014). Jettanacheawchankit *et al* in 2019, affirmed that acemannan at 16 mg/mL (the maximum concentration they used), was not toxic to gingival fibroblasts. These data corresponded with the study of Fogleman *et al*, in 1992 that reported no significant toxicity in oral administration of acemannan to rats for 14 days and to dogs for 90 days (at up to 1500 mg/kg per day).

<https://www.ajol.info/index.php/ijonas/article/view/76833>

https://www.researchgate.net/publication/326462642_Medicinal_plants_used_in_the_treatment_of_sickle_cell_disease_in_Western_Africa

Conclusion on safety: We have used this supplement for some of our beneficiaries at the Foundation for about four years, and have seen no adverse effects from its administration. Notable examples are TS and OS, who are 21 and 23 years old respectively.

PLANS FOR THE DISSEMINATION OF RESULTS

Results will be shared with regulatory health organisations, other research institutions in both the public and private sector for the purpose of implementation and working together to develop better interventions.

CONFIDENTIALITY & PRIVACY (STEPS TO ENSURE THAT)

Patients will be reassured of their safety, privacy and confidentiality. The information gotten will be confidentially kept, with no unauthorised access to information. We will protect the anonymity of the subjects at all times.

RESPONSIBILITY AND LITIGATION

The consent form would state that although no therapy is without its risks, there are no known risks involved in the use of acemannan in animals, or in humans. However, in the event of an adverse effect related to administration of the therapy, the Brain and Body Foundation will bear full responsibility, and will incur any additional medical expenses associated with treatment.

COST AND SOURCES OF FUNDING FOR RESEARCH

The research will be self-funded.

Category	Monthly (NGN)
Supplies (Specific Nutrients)	1,536,000
Standard therapy/routine drugs (folic acid and paludrine)	340,000
Lab tests	200,000
Stipend for Patients	200,000
Collaborating Medical Personnel	1,056,000
- Head pediatrician	- 576,000
- Pediatric nurse and	- 288,000
- Nurse assistant	- 192,000
BBF Personnel (will serve the three hospitals)	2,304,000
- Lead Researcher	- 960,000
- Project Manager	- 576,000
- Research Assistant 1	- 384,000

- Research Assistant 2	- 384,000
Transportation	95,000
Other expenses	50,000
- Stationery	
- Equipment	
Grand Total	5,781,000

The table below shows the monthly expenditure for 100 patients in one Hospital.

Monthly budget for the three hospitals (including BBF staff that will serve all three hospitals) = N 12,735,000

Grand total expenditure for three hospitals in 6 months is N 76,410,000.

REFERENCES

- Adewoyin, A.S. (2015). Management of sickle cell disease: a review for physician education in Nigeria (sub-saharan Africa). *Anemia*. 2015:791498.
- Azghani, A.O., Williams, I., Holiday, D.B. and Johnson, A. (1995). A beta-linked mannan inhibits adherence of *Pseudomonas aeruginosa* to human lung epithelial cells. *Glycobiology*. 5:39-44.
- Badawy, S.M., Cronin, R.M., Hankins, J., Crosby, L., DeBaun, M., Thompson, A.A. and Shah, N. (2018). Patient-Centered eHealth Interventions for Children, Adolescents, and Adults with Sickle Cell Disease: Systematic Review. *Journal of Medical Internet Research*. 20(7):e10940.
- Ballas, S.K. (1998). Progress in Pain Research and Management. Vol. 11. Seattle, WA: IASP Press; Sickle Cell Pain.
- Benjamin, L.J. (2001). Nature and treatment of the acute painful episode in sickle cell disease. In: Steinberg MH, et al., editors. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. Cambridge. pp. 671–710.
- Bozzi, A., Perrin, C., Austin, S. and Vera, F.A. (2007). Quality and authenticity of commercial Aloe vera gel powders. *Food Chemistry*. 103:22–30.
- Christaki, E.V., Florou-Paneri, P.C. (2010) Aloe vera: A plant for many uses. *Journal of Food, Agriculture and Environment*. 8:245–249.
- Cesta, M.F. (2016). Normal structure, function, and histology of the spleen. *Journal of Toxicologic Pathology*. 34(5):455-465.

- Durante, W., Johnson, F.K. and Johnson, R.A. (2007). Arginase: a critical regulator of nitric oxide synthesis and vascular function. *Clinical and Experimental Pharmacology and Physiology*. 34:906-911.
- Embury, S.H., Hebbel, R.P., Mohandas, N. and Steinberg, M.H. (2010). editors. Basic Principles and Clinical Picture. Raven Press (New York). Sickle cell disease. pp. 311–311.
- Femenia, A., Sanchez, E.S., Simal, S. and Rossello, C. (1999). Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis* Miller) plant tissues. *Carbohydrate Polymers*. 39:109–117.
- Fleming, A.F., Storey, J., Molineaux, L., Iroko, E.A. and Attai, E.D. (1979). Abnormal haemoglobins in the Sudan savanna of Nigeria. I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria and survival. *Annals of Tropical Medicine & Parasitology*. 73(2):161-172.
- Fogleman, R.W., Shellenberger, T.E., Balmer, M.F., Carpenter, R.H. and McAnalley, B.H. (1992) Subchronic oral administration of acemannan in the rat and dog. *Veterinary and Human Toxicology*, 34, 144–147.
- Gladwin, M.T., Sachdev, V., Jison, M.L., Shizukuda, Y., Plehn, J.F., Minter, K., Brown, B., Coles, W.A., Nichols, J.S., Ernst, I., Hunter, L.A., Blackwelder, W.C., Schechter, A.N., Rodgers, G.P., Castro, O. and Ognibene, F.P. (2004). Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *The New England Journal of Medicine*. 350:886-895.
- Jettanacheawchankit, S., Sasithanasate, S., Sangvanich, P., Banlunara, W. and Thunyakitpaisal, P. (2009) Acemannan stimulates gingival fibroblast proliferation;

- expressions of keratinocyte growth factor-1, vascular endothelial growth factor, and type I collagen; and wound healing. *Journal of Pharmacological Sciences*. 109:525-531.
- Kato, G.J., Hebbel, R.P., Steinberg, M.H. and Gladwin, M.T. (2009). Vasculopathy in sickle cell disease: biology, pathophysiology, genetics, translational medicine, and new research directions. *American Journal of Hematology*. 84:618-625.
- Lewis, E.J., McDaniel, H.R., Agronin, M.E., Loewenstein, D.A., Riveros, J., Mestre, R., Martinez, M., Colina, N., Abreu, D., Konefal, J., Woolger, J.M. and Ali, H.K. (2013). The Effect of an Aloe Polymannose Multinutrient Complex on Cognitive and Immune Functioning in Alzheimer's Disease. *Journal of Alzheimer's Disease*. 33(2013):393–406
- Liu, C., Cui, Y., Pi, F., Cheng, Y., Guo, Y. and Qian, H. (2019). Extraction, Purification, Structural Characteristics, Biological Activities and Pharmacological Applications of Acemannan, a Polysaccharide from Aloe vera: A Review. *Molecules* (Basel, Switzerland). 24(8):1554.
- Liu, C., Leung, M.Y.K., Koon, J.C.M., Zhu, L.F., Hu, Y.Z., Yu, B. and Fung, K.P. (2006). Macrophage activation by polysaccharide biological response modifier isolated from Aloe vera L. var. chinensis (Haw) Berg. *International Immunopharmacology*. 6:1634–1641.
- Mentzer, W. and Wang, W. (1980). Sickle-Cell Disease: Pathophysiology and Diagnosis. *Pediatric Annals*. 9:10-22.
- Modell, B. and Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. *Bulletin of World Health Organisation*. 86(6):480-487.

- Morris, C.R. (2011). Vascular risk assessment in patients with sickle cell disease. *Haematologica*. 96:1-5.
- Morton, J.F. (1961). Folk uses and commercial exploitation of Aloe leaf pulp. *Economic botany*. 15(4):311-319.
- Nwaoguikpe, R.N., Braide, W. and Ezejiofor, T.I. (2010). The effect of Aloe vera plant (aloe barbadensis) extracts on sickle cell blood (HbSS). *African Journal of Food Science*. 1:58–63.
- Panel CIRE. (2007) Final report on the safety assessment of Aloe andongensis extract, Aloe andongensis leaf juice, Aloe arborescens leaf extract, Aloe arborescens leaf juice, Aloe arborescens leaf protoplasts, Aloe barbadensis flower extract, Aloe barbadensis leaf, Aloe barbadensis leaf extract, Aloe barbadensis leaf juice, Aloe barbadensis leaf polysaccharides, Aloe barbadensis leaf water, Aloe ferox leaf extract, Aloe ferox leaf juice, and Aloe ferox leaf juice extract. *International Journal of Toxicology*. 26(2):1-50.
- Piel, F.B., Patil, A.P., Howes, R.E., Nyangiri, O.A., Gething, P.W., Dewi, M., Temperley, W.H., Williams, T.N., Weatherall, D.J. and Hay, S.I. (2013). Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet*. 381(9861):142-151.
- Porter, M. (2018). Rapid Fire: Sickle Cell Disease. *Emergency Medicine Clinics of North America*. 36(3):567-576.
- Reynolds, T. and Dweck, A.C. (1999). Aloe vera gel leaf: a review update. *Journal of Ethnopharmacology*. 68:3-37.

- Salah, F., El Ghouli, Y., Mandhi, A., Majdoub, H., Jarroux, N. and Sakli, F. (2017). Effect of the deacetylation degree on the antibacterial and antibiofilm activity of acemannan from Aloe vera. *Industrial Crops and Products*. 103:13–18.
- Sierra-García, G.D., Castro-Ríos, R., González-Horta, A., Lara-Arias, J. and Chávez-Montes, A. (2014). Acemannan, an extracted polysaccharide from Aloe vera: A literature review. *Natural product communications*. 9(8):1217-1221.
- Serjeant, G.R. and Serjeant, B.E. (2001). The epidemiology of sickle cell disorder: a challenge for Africa. *Archives of Ibadan Medicine*. 2(2):46–52.
- Surjushe, A., Vasani, R. and Saple, D. G. (2008). Aloe vera: a short review. *Indian Journal of Dermatology*. 53(4):163–166.
- Zhang, L. and Tizard, I. R. (1996). Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. *Immunopharmacology*. 35(2):119–128.