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TOLERANCE STUDY OF "SPIRULINE PLUS" AND EVOLUTION OF BIOLOGICAL PARAMETERS IN PEOPLE LIVING WITH HIV-1 FOR TWELVE MONTHS

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ABSTRACT: Spirulina is an algae used in our country for people living with HIV (PLHIV) and it is known especially for its therapeutic immunostimulatory effects. Spirulina capsules produced locally enriched in zinc and selenium called "spiruline plus" were formulated and given to PLHIV. The objectives of this randomized placebo controlled trial were to assess the tolerance by reporting side effects and to evaluate the clinical and biological outcome of patients after 12 months follow-up. A randomized clinical trial comparing four groups of patients infected with HIV type 1 (HIV-1) and treated with different diets was conducted over 23 months. These groups were: a first group of patients treated only with placebo, a second group treated with "spiruline plus", a third group treated with antiretroviral (ART) and placebo, the fourth group treated with ARTs and "Spiruline plus". Clinical and laboratory data were collected at baseline and at 3, 6, 9 and 12 months after inclusion. Some of the side effects reported in the group receiving antiretroviral therapy and "spiruline plus" decreased with time, and were classified as of low intensity. Biological parameters of the two groups ("spiruline plus" and placebo) with the exception of total protein and total cholesterol did not change significantly ($p > 0.05$) over time. "Spiruline plus" used in association with ARTs, did neither cause side effects nor affect biological parameters in twelve months of follow-up.

INTRODUCTION: Spirulina, blue - green algae, multicellular, rich in vitamins, minerals and pigments, allows nutritional recovery [1-9] and is recommended by FAO [1]. In addition, it is used by people living with HIV for its nutritional and immunostimulatory effects [5, 8, 9].

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However, utilized in association with antiretrovirals (ARTs), there is fear of likely interactions [10].

Zinc and selenium, two antioxidants and immune boosters recommended for persons living with HIV (PLHIV) [11 - 15] were added to the formulation of spirulina capsules called "spiruline plus".

The capsules were given to PLHIV-1 on ARTs in a randomized placebo-controlled trial. The objectives were to assess the toxicity and long-term safety (twelve month follow-up) of "spiruline plus" in association with ARTs.

METHODS:

Type of study: It was a randomized placebo clinical trial comparing four treatment regimens: a first group of patients treated only with placebo, a second group of patients treated only with the "spiruline plus", a third group of patients treated with ARTs and placebo, and a fourth group of patients treated with ARTs and "spiruline plus."

Study population: The study population consisted of men and women infected with HIV-1, from 18-55 years, not suffering from liver or kidney failure and living in Ouagadougou, capital of Burkina Faso. Only non-pregnant women were included in the trial.

Study sites: The trial was conducted in the following approved centers care for PLHIV:

- Day care hospital of University Hospital Yalgodo Ouedraogo (UHYO) of Ouagadougou: one of the three University Hospitals of Burkina Faso. The Center is at the top of the national health pyramid.
- Medical Center of Pissy: Medical Center which is an approved secondary health center for the treatment of PLHIV pyramid;
- Health Centre of African Solidarity Association (ASA). Accredited community center care of PLHIV.
- Laboratory of the National Armed Forces (Camp Sangoulé Lamizana) which is an approved biological center for PLHIV.

The Test products:

1. "Spiruline plus" was the test product. We used spirulina produced in the town of Koudougou (an approved spirulina production center in Burkina Faso), enriched with selenium and zinc. The dosage used was capsules of 420 mg of "Spiruline plus." Each capsule contains 20 mg of selenium and 3 mg of zinc. The "Spiruline plus" was administered to participants at a dose of 6 capsules per day (2 × 3 capsules / day).
2. The placebo consisted of cornmeal with chlorophyll.
3. "Spiruline plus" and placebo were manufactured (blending and capsules) by the Institute for Research in Health Sciences (IRHS) laboratory in its section of the Traditional Pharmacopoeia. Quality control was conducted by the National Public Health Laboratory (NPHL).
4. The Antiretroviral (ARTs): These ARTs were the ones usually taken by patient before the latter is taken into account by our study. The pharmacy of Yalgodo Ouedraogo Teaching Hospital provided the ARTs through the Generic Drug Purchasing Department (CAMEG). In addition, it also made it ample room for storage, management and delivery.

Study procedures:

Ethics: The study was favourably approved of by the National Ethics Committee for Health Research of Burkina Faso. Before each inclusion, the study was explained to the participant who, on approval, signed the informed consent form. Throughout the survey, each participant was free to discontinue their participation.

Pre-inclusion visit: It was conducted a week before the start of inclusions and aimed to:

- Administration of consent and the collection of the informed consent form signatures.
- Verification of clinical and biological criteria for participation in the study. Biologically, it was made a blood sample to confirm the status and type of HIV infection of the patient, assess liver function (transaminases dosage) and renal function (determination of urea, creatinine).
- To collect urine in female participants to search for a possible pregnancy.

Inclusion visit (D0): At baseline, patients fulfilling the inclusion criteria were randomized to one of four treatment groups. Clinical data (assessment of

clinical manifestations: General, pulmonary, Oto Rhino pharyngeal, cardiac, gastrointestinal, neurological, lymphatic, musculoskeletal squeletiques, urogenital and skin), biochemical data (serum electrolytes, glucose, uric acid, total cholesterol, triglycerides, amylase, lactic acid, total protein, total bilirubin and conjugated, AST and ALT), hematologic (blood count, prothrombin time, sedimentation rate, CD4+ T lymphocytes rate), immunological and virological (HIV serology, viral load RNA) were collected.

Tour Day 28 (D28): During this visit, a clinical examination, notification of adverse effects and a sample for verification of biochemical parameters (transaminases, urea, creatinine, glucose, and amylase) were made.

Visits to the 84 th 168 th, 252 th and 365 th day (J84, J168, J252 and J365): Clinical and biological and adverse effects were recorded during the quarterly visits (**Table 1**) data.

TABLE 1 : SUMMARY OF ACTIVITIES

	Day study						
PROCÉDURE	**D-7**	**D0**	**D28**	**D84**	**D168**	**D252**	**D365**
Informed Consent	X						
medical history	X	X					
Pregnancy Test	X			X	X	X	X
HIV test	X						
Randomization		X					
clinical examination	X	X	X	X	X	X	X
Evaluation of Adverse Events			X	X	X	X	X
Laboratory tests	X	X	X	X	X	X	X

D-7: seven days of the inclusion

D0: day of inclusion

D28: twenty eighth day of monitoring

D84: eighty-fourth day of monitoring (third month follow-up)

D168: eight hundred and sixty days of follow-up (sixth month of follow-up)

D252: Two hundred and fifty-second day of monitoring (ninth month follow-up)

D365: three hundred and sixty five days of monitoring (twelfth month follow-up)

Emergency consultations: These consultations are held outside the periods defined by the protocol (excluding protocols consultations) for urgent or necessary cases. During these visits, clinical and laboratory data were also recorded. The relationship between patients' symptoms and treatments were evaluated.

Laboratory tests: In order to carry out these biological tests, the samples were taken in the morning when the patient was on an empty stomach. The blood was collected in a dry tube (without anticoagulant) for biochemical and immunological tests and a tube with EDTA anticoagulant for hematological tests.

Citrate anticoagulant tubes were used for the examinations of the sedimentation rate. All the patients' biological tests on different sites were carried out in the biomedical laboratory of the National Armed Forces (Camp Sangoulé Lamizana).

PLCs BD FACSCount^{TM}, ABX Pentra 60, KONELAB 20, was used respectively for the CD4 count, the realization of the complete blood count and that of biochemical tests.

Management and data analysis strategies: Data were entered using Epi Info (version 3.5.1) software and then converted to Excel (Excel 2003) files. Data analysis was performed with Sigma Stat (Sigma Stat 3.5) software. Adverse events (AEs) were reported and classified according to: grades of severity, causality and evolution of WHO.

- Intensity (0 = no, 1 = Low, 2 = Moderate, 3 = Severe, 4 = severe);
- Causality (0 = No, 1 = Unlikely, 2 = May, 3 = Set, 4 = Unknown)
- Evolution (Cured = 0, the mend = 1 = 2 Continues, Cured with sequelae = 3)

RESULTS:

Diagram of the Study: Patient recruitment was explaining the difference of progressive stages of patients and suspensions at the time of termination of the study. Thus, with 131 subjects enrolled initially, 121, 113, 86 and 66 patients respectively arrived at the third, sixth, ninth and twelfth months of follow-up (**Figure 1**).

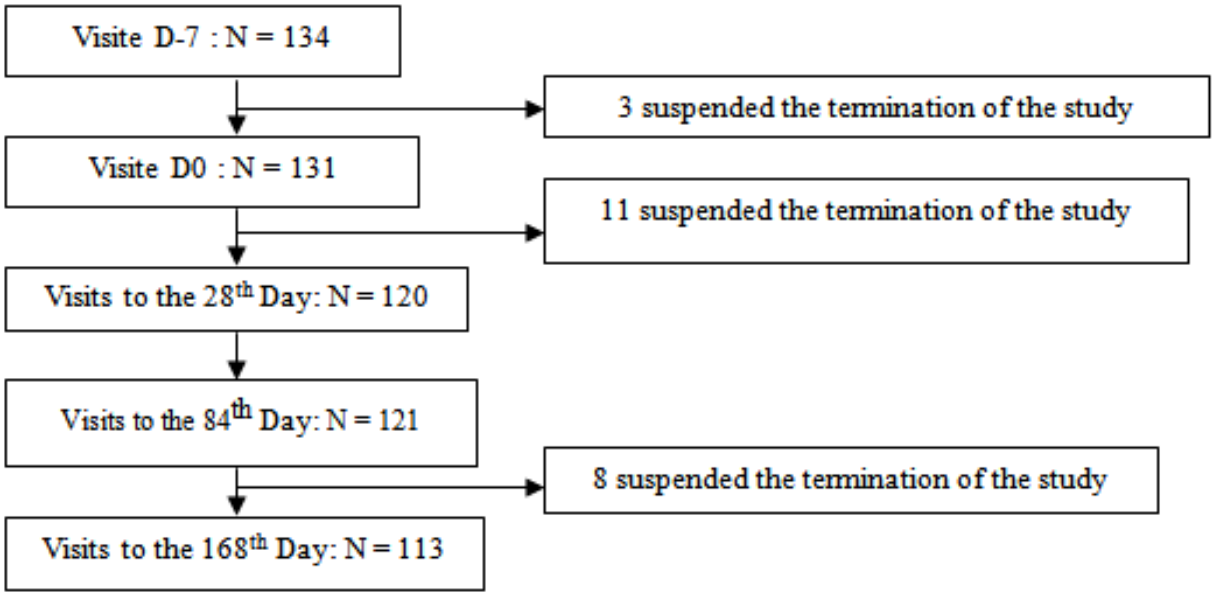

FIGURE 1: FLOWCHART OF THE STUDY

D-7: seven days of the inclusion

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Clinical Results: Adverse effects were reported in each quarter (**Table 2**). At three months of the study, asthenia, three rhinorées, epigastric burning and neuropathy have been reported in patients on ART and "Spiruline plus" against two rhinorées an epigastric burning and neuropathy noted in patients on ART and placebo. Both anorexia, epigastric burning and neuropathy have been reported only in patients on ART and "spiruline plus" in the sixth month after the inclusion. The ninth month two neuropathies have been reported against neuropathy in the twelfth month in patients treated with ART and "spiruline plus".

Biological results: There were no significant changes ($p2 > 0.05$) for each parameter except for total cholesterol and total protein during follow-up time in both regimes (ART+ "spiruline plus" and ART + placebo).

Also, There is no parameter for each significant difference ($p1 > 0.05$) between the two means of the two regimes ("Spiruline plus" + ART and ART+ placebo) in each month of follow-up (**Table 3**).

On neither haematological there was non-significant variations ($p1 > 0.05$) cellular components during months of monitoring, or

significant differences (p2 > 0.05) between the average of the two regimes (ART + "Spiruline plus" and ART + placebo) in each month of follow-up (**Table 4**).

DISCUSSION: The study showed that "spiruline plus" does not interfere with ARTs in the direction of create or increase side effects or disturbances of biological parameters over time. It also showed that adverse events reported were not cumulative over time in persons living with HIV-1 (PLHIV1). The importance of this study was the first to our knowledge to investigate the tolerance and toxicity of Spirulina longer than twelve months in PLHIV-1. However, the discussion knows the limits of comparison of results by the absence of similar studies.

Adverse effects were observed in patients receiving ART and "spiruline plus" but they were not cumulative over time. In effect on the eighteen types of adverse events reported during the twelve month follow-up, only four have been reported in patients who were on ART and placebo. The fourteen other notified patients on ARTs and "spiruline plus" decreased with the time in the third month in September, four in the sixth month, two months and one ninth to the twelfth month. Among these effects, only peripheral neuropathy was notified during successive quarters until the twelfth month. However, its persistence over time cannot be accepted because it was not observed in the same patients during the twelve months (difference therapeutic regimens of patients with neuropathy). This neuropathy can be induced by molecules Stavudine (d4T) and zidovudine (AZT) [16] present in their regimens.

Biochemical and hematological parameters did not change significantly (p > 0.05) over time in patients in both groups (patients on ART regime and "spiruline plus" ART patients and placebo).

Total cholesterol and total protein had significant variations in time and in the same direction in patients in both groups (patients on ART regime and "spiruline plus" ART patients and placebo). The "spiruline plus" compared with the placebo group did not show disturbances or changes in biochemical and hematological parameters of the dose used (2.5 g / day) over time.

TABLE 2: ADVERSE PATIENTS BASED DIETS DURING THE FOLLOW-UP TIME

Group of ART + Placebo	Group of ART + « Spiruline plus »	Group of Placebo	Group of « Spiruline plus »	Regimens	Adverse effects
M3					
	1			AZT+3TC+NVP	Asthenia + aches + fever
2	1			AZT+3TC+NVP	Rhinitis
	1			D4T+3TC+NVP	Rhinitis
	1			TDF+3TC+ NVP	Rhinitis
	1			AZT+3TC+NVP	Precordial
1	1			D4T+3TC+NVP	Epigastric burns
1	1			AZT+3TC+NVP	Neuropathy
M6					
	1			ABC+3TC+LPV/R	anorexia
	1			AZT+3TC+NVP	anorexia
	1			ABC+TDF+LPV/R	Pulmonary
	1			AZT+3TC+NVP	Epigastric burns
M9					
	1			AZT+3TC+EFV	peripheral neuropathy
	1			D4T+3TC+NVP	peripheral neuropathy
M12					
	1			AZT+3TC+EFV	Neuropathie périphérique

M3: third month follow M6: sixth month follow-up M9: ninth month follow-up M12: the twelfth month follow-up.
Abacavir: ABC, Zidovudine: AZT, Lamivudine: 3TC, Nivurapine: NVP, Lopinavir / Ritonavir: LPV/R, Stavudine: D4T

TABLE 3: RESULTS OF BIOCHEMICAL PATIENTS ACCORDING TO TREATMENT GROUP

	AS			AP			p2
	N	Mean	St D	N	Mean	St D	
Total protein							
D0	31	76,65	10,08	31	76,65	10	0,8
M3	30	80,33	18,42	34	82,35	10,6	0,5
M6	27	86,96	9,7	30	85,93	11,6	0,7
M9	30	85,55	10,99	35	85,34	12,4	0,9
M12	22	97,64	22,42	27	91,19	15,5	1
p1		**0,001**			**0,001**		
Urea							
D0	31	3,65	0,7	38	3,74	0,7	0,6
M3	29	3,58	0,86	32	3,78	0,9	0,3
M6	28	3,73	0,91	30	3,78	0,1	0,8
M9	30	3,95	0,83	35	4,04	0,6	0,6
M12	23	3,78	0,64	27	4,13	0,6	0,07
p1		0,4			0,2		
Créatinine							
J0	31	69,06	14,1	37	71,46	14,9	0,5
M3	29	67,52	19,23	33	73,91	15,6	0,1
M6	28	75,04	16,72	30	82,97	62,4	0,5
M9	30	72,32	11,23	35	72,4	11,3	0,9
M12	23	68,04	11,15	27	72,26	13,5	0,2
p1		0,2			0,5		
Glucose							
J0	31	4,27	1,3	35	4,45	0,7	0,4
M3	30	4.42	0,66	34	4,66	2.6	0,6
M6	28	4,26	0,8	30	4,81	2,7	0,3
M9	30	4,31	0,5	35	4,55	0,5	0,08
M12	23	4,49	0,5	27	4,55	2	0,8
p1		0,8			0,9		
AST							
J0	31	24,60	14,6	37	22,7	9	0,8
M3	29	24,28	8,3	29	38,24	74,8	0,1
M6	28	25,93	10,5	29	28,03	46,5	0,3
M9	30	25,77	12,4	35	23,54	8	0,3
M12	23	24,91	10,7	29	22,33	9,9	0,3
p1		0,9			0,2		
ALT							
J0	31	21,85	19,9	36	16,78	6,9	0,1
M3	29	20,86	10,1	32	21,6	17,9	**0,01**
M6	28	20,50	11,6	29	28,03	46,5	0,4
M9	30	21,89	23,7	27	21,83	9,1	0,9
M12	23	20,43	15,67	27	19,7	8,9	0,8
p1		0,9			0,3		
Cholestérol							
J0	30	3,96	1,2	37	3.83	1,4	0,6
M3	28	4,32	1,2	33	4,43	1,6	0,7
M6	28	4,94	0,9	30	4,37	1,4	0,08
M9	30	5,2	1,5	28	5,03	1,5	0,6
M12	22	4,38	1,4	27	5,08	1,1	0,06
p1		**0,003**			**0,004**		
Triglycérides							

J0	31	1,03	0,5	37	1,01	0,6	0,8
M3	29	1,27	0,7	33	1,1	1	0,4
M6	28	1,35	0,9	29	4,45	15,4	0,2
M9	30	1,07	0,7	35	1,06	0,8	0,9
M12	23	9,51	0,4	27	1,4	1,4	0,1
p1		0,2			0,1		

AS: ART + "spiruline plus" AP: ART + placebo. M3: third month follow. M6: sixth month follow-up M9: ninth month follow-up M12: the twelfth month follow-up. p1: variance (Ono ANOVA), p2: t test.

TABLE 4: RESULTS OF HEMATOLOGICAL PATIENTS ACCORDING TO TREATMENT GROUP

	AS			AP			p2
	N	Mean	St D	N	Mean	St D	
White blood cells							
J0	20	5,76	2,2	29	4,98	1,5	0,1
M3	29	5,37	1,5	35	5,36	1,4	0,9
M6	26	4,68	1	28	4,55	1,8	0,7
M9	31	4,95	1,3	35	5,15	2,1	0,6
M12	22	5,23	1,5	27	4,99	1,6	0,6
p1		0,1			0,4		
Red blood cells							
J0	22	3,67	1	22	3,89	2	0,6
M3	28	3,56	0,8	35	3,37	1	0,4
M6	26	3,45	0,4	28	3,33	0,4	0,3
M9	31	3,7	0,9	34	3,66	0,6	0,8
M12	22	3,62	5,4	27	3,89	0,6	0,1
p1		0,8			0,1		
Hemoglobin							
J0	21	1,19	3,2	29	1,30	2,1	0,1
M3	29	1,31	1,7	34	1,25	2	0,2
M6	26	1,2	2,9	28	1,25	1,5	0,4
M9	30	1,32	1,9	34	1,3	1,6	0,5
M12	22	1,22	1,5	27	1,3	1,7	**0,008**
p1		0,1			0,2		
Platelets							
J0	22	226	87,1	29	240	64,3	0,2
M3	29	241	62,4	35	2,37	92,8	0,8
M6	26	237	76,3	28	230	57	0,9
M9	31	234	68,1	36	2,13	65,6	0,6
M12	22	210	62	27	209	61,7	0,5
p1		0,6			0,2		

AS: ART + "spirulina plus" AP: ART + placebo. M3: third month follow. M6: sixth month follow-up. M9: ninth month follow-up M12: the twelfth month follow-up. p1: variance (ono anova). p2: t test.

CONCLUSION: Spirulina enriched in zinc and selenium induced or disruption or significant clinical evolution or biological evolution different from placebo over time at a dose of 2.5 g per day for PLHIV-1. It shows tolerance to this dose with ART over time. Use with ARTs or causes intolerance or biological disturbances in the long term. The enrichment of spirulina with zinc and selenium is recommended as well as unwanted disturbances caused by single spirulina effects are not observed with spirulina enriched in zinc and selenium.

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