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Full length Research paper

Antisickling potential of the ethanol seed extracts of *Vigna unguiculata* and *Vigna subterranean*

Egba I. Simeone[2], Emmanuel N. Tufon[1]*, Ogugua N. Victor[2] and Ndohnui N. Noel[1]

[1]Department of Medical Biochemistry, St Louis University of Health and Biomedical Sciences Mile Three Nkwen Bamenda, Cameroon.
[2]Department of Medical Biochemistry, University of Nigeria, Nsukka, Nigeria.

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Natural plant products have been used in Nigerian folk medicine in the management of sickle cell anemia by inhibiting sickling. This work was therefore aimed at investigating the Antisickling potential of the ethanol seed extract of *Vigna unguiculata* (E_1) and *Vigna subterranean* (E_2) used in the Nigerian herbal medicine with a view of proposing an effective herbal recipe for the management of sickle cell disease. Preliminary phytochemistry, sickling inhibition test, sickling reversal test and polymerization test were carried out using standard methods. The phytochemical analysis indicated the presence of saponins, reducing sugar, carbohydrate, fats and oil, steroids, glycosides, alkaloids and proteins in E_1 and the presence of flavonoids, saponins, carbohydrates, fats and oil, resins, terpenoids, steroids, glycosides, alkaloids and proteins in E_2. The results of the antisickling test showed that E_1 and E_2 had significantly ($p<0.05$) higher antisickling effect than the Hbss control with E_1 showing a higher percentage than E_2. The percentage sickling reversal effect of E_1 was slightly lower than E_2 but their reversal of sickling were significantly higher than the control. The result of the polymerization showed that both extracts significantly ($p<0.05$) increased delayed time before polymerization at 50, 25 and 12% concentrations compared to the control. From the results, the extracts *Vigna Unguiculata* and *Vigna Subterranean* have shown to be therapeutically beneficial in the management of sickle cell disease and thus are strongly recommended by this study to be developed into supplements for the management of sickle cell disease.

Key words: *sickle cell disease, Vigna unguiculata, Vigna subterranean,* polymerization, antisickling, sickling reversal.

INTRODUCTION

Sickle cell disease (SCD) is an inherited hematological disorder characterized by a banana, crescent - moon or sickle shaped human red blood cells (Uwakwe et al., 2002). Sickle cell disease is one of the most prevalent hereditary disorders with prominent morbidity and mortality. While the disease may affect various ethnic groups such as the people of the Hispanic and Middle East descent, it affects those of African descent, the more. The most clinical manifestations are largely due to hemolytic processes leading to severe anemia and vaso-occlusive crises resulting in pain and organ damage (Cotran et al., 1999). Sickling of red blood cells occur as a result of polymerization of deoxygenated Hbs. This abnormality is characterized by painful episodes, chronic anemia, enlarged spleen, serious frequent infections and damage to vital organs (Balgir, 2006). Sickled red blood cells have relatively small oxygen contact area and increased blood viscosity and impede normal circulation in small blood vessels resulting in ischemia and infarction (Carl and Ashwood, 1996). In people with sickle cell disease, these irregular shaped red blood cells become rigid and sticky and die prematurely resulting in chronic anemia (Balgir, 2006). In Nigeria, the number is believed to be up to 4 million and at least 12 million people suffer from sickle cell disease worldwide (Ibrahim et al., 2007). Several therapies have been proposed and many chemical substances investigated for their possible role in the management of sickle cell disease (SCD). Among the many potential agents employed to prevent or reverse sickling include: Hydroxyurea (HU); Erythropoietin,

*Corresponding author. E-mail: tufipiccollo@yahoo.com.

Tucaresol, Ciklervit™ etc. Although hydroxyurea has been found, to be very effective in many patients, in others, it has yielded many pronounced side effects (Charache, 1995). In the search for effective chemotherapeutic agents with less adverse effects on sickle cell disease patients, many researchers have shown the antisickling effectiveness of most nutrients derived from plants and animals and these may provide possible and reliable option for the effective management of sickle cell disease (Uzoegwu, 1996; Nwaoguikpe and Uwakwe, 2005). Natural plant products have been used in Nigerian folk medicine to inhibit sickling and in the management of sickle cell disease and other manifestations of the disease. Several plant parts have been reported to possess anti sickling and sickling reversal properties. However, the search continues for a more effective remedy for the ailment. This work was therefore aimed at investigating the anti sickling potential of the ethanol seed extract of *Vigna unguiculata* and *Vigna subterranean* used in the Nigerian herbal medicine in view of proposing an effective herbal recipe for the management of sickle cell disease.

MATERIALS AND METHODS

Collection and preparation of plant materials

The seeds of *V. unguiculata* (E_1) and *V. subterranean* (E_2) were obtained from the herbal line, Ogbete main market Enugu State, Nigeria. They were authenticated by Mr. Njokuocha of the herbarium section, department of Botany, University of Nigeria, Nsukka; Nigeria. The seeds were air dried under shade at room temperature and pulverized using an electrical grinding machine. The powdered material (500 g) was passed through a 40 - mesh sieve and then macerated in 95% ethanol and filtered using a Whatmann filter paper 125 mm. The filtrate was concentrated to a solid matter to form the stock solution sample using a rotary evaporator. The extracts were stored in the refrigerator at 2 - 8°C.

Blood sample collection

Blood samples (5 ml) used were collected from 25 sickle cell patients of age range 10 - 20 years and of both sexes after securing consent from the individuals or accompanying parents. The blood samples were used within 24 h after collection into sample bottles containing 0.5 mg/ml of EDTA.

Phytochemical analysis

The phytochemical constituents were investigated by the method of Trease and Evans, 1996. Phytochemical tests were carried out to detect the presence of alkaloids, flavonoids, carbohydrates, reducing sugar, glycosides, saponins, tannins, fats and oil, steroids, terpenoids, acidic compounds, resins and proteins.

Determination of anti sickling potentials of the extracts

The anti sickling potential of the extracts was carried out by the method of Barbara, 1980 as modified by Elekwa et al. (2005) as described below. A drop of Hbss blood, a drop of freshly prepared 2% sodium metabisulphite and a drop of the extract were mixed on a clean slide and covered. The cover slit was gently pressed to remove excess mixture and the edges of the cover slip sealed with Vaseline to avoid air from going in. the slides were incubated at 37°C for 30 min and then observed under a microscope using ×10 and ×40 magnification to determine the effect of the extracts on the sickling of the Hbs erythrocytes.

Determination of sickling reversal test

The sickling reversal test was done by the method of Barbara, 1980 as modified by Elekwa et al. (2005) as outlined. Two drops of Hbss blood were mixed with 2 drops of freshly prepared 2% metabisulphite and covered tightly to avoid air from going in. This was incubated for 30 min during which time sickling was induced. Two drops of the buffered extract were added to the mixture. A drop was placed on a clean slide and covered. This was incubated for another 30 min and then observed at ×40 magnification.

Sickle hemoglobin polymerization inhibition test

Polymerization test was carried out using the method described by Noguchi and Schechter (1985). Five microlitres of normal saline (0.9%v/v) was added into different test tubes containing various dilutions of the extracts. Freshly prepared 2% sodium metabisulphite (4.4 ml) and Hbss erythrocytes (0.1 ml) were added into the test tubes simultaneously and mixed. After standardizing with blank (distilled water), the absorbance of the mixtures were read using a spectrophotometer (model, 700D) at 700 nm taken at 5 min intervals for 35 min. Appropriate control experiment was set up excluding the extract.

Statistical analysis

Data entry and analysis were done using SPSS version 15.0 and values were represented as mean ± SD.

Table 1. phytochemical composition of E_1 and E_2.

	E_1	E_2
Flavonoids	-	+++
Saponins	+	+
Tannins	-	-
Reducing sugar	++	-
Carbohydrate	+++	++
Fats and oil	++++	++
Resins	-	++++
Terpenoids	-	+
Steroids	++++	+
Glycosides	+	++++
Alkaloids	++++	+
Proteins	++++	+++
Acidic compounds	-	-
Cyanogenic glycosides	-	-
Anthraquinone glycosides	-	-

Table 2. Anti sickling effect of E_1 and E_2.

	50%	25%	12%	Control
E_1	80.33±1.15	66.66±1.15	42.33±2.51	25.00±2.0
E_2	75.00±2.64	60.00±2.0	41.33±1.15	25.00±2.0

Table 3. The reversal of sickling effect of E_1 and E_2.

	50%	25%	12%	Control
E_1	54.60±5.03	45.33±1.15	33.22±1.15	25.30±1.15
E_2	52.66±2.30	42.00±3.0	30.66±1.15	25.30±1.15

Significant differences were established using independent t-test.

RESULTS

Phytochemical analysis

The results of the phytochemical analysis reveals the presence of saponins, carbohydrate, fats and oil, glycosides, alkaloids, reducing sugar and protein in E_1 and the presence of flavonoids, saponins, terpenoids, steroids, carbohydrate, fats and oil, glycosides, resins, alkaloids and proteins in E_2. These phytochemicals identified have pronounced effects either medicinally or nutritionally (Table 1).

The anti sickling test

The anti sickling effect of extracts E_1 and E_2 obtained were significantly ($p<0.05$) higher than that of the Hbss control with E_1 showing a higher anti sickling potential than E_2 (Table 2).

Reversal of sickling test

The reversal of sickling for E_1 and E_2 were significantly ($p<0.05$) higher than the Hbss control as shown on Table 3. This implies that E_1 and E_2 could potentially be used to reverse sickling in cases where sickling has occurred.

Polymerization of Hbss erythrocytes

The effect of extract E_1 and E_2 on the polymerization of Hbss erythrocyte showed that both significantly increased ($p<0.05$) delay time before polymerization at 50, 25 and 12% concentrations compared to the parallel control (Table 4).

Table 4. The effect of E_1 and E_2 on the polymerization of Hbss erythrocytes measured at 5 min interval for 35 min.

	50%	25%	12%	Control
E_1				
5 min	0.114±0.007	0.170±0.025	0.031±0.013	0.017±0.009
10 min	0.088±0.008	0.024±0.013	0.010±0.002	0.016±0.008
15 min	0.065±0.001	0.051±0.006	0.007±0.002	0.039±0.004
20 min	0.080±0.012	0.080±0.002	0.003±0.002	0.178±0.002
25 min	0.106±0.005	0.080±0.006	0.098±0.009	0.131±0.002
30 min	0.865±0.007	0.665±0.009	0.705±0.013	0.730±0.010
35 min	0.925±0.036	0.680±0.005	0.725±0.004	0.748±0.005
E_2				
5 min	0.026±0.003	0.078±0.002	0.036±0.033	0.017±0.009
10 min	0.003±0.003	0.025±0.004	0.033±0.004	0.016±0.008
15 min	0.002±0.001	0.009±0.001	0.021±0.003	0.039±0.009
20 min	0.044±0.002	0.038±0.002	0.039±0.001	0.178±0.002
25 min	0.035±0.020	0.107±0.012	0.012±0.010	0.131±0.002
30 min	0.740±0.009	0.738±0.024	0.039±0.002	0.730±0.010
35 min	0.747±0.022	0.755±0.007	0.674±0.007	0.748±0.005

DISCUSSION

Phytochemical analysis of the extract showed similar secondary metabolites such as alkaloids, carbohydrates, proteins, fats and oil, saponins, resins, glycosides flavonoids and carboxylic acid which were the active compounds responsible for reversal of sickle erythrocytes by leaves of *Hymenocardia acida* (Ibrahim et al., 2007). This could explain our observation that E_1 showed more potent sickling reversal effect than E_2. Saponins have been demonstrated to possess anti sickling activities (Trease and Evans, 1996; Moody et al., 2003). Flavonoids have been shown to possess indirect anti – oxidant activity. Alkaloids though not proven yet to possess sickling inhibition activity, has been demonstrated to show other positive biological effect that may be of great therapeutic advantage in the management of sickle cell disease. Many of the positive effects of the extracts may no doubt be due to the presence of these secondary metabolites.

The anti sickling and sickling reversal effect of E_1 and E_2 showed marked improvement upon incubation with the extracts compared to the normal saline control. An erythrocyte was considered to be unsickled if the cell was not in the characteristic sickle shape or in crenated holly leaf pattern (Gorecki et al., 1980). The effects of the extracts were significant at the different concentrations used but were dose and tie dependent. This result agrees with the report that sickling activity of the drug tellurite, thiocyanate and hydroxyurea were dose and time dependent (Oyewole et al., 2008). The extracts used in this study achieved a significant decrease ($p<0.05$) in percent irreversible sickle cell upon incubation with sodium metabisulphite pre – sickled erythrocytes thus indicating positive effect in maintaining membrane integrity. The use of sodium metabisulphite to induce sickling is probably a more drastic approach than what actually happens in the vascular system of humans (Egunyomi et al., 2009). It is therefore expected that the extracts may achieve more efficient sickle inhibition *invivo*. The polymerization of Hbss erythrocyte is a major event in the pathophysiology of sickle cell disease. Measuring delay time has been suggested to be the most reliable tool in assessing the effectiveness of a potential anti sickling agent (Hofrichter et al., 1976). The extracts used in this study significantly ($p<0.05$) increased delay time of sickle hemoglobin polymerization at all the three concentrations used with respect to the parallel control.

From the result of this study, we sincerely conclude that *Vigna unguiculata* and *Vigna subterranean* have proven to be therapeutically beneficial and thus can be developed into a supplement for the management of sickle cell disease.

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