In Vitro Anti-Sickling Activity of *Adansonia digitata* Fruits Extract on Sickle Cells

Abstract:

Background: Traditional medicine is used along with synthetic pharmaceutical products to enhance health management. There is a high mortality rate of patients with sickle cell disease. Previous studies have indicated that some medicinal plants have shown anti-sickling activity, indicating a new therapeutic approach to manage people affected by this hemoglobinopathy. This study aimed to assess the in vitro anti-sickling activity of *Adansonia digitata* extract on sickle cells.

Materials and Methods: Blood samples used in the evaluation of the anti-sickling activity of *Adansonia digitata* fruits extract in this study was taken from patients known to have sickle cell disease (HB SS) attending the Sickle Cell Clinic in Khartoum state. The Emmel test was used to assess the anti-sickling activities of the plants.

Results: A significant increase in the percentage of unsickled red blood cells ($p > 0.05$) was observed after incubating red blood cells (RBCs) with 2% sodium metabisulfite in the presence of 1,000, 500 and 250 µg/mL *Adansonia digitata* methanol extract.

Conclusion: This study approved that *Adansonia digitata* fruits extract had a strong anti-sickling activity so it may be used for management of sickle cell disease due to its great amount of phenol and flavonoid.

Keywords: In vitro, anti-sickling activity, *Adansonia digitata*, unsickled red blood cell, sickle cell disease.

Introduction:

Each year, approximately 100,000 children are born with sickle cell disease (SCD) globally, which is a genetic disorder. Although this disease is considered a public health problem in many countries, it is a major burden in Africa, particularly in the tropical regions of western and central Africa [1, 2]. SCD, also known as sickle cell anemia or drepanocytosis, is an inherited illness caused by abnormal hemoglobin levels. SCD causal hemoglobin (sickle hemoglobin or S hemoglobin, Hb S) is caused by a mutation at the sixth position of the beta globin chain, which leads to the substitution of glutamic acid, a polar amino acid, by valine, a non-polar amino acid. This structural modification significantly influences the physical and chemical properties of hemoglobin and hemoprotein, which are responsible for the transport of oxygen from the lungs to other tissues in the body [3, 4]. This mutation decreases the affinity of hemoglobin for oxygen. Under low oxygen tension, mutant hemoglobin polymerizes inside the red blood cells into a gel or further into fibers, leading to a drastic decrease in red cell deformability. Polymerization and precipitation of

Shemoglobin within erythrocytes causes changes in the shape of erythrocytes from their normal globular form to one resembling a sickle. Sickling of blood cells causes precocious hemolysis of erythrocytes and various complications in patients with SS [4].

Traditional medicine has become a popular form of healthcare and has been used alongside synthetic pharmaceutical products to enhance health management. Because of the high mortality rate of patients with SCD, especially children, and because chemotherapy has adverse effects, there is a need for traditional drug development that must embrace not only synthetic drugs but also natural phytomedicines/herbal drugs [5]. The active constituents of medicinal plants and naturally occurring compounds known as anti-sickling agents, which improve the health of sickle cells, are rich in aromatic amino acids, phenolic compounds, and antioxidant nutrients, which are thought to be responsible for their observed anti-sickling action. For sickle cell disease (SCD), the study of antioxidants, especially various anti-sickling agents, is of great importance because different anti-sickling agents have different degrees of effects [6]. Antioxidants (scavengers of free radicals) are believed to be the major components of these anti-sickling agents and add to their potential. Thus, it is believed that the higher the antioxidant property of the anti-sickling agent, the higher its possible anti-sickling effect, as this enables it to reduce the oxidative stress that contributes to sickle cell crisis [7]. The *Adansonia digitata* extract has been used in several applications.

Adansonia digitata is native to Africa, mainly Sudan, Botswana, Namibia, and Mozambique. It is also found in India, and belongs to the Malvaceae family. It is one of the tallest trees in the world and is commonly known as the baobab. Baobab have existed for more than 4,000 years, and is regarded as the “Queen of all carbon storage trees”, commonly referred to as the “upside-d own tree” or the “tree of life”. This is mentioned in Ayurvedic texts under the name of Gorakshi. *Adansonia digitata L.* (Malvaceae) is a universal medicinal and nutritional medicinal plant. This is a multipurpose tree that offers protection and provides
Food, clothing, medicine, and raw materials for many useful items. All parts of the plant are edible. The fruit pulp, seeds, leaves, flowers, roots, and bark of baobab are edible, and have been studied by scientists for their useful properties. *Adansonia digitata* is a large, round, canopied tree with a swollen trunk, approximately 10–25 m in height. The trunk of the tree swells greatly during the rainy season and absorbs 1,000 liters of water. Various nutritional and phytochemical constituents, including vitamin C, steroids, flavonoids, epicatechin, campesterol, tocopherol, adansonin, and amino acids, have been isolated from various parts of the plant. It has many medicinal and nonmedicinal uses. In this study, I assessed the anti-sickling activity of Sudanese *Adansonia digitata* which was collected from Damazin [8]. It has various uses, including astringent, demulcent, diaphoretic, diarrheadysentery, hemoptysis, rheumatic pain, inflammatory ulcers, intermittent fever, and antitrypanosome, anti-diabetic, anti-cancer, and diuretic activities.

1) Diuretic A ctivity[9].

2)The antibacterial activity [10] . 3)
Antidiabetic activity [11].

4)Anti-Rheumatoid Arthritic activity [12].

5)Anti-trypanosomal activity[13].

5) Hepato Protective Activity[14].

As the prevalence of sickle cell diseases continues to increase in Sudan and the economic situation of the population is poor, the treatment and management of sickle cell anemia is very expensive, with adverse effects. Therefore, this study aimed to evaluate whether the extraction of different concentrations of *Adansonia digitata* fruits could be an effective way to combat the sickling process, thus decreasing the cost and side-effects of ordinary treatment.

MATERIALS AND METHODS

This study was experimentally performed in Khartoum State from January to March 2023. The study population was 20 sickle cell anemia samples obtained from patients diagnosed with sickle cell disease (HBSS) admitted to the Jaffer Ebn Auf Hospital.

Inclusion and exclusion criteria

Samples were collected from patients with sickle cell anemia, including both sexes, of different ages. Patients diagnosed with other hereditary disorders, hemoglobinopathy, or cancer were excluded from the study.

Study design and plant materials

This experimental study evaluated the anti-sickling activity of *Adansonia digitata* fruit extracts at concentrations of 1000%, 500%, and 250% according to a previous study by Rayan Hamid et al., who worked at the same concentration for the anti-sickling activity of Moringa oleifera fruit extract [15]. Fresh Adansonia fruits were harvested from an *Adansonia digitata* tree in Damazin. The powder was collected from the fruit and extracted by cold maceration in absolute methanol for 3 days after which it was daily filtrated then extract was allowed to air in an evaporation dish till complete dryness and the yield percentages were 9.92%, calculated as follows:

The weight of the extract obtained from the plant sample × 100 was subjected to anti-sickling assays.

Preparation of Methanolic Extract:

Extraction was performed according to a previously described method. Exactly 100 g of the plant sample was coarsely powdered using a mortar and pestle. The samples were soaked in absolute methanol. Extraction was carried out for 3 days with daily filtration and evaporation of the solvent under reduced pressure using a rotary evaporator apparatus. The sample extract was allowed to air in an evaporating dish until complete dryness, and the yield percentage was calculated to be 20% [16], calculated by the weight of extract obtained/weight of plant sample × 100. Approximately 4 mL EDTA blood samples were obtained from patients and centrifuged at 3,000

rpm for 10 minutes to remove the plasma. The resulting packed erythrocytes were washed 3 times with 1 mL sterile normal saline per 5 mL of blood. The samples were then centrifuged to remove the supernatant. The washed RBC were resuspended in the remaining suspension and used for analysis.

Procedure for anti-sickling activity evaluation

Three diluted solutions in normal saline were prepared from the stock solution of plant extracts as follows (250 µg/mL, 500 µg/mL, and 1,000 µg/mL). In the Emmel test [17], washed erythrocytes were mixed with an equivalent volume of 2% sodium metabisulfite ($Na_2O_5S_2$). Ten microliters of the mixture was spotted on a microscope slide, and 10 µL of the plant extract was added and mixed with the blood mixture. Normal saline (10 µL) was added to one of the slides instead of the plant extract, which served as a control, and all the slides were covered with a cover slip. Paraffin was applied to completely seal the edges of the cover to exclude air (hypoxia), and the slides were incubated for 1 hour. Each slide had been examined under the oil immersion light microscope, and RBCs had been counted in five different fields of view across the slide [16]. The numbers of both sickled and unsickled blood cells were determined, and the percentage of unsickled cells was calculated.

Ethical considerations

Approval for this study was obtained from the Ethical Committee of Alzaeim Alazhari University. The research purpose and objectives were explained to participants in simple words. The participants had the right to participate voluntarily. Data were obtained using privacy.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences software (SPSS) version 21.0 software (SPSS for Windows). Data are reported as mean ± standard deviation (SD), and an independent test was used to compare the mean value of the control and different concentrations of extracts and to calculate *p*-values ($P < 0.05$ was considered significant).

Statistical analysis.

The percentage of unsickled cells was calculated using the formula:

Percentage of unsickling cells = Number of unsickling cells × 100 / Total cells

RESULTS

The results show the mean (SD) of the percentage of unsickled cells for the *Adansonia digitata* extracts at three different concentrations. The highest mean percentage of unsickling was found when blood was incubated with methanol extraction at 1,000 µg/mL (98.38 ± 0.783%), as explained in Table 1.

Table 1 The mean percentage (SD) of unsickled cells for the *Adansonia digitata* extracts in three different concentrations (N = 20).

Extraction	Number	Minimum % of unsickled cells	Maximum % of unsickled cells	Mean	SD
Methanol extraction, 1,000 µg/mL	20	97%	99%	98.38%	0.783
Methanol extraction, 500 µg/mL	20	92%	98%	95.82%	1.416

Methanol extraction, 250 µg/mL	20	87%	96%	92.66%	2.114
Control	20	77%	90%	83.97%	3.960

Table 2 shows multiple comparisons of the mean of percentages of unsickled cells when incubated with *Adansonia digitata* methanol extract in different concentrations (250, 500, and 1,000 µg/mL). The mean percentages were 98.38 ± 0.783%, 95.82 ± 1.416%, and 92.66 ± 2.114%, respectively. Compared with the control (83.97 ± 3.960%), the results were statistically significant (p = 0.022, 0.018, and 0.014, respectively).

Table 2 Multiple comparisons of mean percentage of unsickled cells in methanol extraction and control (N = 20).

(I)	(II)	Mean (I)	(II) Mean	*P*-value

Control	250 µg/mL	83.97%	92.66%	0.014
	500 µg/mL		95.82%	0.018
	1,000 µg/mL		98.38%	0.022

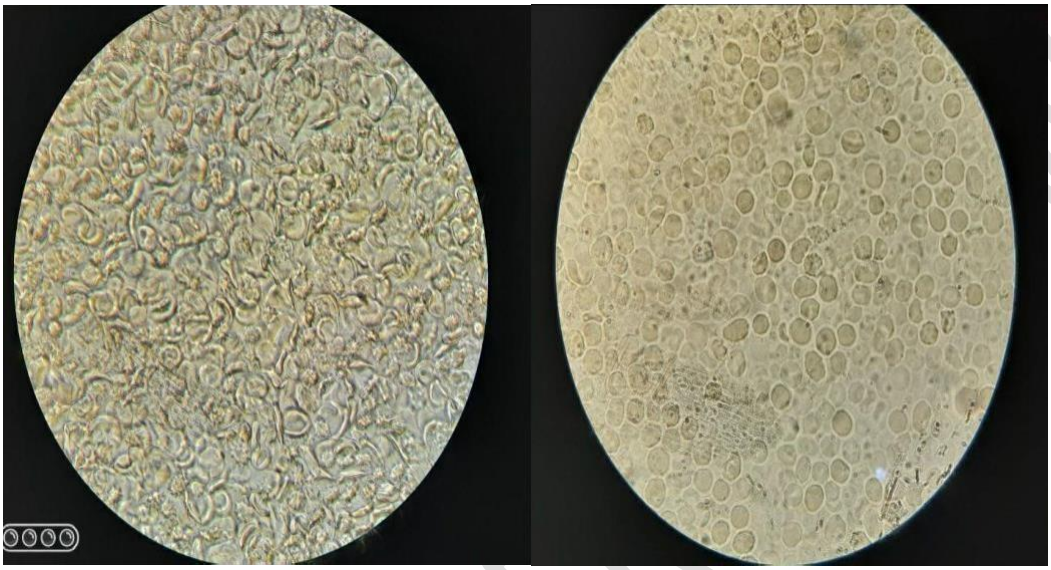
P-value <0.05: Significant difference.

As shown in Table 3, there were no statistically significant differences among the three different methanol extracts of *Adansonia digitata* at different concentrations (1,000, 500, and 250 µg/mL), with p-values of 0.275, 0.133, and 0.213, respectively.

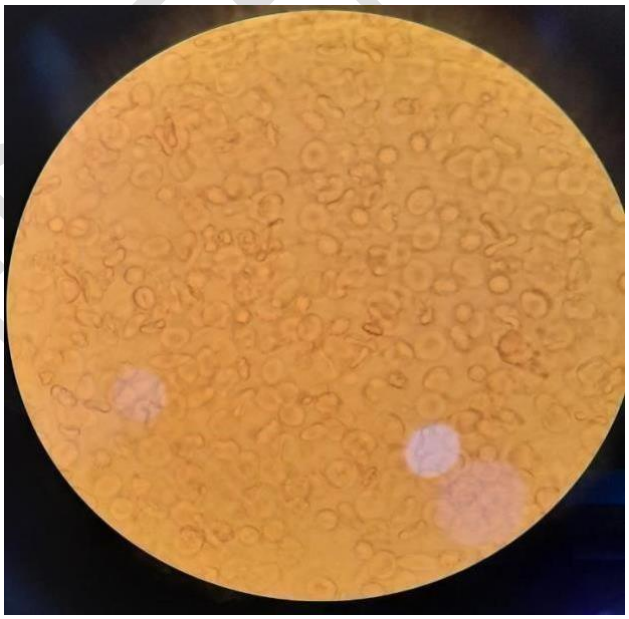
Table 3 Multiple comparisons of mean percentage of unsickled cells in methanolic extraction of *Adansonia digitata*

(I)	**(II)**	**Mean (I)**	**Mean (II)**	***P*-value**
1,000 µg/mL	500 µg/mL	98.38 ± 0.78	95.82 ± 1.41	0.275
1,000 µg/mL	250 µg/mL	98.38 ±0.78	92.66 ± 2.11	0.133
500 µg/mL	250 µg/mL	95.82 ± 1.41	92.66 ± 2.11	0.213

P-value <0.05: Significant difference.



Control **test in 1,000 µg/mL methanol extraction**



Test in 500 µg/mL methanol extraction

Figure 1. Comparison: Morphology with different concentration and control.

DISCUSSION:

Adansonia digitata fruit has high-quality leaves, including proteins, lipids, carbohydrates, ash, vitamin C, traces of calcium and phosphorus, and leaves contain mucilage, which upon hydrolysis gives galacturonic acid and glucuronic acids with small quantities of galactose, rhamnose, glucose , arabinose, and fatty acids (palmitic acid oleic acid, Stearic acid, and linoleic acid) is an essential natural resource with high antioxidant potential because of its richness in polyphenols, mainly flavonoids, and panacea properties [18].

This study demonstrated the anti-sickling activity of the methanol extract of *Adansonia digitata* fruit at different concentrations (250, 500, and 1,000 µg/mL) in sickle cells after incubation for 1 hour. The results were highly significant (*p*-values <0.05). This result agrees with a study done elsewhere; demonstrating that the effect of *Adansonia digitata* fruit on sickle cells is attributed to their high content antioxidant polyphenols and flavonoid, as is shown in a different study [8].

Multiple comparisons of the mean percentage of unsickled cells in methanol extraction in different concentrations (250, 500 and 1,000 µg/mL) confirmed that there were no statistical differences between *Adansonia digitata* extraction concentration, while it seem like there was a different in anti-sickling activity of extraction by increasing the concentration (92.66%, 95.82%, and 98.38%, respectively), but statistically all concentrations had similar anti sickling activity [no statistically significant differences (*P* value < 0.05)].

Previous studies have revealed that *Adansonia digitata* has anti-sickling activity, justifying the use of this plant in traditional Congolese medicine for

SCD management. The extracted anthocyanins showed a good effect on the stabilization of sickle cell membranes and Fe3+/Fe2+ ratio. This plant could be used as a nutraceutical for SCD treatment of sickle cell disease. Indeed, sickle cell anemia is a chronic illness; the best approach in the management of such hemoglobinopathy would be the use of edible medicinal plants to suppress some symptoms of the disease, instead of administering

medication to patients throughout their lives [19]. Another study, performed to detect the supplementary consumption of baobab (*Adansonia digitata L.*) fruit pulp, improved the hemoglobin levels and iron status of schoolchildren in Kenya, and showe d that the hemoglobin levels improved slightly (2.2%), but there was no significant effect of the fruit on improving non-heme iron absorption in most populations [20].

Owing to the scarcity of published data on this issue, few studies have been conducted. None of the traditional recipes that are used in SCD management in Sudan contained *Adansonia digitata* as a constituent. To the best of our knowledge this study is the first document describing the effect of *Adansonia digitata* on blood cells in Sudan.

Conclusion

This study showed that *Adansonia digitata* fruit extract had significant anti-sickling activity; therefore, it may be included in the management of sickle cell disease. When consumed by affected individuals, this plant could be a cost-effective way to combat sickle cell disease complications.

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