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Research Article

In vitro Anti-sickling Activity of Red Grape Juice (*Vitis vinifera* L. var. *Crimson Seedless*) on Human HbSS Erythrocytes

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Abstract

Introduction: Sickle cell disease is characterized by sickle hemoglobin (HbS) polymerization under deoxygenation conditions, causing erythrocyte sickling, hemolysis, and vaso-occlusive complications. Red grape polyphenols exhibit antioxidant properties that may contribute to erythrocyte protection. This study aimed to evaluate the *in vitro* anti-sickling activity of red grape juice (*Vitis vinifera* L. var. *Crimson Seedless*) on HbSS erythrocytes. **Methods:** An experimental *in vitro* study was conducted using blood samples from SS phenotype donors. Sickling was induced with 2% sodium metabisulfite according to the Emmel test. Erythrocytes were treated with three grape juice dilutions (1/10, 1/100, and 1/1000). Sickled erythrocytes were quantified by light microscopy, and sickling inhibition was calculated relative to the control. Results were obtained from six independent biological experiments. **Results:** Red grape juice reduced erythrocyte sickling in a concentration-dependent manner. The highest inhibitory activity was observed at the 1/10 dilution, with 58.52% inhibition, while the 1/100 and 1/1000 dilutions showed lower inhibition rates of 16.03% and 8.24%, respectively. Microscopic analysis showed better preservation of erythrocyte morphology at the 1/10 dilution. **Conclusion:** Red grape juice (*Vitis vinifera* L. var. *Crimson Seedless*) exhibits *in vitro* anti-sickling activity on HbSS erythrocytes. These findings suggest a potential interest in grape-derived bioactive compounds as complementary candidates for sickle cell disease research. Further studies are needed to identify active compounds, assess safety, and confirm efficacy in preclinical and clinical models.

Keywords: Sickle cell disease; SS erythrocytes; anti-sickling activity; *Vitis vinifera*; *Crimson Seedless*; polyphenols; Emmel test.

1. Introduction

Sickle cell disease (SCD) is an inherited genetic disorder characterized by the presence of an abnormal hemoglobin, sickle hemoglobin (HbS), resulting from a mutation in the gene encoding the β-globin chain of hemoglobin. Under deoxygenation conditions, HbS polymerization induces changes in erythrocyte shape and mechanical properties, leading to the characteristic sickle morphology. These alterations promote hemolysis, vaso-occlusive events, and the occurrence of acute and chronic complications that may affect multiple organs [1].

The management of SCD has benefited from significant advances through the use of therapies such as hydroxyurea, which reduces the frequency of some disease-related complications, as well as the development of innovative approaches, including gene therapy [2,3] However, access to these treatments remains limited in many low-resource countries due to their high cost, insufficient availability, and the challenges associated with long-term medical monitoring. In this context, the investigation of accessible natural compounds that may contribute to limiting some mechanisms involved in disease pathophysiology could represent a complementary research avenue.

Oxidative stress contributes to erythrocyte membrane alterations and reduced red blood cell deformability observed in SCD. Natural compounds rich in polyphenols have therefore attracted increasing interest due to their antioxidant properties and their potential ability to interact with cellular membranes. Red grape (*Vitis vinifera* L.) represents an important source of phenolic compounds, including flavonoids, anthocyanins, and other bioactive molecules associated with various biological activities [4,5].

Several studies have highlighted the potential role of polyphenolic compounds or plant extracts in modulating oxidative stress and certain mechanisms

associated with SCD. However, data regarding the direct effect of red grape juice on sickling of SS erythrocytes remain limited. In particular, the anti-sickling activity of the *Vitis vinifera* L. var. *Crimson Seedless* variety has, to our knowledge, not been sufficiently documented in an *in vitro* experimental model.

Therefore, the present study aimed to evaluate the *in vitro* anti-sickling activity of red grape juice (*Vitis vinifera* L. var. *Crimson Seedless*) on erythrocytes obtained from six SS phenotype donors under sodium metabisulfite-induced hypoxic conditions, using the Emmel test.

2. Materials and methods

2.1. Study design and setting

This was an experimental *in vitro* study designed to evaluate the effect of red grape juice (*Vitis vinifera* L. var. *Crimson Seedless*) on erythrocyte sickling from donors with different hemoglobin profiles. Blood samples were collected at the National Blood Transfusion Center (CNTS) in Dakar, Senegal. Laboratory analyses were performed at the Physiology Laboratory of the Faculty of Medicine, Pharmacy and Odontology (FMPO), Cheikh Anta Diop University of Dakar (UCAD), between September and October 2025.

2.2. Participants and blood samples

The study initially included 30 adult donors, divided into three groups according to their hemoglobin profile: HbAA (n = 10), HbAS (n = 10), and HbSS (n = 10). The hemoglobin status of participants was confirmed by hemoglobin electrophoresis.

Blood samples were collected from fresh venous blood drawn into tubes containing ethylenediaminetetraacetic acid (EDTA). After evaluation of sample quality and microscopic preparation conditions, six samples per group were selected for analysis (HbAA: n = 6; HbAS: n = 6; HbSS: n = 6). The remaining samples were excluded due to inadequate transport conditions or prolonged storage time before analysis, which could potentially affect sample quality, preparation integrity, and microscopic slide interpretation.

HbSS samples constituted the main study group for the quantitative evaluation of the effect of grape juice on sodium metabisulfite-induced erythrocyte sickling. HbAA and HbAS samples were subjected to the same experimental conditions to explore potential morphological changes in erythrocytes exposed to sodium metabisulfite, solvent, and different grape juice dilutions. Observations from these two groups were used for morphological comparison purposes only and were not included in the quantitative analysis of sickling inhibition activity.

Participants who had received blood transfusion within the three months preceding sample collection or who had consumed grapes, either as fresh fruit or juice, within 72 hours before blood collection were excluded.

2.3. Ethical considerations

This study received ethical approval from the Research Ethics Committee of Cheikh Anta Diop University (CADU) in Dakar.The study was conducted after obtaining informed consent from all participants. Data confidentiality and sample anonymity were maintained throughout the study.

2.4. Plant material and grape juice preparation

Red grape fruits (*Vitis vinifera* L. var. *Crimson Seedless*) were purchased from local markets and authenticated by a specialist from the Botany Laboratory of the Fundamental Institute of Black Africa (IFAN) in Dakar.

The grape clusters were carefully washed, and the berries were blended. The obtained preparation was allowed to stand for 30 minutes to promote the release of fruit constituents. The mixture was then filtered to obtain grape juice, which was used as the stock solution for the preparation of subsequent dilutions.

Three successive decimal dilutions were prepared from the stock solution using 0.9% sodium chloride (NaCl) solution as solvent. The 1/10 dilution was obtained by mixing 1 mL of grape juice with 9 mL of 0.9% NaCl solution. The 1/100 dilution was prepared by mixing 1 mL of the 1/10 dilution with 9 mL of 0.9% NaCl solution. Finally, the 1/1000 dilution was obtained by mixing 1 mL of the 1/100 dilution with 9 mL of 0.9% NaCl solution.

2.5. Solution preparation and experimental conditions

A 2% sodium metabisulfite solution was freshly prepared by dissolving 2 g of sodium metabisulfite in 100 mL of distilled water. The solution was prepared on the day of experimentation and used according to the established protocol conditions.

For each blood sample, the following experimental conditions were evaluated:

Blood + 2% sodium metabisulfite;

Blood + 2% sodium metabisulfite + 0.9% NaCl;

Blood + 2% sodium metabisulfite + grape juice diluted to 1/10;

Blood + 2% sodium metabisulfite + grape juice diluted to 1/100;

Blood + 2% sodium metabisulfite + grape juice diluted to 1/1000.

The condition containing blood, sodium metabisulfite, and 0.9% NaCl was used as the solvent control to assess the potential influence of NaCl on erythrocyte morphology and to isolate the specific effect of grape juice.

2.6. Evaluation of sickling inhibition activity

The effect of grape juice was evaluated according to the principle of the Emmel test. For each experimental condition, one drop of blood was placed on a clean glass slide and simultaneously mixed with one drop of 2% sodium metabisulfite solution and, depending on the

tested condition, one drop of 0.9% NaCl solution or diluted grape juice.

The preparation was immediately covered with a coverslip while avoiding air bubble formation. The edges of the coverslip were sealed with nail polish to limit gas exchange and maintain deoxygenation conditions. Slides were then incubated for 45 minutes at room temperature.

After incubation, preparations were examined under a light microscope at ×100 magnification. The proportion of sickled erythrocytes was determined by manual counting. Experiments were independently performed on the six selected samples from each group.

2.7. Morphological evaluation of erythrocytes

Erythrocytes were examined to identify morphological changes observed under the different experimental conditions. HbAA samples served as a reference for normal erythrocyte morphology, whereas HbAS samples were analyzed exploratorily to investigate potential specific morphological responses. Observations from HbAA and HbAS groups were described qualitatively and were not included in the quantitative analysis of sickling inhibition activity.

For HbSS samples, an erythrocyte was considered sickled when it exhibited an elongated or crescent-shaped deformation associated with loss of the normal discoid morphology. Representative micrographs were obtained to illustrate the observed morphological changes.

2.8. Calculation of sickling inhibition

The proportion of sickled erythrocytes was expressed as a percentage according to the following formula:

Sickling percentage (%) *= (number of sickled erythrocytes / total number of observed erythrocytes) × 100*

The percentage of sickling inhibition was calculated for each grape juice dilution relative to the solvent control using the following formula:

Inhibition percentage (%) *= [(percentage of sickling in control − percentage of sickling in the presence of grape juice) / percentage of sickling in control] × 100*

2.9. Statistical analysis

Quantitative data obtained from HbSS samples were analyzed from six independent biological experiments (n = 6). Results were expressed as mean ± standard deviation.

Mean proportions of sickled erythrocytes obtained under the different experimental conditions were compared with the control using repeated-measures analysis of variance (ANOVA), followed by post hoc comparisons with p-value adjustment according to the Holm method to account for multiple comparisons. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Morphological analysis of erythrocytes

Microscopic examination of blood samples allowed the assessment of erythrocyte morphology under the different experimental conditions.

HbAA samples exhibited erythrocyte morphology consistent with normal red blood cells. Observations after exposure to the solvent and the different grape juice dilutions did not reveal any particular morphological alterations. HbAS samples were also examined exploratorily to investigate any specific morphological response under the experimental conditions.

In HbSS samples, the addition of sodium metabisulfite induced erythrocyte sickling. In the presence of grape juice, a visual reduction in the proportion of sickled erythrocytes was observed, particularly at the 1/10 dilution. Representative micrographs illustrate the morphological differences observed between the experimental conditions.

3.2. Proportion of sickled erythrocytes under the different experimental conditions

Quantitative results were obtained from the six HbSS samples selected for analysis. The proportion of sickled erythrocytes is expressed as mean ± standard deviation.

The mean proportion of sickled erythrocytes was **81.83 ± 8.93%** in the control condition consisting of HbSS blood, sodium metabisulfite, and 0.9% NaCl. In the condition containing only HbSS blood and sodium metabisulfite, this proportion was **70.53 ± 14.46%**.

In the presence of grape juice, the mean proportion of sickled erythrocytes was **33.94 ± 9.26%** at the 1/10 dilution, **68.71 ± 5.63%** at the 1/100 dilution, and **75.08 ± 4.45%** at the 1/1000 dilution. Therefore, the lowest proportion of sickled erythrocytes was observed with the 1/10 dilution.

Table I. Mean proportion of sickled erythrocytes under the different experimental conditions

Experimental condition	Proportion of sickled erythrocytes (%)	n
HbSS + sodium metabisulfite + 0.9% NaCl	81.83 ± 8.93	6
HbSS + sodium metabisulfite	70.53 ± 14.46	6
HbSS + sodium metabisulfite + grape juice 1/10	33.94 ± 9.26	6
HbSS + sodium metabisulfite + grape juice 1/100	68.71 ± 5.63	6
HbSS + sodium metabisulfite + grape juice 1/1000	75.08 ± 4.45	6

Data are expressed as mean ± standard deviation.
n corresponds to the number of analyzed HbSS samples.

3.3. Percentage of sickling inhibition

The percentage of sickling inhibition was calculated relative to the solvent control condition containing HbSS blood, sodium metabisulfite, and 0.9% NaCl.

The highest inhibitory activity was observed with grape juice diluted 1/10, with an inhibition percentage of **58.52%**. The 1/100 and 1/1000 dilutions showed lower inhibition percentages, corresponding to **16.03%** and **8.24%**, respectively.

Table II. Percentage of sickling inhibition according to grape juice dilution

Grape juice dilution	Inhibition percentage (%)
1/10	58.52
1/100	16.03
1/1000	8.24

Under the experimental conditions tested, the inhibitory activity decreased with increasing dilution of grape juice.

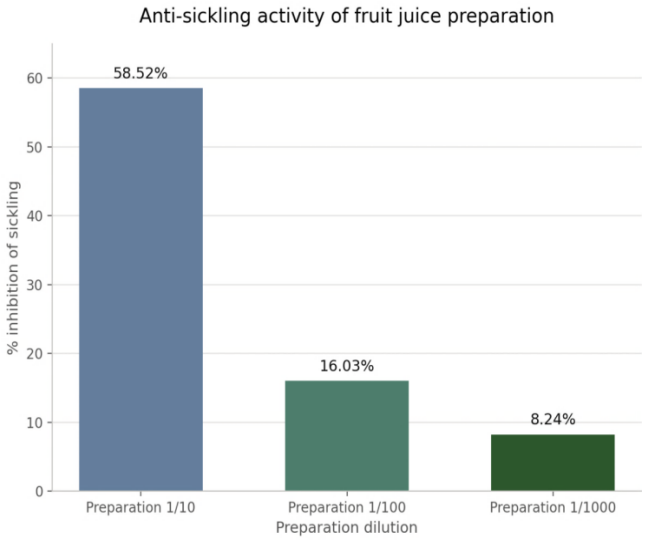

Figure 1. Variation in the percentage of sickling inhibition of sickle erythrocytes according to grape juice dilutions.

3.4. Statistical comparison of experimental conditions

Comparisons between grape juice-containing conditions and the solvent control showed statistically significant differences after adjustment of p-values using the Holm method.

The adjusted p-values were $\mathbf{1.97 \times 10^{-16}}$ for the 1/10 dilution, $\mathbf{8.42 \times 10^{-16}}$ for the 1/100 dilution, and $\mathbf{6.61 \times 10^{-6}}$ for the 1/1000 dilution.

Table III. Comparison of grape juice-containing conditions with the solvent control

Comparison	Holm-adjusted p-value
Grape juice 1/10 vs solvent control	1.97×10^{-16}
Grape juice 1/100 vs solvent control	8.42×10^{-16}
Grape juice 1/1000 vs solvent control	6.61×10^{-6}

The differences observed between each grape juice-containing condition and the solvent control were statistically significant after Holm correction.

4. Discussion

The objective of this study was to evaluate the anti-sickling activity of a red grape juice preparation (*Vitis vinifera* L. var. *Crimson Seedless*) on erythrocytes from homozygous sickle cell disease (SS phenotype) subjects under hypoxic conditions. After incubation of erythrocytes with different concentrations of grape juice for 45 minutes, morphological evaluation using the Emmel test allowed the quantification of the proportion of cells maintaining normal morphology as well as the proportion of sickled erythrocytes.

Our results demonstrated a significant anti-sickling effect of *Crimson Seedless* red grape juice on sickle erythrocytes. Analysis of sickling inhibition percentages revealed a concentration-dependent relationship, with the maximum inhibition observed at the 1/10 dilution (58.52%), whereas the 1/100 and 1/1000 dilutions exhibited weaker effects (16.03% and 8.24%, respectively). This trend was confirmed by statistical analyses, with differences compared with the control remaining significant after Holm correction. These findings suggest that the anti-sickling efficacy of grape juice depends on the concentration of bioactive compounds present in the preparation.

The proportion of sickled erythrocytes observed in the control condition containing physiological saline was higher than that observed with sodium metabisulfite alone. This difference may be related to experimental conditions, particularly the volume and composition of the added medium. However, since physiological saline was used as the solvent control for the different dilutions, the inhibitory effect of grape juice was assessed by comparison with this condition.

These findings are consistent with previous reports describing the biological properties of grape polyphenols, particularly flavonoids and anthocyanins, which exhibit antioxidant, anti-inflammatory, and cell membrane-protective activities [5]. These properties are particularly relevant in the context of sickle cell disease, in which oxidative stress, polymerization of deoxygenated hemoglobin S, and alterations in erythrocyte membrane mechanical properties play major roles in the transformation of red blood cells into sickled cells [6].

Grape phenolic compounds may therefore contribute to limiting erythrocyte sickling through several complementary mechanisms. Their ability to neutralize reactive oxygen species may reduce oxidative stress involved in HbS polymerization [5]. Furthermore, certain polyphenols may preserve erythrocyte membrane properties by modulating lipid and protein interactions involved in cellular rigidity [7]. Anti-inflammatory effects, particularly through modulation of adhesion molecules and cytokines involved in vaso-occlusive processes, may also contribute to the protective effect observed[5].

The concentration-dependent effect observed in our study is also consistent with the pharmacological properties of polyphenols, whose biological activity varies according to their concentration. At higher concentrations, these compounds exhibit greater antioxidant capacity and enhanced interactions with cellular structures, whereas these properties progressively decrease upon dilution [7].

Several previous studies have reported *in vitro* anti-sickling activity of polyphenol-rich plant extracts, particularly those derived from green tea, pomegranate, and turmeric [8]. However, data regarding grape-derived products remain limited, and the anti-sickling activity of the *Crimson Seedless* variety has been poorly investigated. To our knowledge, this study represents one of the first evaluations of the *in vitro* anti-sickling activity of *Crimson Seedless* red grape juice on human HbSS erythrocytes. Our findings provide an original contribution by demonstrating a significant protective effect of this grape juice on SS erythrocytes. These results suggest that red grape may represent a promising source of bioactive compounds worthy of further investigation as complementary approaches in sickle cell disease management.

Nevertheless, this study presents several limitations. First, the experimental model used is an *in vitro* model and does not reproduce all the complex interactions occurring in sickle cell disease patients. Second, the chemical composition of the grape juice preparation has not yet been fully characterized, preventing the identification of the specific molecules responsible for the observed activity. Finally, the absence of cytotoxicity and safety assessments at higher concentrations currently limits the extrapolation of these findings toward therapeutic applications.

Further studies are required to characterize the active compounds of *Crimson Seedless* grapes using analytical approaches such as high-performance liquid chromatography (HPLC), followed by evaluation of their efficacy and safety in preclinical models. In the long term, clinical studies may help explore the potential role of red grape-based nutritional supplementation as a complementary approach to conventional sickle cell disease treatments, particularly hydroxyurea.

5. Conclusion

This study demonstrates the *in vitro* anti-sickling activity of red grape juice (*Vitis vinifera* L. var. *Crimson Seedless*) on human HbSS erythrocytes. The observed effect was concentration-dependent, with a maximum inhibition of erythrocyte sickling of **58.52%** at the 1/10 dilution.

These findings suggest that red grape may represent a valuable source of bioactive compounds with potential to contribute to the limitation of erythrocyte sickling. However, given the *in vitro* nature of the study and the absence of chemical characterization of the grape juice preparation, these results should be interpreted with caution. Further investigations are required to identify the compounds responsible for the observed activity, assess their safety, and confirm their efficacy in preclinical and clinical models.

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Conflicts of Interest: The authors declare that they have no conflicts of interest regarding the publication of this paper.

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