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Research Article

Antisickling Properties of Hydro-Alcoholic Extract of *Ficus abutilifolia* Leaves

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ABSTRACT

Background: The aim of this study was to evaluate the antisickling activity of a hydro-ethanolic extract of *Ficus abutilifolia* leaves using an in vitro model with red blood cells from AS and SS sickle cell subjects.

Materials and Methods: Emmel tests were conducted on blood samples collected from for healthy subjects AA, for healthy subjects AS and for subjects SS using a freshly prepared sodium metabisulphite solution. The average age of the subjects was 18 years. There were 8 women, including 4 with SS, 2 with AS, and 2 healthy subjects, as well as 4 men, including 2 with AS and 2 healthy subjects. In eight experiments, the samples were pretreated with a solution of *Ficus abutilifolia* leaf extract at varying concentrations (1.25 and 2.5 mg/ml). Micrographs were then taken for quantification purposes.

Results: The findings indicate that, in the basal state, SS subjects had a higher sickle cell count (90.15%) compared to AS subjects (73.15%). The *Ficus abutilifolia* leaf extract (EFFA) resulted in a significant reduction in the sickle cell count in SS subjects, with the rate dropping from 90.15% to 20.93% at a concentration of 1.25 mg/ml. In AS subjects, the sickle cell count decreased from 73.15% to 10.75% at the same concentration. However, increasing the extract concentration to 2.50 mg/ml seemed to lessen this effect, with only a slight reduction in the preventive effect on sickling. **** $p < 0.0001$ for inhibitory effect versus control.

Conclusion: These results indicate that the *Ficus abutilifolia* leaf extract exhibits an antisickling activity, which supports its use in traditional medicine.

1. Introduction

Sickle cell disease, also known as sickle cell anemia, is an autosomal recessive genetic disorder. It is caused by abnormal hemoglobin (hemoglobin S), where the glutamic acid is replaced by another amino acid, valine, at the 6th position in the β-globin chain of hemoglobin (Pagnier *et al*., 1985). Sickle cell disease is a major cause of morbidity and mortality, making it a serious public health issue. Its prevalence in Africa is 5 to 7% of the population, with the highest frequency found south of the Sahara (Gentilini et al., 1986). In Senegal, the prevalence ranges from 8 to 10% (Yuma and al., 2013).

To date, although some therapeutic means can improve the prognosis of sickle cell disease, such as allogeneic transplantation, these methods are costly and beyond the reach of poorer countries (Kambale et al., 2013). In Senegal, *Ficus abutilifolia* leaves are used in traditional medicine to treat sickle cell disease (*Personal observations*). However, no scientific proof of the effectiveness of this treatment has been provided. This study was conducted to evaluate the antisickling activity of a hydro-ethanolic extract of *Ficus abutilifolia* leaves.

2. Materials and methods

The study was started after the approval of the university's ethics committee, Cheikh Anta Diop in Dakar.

2.1 Plant Material

The plant material consisted of *Ficus abutilifolia* leaves, which were collected during the rainy season in Kédougou, a region in Senegal. Then they were authenticated under the voucher number IFAN55458 by collaborators from the botany laboratory at IFAN (The Fundamental Institute of Black Africa), Dakar. The *Ficus abutilifolia* leaf powder was obtained after drying for 15 days, shielded from light in room temperature and pulverization and was stored in containers at room temperature (25 to 30°C) in a ventilated room. Ten (10) grams of *Ficus abutilifolia* leaf powder were macerated in a hydro-ethanolic solution (60 ml ethanol with 40 ml distilled water) for 24 hours. After maceration, the organic phase or macerate was recovered and stored at 4°C. The obtained macerate was then filtered using hydrophilic cotton placed in a funnel connected to a suction pump. The filtrate was evaporated to dryness using a rotary evaporator equipped with a water bath heated to 40°C under 4000 rpm, then cooled to 21°C. The dry crude extract obtained after evaporation was stored at -20°C before further steps. From the crude extract, solutions of *Ficus abutilifolia* leaf extract were prepared by dissolving 5 mg of crude extract in physiological saline solution (0.9% NaCl) to obtain a solution at 2.5 mg/ml. This solution was then diluted by half to obtain a solution at 1.25 mg/ml.

2.2 Blood Sampling

Blood samples were obtained in National Blood Transfusion Center with the collaboration of six healthy volunteers and six patients with the AS sickle cell trait and six patients with SS sickle cell disease after they had signed a clear and informed consent form. Three ml of blood samples were taken in EDTA tubes by venipuncture at the elbow crease and stored at 4°C before use.

2.3 Antisickling Activity

To evaluate the antisickling activity, Emmel tests (Emmel VE. 1917) were performed with a freshly prepared sodium metabisulfite solution at 2 % on blood samples collected from for healthy subjects AA, for healthy subjects AS and for subjects SS. The average age of the subjects was 18 years, followed by counting sickle cells under an optical microscope at 40x magnification.

2.4 Basal State: 100 µl of whole blood was incubated with 100 µl of a buffer solution (physiological saline) for 24 hours. After incubation, the red blood cells are mounted between a slide and coverslip in equal volumes with metabisulfite, sealed with nail varnish followed by cell counting at 40x magnification.

2.5 Antisickling Activity of the Extract: 100 µl of whole blood was incubated with 100 µl of the *Ficus abutilifolia* leaf extract solution at 1.25 mg/ml and 2.5 mg/ml for 24 hours. Emmel tests were then performed, followed by sickle cell counting under an optical microscope at 40x magnification.

2.6 Sickle Cell Counting Technique

A count of 500 blood cells (sickle cells and normal cells) was obtained by random counting of multiple micrographs fields at 40x magnification. The ratio of the number of sickle cells to 500 cells per micrographic field captures allowed us to obtain the percentage of sickle cells at the basal state, at 1.25 mg/ml, and 2.5 mg/ml. Four images per sample were analyzed for a total of four samples (n = 4), and the average was calculated. The results were expressed as the percentage of sickle cells.

2.7 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.

3. Results

3.1 Emmel Tests at Basal State

The average sickle cell rate at the basal state (BS) in SS carriers (90.01%) is higher than that in AS subjects (73.15%). No sickle cells were found in normal AA subjects (0%) (Figure 1).

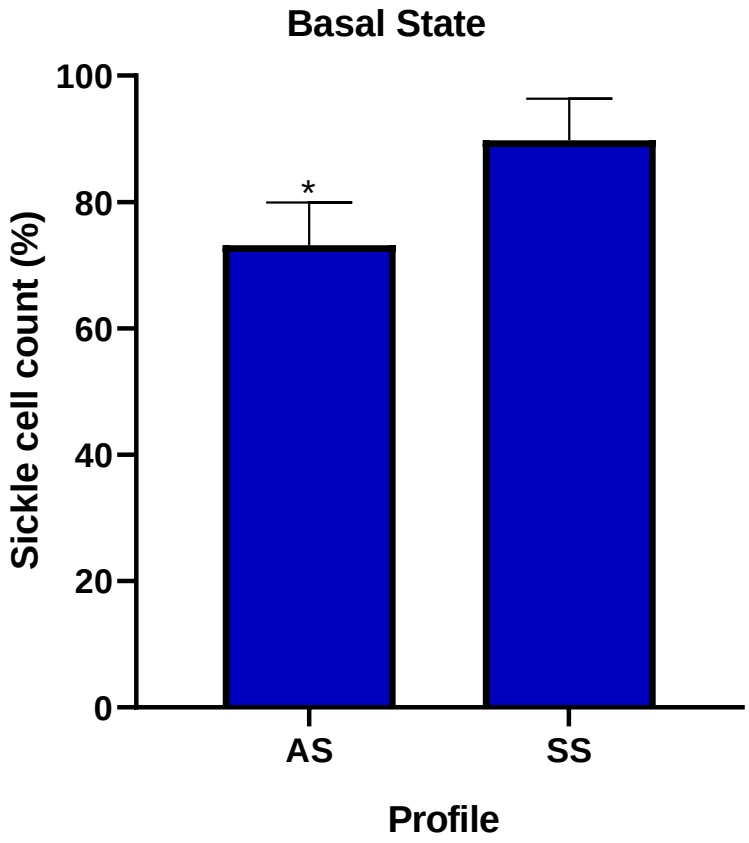

Figure 1: Variation in the sickle cell rate at the basal state (BS) in AS and SS subjects. Results are expressed as the average of for measurements on for different samples. *p < 0.05 for inhibitory effect versus control.

3.2 Antisickling Activity

The *Ficus abutilifolia* leaf extract (EFFA) induces a significant reduction in the sickle cell rate in AS subjects. At the basal state (BS), we observed a sickle cell rate of 73.15%. After adding the extract at a

concentration of 1.25 mg/ml, the sickle cell rate decreased from 73.15% to 10.75%. However, when the dose was increased (2.5 mg/ml), the rate increased from 10.75% to 20.74%, indicating a loss of activity with the increased dose (Figure 2).

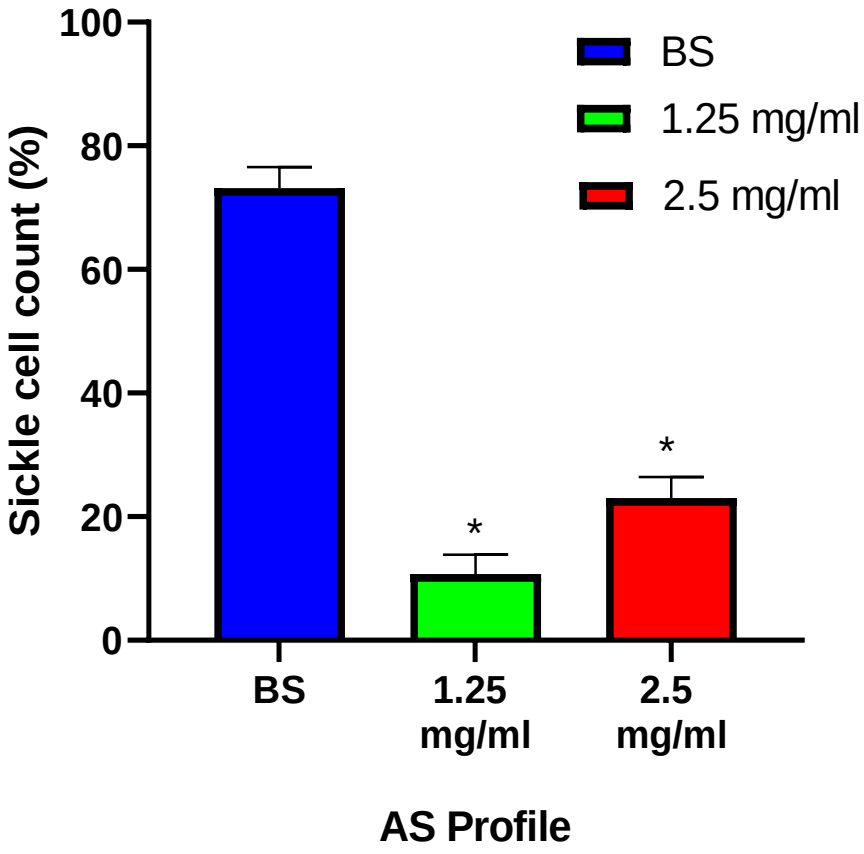

Figure 2: Variation in the sickle cell rate at the basal state (BS) and at different concentrations of the *Ficus abutilifolia* leaf extract (EFFA) in AS subjects. Results are expressed as the average of three measurements on three different samples. **** $p < 0.0001$ for inhibitory effect versus control.

The *Ficus abutilifolia* leaf extract (EFFA) induces a significant reduction in the sickle cell rate in SS subjects. At the basal state, the sickle cell rate was 90.01%. After adding the extract at a concentration of 1.25 mg/ml, the sickle cell rate dropped to 20.93%. However, when the concentration was increased to 2.5 mg/ml, the sickle cell rate slightly increased to 23.01%, indicating a reduction in the preventive effect of the extract at higher concentrations (Figure 3).

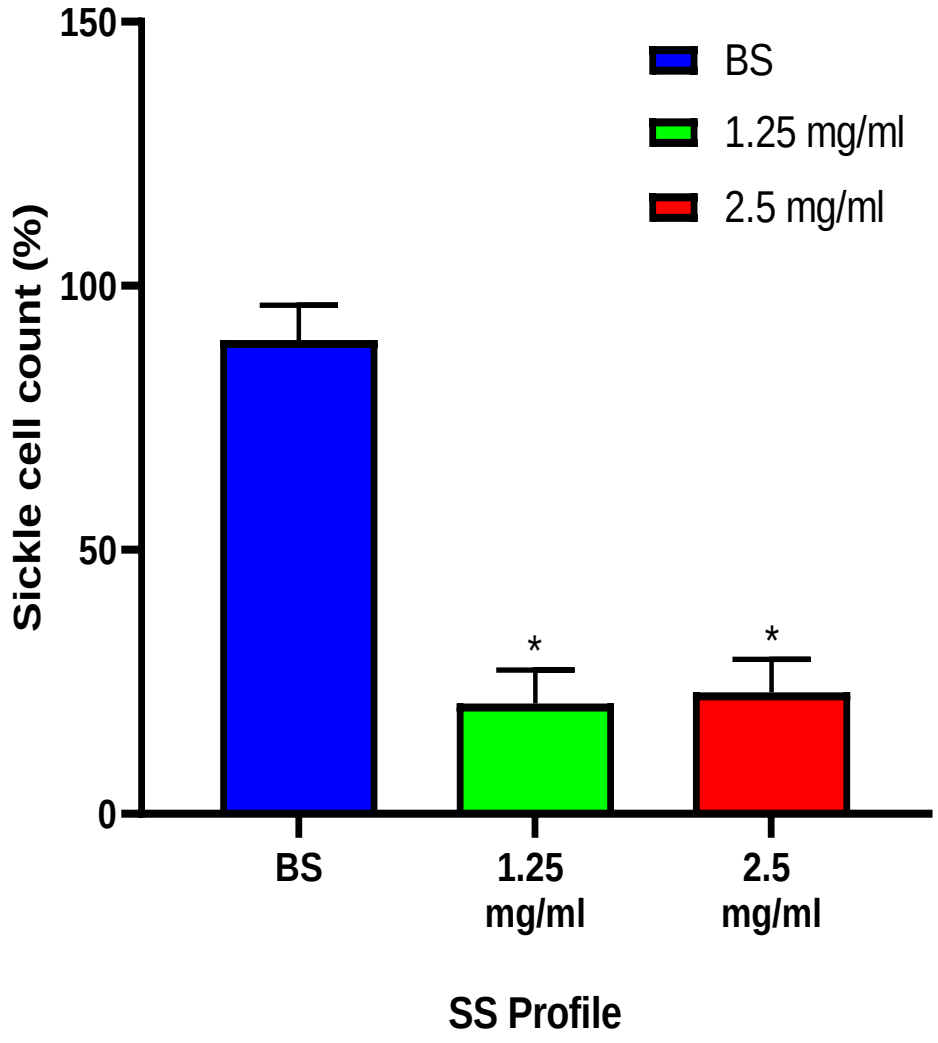

Figure 3: Variation in the sickle cell rate at the basal state (BS) and at different concentrations of the *Ficus abutilifolia* leaf extract (EFFA) in SS subjects. Results are expressed as the average of three measurements on three different samples. **** $p < 0.0001$ for inhibitory effect versus control.

4. Discussion

The results obtained in this study demonstrate significant antisickling activity of the hydro-alcoholic extract of *Ficus abutilifolia* leaves in SS and AS sickle cell subjects. At the basal state, SS subjects had a higher sickle cell rate (90.01%) compared to AS subjects (73.15%), which is consistent with the relative severity of sickling in SS subjects. No sickle cells were observed in normal AA subjects, confirming that sickle cell disease is absent in this population.

The effect of the *Ficus abutilifolia leaf extract* (EFFA) led to a significant reduction in the sickle cell rate in SS and AS subjects. At a concentration of 1.25 mg/ml, a significant decrease in the sickle cell rate was observed in both groups: 73.15% to 10.75% in AS and 90.01% to 20.93% in SS. These results suggest that the extract has a powerful in vitro antisickling effect at this concentration. This effect may occur through several mechanisms, including stabilization of the normal shape of red blood cells or inhibition of sickle cell formation.

However, increasing the concentration of the extract to 2.5 mg/ml appears to diminish this effect, with an increase in the sickle cell rate to

20.74% in AS and 23.01% in SS. This phenomenon could indicate a reverse dose-dependent effect, where a higher concentration of the extract might induce toxicity or a pro-oxidant effect, counteracting the beneficial effects observed at a lower dose. This result highlights the importance of determining the optimal concentration of the extract to maximize its therapeutic effect while minimizing potential adverse effects.

This result corroborates those of Mpiana and his collaborators (Mpiana and al., 2008) for a number of plants such as Adansonia digitata used in traditional Congolese medicine against sickle cell disease. The fact that aqueous or alcoholic extracts are active indicates that the chemical group responsible for this activity is soluble in these solvents. According to the same team, this activity is likely due to polyphenols, particularly anthocyanins (Mpiana and al., 2010). Similarly, studies conducted by Akjie and Fung 1992, Elekwa and al. (2005), Iyamu et al. (2002), Ibrahima and al. (2017), Egunyomi and al. (2009), and Ogunyemi and al. (2008) on plants such as *Centella asiatica*, *Thomandersia hensii*, and *Maesopsis eminii* have shown similar results.

Some studies conducted on the same sickle cell model with extracts containing similar compositions to ours, particularly with the presence of phenolic compounds, have demonstrated antisickling activity. For example, research by Norgonierma B.R. and collaborators on *Ficus gnaphalocarpa*, a species in the same family as *Ficus abutilifolia*, has shown such activity (Nongonierma and al., 2007). Studies *on Ficus umbellata*, another species in the same family as *Ficus abutilifolia*, have also indicated antisickling properties (Diouf and al., 2017). In general, polyphenols are known for their antioxidant capacity (Scalbert and al., 2002). Indeed, studies conducted by Mpiana and al. on Adansonia digitata and *Ficus gnaphalocarpa* (Mpiana and al., 2008; Scalbert and al., 2002), as well as other studies on *Ficus umbellata* by Diouf and al. (Diouf and al., 2017), have shown that the normalization rate, or the percentage of sickled cells returning to a normal shape under hypoxic conditions, increases with the concentration of plant extracts until a maximum threshold is reached, beyond which the normalization rate remains constant regardless of further concentration increases.

In contrast to the study by Mpiana and al. (Mpiana and al., 2008) on the same plant family, our extract in AS and SS individuals (Figure 42) shows that when the concentration increases from 1.25 mg/ml to 2.5 mg/ml, the preventive effect is reduced. This suggests that the plant's effect is concentration-independent and appears to be more effective at lower concentrations. The decreased efficacy of the extract could be due to intracellular dehydration of red blood cells in a highly concentrated environment. It could also be explained by an inverse effect of the extract when its effective concentration is doubled or becomes excessively high. One of the best ways to address these questions is to conduct dose-dependent experiments using different specific fractions of the extract.

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