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Study of the Toxicity of "Spirulina Plus" in HIV1 Infected Patients in Ouagadougou, Burkina Faso

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Abstract

Spirulina is often used in patients being treated with antiretroviral (ART) because of its nutritional and immunostimulatory properties despite the absence of data on the possible consequences of its association with ARTs. The purpose of this study was therefore to assess the safety and toxicity of Spirulina enriched with selenium and zinc ("spirulina plus") in patients treated with ARTs.

From 15 April 2010 to 15 April 2012, a randomized clinical test compared four groups of patients infected with HIV-1 and treated with different diets: a group of patients taking only placebo, a second group treated only with "Spiruline plus", a third group taking ARTs and placebo, the fourth group taking ARTs and "Spiruline plus". Anthropometric, clinical and biological data were collected during the inclusion and during the follow-up (3 and 6 months after the inclusion). One hundred and thirteen (113) patients infected with HIV-1 were shadowed from the inclusion to six months; almost two thirds of whom (63.7%) were taking ARTs. Neither a difference of intolerance nor toxicity was observed between the four groups of treatment. There were no significant changes, biological parameters between the four groups of treatment. The few undesirable side effects reported in the ART group and "Spiruline plus" were classified as having low intensity. "Spiruline plus" results in neither more undesirable side effects nor in toxicity in persons with HIV taking ARTs. It is tolerated with ARTs.

Keywords: Tolerance; "Spirulina plus"; ART

Introduction

Malnutrition is a problem of public health particularly in developing countries. That explains why the United Nations' Organisation for Food and Agriculture (FAO) gave special encouragement to African countries to resort to algae and more precisely microalgae in order to fight against hunger and malnutrition [1]. Spirulina, a multicellular blue alga, enriched with vitamins, minerals, and pigments [2-8] can easily be grown. Consumed since antiquity on the African and American continents, it is nowadays used in all the continents. Burkina Faso, just like other countries, has made the production of spirulina an industry to fight against malnutrition, to contribute to the management of and help to people with HIV. Spirulina is used in persons with HIV because of its nutritional and immunostimulatory effects [2-5].

However, adverse effects reported in previous studies [9-12], raise questions about the possible consequences of its association with antiretroviral [2]. Indeed persons with HIV on antiretroviral treatment, sometimes have problems with toxicity, especially those vital functions (liver, pancreas, heart) and metabolic (hematological disorders, glucose, lipid). Also some of these side effects have been reported in clinical cases [9,10] and pharmacovigilance reports [12] with spirulina. Hepatic toxicities encountered with antiretroviral drugs non- nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors for HIV (IP) have been reported in these reports.

The present study was designed to investigate the safety and toxicity of "Spiruline plus" formula Spirulina locally produced and enriched with antioxidants and immune boosters such as zinc and selenium [13-21] , in people with HIV. In one group of the study, patients had in their therapeutic regimen the following molecules: lamivudine (3TC), abacavir (ABC), zidovudine (AZT or ZDV): stavudine (d4T), efavirenz (EFV), lopinavir/ritonavir (LPV/r) and nevirapine (NVP).

Methods

Type of study

It was a randomized clinical test versus placebo comparing four groups of treatment: a first group of patients was treated only with placebo, a second group of patients only with "Spiruline plus", a third group of patients with ARTs and placebo, and a fourth group of patients with ARTs and "Spiruline plus."

Study population

The study population consisted of men and women infected with HIV-1, from 18 to 55, not suffering from hepatic or kidney failure and living in Ouagadougou, the capital city of Burkina Faso. Only non-pregnant women were included in the test.

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Study sites

The test was conducted in the following accredited centers which have approved of the management of the patients with HIV:

Day care hospital of Yalgado Ouedraogo Teaching Hospital of Ouagadougou (CHUYO): It is one of the three Teaching Hospitals of Burkina Faso. The Center is at the top of the national health pyramid.

Medical Center of Pissy: It is one of the most important health centers of Burkina Faso; it has approved of the management of the patients with HIV;

Health Center of African Solidarity Association (AAS): It is an accredited community center which has approved of the management of the patients with HIV;

Laboratory of the National Armed Forces (Camp Sangoulé Lamizana): It is an accredited biological center for the management of the patients with HIV;

Saint Camille Laboratory: It is a religious laboratory of biomedical analyses for the management of the patients with HIV, in Ouagadougou.

The Test products

"Spiruline plus" was the test product. Our study was concerned with spirulina produced in Koudougou, a town of Burkina Faso (an accredited spirulina production center), enriched with selenium and zinc. The galenic presentation used was in the form of capsules of 420 mg of "Spiruline plus." Each capsule contains 20 mg of selenium and 3 mg of zinc. "Spiruline plus" was administered to participants at a dosage of 6 capsules per day (2 × 3 capsules/day).

Placebo consisted of cornmeal flour plus chlorophyll.

"Spiruline plus" and placebo were manufactured (transformation into capsules) by the Institute for Research in Health Sciences (MEPHATRA/IRSS) in its laboratory section of the Traditional Pharmacopoeia. Quality control was conducted by the National Public Health Laboratory (LNSP).

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 favorably approved 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.

Clinic: At the clinical level, and according to the case report form, the anatomical systems were examined following the normal, abnormal and not examined assessment. The following systems were examined: general systems: ENT, pulmonary, cardiovascular, digestive, neurological, lymphatic, endocrine, musculoskeletal, urogenital, dermatological, other, (to be specified). The undesirable manifestations were classified according to their intensity (Intensity: 0=None; 1=Weak; 2=Moderate; 3=; 4=Very Severe) and their causality (Causality : 0=None; 1=Improbable, 2=Possible, 3=Défined, 4=Unknown).

Pre-inclusion visit: It was conducted one week before the inclusions started and aimed at: Administering the consent and collecting the informed consent form signatures. Verifying the clinical and biological criteria for participation in the study. Biologically, a blood sample was taken to confirm the patient's status and type of HIV infection, to assess hepatic function (transaminases dosage) and renal function (determination of urea, creatinine). Collecting urine in female participants to search for possible pregnancy.

Inclusion visit (D0): During the inclusion, the patients who fulfilled its criteria were randomized into one of the four groups of treatment. The following date were collected: clinical data (assessment of the following anatomical systems: General, pulmonary, ENT, cardiac, gastrointestinal, neurological, lymphatic, musculoskeletal, urogenital and dermatological); biochemical data (production of serum, sodium, potassium, magnesium, calcium, bicabornates, proteins, glycemia, urea, creatinemia, ASAT and ALAT, total cholesterol, triglycerides, lactic acid, total protein, amylase, total and conjugated bilirubin, AST and ALT); hematological data (blood count, prothrombin time, sedimentation rate,)

Day 28 tour (D28): On this visit, the following data were collected: a clinical examination, notification of adverse effects and a sample for verification of biochemical parameters (transaminases, urea, creatinine, glycemia, and amylase).

Visits on the 84th and 168th days (D84, D168): The clinical and biological data and adverse effects as well were collected on the quarterly visits (Table 1). In the present work, for only those patients who were visited, their clinical and biological findings in inclusion visit (D0) and M6 (D168) were selected.

Emergency consultations: These consultations were conducted outside the periods defined by the protocol (outside protocol consultations) for urgent or necessary cases. On these visits, clinical and biological data were also recorded. The relationship between the patients' symptoms and treatments were evaluated.

Biological 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).

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

Table 1: Summary of activities.

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

At the biological level, we could analyze the biological parameters according to the biological manifestations of toxicity of WHO. We defined the grades of toxicity of WHO in the table as well as some definitions of the abbreviations in the tables.

In the validity protocol of the national committee of ethics of health in Burkina Faso, the normal intervals of the biological parameters were defined. Therefore, each parameter was assigned a value which is situated as follows: in the normal interval or normal value, inferior to the value or normal interval, superior to the value or normal interval. We could analyze each one of the biological parameters according to its value or normal or abnormal interval (inferior or superior to the value or normal interval) according to the four groups of treatment, in order just to observe whether there exists any difference between the different groups of treatment as regards the "abnormal values"

The clinical and biological findings of the 168th day visit M6 (D168) were selected for the analysis of the "Spiruline plus"safety and toxicity.

The chief criterion of evaluation was the evolution of creatinine. In the second place, the evolution of other biological parameters and the patients' clinical status were evaluated. The grades of biological toxicities of WHO (2010) [22] (Table 2) were used to assess the toxicities according to the biological parameters.

Light grade or Grade 1: Symptoms which do not prevent, or which hardly prevent, the social and functional usual activities.

Moderate grade or Grade 2: Symptoms which prevent but not very much the social and functional usual activities.

Severe grade or Grade 3: Symptoms which actually prevent the social and functional usual activities).

With a possibility to put at stake the prognosis or Grade 4: Symptoms which prevent from taking care of oneself or a necessity of medical or surgical intervention in order to prevent an organ from being affected for a long period of time, or a permanent handicap or death.

Management and data analysis strategies: The data were typed on Epi Info software (version 3.5.1) and then converted to Excel (Excel 2003) files. The analysis of the data was carried out with the Sigma Stat software (Sigma Stat 3.5) and « Stata version 12 » close to alpha=0.05. In the table, the *p* does indicate the *p* value obtained by the statistical analysis (p=ficher).

All test were considered significant at the $p<0.05$

Results

Diagram of the Study flow and characteristics of the population

Patient recruitment was gradual and explained the level of diversity of their follow-up stage. When the study came to an end, 131 patients of whom 121 and 113 had their visit respectively were included in the third and sixth months of follow-up (Figure 1). Women were more represented (83.7%) than men (16.3%) (Table 3) and the majority (63.7

biological parameters	Light Grade 1	Moderate Grade 2	Severe Grade 3	Up to life-threatening Grade 4
Hematology				
Hemoglobin *N: H: 13-17 g/dl F: 11.5-16 g/dl*	8.0-9.4	7.0-7.9	6.5-6.9	<6.5
Absolute neutrophil count *N: 2000-7000*	1.0-1.5	0.75-0.99	0.5-0.749	<0.5
Platelets *N: 150 000 à 500 000/mm³*	75-99l	50-74.9	20-49.9l	<20l
Biochemistry				
Hypoglycemia *N: <4.10 mmol/l*	3.01-3.55 mmol/l	2.19-3.00 mmol/l	1.67-2.18 mmol/l	<1.67 mmol/l
Hyperglycemia *N: >5.90 mmol/l*	6.44-8.90 mmol/l	8.91-13.88 mmol/l	13.89-27.76 mmol/l	>27.76 mmol/l
Triglycerides *N: 0.68-1.88 mmol*	-	4.52-8.47 mmol/l	8.48-13.55 mmol/l 8.48-13.55 mmol/l	>13.55 mmol/l
Creatinine *N: H: 62-115 µmol/l F: 53-97 µmol/l*	120-180	180-360	360-720	>720
AST *N: <40 µl/l*	50-100	100-200	200-400	>400
ALT *N: <45 µl/l*	50-100	100-200	200-400	>400
Total bilirubin *N: ≤ 20 µmol*	18.7-25.5	27.2-42.5	44.2-85	>85
amylases: *N: <90 µ/l*	55-82.5	82.5-110	110-275	>275

N: Normal=normal value or normal interval

ALT: Alanine aminotransférase ASAT: Aspartate Aminotransférase

- Light grade or Grade 1: Symptoms which do not prevent, or which hardly prevent, the social and functional usual activities;

- Moderate grade or Grade 2: Symptoms which prevent but not very much the social and functional usual activities

- Severe grade or Grade 3: Symptoms which actually prevent the social and functional usual activities)

- With a possibility to put at stake the prognosis or Grade 4: Symptoms which prevent from taking care of oneself or a necessity of medical or surgical intervention in order to prevent an

Table 2: Graduation biological manifestations of toxicity according to WHO 2010.

%) of the patients was being treated with antiretroviral on the one hand, and on the other hand, a minority (36.3 %) was being treated without antiretroviral.

Patients' clinical manifestations

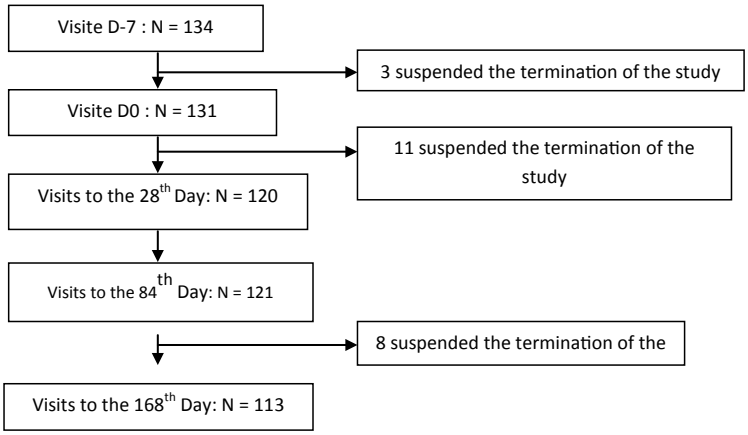
At the clinical level, and according to the case report form, the anatomical systems were examined following the normal, abnormal and not examined assessment. The following systems were examined: general systems: ENT, pulmonary, cardiovascular, digestive, neurological, lymphatic, endocrine, musculoskeletal, urogenital, dermatological, other, (to be specified). The analysis of the clinical manifestations according to the anatomical systems is summarized in Table 4.

The analysis of adverse effects of two groups ("Spiruline plus" and placebo) showed abnormalities in seven anatomical devices (general: ENT, pulmonary, gastrointestinal, neurological, musculoskeletal and dermatological) in patients taking "Spiruline plus" against two anatomical devices (general and dermatological) in patients taking placebo (Table 4). Similarly, no unwanted effects were reported in the placebo group and ARTs. However anorexia, pulmonary and abdominal pain have been reported in patients taking ARTs and "Spiruline plus" (Table 5).

Biological outcomes for patients

The analysis of the undesirable effects noted according to grades of biological manifestations of toxicity of WHO (Table 2) is summarized in Table 6.

Tables 7 and 8 sum up the analysis of each biological parameter



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)

Figure 1: Diagram of the study.

	Placébo	« Spiruline plus »	Total
sex (n=129)			
men	15 (22.1%)	6 (9.8%)	21 (16.3%)
women	53 (77.9%)	55 (90.2%)	108 (83.7%)
Age (n=126)			
<40 years	38 (56.7%)	37 (62.7%)	75 (59.5%)
>40 years	29 (43.3%)	22 (37.3%)	51 (40.5%)
weight (n=117)			
<60 kg	34 (54.0%)	23 (42.6%)	57 (48.7%)
>60 kg	29 (46.0%)	31 (57.4%)	60 (51.3%)
ethnic (n=121)			
Bissa	3 (4.7%)	1 (1.7%)	4 (3.3%)
Dioula	-	1 (1.7%)	1 (0.8%)
Gourounsi	5 (7.8%)	4 (7.0%)	9 (7.4%)
Mossi	47 (73.4%)	39 (68.4%)	86 (71.1%)
Peulh	-	3 (5.3%)	3 (2.5%)
Autres	9 (14.1%)	9 (15.8%)	18 (14.9%)

Table 3: Characteristics of the study population at 6 months follow-up.

	Placebo	« Spiruline plus »	Detail if Abnormal	Placebo	« Spiruline plus »
General device					
Abnormal	1 (1.7%)	1 (1.8%)	cachexy	1	1
Normale	58 (98.3%)	53 (98.2%)			
ORP					
Abnormal	-	1 (1.8%)	decrease visual acuity		1
Normale	59 (100%)	53 (98.2%)			
pulmonary					
Abnormal	-	2 (3.7%)	pulmonary		2
Normale	59 (100%)	52 (96.3%)			
cardiovascular					
Abnormal	-	-			
Normale	59 (100%)	54 (100%)			
digestive					
Abnormal	-	2 (3.7%)	Abdominal pain		1
Normale	59 (100%)	52 (96.3%)			1
neurological					
Abnormal	-	2 (3.7%)	Céphalées+Paresthésie		1
Normale	59 (100%)	52 (96.3%)	neuropathy ringroad		1
lymphatic					
Abnormal	-	-			
Normale	59 (100%)	54 (100%)			
endocrine					
Abnormal	-	-			
Normale	59 (100%)	54 (100%)			
Musculoskeletal					1
Abnormal	-	3 (3.6%)	pains broadcast		1
Normale	59 (100%)	51 (94.4%)	limping		1
Urogénital					
Abnormal	-	-			
Normale	59 (100%)	54 (100%)			
Dermatologie					
Anormale	1 (1.7%)	2 (3.8%)	keratoderma		1
Normale	58 (98.3%)	51 (96.2%)	prurigo	1	

ORP: Oto Rhino pharyngeal.

Table 4: Clinical Events at 6 months follow-up of patients according to the treatment and placebo "Spiruline plus".

ARV+Placebo	ARV+« Spiruline plus »	Molécules	Effets indésirables
	1	ABC+3TC +LPV/r	anorexia
	1	AZT+3TC +NVP	anorexia
	1	ABC+TDF+LPV/r	pulmonary
	1	Azt+3TC +NVP	abdominal pain

3TC: lamivudine ABC: abacavir AZT: zidovudine (appelée aussi ZDV)

d4T: stavudine EFV: éfavirenz LPV/r: lopinavir/ritonavir NVP: névirapine

Table 5: Adverse based regimen of treatment at 6 months follow-up.

		WHO stages of severity of some biological parameters					
biological parameters		No risk	Light grade	Moderate grade	Severe grade	Vital grade	p
amylase	Placebo	2 (4.4%)		43 (95.6%)			0.767
(n=86)	« Spiruline plus »	3 (77.5%)		38(12.5%)			
AST	Placebo	41 (91.2%)		4(8.8%)			0.678
(n=86)	« Spiruline plus »	39 (95.2%)		2 (4.8%)			
ALT	Placebo	42 (93.4%)		3(6.6%)			1.000
(n=86)	« Spiruline plus »	39 (95.2%)		2 (4.8%)			
total bilirubin	Placebo	42 (100%)					NC
(n=80)	« Spiruline plus »	38 (100%)					
glucose	Placebo	38 (82.6%)		8 (17.4%)			0.363
(n=87)	« Spiruline plus »	37 (90.2%)		4 (9.8%)			
hyperglycemia	Placebo	44 (95%)		2(5.0%)			1.000
(n=87)	« Spiruline plus »	39 (95.2%)		2 (4.8%)			
hypoglycemia	Placebo	38 (82.6%)		8 (17.4%)			0.363
(n=87)	« Spiruline plus »	37 (90.2%)		4 (9.8%)			
Triglycérides	Placebo			44 (100%)			NC
(n=85)	« Spiruline plus »			41(100%)			
Créatinine	Placebo	44 (95.6%)		2(2.4%)			1.000
(n=87)	« Spiruline plus »	40 (97.6%)		1 (2.4%)			

NC=not computable p=pFisher ALT: Alanine aminotransférase ASAT: Aspartate Aminotransférase

- Light grade or Grade 1: Symptoms which do not prevent, or which hardly prevent, the social and functional usual activities

- Moderate grade or Grade 2: Symptoms which prevent but not very much the social and functional usual activities

- Severe grade or Grade 3: Symptoms which actually prevent the social and functional usual activities)

- With a possibility to put at stake the prognosis or Grade 4: Symptoms which prevent from taking care of oneself or a necessity of medical or surgical intervention in order to prevent an organ from being affected for a long period of time, or a permanent handicap or death.

Table 6: Biochemical parameters according to WHO stages of severity in the placebo group and "Spiruline plus".

Paramètres biologiques		**ARV+Placebo**	**ARV+« Spiruline plus »**	**Placebo**	**« Spiruline plus »**	**p**
ASAT	<Normal	26(89.7%)	25(89.3%)	16(100%)	12(92.3%)	0.602
(n=84)	Normal	1(3.4%)	3(10.7%)	-	-	
	>Normal	2(6.9%)	-	-	1(7.7%)	
ALAT	<Normal	26(86.8%)	25(96.1%)	16(100%)	12(92.3%)	0.63
(n=84)	Normal	2(6.6%)	1(3.8%)	-	-	
	>Normal	2(6.6%)	-	-	1(7.7%)	
Bilirubine Total	<Normal	2(6.7%)	1(3.6%)	-	-	0.28
(n=86)	Normal	23(76.6%)	25(89.3%)	16(100%)	12(100%)	
	>Normal	5(16.7%)	2(7.1%)	-	-	
Triglycérides	<Normal	-	-	-	-	0.8
(n=85)	Normal	21 (72.4%)	20(71.4%)	14(93.4%)	13(100%)	
	>Normal	8(27.6%)	8(28.6%)	1(6.6%)	-	
Cholestérol total	<Normal	14(50%)	-	8(50%)	2(15.4%)	0.55
(n=87)	Normal	14(50%)	27(96.4%)	8(50%)	11(84.6%)	
	>Normal	2(6.7%)	1(3.6%)	-	-	
Créatininemie	<Normal	-	-	-	-	NC
(n=87)	Normal	29(96.6%)	28(100%)	16(100%)	13(100%)	
	>Normal	1(3.4%)	-	-	-	
Urée	<Normal	-	-	-	-	NC

(n=87)	Normal	30(100%)	28 (100%)	16(100%)	13(100%)	
	>Normal	-	-	-	-	
Amylase	<Normal	10(34.5%)	13(46.4%)	10(62.5%)	5(41.6%)	0.767
(n=85)	>Normal	19(65.5%)	15(53.6%)	6(37.5%)	7(58.4%)	
Calcium	<Normal	3(11.1%)	5(20.8%)	2(12.5%)	2(25%	0.525
(n=77)	Normal	24(88.9%)	19(79.2%)	14(87.5%)	8(75%)	
	>Normal	-	-	-	-	
Magnésium	<Normal	-	-	-	-	NC
(n=77)	Normal	25(92.6%)	20(83.3%)	14(87.5%)	10(100%)	
	>Normal	2(7.4%)	4(16.7%)	2(12.5%)	-	
Sodium	<Normal	1(3.7%)	1(4.2%)	-	-	0.525
(n=77)	Normal	26(96.3%)	23(95.8%)	16(100%)	10(100%)	
	>Normal	-	-	-	-	
Potassium	<Normal	2(7.4%)	-	-	-	NC
(n=76)	Normal	24(88.9%)	23(95.8%)	16(100)	9(100%)	
	>Normal	1(3.7%)	1(4.2%)	-	-	
Bicarbonate	<Normal	21(77.8%)	19(79.2%)	11(73.3%)	8(88.9%)	
(n=75)	Normal	6(22.2%)	5(20.8%)	4(26.7%)	1(11.1%)	
	>Normal	-	-	-	-	
Phosphore	<Normal	1 (3.7%)	3(12.5%)	3(20%)		
(n=75)	Normal	8(29.6%)	7(29.2%)	1(6.7%)	3(33.3%)	
	>Normal	18(66.7%)	14(58.3%)	11(73.3%)	6(66.7%)	
Chlore	<Normal	2 (7.4%)	-	-	-	
	Normal	24(88.9%)	24(100%)	13(86.7%)	6(66.7%)	
	>Normal	1(3.7%)	-	2(13.3%)	3(33.3%)	
Protéine totale	<Normal	3(10%)	-	2(12.5%)	-	0.05
	Normal	5(16.7%)	9(33.3%)	1(6.2%)	2(15.4%)	
	>Normal	22(73.3%)	18(66.7%)	13(81.3%)	11(84.6%)	
glycemia	<Normal	6(20%)	12(42.9%)	6(37.5%)	5(38.5%)	0.326
(n=87)	Normal	22(73.3%)	14(50%)	10(62.5%)	5(38.5%)	
	>Normal	2(6.7%)	2(7.1)	-	3(23%)	

ALT: Alanine aminotransférase ASAT: Aspartate Aminotransférase

Table 7: Biochemical parameters in functions in the treatment group and normal intervals to 6 months follow-up.

according to its value or normal and abnormal interval (less or more than the normal value or interval) according to the four groups of treatment. The calculated variance of the three values (normal, inferior to normal and superior to normal) of each parameter was significant neither inside one group, nor between the four groups (Tables 7 and 8).

Cases of severe or lethal toxicities were not observed in patients during the study. However, no significant toxicities of moderate grades were observed in patients taking "Spiruline plus" and placebo. There was no significant difference between the treatment with " Spiruline plus" and the one with placebo according to the grade of toxicity of WHO 2010 [22] (Table 6).

There were no significant changes of values compared to normal biochemical and hematological parameters. Also, there were significant differences or biological parameters between neither the four groups nor between the ART groups (ART "Spiruline plus" and ART placebo) (Tables 7 and 8) except for the total protein and neutrophils which were higher in patients taking "Spiruline plus".

Calculation of imputability

The method of calculation of imputability by Dangoumeau v7.3 - October 2013 [22], gives nil imputability (Table 9).

There was no presence of objective elements which allow for inclusion of any possibility of exposure to "spirulina plus" (the exposure to spiruline is excluded), there were a few cases of clinical and paraclinical symptoms notified (existing clinical and paraclinical symptoms), the chronolgy of the symptoms cannot be related to "spirulina plus" (incompatible chronology), there were no objective elements of causal characterization: (test, dosage, other cause...) absence, there were no other diagnoses being considered, the bibliography has established a relation of causality.

All these elements taken together give a conclusion of nil imputablity according to the method of calculation of imputability by Dangoumeau v7.3 - October (http://tv.toxalert.fr).

Paramètres biologiques		ARV+Placebo	ARV+« Spiruline plus »	Placebo	« Spiruline plus »	p (variance)
Globules Blancs	<Normal	12 (42.8%)	6(23.1%)	3(18.75%)	3(23.1%)	0.450
(n=83)	Normal	15(53.6)	20(76.9%)	13(81.25%)	9(69.2%)	
	>Normal	1(3.6%)	-	-	1(7.7%)	
Neutrophiles	<Normal	9(32.1%)	8(30.8%)	10(62.5%)	7(53.8%)	0.043
(n=82)	Normal	18(64.3%)	18(69.2%)	6(37.5%)	6(46.1%)	
	>Normal	1(3.6%)	-	-	-	
Lymphocytes	<Normal	4(14.3%)	-	-	-	0.205
(n=82)	Normal	22(78.6%)	25(100%)	14(87.5%)	13(100%)	
	>Normal	2(7.1%)		2(12.5%)		
Monocytes	<Normal	5 (17.8%)	5(19.2%)	-	-	0.356
(n=82)	Normal	10(35.7%)	6(23.1%)	3(18.8%)	3(25%)	
	>Normal	13(46.5)	15(57.7%)	13(81.2%)	9(75%)	
Eosinophiles	<Normal	20(71.4%)	21(84%)	11(68.7)	2(15.4%)	0.07
(n=82)	>Normal	8(28.6%)	4(26%)	5(31.3%)	11(84.6%)	
Basophiles	<Normal	28(100%)	25(96.1%)	16(100%)	13(100%)	NC
(n=83)	> Normal	-	1(3.9%)	-	-	
Globules rouges	<Normal	22(78.6%)	26 (100%)	6(37.5%)	8(66.7%)	0.112
(n=82)	Normal	6(21.4%)	-	10(62.5%)	4(33.3%)	
	>Normal	-	-	-	-	
Hémoglobine	<Normal	6(21.4%)	6(23.1%)	4(25%)	8(66.7%)	0.133
(n=82)	Normal	22(78.6%)	20(76.9%)	12(75%)	4(33.3%)	
	>Normal	-	-	-	-	
Hématocrite	<Normal	17(60.7%)	16(64%)	9(56.2%)	9(69.2%)	0.112
(n=82)	Normal	11(39.3%)	9(36%)	6(37.5%)	4(30.8%)	
	>Normal	-	-	1(6.3%)	-	
CCMH	<Normal	-	1(4%)	-	-	
(n=82)	Normal	2((7.1%)	-	8(50%)	3(25%)	
	>Normal	26(82.9%)	25(96%)	8(50%)	9(75%)	
TGMH	<Normal	-	-	1(6.7%)	-	
(n=82)	Normal	1(3.6%)	4(15.4%)	2(13.3%)	1(8.3%)	
	>Normal	27(96.4%)	22(84.6%)	12(80%)	11(91.7%)	
Plaquettes	<Normal	3(10.7%)	5(19.2%)	4(25%)	-	0.848
(n=82)	Normal	25(89.3%)	21(80.8%)	12(75%)	13(100%)	
	>Normal	-	-	-	-	
VGM	<Normal	-	1(3.8%)	2(12.5%)	1(8.3%)	
(n=82)	Normal	9(32.1%)	10(38.5%)	12(75%)	8(66.6%)	
	>Normal	19(67.8%)	15(57.7%)	2(12.5%)	3(25%)	

Table 8: Hematological features in the treatment group and normal intervals to 6 months follow-up.

Exposure:	Excluding X	Possible	Very likely
Symptomatology, Clinical or paraclinical effect:	Absent	Présent X	
Timeline:	incompatible X	Compatible	evocative
Objectives causal characterization elements: test, measurement, otherwise…	Presence of contrary elements	Absence of evidence X	Presence of evidence
differential diagnoses:	Diag. other confirmed	Diag. other not considered X	Diag. other discarded
Link extrinsic (references):	Table X never described	possible link	probable link

Conclusion: nil imputablity

Table 9: Method of accountability spirulina calculation according Dangoumeau v7.3 - October 2013.

Discussion

Spirulina, an alga which contains nutritional and immunostimulatory properties, is commonly used in malnourished and immunocompromised patients. The study showed that the use of spirulina enriched in zinc and selenium ("spirulina plus") in patients infected with HIV-1 involves no additional toxicity. Similarly, the results showed that the use of "spirulina plus" does not result in undesirable additional, clinical and biological effects in patients with HIV-1. On the contrary, side effects were less noticed in patients taking ARTs and "spirulina plus" than those taking only "spirulina plus". Which would show the interest of the ART and "spirulina plus" association.

A special feature of this study is its being the first of this kind to have been able to shadow patients with HIV-1 in order to assess "spirulina plus" toxicity and tolerance in this vulnerable population to our knowledge. However, the discussion knows the limits of comparisons for lack of similar previous study.

The majority of patients in the study were female. Burkina Faso's population consists of 52% of women and 48% men explain that.

Clinically

Clinically, the manifestations of adverse effects noted are not those expected with antiretrovirals used: lipoatrophy caused by molecules such as stavudine (d4T) and zidovudine (AZT) were not observed. Oxidative stress induced by molecules of a mitochondrial dysfunction has be attenuated by the oxidizing effect of the "spiruline plus". The gastrointestinal intolerance, headache, skin problems caused mainly led by AZT were each observed in one patient under "Spiruline plus" but not in patients ' Spiruline plus" and AZT (Tables 4 and 5). This would suggest that AZT and "Spiruline plus" does not lead to a potentiation of adverse effects expected by AZT but instead could neutralize them. Also other undesirable clinical manifestations of ARV molecules used in our study were not observed. This is the case of hepatitis that can cause mainly by NVP, EFV and ABC molecules, pancreatitis and peripheral neuropathy engendered d4T. The dose of "Spiruline plus" used in our study could explain the tolerance set. Any time it is confirmed with a larger sample than ours in a long time.

Biologically

The percentages of grades moderate biological manifestations of patients "Spiruline plus" decreased compared to placebo (Tables 6 and 7). It can be suggested that attenuation of the toxicity of ARVs by the "Spiruline plus". Biological problems sometimes caused by ARVs used have not been observed with patients "Spiruline plus" and ARV: Lipid disorders such increase in triglycerides and total cholesterol, and increased transaminases that cause molecules such as NVP and EFV, have not been observed with patients "Spiruline plus" and ARVs. Hematological disorders kinds cytopenia and anemia caused by AZT, have not been observed with patients "Spirulina plus" and ARVs. Also increased percentages above or below the normal laboratory values in patients "Spiruline plus" versus under "Spiruline plus" and ARVs (Tables 7 and 8), strengthen the suggestion of attenuating toxicity ARV spoke " Spirulina more».

Undesirable effects reported only in patients taking "Spiruline plus" and not in those taking placebo (Table 4), would make one say that "Spiruline plus" would lead to unwanted effects of low intensity especially pulmonary, gastrointestinal, neurological, and musculoskeletal However these undesirable effects are reduced if "Spiruline plus" is associated with ART: as a matter of fact, fewer undesirable effects were noticed in patients taking ARTs and "Spiruline plus" (manifestations: pulmonary manifestations, a broncho-pneumopathy type and gastrointestinal manifestations, a type of abdominal pains) than in those patients taking only "Spiruline plus" (general manifestations, ENT, pulmonary, gastrointestinal, neurological, musculoskeletal and dermatological).

The significant variations of the values of the proteins and polynuclear nutrophils could be explained by the immunostimulatory properties of spirulina. As a matter of fact, a realization of electrophoresis of proteins should have confirmed that. Hepatic, dermatological and rhabdomyalyse toxicities noticed in clinical studies [9-11] as well as hepatic, dermatological, hematological, nephrological toxicities and electrolytic problems referred to in pharmacovigilance reports [12] with simple spirulina were observed neither in patients taking "Spiruline plus" nor only in those taking ARTs and "Spiruline plus". The enrichment of spirulina with zinc and selenium as well as the posology used, could possibly explain these differences. Similarly, the same undesirable effects noted with simple spirulina types such as the following: hypoglycaemia, reduction of triglycerides and total cholesterol [23-34], hepato toxicity through increase in transaminases [11,12], increase in hemoglobin [3,7,31], were not observed in patients taking "Spiruline plus" only. This presumably would confirm the important role of oligo elements (zinc and selenium) when added to spirulina.

Conclusion

"Spiruline plus" does not result in unpleasant significant effects or death in people with HIV-1. It does not interfere with biochemical and hematological parameters. The study confirms neither an increase in hemoglobin and blood elements nor a decrease in glycaemia nor hepatic toxicity noted in previous studies with single spirulina.

The results did not show a difference in treatment between the four different groups of the study. On the other hand, they give evidence for tolerance of "Spiruline plus" with ARTs as well as the importance of zinc and selenium when added to a dose of 2.5 g per day of "Spiruline plus". Effects or events observed are attributable to "Spiruline plus".

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