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Full Length Research Paper

In vitro reversal of deformity and inhibition of aggregation of sickle red blood cells by two Congolese herbal medicines

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The effects of aqueous extracts from the trunk bark and branches of *Ceiba pentandra* and from the root bark of *Quassia africana* Baill., which are claimed to overcome the clinical events of the sickle cell anemia (SCA) in Democratic Republic of Congo (DRC), were investigated *in vitro* on the red blood cells (RBC) deformity and aggregation. Blood samples from SCA patients and from healthy persons were treated with 2% sodium metabisulfite to induce hypoxia and sickling of erythrocytes, and then, were incubated with the drug extracts. It was found that extracts, used separately or together, reversed the induced deformity of RBC. On the other hand, aggregates of RBC were incubated with the plant extracts and the action was evaluated by microscope examination, which showed that cells became dispersed and isolated, while they remained stacked in the samples not treated. Sickling of RBC is a major factor among others, which are implicated for initiating the events of sickle cell crises as well as the increasing red blood cells adhesiveness observed in increased blood viscosity. These observations could support the use of the two medicinal drugs to deal with the clinical events of SCA.

Key words: Sickle cell anemia (SCA), red blood cell (RBC), deformity, hypoxia, aggregation, hemoglobin S, hemoglobin A, blood viscosity, hemolysis, *Ceiba pentandra*, *Quassia africana* Baill.

INTRODUCTION

Deformity of the red blood cells is the mechanism initiating pathologic manifestations, which are the source of several complications in sickle cell anemia (SCA). Red cells have a tendency of losing their elasticity and are unable to flow through narrow capillaries, leading them to become stuck in blood vessels. This deprives the downstream tissues of oxygen and causes ischemia and infarction, which may lead to organ damage, such as stroke (Platt, 2000; Wikipedia, 2007; Lonergan et al., 2001). In addition, aggregation of red blood cells is another factor implicated in the pathophysiology in SCA and may influence the blood viscosity as a result (Martorana et al., 2007; Saldanha, 2002). Increased blood viscosity as well as alteration of membrane viscosity of red blood cell (RBC) are reported in SCA, playing a role on the disease defect and making worse the clinical complications (Chien et al., 1970; Wendell et al., 2000).

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Abbreviations: RBC, Red blood cell; **SCA,** sickle cell anemia; **Hb-SS,** related to sickle cell anemia subject with affected hemoglobin SS; **Hb-AA,** related to healthy subject with normal hemoglobin AA.

SCA affects millions of people worldwide, mostly in Africa where the sickle trait frequency varies from 15 to 25% (Serjeant, 1994) and a greater number of people rely on traditional medicines to deal with the disease due to the high costs of lifelong modern treatments. The practice of traditional medicine is widespread in the world and a variety of activities and projects in medicinal plants are being conducted and promoted by several organizations worldwide (Hoareau and DaSilva, 1999). Many plants are being used as traditional medicines, but most of them have not yet been scientifically studied to prove or determine their efficacy.

The drugs used in this study are prepared and administered since many years to SCA patients by the "Centre de Phytothérapie Moderne NIECA", an officially recognized medical centre located in Kinshasa (DRC), named BEAT-SS for that prepared from *Ceiba pentandra* and DOCABE for *Quassia africana* (Baill). *C. pentandra* is a big tree generally found in rainforest tropical zones, and is widely used in herbal medicine in West and Central Africa, in South America, in West and South East Asian countries (Burkill, 1985). This plant is reported to have hypoglycemic (Ladeji et al., 2003; Dzeufiet et al., 2006, 2007), antidiarrheic (Abena et al., 2008), and antifungal (Nwachukwu et al., 2008) properties. *Q. africana* (Baill.) is a small tree of the lowland rainforest in the transition zone from evergreen to semi-deciduous forest (FAO, 1986); it has been found to have antiviral (Apers et al., 2002) and antipaludic (Lohombo et al., 2003) activities; in African folk medicine, the decoction of the bark and leaves is used for gastro-intestinal conditions and as a vermifuge, the root is used to treat bronchial illness, as a febrifuge and as anti-rheumatic (FAO, 1986).

BEAT-SS is dispensed by the Centre NIECA to SCA patients to relieve the clinical manifestations. DOCABE is administered in association with BEAT-SS in case of painful crises and is considered to have an anti-inflammatory effect. According to this centre, improvement of clinical symptoms has been observed during and after the treatment; especially referring to fatigue, acute pains, swelling in hands or feet, and skin pallor. Moreover, non-recourse to blood transfusion is done for a long period after cessation of medication. However, these activities have not yet been investigated. The aim of this work was to examine by *in vitro* experiments some scientific parameters in order to understand the use of these two traditional medicines as anti-sickling drugs in the management of SCA.

MATERIALS AND METHODS

Blood samples

Blood samples from SCA patients used in this study were provided under the authorization of the Congolese ethic committee (N° d'approbation: ESP/CE/048/2009) by the "Centre de Phytothérapie Moderne NIECA" and the "Centre de Médécine Mixte et d'Anémie SS," located both in Kinshasa (DRC). Patients selected were those who did not receive blood transfusion two months before donating blood. Adults with known hemoglobin AA type (Hb-AA), mostly laboratory members, donated samples from healthy subjects. Blood was introduced in a plastic tube containing anticoagulant (Ethylenediaminetetraacetic acid (EDTA) 10%: 0.05 ml for 3 ml of blood). The samples were placed in an icebox for commuting to the laboratory and were kept at 4°C before the experiment.

Extracts preparation

Dried powdered plant materials were provided by the "Centre NIECA" and plant voucher specimens were identified at the "Institut National d'Etudes et des Recherches Agronomiques" (INERA) of Kinshasa University (DRC). 1000 ml of aqueous extracts from each plant were separately prepared according to the phytotherapist know-how, by boiling for 5 min in distilled water, respectively, 50 g of dried powder from the bark of trunk and branches of *C. pentandra* and 100 g of dried powder from the bark of roots of *Q. africana.* The prepared decoctions were filtrated and lyophilized to yield 3.5 and 4.3 g of dried powder for *C. pentandra* and *Q. africana*, respectively. The lyophilized substances from the two extracts were used to prepare, for each, a stock extract solution of 1 mg/ml using distilled water. The solutions were further filtrated on a sterile Millex HA filter Unit of 0.45 µm Millipore diameter and were kept at 4°C in a freezer for use in subsequent analysis.

Evaluation of RBC hemolysis after extracts addition

Addition of extracts may induce the hemolysis of erythrocytes, which could be detected by reduction on the RBC number as compared to a control sample where extracts are not added. The effect of high concentrations of the two extracts on the presumed hemolysis of the RBC was evaluated by using erythrocytes count test. Blood samples from a sickle cell patient and from a healthy subject were diluted with Hayem's solution 1/100 (Na_2SO_4, 2.5%; NaCl, 0.5%; and $HgCl_2$, 0.25%), and then samples were incubated separately with the two extracts in different concentrations for 2 or 24 h. The number of erythrocytes from each sample was determined by cell count under microscope using Neubauer hemocytometer. The concentrations of the extracts used were 20, 100, 500, and 2500 µg/ml for BEAT-SS; and 5, 25, 125, and 625 µg/ml for DOCABE.

Inhibition of deformity of sickled RBCs

Deformity or sickling of RBC is induced by hypoxia condition created by the addition of sodium metabisulfite ($Na_2S_2O_5$, 2%) on the blood samples from SCA patients (Hb-SS) according to Emmel test (Murayama and Nalbandian, 1973). To evaluate the action of the extracts on the inhibition of deformity of sickled erythrocytes, the previous samples in hypoxia condition were treated separately with the two extracts in different concentrations. After incubation, sickled erythrocytes (elongated cells) will regain the normal round form: this activity was expressed as the inhibition of deformity or the normalization of sickled RBC.

A determined volume of the blood sample from a SCA patient was mixed with an equivalent volume of sodium metabisulfite (2%). The mixture was incubated for 1 h at room temperature to complete the sickling. Observations under microscope confirmed that sickling of RBC begin to occur at least 30 min after the addition of sodium metabisulfite. Thus, the duration of 1 h of incubation was quite enough to complete sickling for the majority of RBC. This constituted the control sample of Hb-SS in hypoxia condition. Another control sample in oxygenated condition was observed

before the addition of sodium metabisulfite to Hb-SS.

After 1 h of incubation of blood with sodium metabisulfite, an equivalent volume of the extract in different concentrations was added to that of the previous mixture (blood with sodium metabisulfite) and incubated again for 30 or 120 min more at room temperature. A specimen was taken for microscopic examination and was pictured (LCD-microscope Bresser, GmbH & Co. KG; and Olympus microscope CKX41 with camera DP50). Cells were counted to determine the number of both sickled cells (distorted and elongated RBC) and normal cells (round shaped RBC). The total cells number was deducted by the summation of sickled and normal RBC. The percentage of normal RBC was obtained by calculation and it represents the ratio of normal rounded cells in the total cells numbered. The concentrations used were 10, 50, 100, 200, 400, and 500 µg/ml for BEAT-SS; and 1, 5, 10, 50, 100 and 200 µg/ml for DOCABE. Used in association, the two extracts were incubated together with blood samples in couples of concentrations as shown in Table 2. The same experiment was conducted on blood samples provided by healthy subjects.

Inhibition of red blood cells aggregation

Blood samples were allowed to stand for 48 h at 4°C after their arrival in the laboratory. After this rest, aggregates of red RBC were observed on microscope for both Hb-SS (Figure 2A) and Hb-AA samples. Addition of an equivalent volume of physiological saline (NaCl, 0.9%) to blood was required to disperse these aggregates. Aggregates were not dispersed on samples from Hb-SS (Figure 2B), but this was not the case with samples from Hb-AA (Figure 2C). After these observations, a volume of Hb-SS blood sample with aggregates of RBC (Figure 2A) was incubated for 30 min or 120 min with an equivalent volume of the extracts in different concentrations, then they were examined under microscope. Two controls Hb-SS blood samples with aggregates of RBC were made up, as described earlier: one with blood alone and the other with blood mixed with the physiological saline. The extract's concentrations in the sample tests were 100 and 200 µg/ml for BEAT-SS, 50 µg/ml for DOCABE, and in association of the two extracts, 200 µg/ml for BEAT-SS and 50 µg/ml for DOCABE. The same experiment was conducted on blood samples provided by a healthy subject.

Furthermore, to verify if the dispersion of the aggregated RBC from Hb-AA after addition of saline (Figure 2C) occurred independently of the red blood cells density, the experiment was repeated using normal density of RBC from Hb-AA blood samples by avoiding dilution with saline solution. Extracts in different concentrations were added to blood samples in small volume (1 volume of extract for 19 volumes of blood), and then they were incubated for 2 h at room temperature. The extracts concentrations were 50, 100, and 200 µg/ml for BEAT-SS; and 5, 10, and 50 µg/ml for DOCABE.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD) and were analyzed for statistical significance using analysis of variance (ANOVA) and Bonferroni's multiple *t*-test. P-values of less than 0.05 were considered to be statistically significant.

RESULTS

Effect of extract on the red blood cells hemolysis

Table 1 reports the results of different concentrations of the two plants extracts action on the number of erythrocytes after incubation at room temperature with the blood samples from sickle cell patients and healthy subjects.

In the case of BEAT-SS extract, a decrease on the number of erythrocytes was not noticed after incubation for 2 or 24 h of the blood samples with the extract from 20 to 500 µg/ml in the two groups of blood samples (Hb-SS and Hb-AA), denoting that hemolysis of RBC did not occur with addition of BEAT-SS at these concentrations. A significant decrease in the number of erythrocytes was noticed with the concentration of 2500 µg/ml in both blood samples (Hb-SS and Hb-AA) after 2 h of incubation and more again after 24 h, denoting that high concentration of BEAT-SS (2500 µg/ml) induced destruction (hemolysis) of a part of the population of RBC after 2 h of exposition, and more after 24 h. Also, concerning DOCABE extract, no decrease in the erythrocytes number was observed for the two groups of samples (Hb-SS and Hb-AA) after 2 or 24 h of incubation with the extract in the concentrations from 5 to 625 µg/ml, except for a significant decrease observed after 24 h of incubation with the highest concentration of 625 µg/ml, solely with the blood sample from Hb-AA. This denotes that DOCABE extract did not induce hemolysis of RBC in the used concentrations, except for 625 µg/ml, solely after 24 h of incubation for Hb-AA.

Effect of the extracts on the erythrocyte's deformity

Pictures taken from slides test of treated samples for the examination of the inhibition of deformity by the two extracts are as shown in Figure 1. After images examination, the total number of RBC, including sickled cells (SC or distorted RBC) and normal cells (NC or round shaped RBC), was determined by count and the percentage of normal RBC, compared to the total number of RBC in the sample, was deduced by calculation (Table 2).

In the control samples from Hb-SS, it was observed that RBC did not exhibit the deformity on the shapes (Figure 1A1), when in the oxygenated condition (or before the addition of sodium metabisulfite on the blood sample from Hb-SS). Deformity of RBC was observed 1 h after hypoxia by the addition of sodium metabisulfite and most of the RBC lost the round shape and became distorted (Figure 1A2). Control RBC from Hb-AA did not display any shape's change after addition of sodium metabisulfite in the same conditions (Figure 1A3). Furthermore, blood samples from Hb-SS which have been incubated with BEAT-SS extract after induction of hypoxia (sodium metabisulfite addition for 1 h) displayed an appreciable reversing effect on the induced deformity, as shown in Figure 1B1, B2, and B3, respectively for BEAT-SS 50, 100, and 200 µg/ml. It appeared that this reversing action depended on the concentration of the

Table 1. Effect of the extracts on the erythrocytes number after extracts treatment for 2 or 24 h of incubation.

Extract concentration (µg/ml)	Hb- SS		Hb-AA	
	2 h incubation	24 h incubation	2 h incubation	24 h incubation
Control	3.4	4.0	5.3	4.5
BEAT-SS 20	3.6	3.7	5.8	4.7
BEAT-SS 100	3.8	3.2	5.2	4.8
BEAT-SS 500	3.6	3.3	4.6	4.7
BEAT-SS 2500	2.1*	1.6**	3.8*	3.2**
DOCABE 5	3.9	4.1	5.7	4.9
DOCABE 25	3.7	3.4	5.0	4.6
DOCABE 125	3.9	3.9	5.1	4.6
DOCABE 625	3.9	3.8	5.0	3.3**

To evaluate the effect of the extracts on the hemolysis of RBC, Hb-SS and Hb-AA blood samples were incubated with the extracts in different concentrations for 2 or 24 h, then the number of erythrocytes from each sample was determined by erythrocyte count test under microscope using Neubauer hemocytometer. Values are mean of four counts and are expressed as cell number $\times 10^6$ mm^{-3} (Normal values are 4.5~6.5 $\times 10^6$ mm^{-3} for male and 3.9-5.6 $\times 10^6$ mm^{-3} for female). Significant difference from the corresponding control, *P < 0.05, **P < 0.01.

Table 2. Ratio of normal RBC after induction of hypoxia in Hb-SS blood samples and treatment with the extracts for 30 and 120 min.

Sample treatment (µg/ml)	30 min incubation			120 min incubation		
	SC	NC	Ratio-NC (%)	SC	NC	Ratio-NC (%)
Control in oxygenated state	6	106	94.6	6	115	95.0
Control in hypoxia (1)	144	29	16.8	131	26	16.6
BEAT-SS 10	160	10	5.9	84	5	5.6
BEAT-SS 50	73	4	5.2	72	19	20.9
BEAT-SS 100	34	140	80.5	41	172	80.8
BEAT-SS 200	32	113	77.9	24	131	84.5
BEAT-SS 400	29	190	86.8	44	71	61.7
BEAT-SS 500	91	25	21.6	54	44	44.9
Control in hypoxia (2)	138	13	8.6	181	30	14.2
DOCABE 1	51	4	7.3	31	36	53.7
DOCABE 5	107	5	4.5	57	22	27.8
DOCABE 10	44	85	65.9	17	41	70.7
DOCABE 50	11	165	93.8	5	85	94.4
DOCABE 100	83	26	23.9	37	49	57.0
DOCABE 200	47	117	71.3	28	69	71.1
Control in hypoxia (3)	106	9	7.8	150	7	4.5
BEAT-SS 20 µg/ml + DOCABE 1	191	8	4.0	53	17	24.3
BEAT-SS 100 µg/ml + DOCABE 5	198	11	5.3	28	4	12.5
BEAT-SS 200 µg/ml + DOCABE 50	35	153	81.4	16	91	85.0
BEAT-SS 400 µg/ml + DOCABE 100	66	45	40.5	39	34	46.6

Sodium metabisulfite ($Na_2S_2O_5$) 2% was added to Hb-SS blood sample and set aside for 1 h to induce hypoxia and deformity of RBC. Then, extracts were added and incubated at room temperature for 30 or 120 min. Specimens were taken for microscope examination. Control in oxygenated state is the sample from Hb-SS not treated by sodium metabisulfite. Controls in hypoxia (1), (2), and (3) are samples where sodium metabisulfite was added without further incubation with the extracts. All other samples were incubated with the extracts in indicated concentrations, after a previous induction of hypoxia for 1 h by 2% sodium metabisulfite. SC represents the number of Sickled Cells (distorted shape RBC); NC represents the number of Normal Cells (rounded shape RBC) and Ratio-NC (%) is the ratio of Normal Cells, which is the percentage of normal RBC in the total numbered RBC (SC + NC) in the optical field. Ratio-NC (%) = [NC / (SC + NC)] × 100%.

extract, but that the incubation time (30 or 120 min) of blood samples with the extract did not have an influence on the recovery from the RBC deformity. These observations could be later justified by cell count (Table 2) which indicated that the ratio of normal cells was 77.9% for 30 min incubation and 84.5% for 120 min with 200 µg/ml of BEAT-SS. Shape recovery was noticed with BEAT-SS extract beginning from 100 µg/ml of concentration.

Images examination of the incubation of Hb-SS blood samples with DOCABE extract after hypoxia revealed that sickle RBC recovered from elongated form to the rounded one, starting from the concentration of 10 to 50 µg/ml (Figure 1C1, C2, and C3). At 50 µg/ml, this reversing action of DOCABE was maximal as confirmed by cell count reported on Table 2. Here, again, incubation time did not affect the result as observed with BEAT-SS. In the case of DOCABE, cell count confirmed that 93.8 and 94.4% of red blood cells were round-shaped after 30 and 120 min of incubation with 50 µg/ml of DOCABE extract respectively (Table 2). The two extracts used in association showed positive result in reversing the deformity of sickled RBC for the couple of concentration of 200 µg/ml for BEAT-SS and 50 µg/ml for DOCABE (Figure 1D3), and cell count indicated that the percentage of round-shaped RBC was 81.4 and 85% after incubation of the two extracts for 30 and 120 min respectively (Table 2). The other couples of concentrations did not exhibit satisfactory results for reversing the deformity of sickled RBC (Table 2 and Figure 1D1 and D2). The same experiments as described herein were equally conducted, too, with blood samples from Hb-AA subjects. Here, microscope images did not exhibit change on the RBC morphology after the same treatments; neither with sodium metabisulfite (Figure 1A3) nor with both drugs used separately or in association. Cells remained round-shaped in all cases (data not shown).

Inhibition of the aggregation of RBC

After images examination on microscope, it was noticed that both Hb-SS blood samples and Hb-AA blood samples (Figure 2A) showed cloudy aggregates of RBC after 48 h of rest. The addition of an equivalent volume of physiological saline solution (NaCl, 0.9%) did not disperse these aggregates in the case of Hb-SS (Figure 2B), RBCs were still stacked and aggregates remained, whereas RBC aggregates from Hb-AA blood samples were dispersed in the same condition (Figure 2C).

After addition of the extracts on the Hb-SS samples, which has displayed cloudy aggregates of RBC, it was observed that aggregations of RBC were dispersed after incubation with BEAT-SS 200 µg/ml (Figure 2D) and DOCABE 50 µg/ml (Figure 2E) used separately. The dispersion of RBC was most appreciated when DOCABE extract was used alone and in association with BEAT-SS extract (Figure 2F). Concerning Hb-AA samples, RBC remained dispersed in all cases after the aforementioned treatments (data not shown).

On the other hand (Figure 3), Hb-AA samples in normal density showed aggregated RBC in the control sample before addition of the extracts (Figure 3A), but these aggregations were dispersed in the samples where BEAT-SS (Figure 3B) and DOCABE (Figure 3C) were added despite the high density of RBC.

DISCUSSION

This study was conducted with the purpose to evaluate the *in vitro* action of two extracts prepared from *C. pentandra* and *Q. africana* Baill. on the deformity of sickled RBCs. Microscopic observations could suggest that the two extracts reversed the deformity of sickled erythrocytes in hypoxia conditions. The two extracts used, separately displayed a similar activity. It was estimated that 200 µg/ml for BEAT-SS and 50 µg/ml for DOCABE were suitable concentrations for reversing the deformity of sickled RBC. When the two extracts were incubated together with the sickled RBC in the aforementioned concentrations, the recovery from the sickling was not particularly different as compared to those of individual extracts alone. The sickling of RBC, which involves hemoglobin S polymerization, is a characteristic of SCA and this phenomenon leads to the pathophysiology of episodic acute pains. The inhibition of the deformity of sickled RBC is an important parameter, which may support in a preliminary stage, the use of these crude drugs in the management of the SCA.

Abnormalities implication of the adherence of sickle RBC to endothelium have been reported by several studies (Mohandas and Evans, 1984; Hoover et al., 1979); these abnormalities may also include interactions between sickle RBCs, platelets, leukocytes, and plasma constituents (Harlan, 2000; Hebbel et al., 1980; Hebbel, 1977), since they could be strongly affected by plasma factors and membrane changes on the surface of sickle RBC. Enhanced RBC aggregation under physiological conditions could be noticed in low or non-flow conditions where RBC adheres face to face to form reversible cell-to-cell contact leading to aggregations (Kavitha and Ramakrishnan, 2007). SCA is a disease among others in which, variation in blood viscosity is seen and is significantly higher than the normal viscosity. Sickle RBCs confer a major effect on the viscosity of blood in SCA because they are less deformable than normal red cells; and increase in blood viscosity is in part induced by the increase of erythrocytes aggregation (Thurston et al., 2004). In our experiment, this could be confirmed by the fact that the *in vitro* dispersion of the aggregated RBC by physiological saline was less obvious in the case of Hb-SS samples (Figure 2B) than in Hb-AA samples. This leads to the supposition that sickle RBC aggregates more

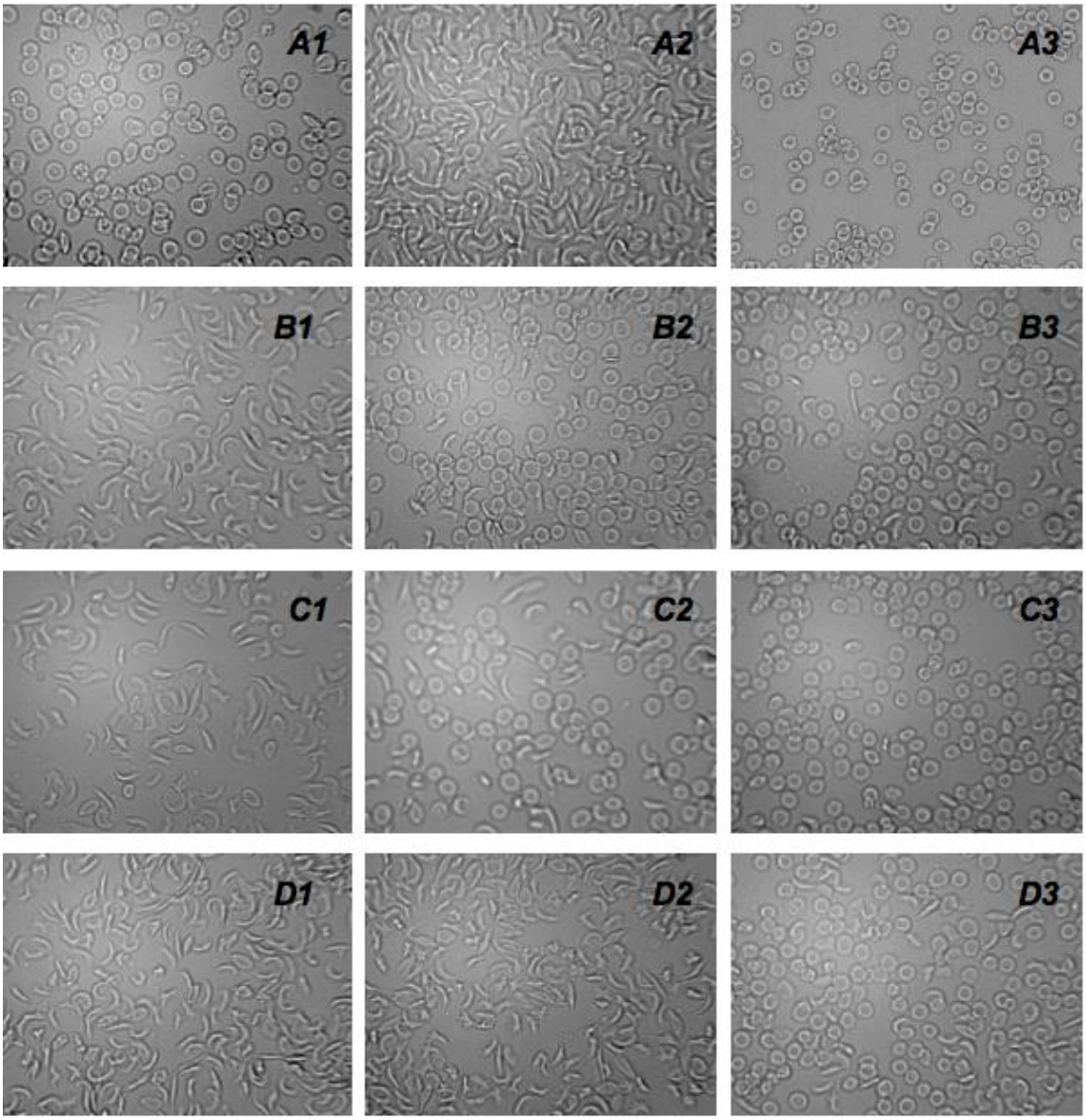

Figure 1. Effect of extracts on the reversing action of induced deformity of sickled red blood cells. Pictures were taken from microscope observation of RBC before and after inducing hypoxia followed by the treatment with the extracts. Blood samples from SCA patients (Hb-SS) were incubated with 2%$Na_2S_2O_5$ to induce hypoxia and sickling (deformity) of RBC. Samples were treated then with extracts in different concentrations. Group A displays control samples: *A1* and *A2*, RBC from Hb-SS respectively before (oxygenated state) and after addition of $Na_2S_2O_5$ (hypoxia); *A3*, RBC from healthy subjects (Hb-AA) after addition of $Na_2S_2O_5$. Group B displays samples which have been incubated with BEAT-SS extract after deoxygenation and sickling of RBC, B1: 50 µg/ml, B2: 100 µg/ml, and B3: 200 µg/ml. Group C displays samples incubated with DOCABE extract after hypoxia as explained above, C1: 5 µg/ml, C2: 10 µg/ml, and C3: 50 µg/ml. Group D displays samples incubated with both extracts together, D1: BEAT-SS 20 µg/ml with DOCABE 1 µg/ml, D2: BEAT-SS 100 µg/ml with DOCABE 5 µg/ml and D3: BEAT-SS 200 µg/ml with DOCABE 50 µg/ml.

strongly each other than RBC from Hb-AA samples (Figure 2*C*). The effect of the two extracts on the aggregation of RBC in the present experiment showed that the two extracts might prevent RBC aggregation, either when they are used separately or together (Figures 2D, E, and F and 3B and C) for both cases (HB-SS and Hb-AA); suggesting that the two extracts might contribute to reduce blood viscosity. Thus, the two drugs could be beneficial for preventing aggregations of RBC, which could be implicated in the abnormal adhesion of each other, a phenomenon among others influencing blood viscosity and initiating the cascade of micro vascular

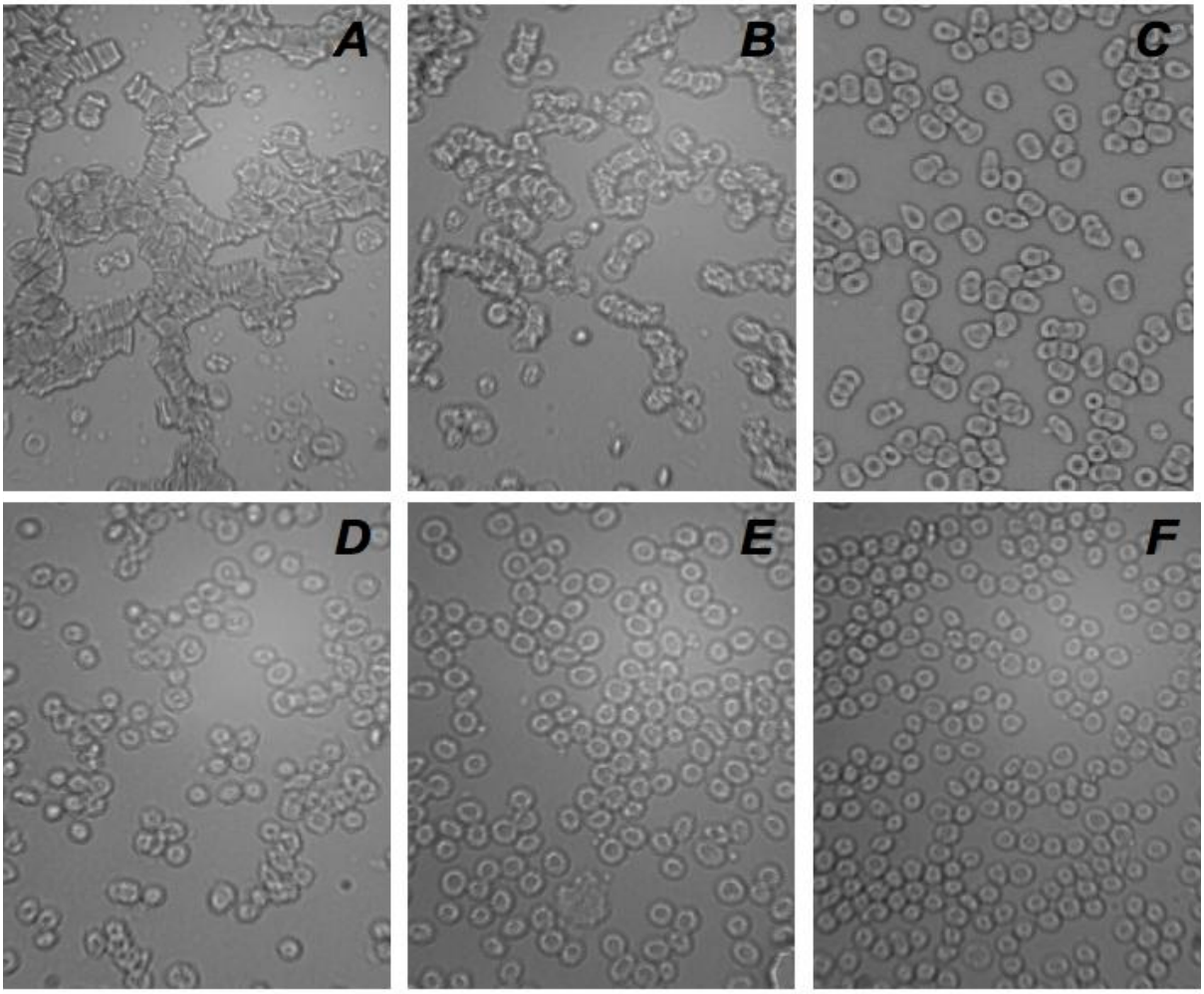

Figure 2. Effect of extracts on the inhibition of aggregated sickle red blood cells. Blood samples were allowed to rest for 48 h at 4°C, then equivalent volume of physiological saline NaCl 0.9% as control or extract diluted in physiological saline was added and incubated for 30 min. A: aggregates of RBC observed before saline addition for either Hb-SS or Hb-AA samples. B: aggregates of RBC from Hb-SS remaining after addition of equivalent volume of saline to blood sample. C: RBC from Hb-AA after addition of equivalent volume of saline to blood sample. D: RBC from Hb-SS after addition to blood of equivalent volume of BEAT-SS extract diluted in saline (200 µg/ml). E: RBC from Hb-SS after addition to blood of equivalent volume of DOCABE extract diluted in saline (50 µg/ml), and F: RBC from Hb-SS after addition to blood of equivalent volume of BEAT-SS extract and DOCABE extract in association.

occlusion.

This study suggests that the two extracts might not affect the viability of the RBC due to the fact that hemolysis of RBC did not occur in the concentrations which are reported to produce satisfactory results in the present investigation; in this case, the concentration of 200 µg/ml for BEAT-SS and 50 µg/ml for DOCABE. Furthermore, high concentrations (2500 µg/ml for BEAT-SS and 625 µg/ml for DOCABE) give some indications about the toxic dose of the two extracts on the red blood cells.

This study may be regarded as an exploratory analysis contributing to the safety profile of crude medicines from *C. pentandra* and *Q. africana* in the management of SCA. Hence, further investigations need to be carried out on these plants to discover potential new lead compounds for the management of SCA.

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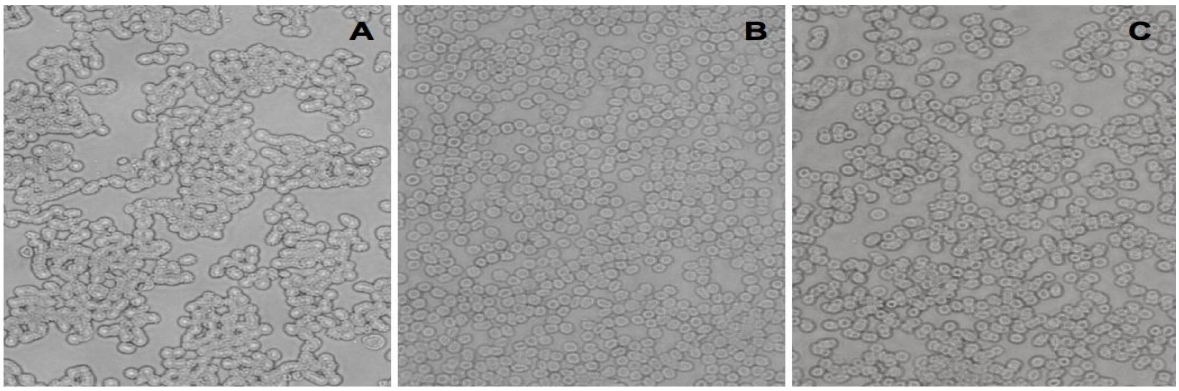

Figure 3. Effect of extracts on the inhibition of aggregated Hb-AA RBC in normal density. Hb-AA blood sample was allowed to stay for 48 h and a specimen was taken for microscopic examination (A). Then, extracts were added to the blood samples without diluting the blood. Pictures show aggregated RBC in normal density after 48 h of blood stay (A), after addition of BEAT-SS 200 µg/ml (B), and DOCABE 50 µg/ml (C).

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