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RESEARCH ARTICLE

Study of the antidrepanocytary properties of an extract of *Ficus umbellata* leaves

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ABSTRACT

Background: Sickle cell disease is the most common symptomatic hemoglobinopathies in the world, especially in Black Africa. Herbal medicine becomes an alternative for the treatment of this condition, especially as the African pharmacopeia in general and Senegal, in particular, is widely rich plants with anti-sickle interesting properties. **Aim and Objective:** Our study aimed to evaluate the antifalcémiante activity of the extract of leaves of *Ficus umbellata*. **Materials and Methods:** Two solutions hydroethanolic extract of leaves of *F. umbellata* dosed at 2.5 mg/L and 5 mg/L were prepared. Then, test Emmel was performed with and without the solution of the extract on blood samples from carriers of the sickle cell trait AS, SS, and subjects not having the move. Sickle cell counting by light microscopy at a magnification of 100 was followed. **Results:** At baseline in AS subjects, we have a sickle rate of 47%, after adding the extract to 2.5 mg/ml, we have a sickle rate of 18.1%, and after adding the extract 5 mg/ml, the rate rose to 12%. Similarly, in subjects trait carriers SS at baseline, we have a sickle rate of 72.7%, after adding the extract at concentrations, 2.5 and 5 mg/ml rates increased, respectively, 32.5% and 20%. **Conclusion:** The results showed that the leaf extract of *F. umbellata* induced a significant decrease in sickle rates. This activity is dose-dependent for preventing sickling is sharper at high concentrations.

KEY WORDS: Prevention; Sickle Cell Disease; Sickle Cell Anemia; *Ficus umbellata*

INTRODUCTION

Sickle cell anemia is a genetic disease that particularly affects tropical regions.

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Several treatment methods have been devised to relieve the sick, among other grafting bone marrow gene therapy, repeated blood transfusions, taking hydroxyurea, etc.[1-11] The use of medicinal plants seem yet to give a little more hope. Indeed, several plants used in traditional African medicine against this genetic disease have shown *in vivo* activity antifalcémiante.[1] That's the whole point of our study is conducted on a plant, *Ficus umbellata* used by people in northern Senegal to treat sickle cell anemia.

In the first part, do a literature review on sickle cell disease and *F. umbellata*.

In the second part, study the properties antifalcémiantes hydroethanolic extract of the leaves of *F. umbellata*.

MATERIALS AND METHODS

The study was started after the approval of the university's ethics committee Cheikh Anta Diop in Dakar; blood samples were obtained from AA subjects and other carriers of sickle cell traits known AS and SS by venipuncture at blood transfusion center of Dakar. We have obtained the prior informed consent of patients who voluntarily gave their blood for the needs of the study.

The plant material consists of leaves of *F. umbellata* whose harvest and authentication and was conducted at the Botanical Laboratory of the Dakar IFAN on a tree specimen Dindefelo in the region of Kedougou Senegal.

The powder drug obtained after spraying is kept at room temperature (25°–30°) in a ventilated room.

Is introduced 10 g of powder of leaves of *F. umbellata*, obtained after crushing by the crusher RM100, Erlenmeyer flasks in a previously weighed to 0. Measure 100 ml of hydréthanolique solution is added into the flask. The latter is vigorously stirred using a magnetic bar during 24. It should be noted that the flask is covered with aluminum foil to protect the photosensitive molecules. After maceration, the organic phase or macerate is recovered and stored at + 4°C to block any biochemical reactions. After this step, the macerate is filtered with cotton wool placed in a funnel connected to a suction pump which accelerates the filtration. After a few minutes, we get only a liquid solution. The filtrate obtained is evaporated to dryness using a rotary evaporator under the following conditions: Temperature of the water bath 40°C, cooling temperature 21°C *Ficus umbellata*, and the number of rotation 4000 r/min. Thus, the evaporation results in obtaining a dry crude extract ethanolic leaves of *F. umbellata*. The dried crude extract is kept at a temperature of −4°C in the refrigerator before the final step of the analysis.

Preparation of the Solution of *F. umbellata*

Introduce 5 mg of aqueous-ethanolic extract of leaves of *F. umbellata* dissolved in 1 ml of a physiological saline solution (9/1000 NaCl). This allowed us to have a stock solution of *F. umbellata* 5 mg/ml. The latter is vigorously stirred.

Then, we make a ½ dilution of the mother with saline. This allowed us to have a daughter solution *F. umbellata* 2.5 mg/ml. The latter is vigorously stirred.

In search of antifalcémiante activity tests, Emmel is made with sodium metabisulphite solution prepared extemporaneously in about AA AS SS followed by counting of the sickle optical microscope at a magnification of 100.

Basal State

100 .mu.l whole blood are mixed with 100 .mu.l of a buffer solution (physiological solution) and then incubated for 24 h. After incubation, test Emmel is made followed by counting under magnification 100.

Antifalcémiante Activity of the Extract

100 .mu.l of whole blood are incubated with 100 .mu.l of a solution containing 2.5 mg/ml–5 mg/ml for 24 h. We then realized Emmel tests followed by a count of sickle optical microscope 100.

Count Technique Sickle

A number of 500 blood cells (sickle cells and normal cells) are obtained after random counting multiple captures microscopic fields with the objective ×100. The ratio of the number of sickle cells/500 cells allowed us to get the percentage of sickled cells at baseline, 2.5 mg/ml and 5 mg/ml.

Three determinations were performed ($n = 3$) and the mean ± STD is calculated.

The results are expressed in sickle cell ratio (%) operated by the software Prism GraphPad.

RESULTS

Figures 1 and 2, respectively, following micrographs give blood only AS and AS blood in the presence of hydroethanolic extracts of *F. umbellata*. The sickle average rate at baseline of sickle cell trait SS (72.7%) is higher than that of sickle AS (47%). In normal AA, subjects not found in sickle cells (0%)

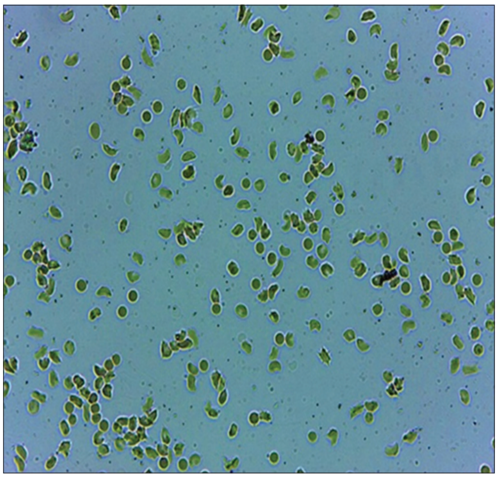

Figure 1: Optical micrograph of untreated sickle AS (baseline) (0.9% NaCl; 2% $Na_2S_2O_5$; ×100)

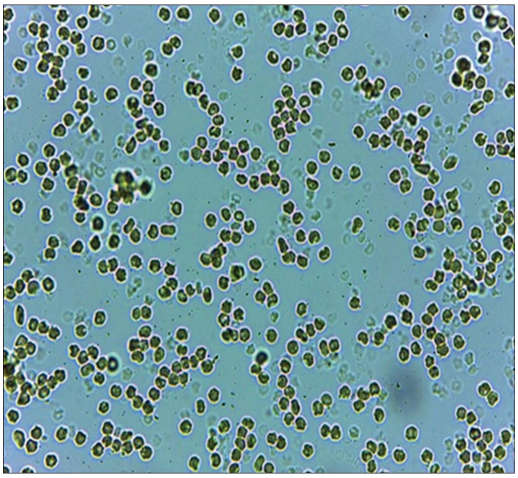

Figure 2: Optical micrograph of sickle cells treated with AS extracts hydroethanolic (2.5 mg/ml) (0.9% NaCl; 2% $Na_2S_2O_5$; ×100)

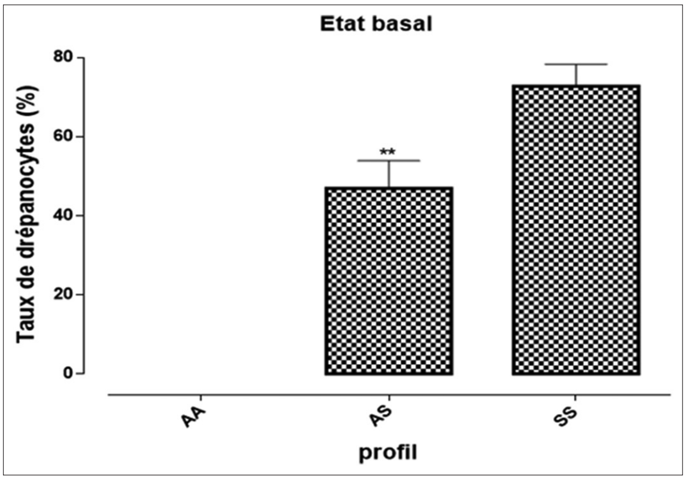

Figure 3: Change in sickle cell levels at baseline in AA, AS, and SS. Results are expressed as mean ± STD three measurements on three different samples

[Figure 3]. The extract of leaves of *F. umbellata* induced a significant decrease in sickle cell levels in AS subjects. In fact, at baseline we have a sickle rate of 47%, after adding the extract to 2.5 mg/ml, we have a sickle rate of 18.1%, and after adding the extract 5 mg/ml, the rate rose to 12% [Figure 4].

DISCUSSION

Comparing Figures 3 and 4, we can say that the hydroethanolic *F. umbellata* extract has the ability to put the sickle erythrocytes in their normal form because the two micrographs were performed in hypoxic conditions. This shows that this plant has an antifalcemic activity *in vitro*. Indeed at baseline in subjects AS, we have a sickle rate of 47%, after adding the extract to 2.5 mg/ml, we have a sickle rate of 18.1%, and after addition of extract 5 mg/ml, the rate rose to 12%. Similarly, in subjects trait carriers SS at baseline, we have a sickle rate of 72.7%, after adding the extract at concentrations, 2.5 and 5 mg/ml rates increased, respectively, 32.5% and 20%.

These results corroborate those of Nongonierma *et al*.[10] regarding the *Ficus gnaphalocarpa* which belongs to the same family as *F. umbellata*. However, it has assessed the anti-sickle cell activity compared to pentoxifylline; likewise, this result corroborates those of Mpiana *et al*.[5-9] for many of the plants used in traditional medicine against Congolese sickle cell disease. The fact that the hydroalcoholic extract or active indicates that the chemical group to the base of this activity is soluble in the solvents used. The antifalcémiante property is generally attributed to the inhibition of the polymerization of hemoglobin S. Moreover, Mpiana *et al*.[5-9] have obtained results showing that it is the anthocyanins which are natural to the base antifalcémiante the activity of a plant su same kind, namely capensis ficus. Indeed, anthocyanins are known to have the ability to interact with proteins. Their possible interaction with hemoglobin S may compete with the polymerization of the hemoglobin and thus prevent the sickling of sickle

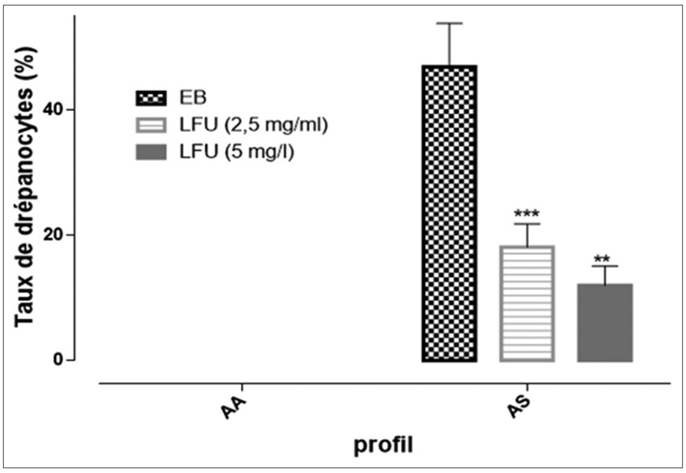

Figure 4: Change in sickle rate at baseline and different concentrations of the extract of leaves of *Ficus umbellata* in AS. Results are expressed as mean ± STD three measurements on three different samples

cells. For the polymerization is one of the determinants of sickling.[3] Anthocyanins are also known for their antioxidant properties, they may also act on the Fe3 + ratio/Fe + 2 which is elevated in the sickle cell or the stability of the erythrocyte membrane (Mian *et al*., 1977; Kahkoonen *et al*., 2003).[4] It is, in fact, known that the formation of tactoids intraerythrocytic is the basis of a phenotypic modification of red blood cells of the blood SS.[2] As against the chemical screening performed on leaves of *F. umbellata* shows that it contains tannins, flavonoids, and cardiac glycosides.

In summary, our study has provided scientific evidence on the anti-sickle cell effect of *F. umbellata* leaf extract already used in traditional medicine. On the other hand it would be interesting to make a chromatographic separation of the total extract and see if the *F. umbellata* also contains anthocyanins like most of the plants having this same activity. Other research will also be able to test the tannins, flavonoids, and cardiotonic heterosides of this plant to see with greater precision all the active molecules. Finally, exploring the mechanisms involved will allow great progress.

CONCLUSION

Our study aimed to evaluate the antifalcémiante activity of the extract of leaves of *F. umbellata*. The results show that the water-ethanol extract of leaves of this plant has antifalcémiantes vitro properties. Indeed at baseline in subjects AS, we have a sickle rate of 47%, after adding the extract to 2.5 mg/ml, we have a sickle rate of 18.1%, and after adding the extract at 5 mg/ml, the rate rose to 12%. Similarly, in subjects trait carriers SS at baseline, we have a sickle rate of 72.7%, after adding the extract at concentrations, 2.5 and 5 mg/ml rates increased, respectively, 32.5% and 20%. It would be interesting for further research on the chemistry, toxicity, and therapeutic doses to better popularize the use of the plant.

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