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# "HÉMODYA": A PHYTOMEDECINE FOR SICKLE CELL DISEASE MANAGEMENT IN CAMEROON

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## ABSTRACT

The present study, evaluates the role of "hémodya" as a phytomedicine in the management of sickle cell disease. The phenolic content, free radical, and reactive oxygen scavenging ability of "hémodya" were measured. The quality of hémodya's antioxidant was evaluated in the prevention of LDL oxidation *in vitro*. Blood samples of sickle cell patients were incubated with "hémodya" for one hour to study the migration of membrane and plasma lipids. "Hémodya" showed some antioxidant capacity by scavenging free radicals and reactive oxygen species *in vitro*. "Hémodya" was also effective than alpha tocopherol in preventing the oxidation of low-density lipoprotein cholesterol (LDL-c). "Hémodya" reducing the rigidity of the erythrocyte by reducing its cholesterol content. That antioxidant activity of "hémodya" may be applicable in the reduction of oxidative stress posed to the lipids during sickling crises.

**KEYWORDS**: "Hémodya", heamoglobin, membrane lipids, reactive oxygen species, Sickle cell disease, phenolics.

## INTRODUCTION

Sickle cell anaemia is a genetic disorder resulting from the inheritance of two abnormal allelomorphic genes that control the formation of the β-chains of haemoglobins. It is one of

the most prevalent hereditary disorders with prominent morbidity and mortality. [1-2] In the pathophysiology of sickle cell disease, increased oxidant susceptibility of sickle red blood cells (RBC) has been demonstrated to play a major role. [3] The exploration of classical lipid parameters and oxidized LDL in the sickle cell disease shows that in addition to the qualitative abnormality of heamoglobin, an abnormal lipid profile which includes a decrease in HDL cholesterol level, an increase in triglycerides, in TG/HDL-c ratio and a high rate of oxidized LDL is observed in these patients. [4] About 40 % of the black population in Africa are carriers of the sickle cell gene. [1] Sickle cell disease is characterized by haemoglobin polymerization, erythrocyte rigidity, haemolysis, and microvascular occlusion resulting from deoxygenation of intra-erythrocytic haemoglobin S. Ischemia-reperfusion injury, plasma haemoglobin-mediated nitric oxide consumption, and free radical generation activate systemic inflammatory responses. [5] Thus, substances with antioxidant properties in scavenging free radicals may play a role in the prevention of the sickle cell crisis.

In developing countries, traditional healers and their plant medicines provide the major health care to the majority of people in a curative rather than a preventive approach. Today, there is a vast literature on the use of traditional medicine with botanist reporting description of plants used for different disease treatments, the phytochemist on the chemical constituents and the pharmacologist on the effectiveness of particular plant compound or extracts. Some plant extracts have earlier been used for the management of Sickle cell disease. *Cajanus cajan* has extensively been studied for its antisickling activity [6-7-8-9-10-11] with the main active compound being phenylalanine. [1] Moody et *al*. (2003) also reported on the potential anti-sickling activity of a Nigerian herbal formulation (ajawaron HF) with the main plant being *Cissus populnea*. The use of medicinal plants and their products in the treatment of different disease condition is gaining grounds in Cameroon. “Hémodya” is a phytomedicine made from a combination of medicinal plants that are used for the treatment of numerous infectious diseases. “Hémodya” has been in use for the management of sickle cell disease in Cameroon for a long time now (upward of 14 years). According to testimony’s of many patients, "hémodya" reduces the frequency of thier crises and improves their general state. It reduces the rate of haemoglobin S to the profit of the synthesis of A2 and F haemoglobin.[12] The oral administration of "hémodya" does not induce any toxics acute and subacute effects.[13] The qualitative screening phytochemical of “hémodya” showed the presence of phenolic compounds like gallic and catechic tannins, flavons, leucoantocyans and coumarines. “Hémodya” content also quinones, anthranols, alkaloids, cardiotonic heterosides,

fat acids sterols and terpens. The antisickling effect of "hémodya" were investigated shows the ability to inhibit polymerization of sickle cell heamoglobin (HbS), improve the $Fe^{2+}/Fe^{3+}$ ratio and lower the activity of lactate dehydrogenase (LDH) in blood plasma. [14]

The purpose of this study was to evaluate the antioxidant activity of "Hémodya" and its effect on some plasma biochemical parameters of sickle cell patients.

## MATERIAL AND METHODS

"Hémodya", a decoction form containing 3 medicinal plants barks: *Cassia siamea* Lam (Euphorbiaceae), *Delonix regia* le Flamboyant (Caesalpiniaceae), *Garcinia cowa* Rox (Guttiferae) was provided by the "Pr. ETAME foundation", P.O Box: 14709 Yaoundé-Cameroon.

### In vitro human study

The study was performed with informed consent and approved by the Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon Ethics Committee. Seven sickle cell patients consulting in the out patients unit of the University Teaching Hospital, Yaoundé, Cameroon participated in the pilot study. Human whole blood was collected from sickle cell patients of both sex (12-30 years) by venu puncture into EDTA tubes and treated as follows: To 0.5 ml of blood in a test tube was added either "hémodya" (test) or physiological saline (serving as a control) in increasing volumes. The tubes were then incubated for 1 hour at 37 $^0$C, centrifuged at 3000 rpm and the supernatant obtained was analysed for total cholesterol, triglycerides, HDL cholesterol using Sigma kits. LDL cholesterol was calculated using the formula of. [15] The residue (red blood cell) was then washed three times with physiological saline and then lysed by the addition of cold distilled water. This was then centrifuged, the residue (red blood cell membrane) was then collected, and the membrane lipids extracted and purified as described by. [16] The purified membrane lipids were then analysed for total lipid [17] and cholesterol. [18]

### Determination of phenolic concentration of "Hémodya"

500 mg of "Hémodya" was accurately weight and carefully boiled with 10 ml of methanol for 2 hours at 90 $^0$C in order to eliminate vitamin C. After centrifugation, the supernatant was stored at -20 $^0$C until used. The phenolic concentration in the supernatant was assessed using Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO) diluted 5 times as described in. [19] Catechin was used as standard.

**Antioxidant activity of hemodya (Radical and reactive oxygen species scavenging activity).**

The extracted "Hémodya" was thawed and a range of concentrations (1-10 mg/ml) prepared to be used for the radical scavenging activity.

**DPPH scavenging activity**: The free radical scavenging activity of "Hémodya" was assayed by DPPH radical as earlier described. [20] The percentage (%) radical scavenging activity of "hémodya" was calculated as follows: % radical scavenging effect = $[(Abs_1-Abs_2)/Abs_1]$ x 100. Where $Abs_1$ is the absorbance of the control, and $Abs_2$ is the absorbance of "Hémodya".

**Superoxide radical scavenging activity**: The method of [21] was used to measure superoxide radical scavenging activity of "Hémodya". The superoxide radical was generated in a PMS-NADPH system by oxidation of NADPH and assayed by the reduction of NBT.

**Hydroxyl radical scavenging activity**: The hydroxyl radical scavenging activity of "hémodya" was determined by applying the method of [22] with modification on the composition of the reagents. The reaction mixture consisted of $FeCl_3$ (300 µM), EDTA (780 µM), 2-deoxiribose (2.8 mM), ascorbic acid (300 µM), $H_2O_2$ (4mM) and aliquots of "hémodya in a final volume of 1 ml. All the reagents were dissolved in potassium phosphate buffer (20 mM, pH 7.4). This was then incubated at 37$^oC$ for 1 hour. After incubation, 1 ml of TCA (2.8 %) and TBA (1 %) were added to the reaction mixture and incubated at 100 $^oC$ for 20 minutes. The absorbance was read spectrophotometrically at 532 nm.

**Nitric oxide scavenging activity**: Hemodya was assayed for nitric oxide scavenging activity by the method of. [23] This method measures the concentration of nitrite produced by the reaction of nitric oxide and oxygen at 546 nm.

**Metal chelating activity**: The chelating of ferrous ions by "hémodya" was determined by the method of. [24] The reaction was initiated by the addition of ferrozine and absorbance measured at 562 nm.

**Effect of "Hémodya" in the prevention of LDL cholesterol oxidation**:

Low-density lipoprotein (LDL) was isolated from plasma of nomocholesterolemic individual as earlier described by. [19] The LDL was used for the measurement of $IC_{50}$ (polyphenolic concentration of "hémodya" that will inhibit 50% of LDL from oxidation as compared to the blank) [25] and Lag-time. [26] Vmax and CDmax were calculated from the lag-time curve. [27]

**Statistical Analysis**

Measurements were carried out in triplicates and the results are presented as mean ± standard deviation (SD). Kruskal-Wallis One Way Analysis of Variance on Ranks was employed in dose comparison. Student Newman-Keuls Test was used to determine significant difference between groups and doses ($P<0.001$). The SigmaStat version 3.01 package was used for these analyses.

## RESULTS AND DISCUSSION

Table 1 presents the effect of incubation of whole blood of both sickle cell and normal subjects with "hémodya" (at different doses) as compared to their respective control on lipid parameters. Sickle cell disease results to a decrease in the plasma lipid. [28]shown that plasma triglyceride concentration changes when there is an alteration on membrane lipids. At the beginning of the study it was observed that plasma triglyceride of HbSS was lower than HbAA subjects, though not significantly different. Incubation of blood samples with "hémodya" did not have any significant ($p>0.05$) effect on the triglyceride concentration of both HbSS and HbAA when compared to their control (NaCl). [29] reported that red blood cell of sickle cell patient easily transform their cholesterol to LDL-cholesterol during diffusion through the tissues to deliver oxygen. Thus resulting to decreased plasma total cholesterol and increase membrane cholesterol that may account for the rigidity of sickle red cell. Similar results were obtained in the present study. However, when "hémodya" was incubated with blood samples (HbSS and HbAA), a significant ($p<0.05$) increase in the plasma total cholesterol and HDL-cholesterol was observed in both HbSS and HbAA though the increase was not dose related. Similarly, an increase was observed in the plasma LDL-cholesterol concentration.

In sickle cell disease, deoxygenation of intra-erythrocytic haemoglobin SS leads to haemoglobin polymerization, erythrocyte rigidity, haemolysis, and microvascular occlusion. [4]

Some red blood cells in patients with sickle cell anaemia have an elevated density and possess an abnormal membrane. [28] observed that there is an exchange of lipids between the plasma and the red blood cell membrane of sickle cell disease during treatment, thus normalizing the abnormal membrane by improving on fluidity. In the present study, treatment with "hémodya" had a significant ($p<0.05$) decreasing effect on membrane lipids (Table 2). When "hémodya" was incubated with whole blood (1 hr), a significant decrease ($p<0.05$) in

the RBC membrane total lipids of both HbSS and HbA2 subjects was observed (Table 3). "Hémodya" had little or no effect on the membrane cholesterol of HbSS with significant increase observed only at 12 µl incubation. On the contrary, "hémodya" induced a decreasing ($p<0.05$) effect on the cholesterol of HbA2 subjects though not dose related.

Oxidative phenomena play a significant role in the pathophysiology of sickle cell disease. In 1982, [30] demonstrated that sickle RBCs produce greater quantities of superoxide radical ($O_2^{-\cdot}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($^{\cdot}OH$) than do normal RBCs. In the presence of an $O_2^{-\cdot}$ generating system, Fe(III) is reduced to Fe(II) with subsequent formation of $^{\cdot}OH$ from $H_2O_2$. [31] The hydroxyl radical oxidizes unsaturated esterified membrane lipids, resulting in changes in fluidity of the bilayer. Additionally, there is increased ion permeability, inactivation of membrane enzymes and receptors, and covalent cross-linking of lipid and protein membrane constituents. [32] As measured by Folin Ciocalteu reagent, "hémodya" had a phenolic concentration of 4.91± 0.98 mg/g (catechin equivalent). "Hémodya" also showed a strong radical scavenging and metal chelating activity (Table 3). At a low dose of 1 mg/ml, "hémodya" could scavenge 91.21 % of DPPH free radical, 43.39 % of $SO_2^{-\cdot}$ and 23 % metal chelating activity. This activity as observed was dose related with highest dose being the most effective. Thus, hemodya can prevent sickle cell crisis base on its radical scavenging and metal chelating activity.

Sickle RBCs exhibit increased levels of TBARSs at baseline [33-34], suggesting that they are targets for oxidative stress. In studying the preventive role of "hémodya" on the LDL-cholesterol oxidation mediated by copper II ion, it was observed that the $IC_{50}$ was significantly ($p<0.001$) small (six times) compared to the vitamin E standard (Table 4). That is to say, a low concentration of "hémodya" will inhibit 50 % oxidation of LDL as against a higher concentration for vitamin E. "Hémodya" also more than doubled the lag-time of LDL-cholesterol compared to vitamin E and control LDL-cholesterol. The longer lag-time means in the presence of "hémodya" it will take the LDL a long time to undergo oxidation. Thus, the lower the $IC_{50}$, the longer the lag-time and the better the antioxidant. This is justified by the significantly slow reaction rate (Vmax) and reduced concentration of conjugated diene (CDmax) formed in the LDL incubated with "hémodya" as against the control LDL and the LDL incubated with vitamin E standard. These suggest that "hémodya" contains polyphenolic antioxidants that acts through a mixed mechanism such as binding copper ions

from solution, scavenging of free radicals from solution, blockage of the copper-LDL binding site, or by catalysing non-radical decomposition of conjugated dienes as they are formed.

**Table 1. Effect of "hémodya" on lipid parameters of whole blood of sickle cell and normal subjects.**

| Lipid parameters | Blood Type | Control NaCl (9 %) | | | "hémodya" | | |
|---|---|---|---|---|---|---|---|
| | | 24µl | 30µl | 48µl | 24µl | 30µl | 48µl |
| **Triglyceride (g/l)** | HbSS | 1.31 ±0.38 | 1.35 ±0.37 | 1.49 ±0.47 | 1.62 ±0.44 | 1.38 ±0.68 | **1.82 ±1.07** |
| | HbAA | 1.10 ±0.44 | 1.24 ±0.39 | 1.31 ±0.46 | 1.61 ±0.76 | 1.38 ±0.60 | **1.58 ±0.82** |
| **Total cholesterol (g/l)** | HbSS | 1.31 ±0.38 | 1.25 ±0.33 | 1.48 ±0.29 | 2.09 ±0.45* | 2.44 ±1.24* | **2.70 ±0.41*** |
| | HbAA | 1.19 ±0.32 | 1.25 ±0.21 | 1.41 ±0.37 | 2.21 ±0.82* | 2.15 ±0.58* | **2.70 ±0.52*** |
| **HDL-cholesterol (g/l)** | HbSS | 0.65 ±0.2 | 0.67 ±0.14 | 0.90 ±0.24 | 0.99 ±0.11* | 1.17 ±0.16* | **1.40 ±0.33*** |
| | HbAA | 0.67 ±0.28 | 0.67 ±0.34 | 0.84 ±0.17 | 1.16 ±0.62* | 1.15 ±0.08* | **1.37 ±0.36*** |
| **LDL-cholesterol (g/l)** | HbSS | 0.70 ±0.28 | 0.72 ±0.15 | 0.77 ±0.37 | 0.86 ±0.30 | 0.91* ±0.37 | **0.98* ±0.55** |
| | **HbAA** | **0.70 ±0.23** | **0.76 ±0.27** | **0.86 ±0.25** | **0.89 ±0.49** | **0.95 ±0.27** | **1.03 ±0.45** |

*Significant different compared to corresponding control ($p<0.05$), HbSS = sickle cell subject, HbAA = normal subject.

**Table 2. Effect of "hémodya" on RBC membrane lipids of sickle cell and normal subjects**

| Lipid parameters | RBC type | Control NaCl (9 %) | | | | "hémodya" | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 12µl | 24µl | 30µl | 48µl | 12µl | 24µl | 30µl | 48µl |
| **Total lipids (mg/ml )** | HbSS | 1.42 ±0.40 | 0.77 ±0.2 | 0.80 ±0.40 | 0.66 ±0.32 | 0.84 ±0.17* | 0.43 ±0.11* | 0.42 ±0.06* | **0.34 ±0.10*** |
| | HbAA | 1.53 ±0.16 | 1.01 ±0.30 | 0.97 ±0.40 | 0.90 ±0.20 | 0.84 ±0.10* | 0.46 ±0.08* | 0.42 ±0.07* | **0.32 ±0.07*** |
| **Cholesterol (mg/ml )** | HbSS | 0.07 ±0.02 | 0.07 ±0.03 | 0.07 ±0.02 | 0.05 ±0.03 | 0.17 ±0.08* | 0.09 ±0.05 | 0.06 ±0.03 | **0.043 ±0.21** |
| | **HbAA** | **0.29 ±0.10** | **0.29 ±0.01** | **0.16 ±0.03** | **0.10 ±0.06** | **0.23 ±0.08** | **0.13 ±0.05*** | **0.10 ±0.05*** | **0.07 ±0.04*** |

*Significant different compared to corresponding control ($p<0.05$)

**Table 3. Radical scavenging activity of "hémodya".**

| "hémodya" / Radicals | 1mg/ml | 2mg/ml | 4mg/ml | 8mg/ml |
|---|---|---|---|---|
| **DPPH (%)** | 91.21± 2.82 | 93.97± 1.87 | - | - |
| **$SO_2^{\cdot\cdot}$ (%)** | 43.39 ±1.06 | 67.43 ±3.10** | 85.03± 1.86** | - |
| **$NO^{\cdot}$ (%)** | - | 45.25 ±2.76 | 53.50± 3.33** | **85.76± 1.30**** |
| **$OH^{\cdot}$ (%)** | - | 48.97 ±1.39 | 66.22± 1.28** | **76.79 ±1.08**** |
| **Metal chelation (%)** | **23.01±0.96** | **47.72 ±1.01*** | **71.63 ±1.14**** | **75.48± 2.29**** |

Significantly higher compared to lower dosages, (**$p<0.001$, *$p<0.05$).

**Table 4. Effect of "hémodya" (1 µM) in the prevention of LDL-cholesterol from copper mediated oxidation.**

| Parameters | $IC_{50}$ (µM) | Lag-time (min) | Vmax (µmole CD/min.g LDL) | CDmax (µmole/g LDL) |
|---|---|---|---|---|
| **Control LDL** | - | 120±8.4 | 1.26±0.32 | **87.17±2.8** |
| **"hémodya" +LDL** | 0.27±0.02** | 420±30** | 1.13±0.10* | **73.12±4.1*** |
| **Vit. E + LDL** | **1.83±0.51** | **198±15** | **1.16±0.43** | **84.26±3.0** |

Significantly different from control and Vit. E standard (**p<0.001, *p<0.05)

## CONCLUSION

"Hémodya" is a phytomedicine that can play a role in the management of sickle cell disease by reducing the concentration of membrane cholesterol, which may stem from its antioxidant activity. All these may result to the amelioration of the structure and functional capacity of the sickle red cells.

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