

EFFECTS OF AQUEOUS EXTRACTS OF *GARCINIA KOLA* SEEDS ON MEMBRANE STABILITY OF HbAA, HbAS AND HbSS HUMAN ERYTHROCYTES

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ABSTRACT

The effects of water-extract of the seed of *Garcinia kola* (Henckel), a popularly consumed seed known as 'bitter kola' in Nigeria, on the stabilities of erythrocyte membranes from HbAA, HbAS and HbSS blood donors were determined. The median corpuscular fragility (MCF) of erythrocytes from the three genotypes were 3.6 ± 0.1 , 3.4 ± 0.1 and 2.8 ± 0.2 , expressed in [NaCl] g/l for HbAA, HbAS and HbSS, respectively. The aqueous extract from *G. kola* seeds stabilized the membranes from HbAA, HbAS and HbSS erythrocytes by 22, 17 and 25% respectively. Phenylalanine, a known anti-sickling agent, stabilized the HbSS membrane by 17.85%. Viscosity studies indicated that the HbSS blood was more viscous and the aqueous extract from *G. kola* seeds decreased the viscosity of the HbSS blood, producing a statistically different ($p < 0.5$) value from that for HbAA and HbAS blood. The extract showed pronounced reversion of 2% sodium metabisulphite-induced sickling. Similar effect was observed with phenylalanine at 400 μ M. These findings present support for the possible use of the aqueous extract of *G. kola* seeds for the management of sickle cell crisis.

Key words: 'bitter kola', osmotic fragility, membrane stability, erythrocyte

INTRODUCTION

The plasma membrane is a semi-permeable barrier that serves to maintain the integrity of a living cell. The stability of this covering influences the flow of materials in and out of the cell and, in addition to the nature of the components of the membrane, the erythrocyte membrane stability is achieved for the most part through unidirectional activated transport of ions (Kyte, 1971). This vectorial transport is performed by the ion pumps (Na^+ , K^+ -ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase) that were shown to occur at different activity levels in human erythrocytes of HbAA, HbAS and HbSS genotypes (Ibeh et al, 1992). This finding lent support to the deduction that the membranes of these erythrocytes had varying stabilities. It also supported the suggestion that pharmacological agents that alter membrane stability could be beneficial in controlling sickling (Dean and Schechter, 1978).

The seed of *G. kola* (popularly known as 'bitter kola' in Nigeria) has been ascribed many trado-medical applications (Uphof, 1968; Willis, 1973), and is widely consumed for cough suppression, anti-tumor/anti-microbial activity, as an aphrodisiac (Harley, 1970), as well as an anti-hepatotoxic agent (Iwu, 1985). Preliminary report (Elekwa, unpublished) suggested some form of relief to sicklers when this seed is chewed. The

present report addresses the possible effect of the water extract of this popular seed on the stability of the erythrocyte membrane. The findings would have far reaching effect on the continued search for remedies to the nagging sickle cell disease.

MATERIALS AND METHODS

Heparin was purchased from Sigma Chemical Co. USA, while phenylalanine was from Merck, W. Germany. All other reagents were of analytical grades.

Blood sample collection

Blood was collected from volunteers who came to the Sickle Cell Clinics at the University of Port Harcourt Teaching Hospital and the University of Nigeria Teaching Hospital, Enugu, as well as the Medical Center of the University of Port Harcourt, by veni-puncture into lithium heparinized sterile tubes. The different blood samples were genotyped using standard electrophoretic procedure. Blood samples were stored in the refrigerator and used within 24 hours after collection.

Preparation of *G. kola* seed aqueous extract

The seeds of *G. kola* were purchased from a market at Umuahia, Abia State, Nigeria

250 ml) for 24 hours, after which the mixture was filtered first through a clean white cloth and then with Whatman #2 filter paper.

Determination of Osmotic fragility

This followed the procedure developed by Parpart et al (1947), using saline buffer at pH 7.4. A 10 g/l solution was made from 100 g/l NaCl stock and dilutions equivalent to 9.0, 7.0, 6.0, 5.5, 5.0, 4.0, 3.0, 2.0 and 1.0 g/l NaCl were made to 50 ml (final volume).

A 0.05ml aliquot of blood sample was added to 5 ml of the hypotonic solution, and immediately mixed by inverting several times. The tubes were allowed to stand for 30 minutes at room temperature. The contents were re-mixed and centrifuged for 5 minutes at 1500 g. The absorbance of the supernatant was read at 540 nm using 9.0 g/l NaCl tube as blank. This procedure was repeated for the different blood genotypes of HbAA, HbAS and HbSS, with five samples per genotype. Each blood sample was used thrice and an average value taken.

For the effect of the aqueous extract of *G. kola* seeds, 0.5 ml of 100g/250 ml extract was used and 4.5 ml of hypotonic solution was added. The final NaCl concentration was maintained as with the control by using appropriate stock NaCl solutions. A 0.05 ml aliquot of blood sample was added and the mixture treated as before. The total volume was 5.05 ml, as with the control.

Determination of blood viscosity

Blood viscosity was determined using an Ostwald viscometer and the time taken for the liquid to fall from the top to the bottom mark on the wall of the viscometer is recorded. Three milliliters of distilled water was mixed with 2 ml normal saline, and the mixture introduced into the viscometer. Using a stopwatch, the time taken for the meniscus to pass from the top to the bottom mark was determined. The test blood samples replaced the distilled water for the test experiments. All readings were carried out at laboratory temperature of 30°C.

Table 1. Median corpuscular fragility (MCF) values and % stabilization for HbAA, HbAS and HbSS erythrocytes in the absence/presence of aqueous extract of *G. kola* seeds [100 g/250 ml (w/v)]. Data represent mean \pm SD of five triplicate determinations.

Genotype	MCF expressed as [NaCl] g/l		% Stabilization
	Control	+ Extract	
HbAA	3.6 \pm 0.1	4.4 \pm 0.1	22.22
HbAS	3.4 \pm 0.1	4.0 \pm 0.1	17.65
HbSS	2.8 \pm 0.2	3.5 \pm 0.1	25.00

Table 2. Median corpuscular fragility (MCF) values and % stabilization for HbAA, HbAS and HbSS erythrocytes in the absence/presence of phenylalanine (400 μ M, final concentration). Data represent mean \pm SD of five triplicate determinations.

Genotype	MCF expressed as [NaCl] g/l		% Stabilization
	Control	+ Phe	
HbAA	3.6 \pm 0.1	4.3 \pm 0.1	19.44
HbAS	3.4 \pm 0.1	3.8 \pm 0.1	11.77
HbSS	2.8 \pm 0.2	3.3 \pm 0.1	17.85

Phe = phenylalanine

Table 3. Effects of water extract of *G. kola* seeds [100 g/250 ml (w/v)] and Phenylalanine (400 μ M) on the viscosity of blood samples.

Incubation Time (minutes)	Viscosity ($\times 10^{-3}$ Pa-S)						
	+ <i>G. kola</i>				+Phenylalanine		
	0	30	60	90	30	60	90
HbAA (n=5)	1.7	1.5	1.3	1.3	1.6	1.4	1.4
HbAS (n=5)	2.1	1.8	1.7	1.7	1.8	2.0	2.0
HbSS (n=5)	3.4	2.5	1.9	1.5	2.6	1.8	1.3

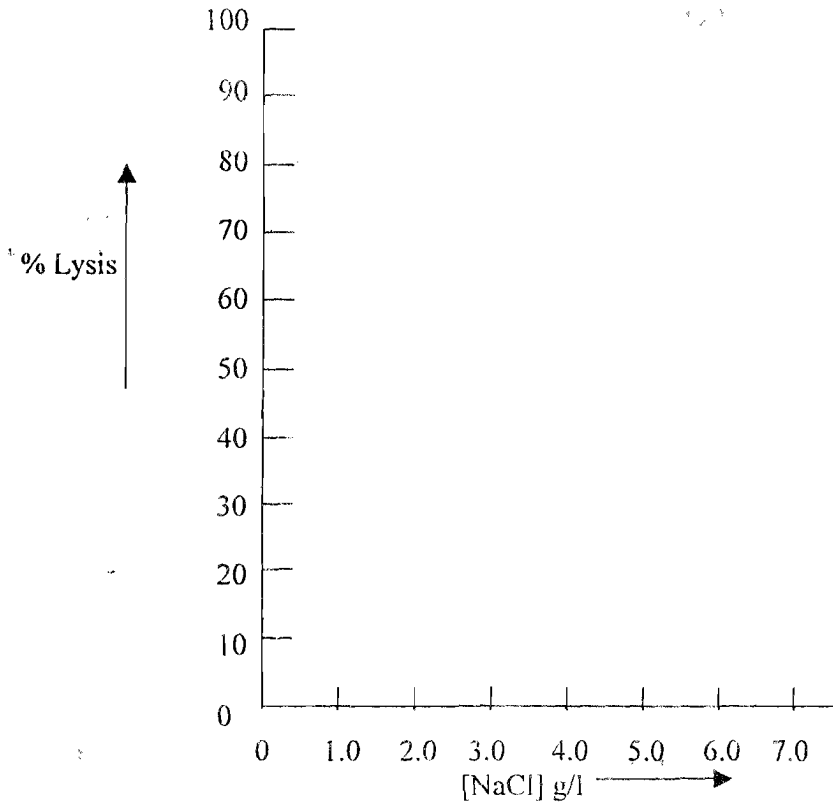
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Figure 1. Osmotic Fragility plot for HbAA, HbAS and HbSS blood samples. Data represent mean \pm SD of five duplicate determinations (●-●, HbAA; ○-○, HbAS and ■-■, HbSS).

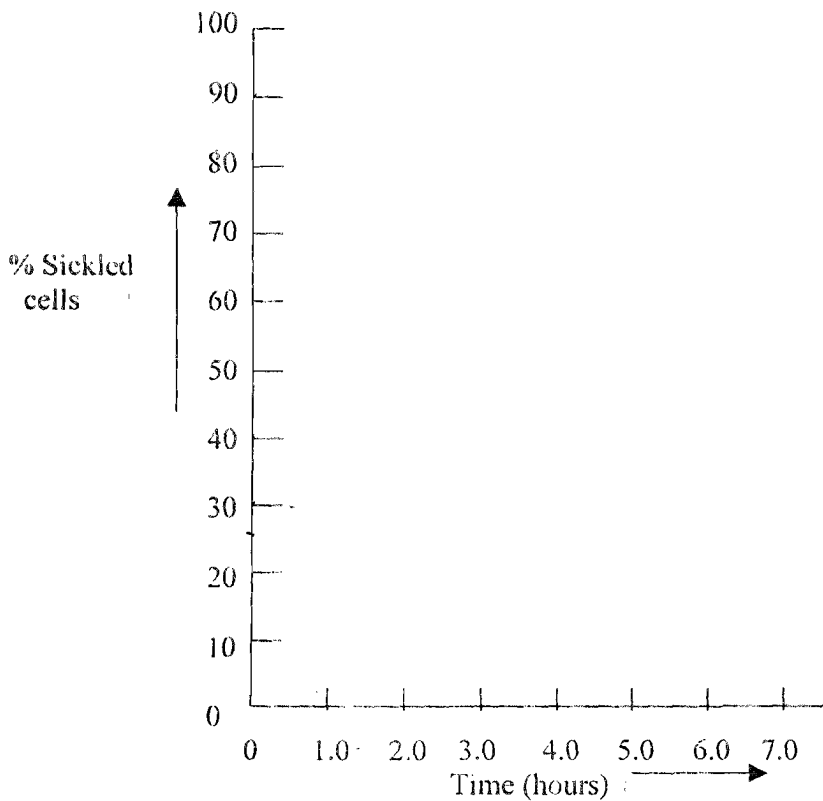


Figure 2. The reversion of sickling induced by 2% sodium meta-bisulphite by water extract of *G. kola* seeds. Data represent mean \pm SD for triplicate determinations (●-●, Control; ■-■, With extract).

and properly identified at the National Root Crops Research Institute, Umudike. The testa was peeled off and the white seeds chopped into

small pieces. They were air-dried and homogenized with an electric blender. The coarse product was then soaked in distilled water (100g /

Effect of aqueous extract and phenylalanine on blood viscosity

The reaction medium contained 0.2 ml of extract (100g / 250ml), 3.0 ml of blood sample and 1.8ml saline. These were incubated at 37°C for 30, 60 and 90 minutes. The viscosity was determined using the Ostwald viscometer as detailed above. The mean of five samples per genotype was calculated. The same procedure was used to determine the effect of phenylalanine at 400 μ moles (final concentration in test mixture) by replacing the extract with 0.2 ml of 10 mM phenylalanine solution.

Determination of in vitro sickling/reversal of sickling

Three milliliters of confirmed HbSS blood was diluted with 0.15M phosphate buffer and mixed with 3 ml of 2% sodium meta-bisulphite. A drop from the mixture was spotted on a microscopic slide, and covered with a cover slip. Petroleum jelly was used to seal the edges completely to exclude air. Under the microscope, four hundred cells were counted at every 10 minutes for 60 minutes. At each count, the numbers of sickled cells were noted and the percentage of sickled cells calculated. For the reversal of sickling, a 1:20 dilution of artificially sickled cells was made. One milliliter of *G. kola* extract (100 g/250 ml) was added to 5 ml of the diluted artificially sickled blood. A drop of the mixture was fixed on the microscope at an objective magnification of x 40. Two hundred cells were counted at each interval of 60 minutes for 3 hours. The percentage of sickled cells was determined on a time-dependent basis.

Statistical analysis

The student's T-distribution table as adapted from Pearson and Harley (1966) was applied with test of significance taken at the cut-off of 95% confidence level ($p = 0.05$ to 0.001).

RESULTS AND DISCUSSION

The result for the osmotic fragility tests for the three different genotypes is shown in Figure 1, which presents a plot of % lysis against [NaCl] g/l. The median corpuscular fragility (MCF), which is the concentration of saline causing 50 % haemolysis, obtained from this graph were 3.6 ± 0.1 , 3.4 ± 0.1 and 2.8 ± 0.2 [NaCl] g/l, for HbAA, HbAS and HbSS, respectively. The results indicate a stability order of HbAA>HbAS>HbSS. The activity of Na^+ , K^+ -ATPase, one of the ion pumps suggested to be involved with maintaining the stability of the erythrocyte membrane, had

been shown to be less than 50% of the normal for sickle erythrocytes (Ibeh et al, 1992). The order of stability established in our studies support this earlier finding. When aqueous extract of *G. kola* was present during this determination, the three genotypes showed decreased MCF which is indicative of increased stability to the erythrocyte membranes (Table 1). The values of MCF were 4.4 ± 0.1 , 4.0 ± 0.1 and 3.5 ± 0.1 for HbAA, HbAS and HbSS, respectively, producing % stabilization of 22.22, 17.65 and 25.00, respectively.

Phenylalanine, a known anti-sickling agent (Noguchi and Schechter, 1977; Ekeke and Shode, 1990), at 400 μ moles, increased the MCF values for the different genotypes as indicated by Table 2. The MCF values were 4.3 ± 0.1 , 3.8 ± 0.2 and 3.3 ± 0.1 , for HbAA, HbAS and HbSS, respectively. The % stabilization were 19.44, 11.77 and 17.85, respectively, for HbAA, HbAS and HbSS blood samples. When compared with the effect seen with the phenylalanine, a somewhat better stabilization was seen with the *G. kola* extract. Blood viscosity measurement showed that HbSS blood was more viscous than the other two genotypes (Table 3). The *G. kola* extract reduced the viscosity of the HbSS blood, bringing it close to the normal. The result of the reversion of sickling by the *G. kola* extract is shown in Figure 4, and is similar to the observations of Sofowara and Isaacs-Sodeye (1971), Isaacs-Sodeye (1973) and Sofowara (1975) for extracts of *Fagara zanthoxyloides*¹ roots (another popular trado-medical plant of wide use in Nigeria).

The results of our findings in this report show that the aqueous extract of *G. kola* might have potential use in the therapy of sickle cell disease.

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