Original Article

Antisickling Effect of *Ficus vallis-choudae*: A Potential Inhibitor of Erythrocyte Morphological Alterations in Sickle Cell Disease

[1]Sène Mbaye, [2]Diouf Ibrahima, [3]Ba Awa, [4]Diop Doudou, [1]Niasse Rokhy, [5]Ba Fatoumata, [1]Diaw Ndeye Absatou, [1]Kane Modou Oumy & [1]Sarr Mamadou

[1]Laboratory of Pharmaceutical Physiology, Faculty of Medicine, Pharmacy, and Dentistry, Cheikh Anta Diop University, BP 5005, Dakar, Senegal, [2]Unit of Training and Research in Health Sciences, Assane Seck University of Ziguinchor, Senegal, [3]Faculty of Health and Sustainable Development, Alioune Diop University, Bambey, Senegal, [4]Laboratory of Botany, Basic Instute of Black Africa, University Cheikh Anta Diop of Dakar, Dakar, Senegal, [5]UFR Santé, Université Gaston Berger, Saint-Louis, Sénégal

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Abstract

Background: The aim of this study is to evaluate whether a hydroethanolic extract of *Ficus vallis-choudae* (EFFV) leaves can prevent the sickling of red blood cells in individuals with sickle cell carriers(AS) and homozygous sickle cells (SS).

Material and Methods: Ten grammes of *Ficus vallis-choudae* leaf powder were macerated in 100 ml of a 60% hydroethanolic solution. After filtration, the macerate was subjected to rotary evaporation until a dry crude extract was obtained. This dry extract was reconstituted in water to prepare two solutions with concentrations of 5 mg/ml and 2.5 mg/ml. Emmel tests were performed following incubation of the extract solutions with blood from individuals with the AS sickle cell trait, SS homozygous sickle cell disease, and normal AA controls. The percentage of sickled cells was determined using optical microscopy.

Results: The hydroethanolic extract of *Ficus vallis-choudae* (EFFV) significantly reduces sickle cell formation in both AS and SS individuals. This antisickling effect is dose-dependent.

Conclusion: Our results demonstrate that the hydroethanolic crude extract of *Ficus vallis-choudae* (EFFV) could have beneficial effects in the context of sickle cell disease. These findings offer promising prospects for the development of cost-effective treatments for this condition.

Keywords: Sickle cell disease, Antisickling, *Ficus vallis-choudae.*

Introduction

Sickle cell disease is a genetic disorder of haemoglobin caused by a mutation in the beta-globin gene of red blood cells, resulting in the substitution of glutamic acid with valine at position 6 of the beta chain. This mutation produces sickle haemoglobin (HbS), which, in its deoxygenated form, polymerises to form intracellular fibres that stiffen and deform red blood cells, giving them their characteristic sickle

Corresponding Author: Professor Mbaye SENE

E-mail: mbaysen@yahoo.fr Or mbaye2.sene@ucad.edu.sn

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orcrescent-shaped[1]. These abnormal cells disrupt the physiology of the body, particularly through repeated vascular occlusions and oxygen deprivation in various organs (hypoxia). The distribution of the sickle cell gene has been well characterised for many years; it is the most common genetic disease globally, with over 50 million carriers. The highest frequencies are found in Africa, particularly in regions with a high prevalence or historical burden of malaria [2]. Senegal is notably affected, with a sickle cell prevalence of 10%, which can reach up to 40% in certain Central African countries. Each year, approximately 1,700 children are born with sickle cell disease in Senegal [3, 4, 5]. Despite several therapeutic optionssuch as hydroxyurea, blood transfusions and bone marrow transplants, as well as emerging drugs such as voxelotor, crizanlizumab and L-glutamine, developed to combat sickle cell disease, most fail to achieve the desired success. These treatments are often costly, may cause adverse effects, and remain largely inaccessible to low-income populations. In many communities, medicinal plants are used to manage the condition and relieve pain, with numerous studies demonstrating the safety, efficacy, and quality of some traditional remedies, particularly in reducing acute pain crises [6, 7]. This context forms the basis of our study, which investigates *Ficus vallis-choudae*, a plant from the Senegalese pharmacopoeiawhose richness in polyphenols has been demonstrated[8]. The aim is to determine whether a hydroethanolic extract of its leaves (EFFV) can prevent the sickling of red blood cells in individuals with sickle cell trait (AS) and those with homozygous sickle cell disease (SS).

Materials and Methods

Study Population

This study was approved by the ethics committee of Cheikh Anta Diop University in Dakar. Participants were male and female adults aged between 25 and 45 years, recruited from the haematology department of the National Blood Transfusion Centre in Dakar after giving free and informed consent. They were divided into three groups of 15 individuals: the "healthy" control group included adults without the sickle cell trait (AA); the second group comprised individuals with the sickle cell trait (AS); and the third group consisted of individuals with homozygous sickle cell disease (SS).All these subjects were subjected to a comprehensive clinical examination and additional examinations including: an interrogation, a hemoglobin electrophoresis to determine the AS and SS profiles.

Blood Samples

For each patient, blood was collected into an EDTA tube in accordance with the study protocol. Samples were obtained by venous puncture at the elbow crease and were kept cool (at +4 °C) if not used immediately.

Plant Material

The plant material consisted of leaves of *Ficus vallis-choudae*, which were harvested and authenticated in April 2020 by Dr Doudou Diop (IFAN Botanical Laboratory, Dakar), based on field observations in the Kédougou region. The powdered plant material was obtained after drying and was then stored at room temperature (25–30 °C) in a well-ventilated room.

Extraction

The aim is to introduce 10 grams of *Ficus vallis-choudae* leaf powder, obtained after grinding with an RM100 grinder, into an Erlenmeyer flask previously calibrated to zero. Then, 100 ml of hydroethanolic solution (40/60) is measured and added to the flask. The mixture is vigorously stirred with a magnetic stirrer for 24 hours. It should be noted that the Erlenmeyer flask is covered with aluminium foil to protect photosensitive molecules. After maceration, the organic phase (macerate) is recovered and stored at +4 °C to inhibit potential biochemical reactions. The macerate is then filtered using hydrophilic cotton placed in a funnel connected to a suction pump to accelerate the process. After a few minutes, a purely liquid solution is obtained. The resulting filtrate is evaporated to dryness using a rotary evaporator under the following conditions: water

bath temperature 40 °C, cooling temperature 21 °C, and rotation speed of 4000 rpm. This process yields a hydroethanolic dry crude extract of *Ficus vallis-choudae* leaves (EFFV). The dry extract is stored at −4 °C until the final analysis stage.

Preparation of Extract Solutions

Five mg of hydroethanolic dry extract of *Ficus vallis-choudae* leaves are dissolved in 1 ml of a physiological saline solution (9 per 1000 NaCl) and then vigorously homogenised. This results in a 5 mg/ml stock solution. The stock solution is then diluted by half with saline solution to obtain a 2.5 mg/ml EFFV daughter solution. The mixture is vigorously agitated.

Characterization of Antisickling Activity

An Emmel test was performed on the subjects from all three groups, using a 2% sodium metabisulphite ($Na_2S_2O_5$) solution prepared extemporaneously. It consists of observing red blood cells (red blood cells) under a microscope subject to deoxygenation (absence of oxygen). 100 µl of whole blood (AA, AS, and SS) were incubated with 100 µl of an EFFV solution at concentrations of 5 mg/ml and 2.5 mg/ml for 24 hours. The Emmel test was then performed, followed by an optical microscope sickle cell count (X 100). An average of 500 blood cells (both sickle and normal cells) was counted after randomly capturing several fields. The number of sickle cells was then counted in the basal state and in the presence of different concentrations of EFFV extracts (5 mg/ml, 2.5 mg/ml). The average number of sickle cells per field was calculated. The ratio of sickle cells to total cells (out of 500) allowed the percentage of sickle cells to be determined at baseline, at 2.5 mg/ml, and at 5 mg/ml. Four determinations were made (n = 4) for each subject, and the average was calculated.

Statistical Analysis

The results are expressed as means ± SEM of 4 experiments. Statistical significance was determined through a one-way analysis of variance (ANOVA) followed by Bonferroni's test or with Student's t test for paired data as required. Statistical analysis was performed using GraphPad. Prism version 8.0.1 ® for Windows (GraphPad Software, San Diego, Calif., USA). Values of *p < 0.05 were considered statistically significant.

Results

In the Basal State

The mean basal rate of sickle cells in SS homozygous individuals (85.5% ± 1,73) is higher than that observed in carriers of the sickle cell trait AS (67.81% ±1,45). In contrast, no sickle cells were observed in normal AA subjects (Fig. 1).

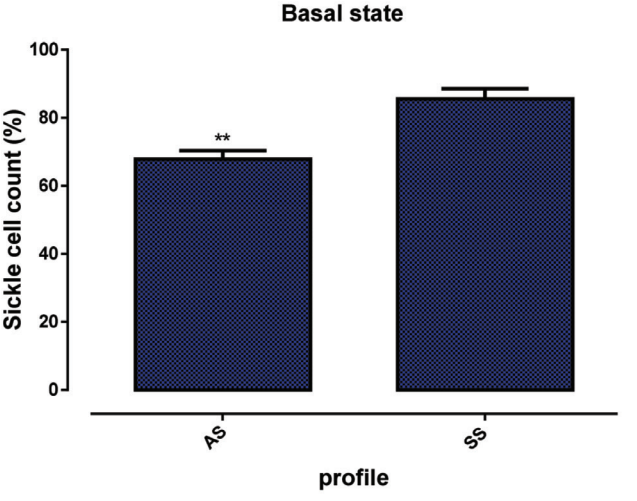

Figure 1: Variation in the sickle cell rate at the basal state (BS) in AS and SS subjects. Results are expressed as the average of for measurements on for different samples. **p < 0.01 for inhibitoryeffect versus control.

In AS Sickle Cell Trait Carriers

The hydro-ethanolic extract of *Ficus vallis-choudae* (EFFV) leaves induces a reduction in sickle cell rates in AS subjects. At baseline, the sickle cell rate was 67.81% ±1,45; following treatment with EFFV at concentrations of 2.5 mg/ml and 5 mg/ml, the rates decreased to 58.73% ± 1,73 and 23.96% ± 1,15, respectively (Fig. 2).

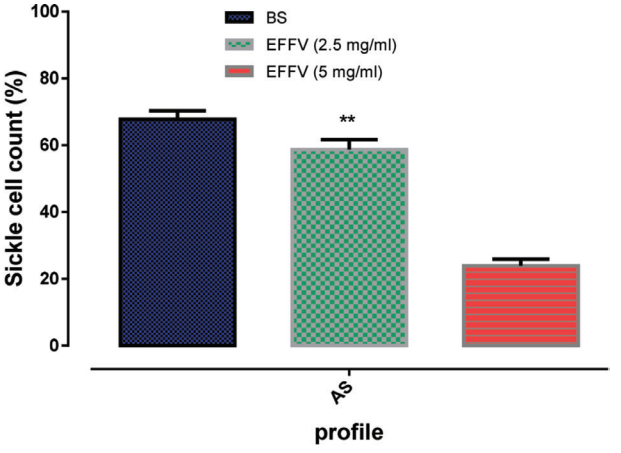

Figure 2: Variation in the sickle cell rate at the basal state (BS) and at different concentrations of the *Ficus vallis-choudae* leaf extract (EFFV) in AS subjects. Results are expressed as the average of three measurements on three different samples. ** p < 0.01 for inhibitoryeffect versus control.

In Sickle Cell SS

The hydro-ethanolic extract of *Ficus vallis-choudae* (EFFV) leaves leads to a reduction in the sickle cell rate among SS subjects. At baseline, the sickle cell rate was 85.5% ± 1,73; following treatment with EFFV at concentrations of 2.5 mg/ml and 5 mg/ml, the rate decreased to 28.33% ± 2,02 and 17.33% ± 1,45, respectively (Fig. 3).

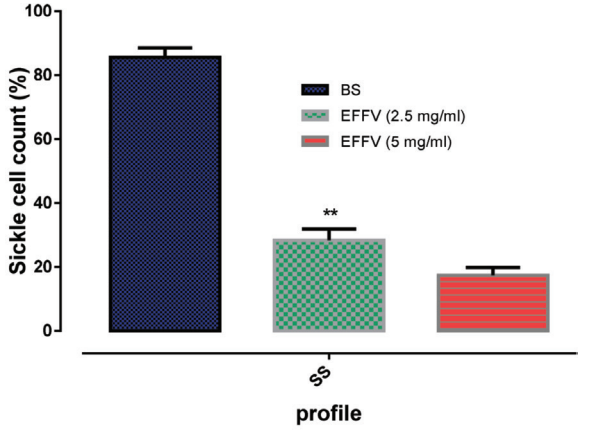

Figure 3: Variation in the sickle cell rate at the basal state (BS) and at different concentrations of the *Ficus vallis-choudae* leaf extract (EFFV) in SS subjects. Results are expressed as the average of three measurements on three different samples. ** p < 0.01 for inhibitoryeffect versus control.

Discussion

The aim of this study was to investigate the antisickling properties of a hydro-ethanolic extract of *Ficus vallis-choudae* leaves (EFFV) in subjects with sickle cell trait (AS) and homozygous sickle cell disease (SS). Our results showed that EFFV can prevent the in vitro sickling of red blood cells under hypoxic conditions. The most pronounced effects were observed at a concentration of 5 mg/ml, which could support its traditional use in the management of sickle cell disease.

Previous studies have demonstrated anti-nociceptive and anti-inflammatory activities of a methanolic extract of *Ficus vallis-choudae* stems. These properties are beneficial in managing sickle cell crises ([9]). A phytochemical analysis of the plant's leaves revealed the presence of flavonoids, glycosides, alkaloids, tannins, and saponins ([10]). The presence of alkaloids may explain EFFV's antisickling activity, supporting the findings of Dembele et al., who reported that the anti-sickling effect of *Zanthoxylum zanthoxyloides* was due to alkaloids ([11]).

Our results are consistent with those of Nongonierma et al. regarding *Ficus gnaphalocarpa*, a species in the same genus as *Ficus vallis-choudae* ([12]). Other studies using similar sickle cell models and extracts containing phenolic compounds have also demonstrated antisickling activity ([13, 14, 15]).

Some authors suggest that the antimalarial activity of certain extracts is due to anthocyanins.

The properties of anthocyanins to adsorb themselves on proteins would block the polymerization of the desoxyhemoglobin S in tactoids; this could reduce the sickling process and thus, induce the return to the normal biconcave form of the erythrocytes as the Emmel test reveals it. The sickling modifies the membrane flexibility, which would make it more fragile and would increase the precocious risk of hemolysis ([16,17]).

The phytochemical investigation of *Ficus vallis-choudae* figs led to the identification of a novel ceramide, nkwenamide, along with seven known

compounds. Although the methanolic extract exhibited urease, α-glucosidase inhibition, and antioxidant activity, most of the isolated compounds were primarily active against urease and α-glucosidase and reactive oxygen species. These findings support the inclusion of *Ficus vallis-choudae* in the *Cameroonian pharmacopoeia* ([18, 19]).

Although previous studies have identified chemical constituents in the bark of *Ficus vallis-choudae*, further investigations are needed to assess the bioactive components of the hydro-ethanolic leaf extract. Such studies would help determine which specific fractions are most active and clarify the mechanisms involved. Additional phytochemical investigations on various parts of this plant are warranted, given its important role in traditional medicine.

Conclusion

The findings demonstrate that the hydro-ethanolic extract of *Ficus vallis-choudae* leaves is capable of preventing red blood cell sickling, resulting in a significant reduction in the proportion of sickle cells. As this reduction appears to be concentration-dependent, a dose-response toxicity study is warranted to determine the therapeutic range and to identify any doses that could be harmful or affect organ function. This study supports the potential of *Ficus vallis-choudae* as a promising and accessible option for the management of sickle cell disease.

Conflict of Interest: The authorsdeclarethatthey have no conflict of interest.

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Ethical Clearance: All manipulations were carried out after the approval of the ethics committee of the Cheikh Anta Diop University of Dakar (UCAD), at the laboratory of pharmaceutical physiology of the faculty of medicine, pharmacy and dentistry of the Said university.

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