Scientific Research and Essays Vol. 5(24), pp. 3803-3807, 18 December, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2010 Academic Journals

Review

The use of phytomedicines as effective therapeutic agents in sickle cell anemia

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Accepted 19 May, 2010

Medicinal plants are parts of a plant or the whole plant that possess healing properties and unlike orthodox (synthetic) medicines, which may have adverse side effects, medicinal plant formulations are considerably cheaper and safer to use. In this review, selected medicinal plants with anti-sickling properties which are currently in use for the management of sickle cell anemia are highlighted, their methods of extraction, the various methods of analyzing herbal extracts for anti-sickling activity via efficacy tests and analyses and research findings are also discussed.

Key words: Phytomedicines, Fagara, Niprisan, ajawaron HF, *Cajanus cajan.*

INTRODUCTION

Phytomedicines are medicines derived from plant sources. These are commonly referred to as medicinal plants. It is widely known in ethnomedicine that the parts of a plant or the whole plant can posses healing properties. For instance, the bark of *Rauwolfia mombasiana* is used for the treatment of malaria. The root of the same plant is used for the treatment of fevers and anxiety states. The roots, stem and leaves of another species of this plant, *R. vomitoria*, are used for fevers, sedative in maniac syndromes and emetic (Iwu, 1993). It is acknowledged world-wide that traditional medicine can be explored and exploited to be used along-side synthetic pharmaceutical products for enhanced health management. Due to the high mortality rate of sickle cell patients, especially in children, and since chemotherapy has its adverse effects, there is need for rational drug development that must embrace not only synthetic drugs but also natural products (phytomedicines/herbal drugs), naturally occurring anti-sickling agents which can be obtained from our vast forest resources and can be used to effectively manage the sickle cell patient and treat the anemic condition accompanying this disorder. Unlike orthodox (synthetic) medicines, which may have adverse side effects, medicinal plant formulations are considerably cheaper and safer to use (Sofowora, 1984).

Presently, first-line clinical management of sickle cell anemia include use of Folic acid, amino acids (as nutritional supplements), Penicillin – prophylaxis (helps prevent infection) and anti-malarial prophylaxis (helps prevent malaria attack) e.g. PaludrineR in varying doses in childhood, adulthood and pregnancy. The faulty 'S' gene is not eradicated in treatment, rather the condition is managed and synthesis of red blood cells induced to stabilize the patient's hemoglobin level. Further management and treatment of this disorder with compounds or techniques which directly affect the hemoglobin (Hb) molecule (e.g. Hydroxyurea, Bone Marrow Transplantation and Blood Transfusion) are very expensive and out of reach of the masses and besides may expose the patient to mutagenicity, iron overload and other fatal risks (Brittain and Han, 2004; Steinberg, 2004; Amrolia, 2003; Sauntharajah and Maziarz, 2003; Nagel, 1998).

Attempts to find alternative, cheaper and less toxic therapies, led to the scientific discovery of antisickling properties of some medicinal plants such as *Cajanus cajan* seeds, *Zanthoxyllum zanthoxyloides* (Fagara) root, *Carica papaya* unripe fruit and *C. papaya* leaves and also *Parquetina nigrescens* whole plant extracts which boost blood volume- all these are locally used by traditional healers in Nigeria for diverse herbal remedies (Oduola et al., 2006, 2007a,b; Ogunyemi et al., 2008; Imaga et al., 2009; Kade et al., 2003; Ekeke and Shode, 1985; Sofowora et al.,1975). Various works have identified a number of herbal applications that have ameliorating effects on sickle cell disorders. Quality control tests,

patenting products, clinical trials, public acceptability campaigns, etc, are a few of the things to be noted though in preparing such herbal drugs to ensure wide acceptability. This review aims at highlighting the Phytomedicines with antisickling properties in use for the management of Sickle Cell anemia and showing the various methods of analyzing herbal extracts for antisickling activity and the use of biochemical research to explain the observed antisickling effects.

BRIEF HISTORY AND CLINICAL MANAGEMENT OF SICKLE CELL DISEASE

Sickle Cell disease has been known to the peoples of Africa for hundreds of years. In West Africa, various ethnic groups gave the condition different names (Harvard Educational website). In Western Literature, the first description of the disease was by a Chicago Physician, James B. Herrick, who noted in 1910 that a patient of his from the West Indies had an anemia characterized by unusual red cells that were “sickle shaped”. In 1927, Hahn and Gillespie showed that Sickling of the red cells was related to low oxygen. In 1940, Sherman, a student at Johns Hopkins Medical School, noted a birefringence of deoxygenated red cells, suggesting that low oxygen altered the structure of the hemoglobin in the molecule. Janet Watson, a pediatric hematologist in New York, suggested in 1948 that the paucity of sickle cells in the peripheral blood of newborns was due to the presence of fetal hemoglobin in the red cells, which consequently did not have the abnormal sickle hemoglobin seen in adults.

Using the new technique of protein electrophoresis, Linus Pauling and Harvey Itano showed in 1948 that the hemoglobin from patients with sickle cell disease is different from that of normal. This made sickle cell disease the first disorder in which an abnormality in a protein was known to be at fault. In 1956, Vernon Ingram and J.A. Hunt, in England, sequenced sickle hemoglobin and showed that a glutamic acid at position 6 was replaced by a valine in sickle cell disease. Using the known information about amino acids and the codons that coded for them, he was able to predict the mutation in sickle cell disease (SCD). This made SCD the first genetic disorder whose molecular basis was known. In 1984, bone marrow transplantation in a child with sickle cell disease produced the first reported cure of the disease. The transplantation was done to treat acute Leukemia. The child’s SCD was cured as a side-event. The procedure nonetheless set the precedence for later transplantation efforts directed specifically at sickle cell disease (Harvard Educational website).

Hydroxyurea became the first (and only) drug proven to further complications from the Sickle cell disease in the ‘Multicenter study of hydroxyurea in sickle cell anemia’ completed in 1995. However, hydroxyurea is potentially mutagenic and carcinogenic (Buchanan et al., 2004). Questions remain about the clinical benefits of Hydroxyurea in HbSC disease, so that a decision to treat patients with this genotype is a matter of clinical judgement. Generally, hydroxyurea is known to increase levels of HbF in most SCD patients. Decitibine, a less-toxic analog of 5-azacytidine, may affect HbF levels by causing hypomethylation of the γ-globin genes. It is used in Sickle Cell Anemia patients who failed to respond to Hydroxyurea (Bunn, 1997).

PHYTOMEDICINES WITH ANTISICKLING PROPERTIES

A number of Phytomedicines are used in the treatment and prevention of SCD. It has been discovered that the active constituents of most antisickling herbs are the Phenylalanine and hydroxybenzoic acid components (Onah et al., 2002) as well as carjaminose (in the case of *C. cajan* seeds). A study done by Acquaye et al. (1982) showed that L-Phenylalanine benzyl ester (Phe-Bz) and a number of ester analogues prevent sickling of erythrocytes from SCD patients. It was also discovered that Phe-Bz and its analogs caused an increase in cell volume and suggested that the direct effects of their hydrolysis products on HbS probably act in concert to bring about the anti-sickling effect.

Zanthoxyllum (Fagara) *zanthoxyloides*

Root extracts of fagara (Yoruba: “orin-ata”) with o-hydroxybenzoic acid as active constituent, have been shown to have anti-sickling properties and esterase activity. Saline and alkaline extracts of fagara have been investigated and found to have antiprotease and membrane stabilizing activities (Oyedapo and Famurewa, 1995). Results have shown that the fagara extract is potent and relatively safe for use in the management of painful episodes experienced by sickle cell patients.

Cajanus cajan

The aqueous methanolic extract (3:1, v/v) of the seeds of *Cajan* (pigeon pea- a major constituent/base of a herbal formula, CiklaviteR, used in the Management of Sickle Cell disease in Nigeria) has been found to posses significant anti-sickling activity in concentration-dependent doses. Phytochemical screening of the extract revealed the presence of free amino acids (phenylalanine most active), phenolic compounds (p-hydroxybenzoic acid), tannins, globulins and saponins (Onah et al., 2002). It has also been found to be potent and relatively safe for use in preventing sickle cell crisis.

Niprisan (Nix-0699)

The drug Nix-0699 is a product of the extracts of four different kinds of plants: the seeds of *Piper guineense*, the stems of *Pterocapus osun*, the fruit of *Eugenia caryophyllum* and the leaves of *Sorgum bicolor* (Iyamu et al., 2002). The herbal medicine formulation has been used locally among folk groups in Nigeria to prevent painful crises that are associated with SCD. *In vitro* and *in vivo* (using Transgenic, Tg mice models) protocols have established the efficacy of Niprisan as a potential antisickling drug.

Ajawaron HF

The anti-sickling activities of the extracts of the roots of a plant *Cissus populnea* L. (CPK), a major constituent of an herbal formula Ajawaron HF used in the management of sickle cell disease in Southwest Nigeria, has been examined and found to posses anthraquinone derivatives, steroidal glycosides and cardiac glycosides. The herbal formula (HF) exhibited a relatively high antisickling activity compared with p-hydroxybenzoic acid and n-saline controls (Moody et al., 2003).

ANALYTICAL METHODS FOR ANTISICKLING ACTIVITY

Plant collection and extraction

Selected plants are harvested, authenticated, voucher numbered and deposited in a herbarium. Collected plants are cut into bits, air-dried or oven-dried (under controlled temperature of not more than 40°C) and milled/ground. The ground herb goes through one of various extraction methods including: decoction, infusion, percolation or by soxhlet extraction. Various solvents could be used for extraction such as water, alcohols, pet ether, ethyl acetate, hexane, and other solvents depending on the plant constituents desired.

Phytochemical screening and proximate analysis

The extracts are subjected to phytochemical screening and proximate analysis tests to ascertain their constituents and nutrient compositions. As part of the phytochemical screening, thin layer chromatography [TLC] is done on the crude extract to confirm the presence and types of plant metabolites such as of alkaloids, glycosides, tannins or saponins (Evans, 1996). Further High Performance Liquid Chromatography is carried out to identify and further characterize the active constituents present.

Sample collection and preparation

According to methods of Acquaye et al. (1982), whole blood samples from sickle cell disease patients and normal subjects are collected into heparinized tubes. The cells are washed with ice-cold incubation medium consisting of buffered Tris-HCl [pH 7.2.] and other physiological salts. Ekeke and Shode (1985) modified the Acquave et al method by the use of EDTA bottles for blood collection and they worked with fresh blood.

Toxicological analysis

Toxicological assessment of the effects of the herbal extracts/ phytomedicines can be carried out *in vivo* on normal and transgenic mice models. Minimum lethal dose (LD50) of the extract is performed to determine the dose that would kill 50% of animal population. Sub-acute toxicity test to determine toxic level of the plant extracts is also carried out. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), bilirubin and creatinine assays of serum samples, as well as histological analysis of selected organs are used to ascertain the toxic profiles of the extracts (Imaga et al., 2009).

Sodium-metabisulphite (SMBS) test

This involves the typical antisickling and desickling experiments (Acquaye et al., 1982; Imaga et al., 2009). Red cells are incubated with the extract after which 2% sodium metabisulphite is added to aliquots of the previous mixture. The ability of the extract to inhibit sickling/delay sickling time is measured. In the desickling experiment, otherwise called 'reversal of sickling' (Ekeke and Shode, 1985), there is an initial bulk sickling of the red cells with 2% sodium metabisulphite solution. Then aliquots of this mixture are incubated with varying doses of extract and the ability of the extract to reverse the sickled cells is measured by counting under a microscope.

Membrane stabilizing tests

The effect of the herbal extracts on erythrocytes is also analyzed using the osmotic fragility test, which reveals appreciable membrane protective effects of the herbs and its inhibitory action on hemolysis of red blood cells. This is a definite and quantitative means of analysis. Analyses are done using the methods of Oyedapo and Famurewa (1995). To 10 ml reaction vessels containing 4 ml of different concentrations (0.00 to 0.85 %) of buffered saline pH 7.4, 1 ml of concentrations of each plant extract (1 to 5 mg/ml) and 0.05 ml HbSS blood is added. The

mixture is left to incubate at room temperature for 30 min and then centrifuged at 2000 rpm for 5 min. The supernatant is collected and read at 540 nm, against 0.85% buffered saline concentration as blank. The mean corpuscular fragility is then calculated to obtain the most effective herbal extract concentration.

EFFECT OF PHYTOMEDICINES ON ERYTHROCYTES

Fagara *zanthoxyloides*

Oyedapo and Famurewa (1995) carried out investigations into membrane stabilizing activity of fagara and two other plant extracts and reported that fagara exerted a potent membrane stabilization at the highest concentrations tested. This action is significant in explaining the anti-inflammatory property of fagara and its use in the management of pain in Sickle cell crisis. The active constituent in fagara, ortho-hydroxymethyl benzoic acid, is responsible for this antisickling and red cell membrane protection against hypotonic hemolysis and heat-induced lyses *in vitro*.

Cajanus cajan

Ekeke and Shode have shown that *Cajanus cajan* seed extract exerts significant inhibitory effect on sickling. The extract was also found to rapidly reverse already sickled cells using protocols explained earlier. Onah et al. (2002) showed that the antisickling activity of crude extracts of *cajan* displayed a hyperbolic curve similar to that reported by Ekeke and Shode (1985). They went on to show that the reversal of sickling with the extract was concentration-dependent. They also reported an average half-life of the extract indicative of a reasonable duration of action of the extract. This was explained by the high polarity of the active constituent(s) that must be actively transported across the membrane barrier of the erythrocyte before interacting with the hemoglobin (Hb) molecule. The hemoglobin molecule is believed to have a high affinity for most substrates that reverse the sickling phenomenon and the configuration of the region of Hb where antisickling agents bind has been determined by Abraham et al. (1982).

Niprisan

Iyamu et al. (2002) have reported their findings on Niprisan; Treatment of SS cells with Niprisan reduced the degree of sickle cell formation in a dose-dependent manner. Also under hypoxia conditions, Niprisan enabled a reversal of sickled cells to the original discoidal shape. Niprisan does not induce Red Blood Cell lysis, formation of MADH or met-Hb formation. The drug was therefore found to have a potent antisickling effect though not explainable by the slightly reduced P50 value. Niprisan increased the oxygen affinity of HbS and may apply a double-target hit on HbS polymerization in attenuating SS cell sickling. There was lack of swelling of cell volume and the drug was effective at low concentration, thus indicating that the effect of the drug is probably not at the cell membrane level. The solubility and gelatin tests showed a delay in deoxy-Hb polymerization which suggests that the antisickling effect of Niprisan is achieved through direct interaction with HbS molecules.

Conclusion

As a cheaper and safer alternative to treatment and management of sickle cell disease patients, phytomedicines are fast becoming the drug of choice here in Nigeria. The orthodox drug in use, Hydroxyurea results in outright treatment of the disorder through the stimulation of HbF synthesis which is antagonistic to the HbS gene. But as earlier explained the drug is mutagenic and carcinogenic though risks of complications from SCD far outweigh these toxic effects.

However, this review has highlighted the herbal formulations/ phytomedicines in use currently in Nigeria that are effective in keeping the patient out of crisis and enables them live stable lives in the society even though the faulty S gene is not eradicated but rather managed. This does not undermine the fact that SCD individuals can also live comfortable lives with stable nutritive diets, fortified with vitamins, minerals and amino acids to ensure a crisis-free life.

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