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Impact of the Daily Intake of Powder of the Moringa Oleifera Leaf on the Evolution of CD4 in the Elderly Aged 18 and Over Living with HIV AIDS in Sarh/Chad

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Abstract* - Malnutrition is a common clinical problem in patients infected with HIV and causes immune suppression and depletion of CD4 T cells. The powder from the leaves of *Moringa oleifera* (MO) through numerous studies has shown nutritional and therapeutic virtues. Also the administration of this vitamin complex associated with mineral salts to a patient would be simple and practical than that of conventional micronutrients. The objective of this study was to measure the impact of the daily intake of *Moringa oleifera* leaf powder on the cells of the immune system, more particularly the CD4 T lymphocytes (CD4 LT) of people aged 18 years and over living with HIV AIDS at Notre Dame des Apôtres Maingara Hospital in Sarh / Chad. This was a comparative case-control study that took place over a period of six (06) months. The study included 100 undernourished people living with HIV / AIDS aged 18 or under followed at the level of the People Living with Human Immunodeficiency Virus (PLWHIV) department. Patients were enrolled in two groups. Each patient received 15g / day of *Moringa oleifera* leaf powder (group A) or NDA enriched flour (group B) during the 6 months. The patients also benefited from a clinical follow-up (with the measurement of the biological parameters (CBC, TCD4 lymphocytes) at inclusion and after six months. The results obtained showed that there was a nutritional recovery of 52.97 % for the groups supplemented with *Moringa oleifera* leaf powder versus 20.23% for the enriched flour NDA. We also observed no statistically significant difference in the mean of lymphocytes in group B (enriched flour NDA) (p0.975) The improvement in the CD4 LT count observed in patients supplemented with *Moringa oleifera* also reflects an improvement in the immune status of PLHIV which is normally in decline after infection with HIV oxidative stress. These micronutrients (vitamins, Mg, phycocyanin) have immuno stimulatory properties which would allow a gain or stabilization of the number of CD4 lymphocytes in groups supplemented with *Moringa oleifera* leaf powder. Analysis of the blood count reveals that the consumption of *M. oleifera* allowed a significant increase (p≤ 0.000) in the values of red blood cells (RBC), hemoglobin level (THb), hematocrit (Hte) and (p≤ 0.001) of the mean globular volume (MCV) as well as the parameters of the leukocyte line (macrophages, monocytes and dendritic cells which express this CD4 receptor) which are also major players in the immune system. In control subjects, blood count parameters did not change significantly. *Moringa Oleifera* leaf powder is said to offer enormous benefits in nutritional recovery (weight gain), stabilization or gain of lymphocytes thus contributing to the well-being of PLHIV.

Keywords: Undernutrition, *Moringa oleifera*, NDA enriched flour, CD4 T lymphocytes, people infected with HIV / AIDS (PLHIV).

I. INTRODUCTION

The pandemic of the human immunodeficiency virus and the acquired immunodeficiency syndrome (HIV / AIDS) is today a real public health problem on a global scale. According to the program United Nations Joint Committee on HIV / AIDS (UNAIDS), more than 25 million people around the world have died of AIDS since the first cases appeared in 1981, making it one of the deadliest epidemics in the world history of humanity [1].

In 2016, an estimated 36.7 million people living with HIV (PLHIV) worldwide, including 34.8 million adults (17.8 million women) and 2.1 million children under 15 years old. In the same year 1.8 million people were infected and 1 million died as a result of AIDS-related illnesses.

Sub-Saharan Africa now has 25.5 million PLHIV in 2016 and remains the most affected region[2]. It also concentrates nearly two-thirds of new HIV infections in the world.

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In Chad, the national HIV prevalence is 1.6% in the adult population and estimated that 170,000 people were infected with HIV / AIDS in 2015, including 23,000 children [3].

In developing countries, malnutrition stems in part from poverty which restricts access to food of sufficient quality and quantity to meet daily protein-energy needs [4]. The numerous civil conflicts, the difficulties of governance, the food shortage and the increase in the price of food all contribute to swelling the ranks of the malnourished[5,6,7]. The presence of many infectious processes, including HIV, also exacerbates this disorder among these populations. In addition, people living with HIV have increased energy needs, and difficulty in ingesting and digesting food. This is why the WHO recommends medical, psycho-social and nutritional care [8].

Indeed, nutrition is a key element in slowing the progression of the disease and prolonging the life of PLHIV[8].

Malnutrition through undernutrition is accompanied by weight loss and deterioration in general health. Weight loss is due to reduced food intake due to lack of appetite and the opportunistic infections that cause diarrhea. Undernutrition impairs cellular immunity by reducing phagocytic function, antibody production, complement and the number of total lymphocytes[9]. It has been shown that undernutrition is strongly correlated with disease progression [10,11]. In developed countries, it is the low protein intake, the lack of energy, micronutrients and vitamins that lead to malnutrition. This is because most foods are very imbalanced in nutrients.

The enhancement of plant resources rich in proteins and micronutrients, accessible at a lower cost, is a strategy for effectively combating nutritional deficiency and strengthening the immune system [12]. In addition, several studies have highlighted the exceptional nutritional qualities of *M. oleifera* Lam leaves (native to India) which are used in food in Asia and Africa [12,13]. Studies have shown the efficacy of these leaves in the prevention, correction of malnutrition and associated diseases although they contain anti-nutritional factors such as phytates and oxalates [13]. They can therefore constitute a food supplement for malnourished subjects because of its richness in proteins, vitamins (A, B, C, E) and mineral salts (Ca, K, Mg, P, Iron, Zn, Se, Cu, Mn , Na, Cl) and position itself as a tonic, fortifying and stimulating product for the immune system for subjects living with HIV / AIDS[13,14].

Many therapeutic virtues are attributed to *M. oleifer*a which is used in traditional medicine for the treatment of metabolic, inflammatory, infectious, parasitic diseases, cancer and also for the purification of water [13,14].

Despite all the work done on *M. oleifera*, there is currently a lack of epidemiological data to clarify the exact effect of the use of its leaves on certain biological parameters.

The main objective of the present study is to assess the impact of the consumption of Moringa leaf powder on the evolution of CD4 cells and the recovery of undernourished people living with HIV / AIDS aged 18 and over. Our Lady of the Apostles Hospital in Maïngara Sarh in Chad.

II. MATERIALS AND METHODS

2.1 Plant material

The leaves used during this study are harvested from the plants of M.oleifera "Chadian ecotype" obtained strewn with seeds, cultivated by the Sisters of Our Lady of the Apostles on an area of 500 square meters in the virgin domain of their concession. in Sarh. Their identification was confirmed at the Laboratory of Botany and Plant Ecology of the University of N'Djamena (Capital of Chad).

2.2 Preparation of *M. oleifera* powder

The fresh leaves of *M. oleifera* are quickly washed twice in drinking water to remove dust, then in 1 tub of water with sodium hypochlorite added to a final concentration of 1% for 3 to 5 min. Finally, they are washed again with tap water and then drained for 30 min. The leaves are then spread out on filter paper and left to dry in a room protected from sunlight and dust for 4 days (Fig1).

Figure 1: The leaves of *M. Oleifera* washed and laid out for drying on a bench out of direct sunlight

The dry leaves are powdered in an artisanal mill. The powder is packaged in non-transparent and hermetically sealed

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boxes and stored in a dry place so as not to lose the nutritional virtues (vitamins and minerals very sensitive to heat) (Fig 2).

Figure 2: Box containing the ready-to-use *M. Oleifera* leaf powder

2.3 Setting, type and study population

The present study took place in Chad, in the Commune of Sarh, Department of Barh-kôh, Region of Moyen-Chari, health district of Sarh and specifically in the service of PLHIV at Notre Dame des Apôtres Hospital in Maïngara.

This is a comparative case-control study that took place over a period of 6 months (January 15 to June 15, 2019).

Our study population consisted of subjects of both sexes over the age of 18, living in the city of Sarh, infected with HIV. Their HIV status is pre-established when they enter the HNDA and is confirmed later when they are included in our study.

Were included in this study:

- HIV-positive patients between the ages of 18 and 65 who are willing to use the study product as directed by the physician, adhere to the follow-up schedule and study procedures, and have provided informed consent as evidenced by the signature of a form established for this purpose.
- HIV-positive patients aged 18 to 65 with a BMI less than 18.40 and MUAC.

The following were excluded from this study:

- Patients under 18 and presenting associated pathologies (heart disease, cancer) or having violated the protocol.
- Pregnant women and people who have refused to participate or who have withdrawn voluntarily; finally, those excluded by decision of the doctor for reasons of safety and / or the well-being of the patient.

A total of 185 patients were followed until the end of the study. This study population was separated into 2 separate groups for study purposes. So we have the:

- Group A (Case), made up of patients who each receive 15 g of *M. Oleifera* powder distributed over the three daily meals for 6 months.
- Group B (negative controls), made up of patients who receive the enriched flour NDA.

2.4 Biological parameters (leukocyte line and erythrocyte line)

Four checkups were done at the start and evaluated every two weeks until the end of the *M. oleifera* daily consumption study. All assays are performed on the Lisa 500 plus automated system (BiocodeHycel, France) with Spinreact reagent kits (Sant Esteve de Bas, Spain).

- Blood sampling and processing of blood samples

Blood was collected from all study participants in EDTA tubes for blood count and CD4 lymphocyte assay.

2.5 Procedure for carrying out laboratory examinations

When the patients were recruited, a 5 ml blood sample was taken on a dry tube for confirmation of the serological status. The tests used were the "Determine" (Abott l aboratories, Gennany) the Standard Diagnostic (SD) Bioline (BiolineLaboratories, USA), in accordance with the HIV screening algorithm in force in Chad. At each biological follow-up, we took a blood sample on an EDTA tube (5 ml for CD4) on a dry tube (5 ml for biochemistry).

We used a spectrophotometer for the CD4 T lymphocyte assay.

2.6 Preparation of blood samples and monocytic depletion

- The blood taken in a tube with k3EDTA or ACD anticoagulant is mixed gently by inversion. Then stirred for 5 min at room temperature on the Dynal MX1 rotary stirrer.
- After having identified a Dynal T1 microtube for each sample, introduce into these tubes 350 microliter of PBS then 125 ml of whole blood from the patient and finally 25 ml of anti-CD14 monoclonal antibody. Close the micro tubes hermetically and let the micro tubes Tl gently shake for 10 min at room temperature on the stirrer.

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- Collect the Dynal Tl micro tubes and position them correctly on the magnetic rack.
- Dynal. Incubate for 2 min at room temperature.
- Number other Dynal T2 micro tubes. Deposit 200 ~ of BPS.

2.7 CD4 assays

The standard measurement of the CD4 count is done using a FACS count (Flow Cytometry Assay) The FACS Count uses a pair of tubes, one determines the absolute number of helper / inducer T lymphocytes (CD4 / CD3) and the other determines the absolute number of suppressor / cytotoxic T lymphocytes (CD8 / CD3). Its interest is to assess the degree of immune deterioration and the speed of progression to the AIDS stage; identify, using clinical manifestations, the appropriate time to initiate antiretroviral therapy; consider starting preventive treatments for opportunistic infections; and to assess therapeutic efficacy. It was chosen in order to determine the immune status of patients, to take measures in the management of the onset of opportunistic infections. According to the WHO classification (2009), the variable was categorized as follows: <100 cells / mm^3, 100-200 cells / mm^3, 200-350 and ≥ 350 cells / mm^3. The threshold of 200 cells / mm3 makes it possible to identify patients with the greatest risk of developing opportunistic infections and progressing to an AIDS stage (Phair et al., 1990, Hogg et al., 2001). The variable was measured twice during the study.

2.8 Data analysis

The data were entered into Excel and word software as the study progressed. These data were analyzed using Excel 2010, Microsoft Word and Statistical Package for Social Sciences (SPSS) version 16.0 software. The threshold of statistical significance was set at p <0.050.

The results were presented as tables, histograms and graphs from Excel 2010 and SPSS 16.0.

III. RESULTS AND DISCUSSIONS

3.1 White blood cell line

Table 1: Leukocyte Line Parameters of Study Subjects

	Cas		P-Value	Témoins		P-Value
PARAMETRES	**AVANT n = 54**	**APRES n = 54**		**AVANT n = 45**	**APRES n = 45**	
GB (mm^3)	**4,34±1,3**	**4,89±1,2**	**0,000**	**4,17±0,9**	**4,44±1,4**	**0,323**
Mono**(mm^3)**	**8,30±5**	**10,22±4,6**	**0,016**	**8,47±5,2**	**8,50±4,94**	**0,259**
Lympho**(mm^3)**	**41,6±12,1**	**0,2±10,5**	**0,000**	**40,51±12,1**	**43,77±12,1**	**0,983**
Gra	**45,17±13,7**	**50,90±1**	**0,004**	**44,77±13,9**	**45,95±13,0**	**0,675**

After supplementation with *M. oleifera*, the results (Table 1) show that the values of GB and LYMPHO (p <0.001), Mono and Gra (p <0.01) are significantly different. In the controls, the parameters of the leukocyte line did not show a significant difference.

After 6 months of moringa powder supplementation, the mean peripheral blood CD4 count increased from 527.782 ± 328.307 to 603.913 ± 280.943 in the experimental group (under Moringa powder), but not significantly (p = 0.079). This increase was greater in patients with a CD4 count below 200. On the other hand, in the control group, no increase in CD4 lymphocytes was observed.

Table 2: Variation in the average number of CD4 lymphocytes per group and according to the type of supplementation over the 6 months

Variables	**Moringa (M0)**	**Moringa (M1)**	**P-Value**	**FHNDA (M0)**	**FHNDA (M1)**	**P-Value**
CD4< 200	119,25± 19,25	146,16± 22 ,16		154,88± 26,987	69,61 ± 36,449	
200< CD4< 400	312,7 ± 52,06	323,85± 28,16	**0,079**	319,6 ± 39,9	293,53 ± 45,04	**0,975**

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CD4> 400	737,84± 152,56	746,54± 158,87		607,09± 147,727	512,66 ± 116	

3.2 Red blood cell line

Table 3: Red blood cell line parameters

	Cas		P-Value	Témoins		P-Value
PARAMETRES	**AVANT**	**APRES**		**AVANT**	**APRES**	
	n = 54	**n = 54**		**n = 45**	**n = 45**	
GR ($10^6/m^3$)	3,50±**0,5**	4,03±**0,5**	**0,000**	**3,56±0,5**	**3,71±0,6**	**0,226**
THb(g/dl)	10,13±**1,5**	**12,04±1,3**	**0,000**	**10,00±1,4**	**10,41±1,5**	**0,110**
Hte(%)	34,43±**5 ,7**	40,07±**12,6**	0,001	33,90±**5,2**	**35,16±5,7**	**0,322**
VGM (fl)	97,46±**12,04**	102,56±**9,9**	**0,001**	**98,68±12,62**	**98,95±10,7**	**0,909**

The results shown in Table 3 show significant differences observed after the use of *M. oleifera* for GR and THb (p <0.001) and for THb2 and VGM (p = 0.001). On the other hand, in the control subjects, no significant difference was observed.

IV. DISCUSSION

The study of the impact of *M. oleifera* powder supplementation on the erythrocyte and leukocyte lines revealed the following phenomena.

In fact, in the leukocyte line, a non-significant increase in the mean number of CD4 is observed in the *M. oleifera* group as under flour HNDA (P = 0.079 and 0.975 respectively). This average decreased in patients on HNDA Flour.

Observation of our results shows that *M. oleifera* powder contributes to an increase in the number of CD4 / µL and to its maintenance. Indeed, *M. oleifera* powder is a good source of b-carotene (convertible into vitamin A.) but also of mineral salts (zinc and magnesium). These micronutrients have a positive effect on the cellular immune system, which corroborates with the data in the literature.

This increase in the CD4 count after consumption of *M. oleifera* powder also reflects an improvement in the immune status of PLHIV which is normally in decline after HIV infection [15,16,17].

In addition to CD4 T lymphocytes, macrophages, monocytes and dendritic cells which express this CD4 receptor are also target cells of the AIDS virus [18]. The drastic decrease in their level following HIV infection is also a sign of profound immunodeficiency.

Our study observed an increase in their level after supplementation with *M. oleifera* powder, which again reflects a positive impact of our plant on the failing immune system of PLHIV.

Indeed, this effect of strengthening the immune system following supplementation with powdered leaves of *M. oleifera* could be explained by the improvement of the general condition of PLHIV subjected to the diet containing *M. oleifera*. Thus, the nutritional recovery of this group of PLHIV which induces a correction in the level of leukocyte cells is a good explanation for the positive impact observed on the immune system, since malnutrition is considered to be the main cause of immune deficiency in planetary scale [19].

Regarding the erythrocyte line, our study observed an improvement in the level of red blood cells and hemoglobin in patients receiving supplementation with *M. oleifera* powder. These results reflect a correction of a possible anemia which is the most frequent type of cytopenia in PLWHIV. It should be noted that anemia can have multiple etiologies, including iron or micronutrient deficiency, which affects the reproductive capacity of the bone marrow and thus decreases erythropoiesis. Hypochromic microcytic (mean corpuscular volume (MCV) <80) anemia mainly suggests iron deficiency [20].

Thus, this correction of anemia in PLWHIV by *M. oleifera* powder could be explained by the presence of vitamins and micronutrients, in particular iron in the powder of *M. oleifera*. This richness of our plant in micronutrients is corroborated by the work of [12]. Who describes *Moringa Oleifera* as a legume very rich in proteins (25-44%), essential amino acids, vitamins and minerals, especially in calcium, sodium, potassium, iron, magnesium, zinc and selenium. The work of Makkar and Becker [21], also confirms this important

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nutritional value of our plant, by affirming that the contents of essential amino acids present in *Moringa* leaves are higher in amino acids recommended in the protein benchmark for a 2-5-year-old child and are comparable to those for soybeans. The vitamin contents of *Moringa* leaf flour are of the order of 204.3 mg, 28.5 mg, 221.16 mg, 88.6 mg, 187 mg and 1220 mg / kg of dry matter respectively for vitamins A, B1, B2, B3, C and E.

V. CONCLUSION

This study of the impact of moringa powder (*Moringa Oleifera*) on the evolution of CD4 LT in PLWHIV during the daily intake of moringa powder for 6 months allowed us to observe in addition to nutritional recovery, a marked improvement in the levels of cells of the immune system in general, and more precisely of CD4 LT, which are the cells most affected during HIV infection, which drastically drops their level.

Thus, this proven immuno-stimulating activity of *M. Oleifera* places this plant as a good food candidate for the nutritional and therapeutic care of PLWHIV.

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