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Effects of spirulina supplementation on selected anthropometric, biochemical, and hematological parameters of HIV-infected adults in Ouagadougou, Burkina Faso

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ABSTRACT

The objective of the study was to assess the impact of spirulina supplementation on selected anthropometric, biochemical, and hematological parameters of HIV-infected adults in Ouagadougou. This quasi-experimental two-arm pilot study was conducted with adults infected with HIV-1 in Ouagadougou, Burkina Faso. A group of 50 participants received a 10 gram daily spirulina supplementation in addition to antiretroviral treatment (ART) while a 50 participant control group received only ART. At 9 months of follow-up, the mean values of mid-upper arm circumference (MUAC) and hemoglobin were significantly higher and creatinemia was lower in participants assigned to spirulina supplementation compared to those in the control group (p = 0.007, 0.002, and 0.01 respectively). At 6 months of follow-up, a significant decrease in gammaglobulins was observed in the intervention group as compared to the control group (p=0.04). There was no difference in the mean serum concentration of alanine transaminase (ALT) or amylasemia between the two groups.The daily 10 gram spirulina supplementation used in our study had a positive effect on several vital biological parameters in HIV-infected adults. Randomized clinical trials on large samples with longer follow-up periods will be necessary to test these assumptions.

Keywords: Spirulina, HIV adult patients, anthropometric, biological parameters.

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INTRODUCTION

Malnutrition, accompanied by a degradation of the subject's general condition as a result of protein and micronutrient deficiencies, is a common concern for those infected with the human immunodeficiency virus (HIV) (Shalini et al., 2012). According to the WHO, at stages III and IV of the infection, these protein deficiencies result in significant weight loss, often greater than 10% of total body weight (Chevalier, 2003).

The food situation in Africa, and particularly in Burkina Faso, is troubling (MAHRH, 2009) and characterized by chronic under-nutrition resulting in cases of acute and chronic malnutrition and an elevated prevalence of certain micronutrient deficiencies such as iron and vitamin A (Méda et al., 2000; INSD, 2011). In the context of local HIV prevalence, estimated at 1.2% (UNAIDS, 2012), these nutritional deficiencies compromise national response strategies to the epidemic.

Spirulina (*Oscillatoriceae spirulina platensis*), an edible microalgae, is naturally rich in proteins, which make up 50-70% of its dry weight (Falquet et Hurni, 2006). In addition, it contains a wide variety of vitamins (beta carotenes, vitamin B12, vitamin E, etc.), essential fatty acids (Omega-3, Omega-6), carbohydrates (15-25%), and other trace elements (Falquet et Hurni, 2006; Charpy et al., 2008). Given this fact, spirulina has received significant attention in the fight against certain forms of anemia (iron deficiency, pernicious), xerophthalmia, HIV, and nutritional deficiencies (Simporé et al., 2007; Selmi et al., 2011; Teas et al., 2012). Animal models have shown that, as a result of its chemical composition, this alga has an important role to play in numerous biological and nutritional activities (Diadelis et al., 2002; Paloma et al., 2008; Chu et al., 2010). However, a study carried out in the Central African Republic (CAR) on the effects of spirulina supplementation (10 grams/day) on the clinical and biological parameters of HIV-infected adults showed no significant impact after 6-months of follow up (Yamani et al., 2009). That being said, nutritional rehabilitation experiments using spirulina for people living with HIV (PLHIV) are extremely rare and often have short follow-up times. Moreover, the impact of long-duration use on biological parameters (transaminases, creatinemia, and hemoglobin) remains relatively unstudied.

Despite a lack of evidence for its potential benefits, many PLHIV in Burkina Faso consume spirulina to slow the progression of the disease or to better handle the side effects of antiretroviral (ARV) treatment (Sawadogo et al., 2004). Following the joint decision of the clergy of Burkina Faso and the Catholic Organization for Development and Solidarity (OCADES) to construct as pirulina production plant in the country and to promote the consumption of this alga, the Ministry of Health of Burkina Faso encouraged a study on the topic. The objective of this study was to evaluate the impact of daily spirulina supplementation on the anthropometric, biochemical, and hematological parameters of adult PLHIV.

MATERIALS AND METHODS

This quasi-experimental open pilot study had two arms and included a total of 100 adults living with HIV-1 at WHO defined stages III or IV of the infection (WHO, 2006). Activities were conducted from January 2008 to February 2010 in two health facilities responsible for the management of HIV-infected individuals in Ouagadougou, Burkina Faso. Participants who received supplementation were recruited from the *Centre d'Accueil Notre Dame de Fatima* (CANDAF) while controls came from the Bogodogo district hospital. Spirulina was

produced at the Nayalgué farm located in the Centre-Ouest region of Burkina Faso and packaged in 10 gram packets.

Participant enrollment

Participants were enrolled from records of patients living with HIV obtained from the two health facilities mentioned above. The objectives, procedures, and requirements of the study were explained to the individual before asking for their consent to participate. After obtaining consent, an initial clinical examination and blood test were performed to determine baseline biochemical and hematological parameters.

Study procedure and follow up of participants

The two groups were monitored over the course of nine months. The first group of 50 participants received a total of 10 grams of spirulina per day taken in two doses, one at lunch and the other at dinner. The second group of 50 patients served as a control. In accordance with national HIV treatment guidelines, both groups received first-line ARV treatment (2INTI+1INNTI) and cotrimoxazole (CNLS-IST, 2009).

Clinical and biological follow-up was conducted monthly for the first three months of the study then on a quarterly basis until the ninth month. Clinical follow-up consisted of clinical examinations and measurements of anthropometric parameters. Biological follow-up involved determining hemoglobin, albumin, alanine transaminase (ALT), amylase, and creatinin serum levels. In addition, these examinations allowed the investigators to verify adherence to supplementation, monitor spirulina and ARV tolerance, and served as an opportunity to distribute the participants' monthly stock of spirulina.

Adherence to spirulina supplementation was assessed by counting the number of spirulina packets remaining on the day of the monthly appointment. Participants who did not come in within three business days following the date of their appointment were contacted by phone or at their home by their doctor or his aide in order to inquire about their health. Those participants, who could not be found within the following seven days, other than in cases of death, were reported as lost to follow-up. Adherence to ARVs was determined at each visit by asking the participant how often they took their medication and by counting the number of pills brought back on the day of the visit.

Data collection

Data were collected using a case report form including information on socio-demographic and clinical characteristics, anthropometric measurements, adherence to ARVs and spirulina supplementation, side effects, death and reasons for dropouts, and results of biological tests.

Data analysis

Data were entered and validated using EpiData software (http://www.epidata.dk/,), then analyzed using SPSS version 17.0 (International Business Machines Corporation, Armonk, New York, USA). Pearson Chi-square tests was used to compare proportions between the two groups of participants. Comparisons of the means and medians of the biological parameters of the groups were performed using the Student's t-test or the Mann-Whitney U test. Incidence density ratios were estimated and tested with a Poisson regression. A threshold of $p<0.05$ was considered statistically significant.

Ethical considerations

Participation in the study was free and voluntary and written informed consent was obtained before inclusion. Participants were free to discontinue participation in the study at

any time. All participants received financial support to compensate for the additional travel required by the study and for the management of side effects. Before implementation, the study received approval from the Ethics Committee for Health Research of Burkina Faso.

RESULTS

A total of 100 participants were included in our study divided into an intervention and a control group of 50 individuals each. Figure 1 shows the participants' follow-up charts. Participants who received spirulina supplementation had a mean age of 37.36 ± 8.25 years while the control group had a mean age of 38.28 ± 9.18 years (p = 0.60). The socio-demographic characteristics of participants are presented in Table 1. By the end of the study, there had been a significantly greater number of dropouts among those taking spirulina (9 cases, 18%) than those in the control group (1 case, 2%) (p = 0.01). Digestive intolerance

Table 1: Characteristics of patients in both groups at inclusion.

	Spirulina group (n = 50)	Control group (n=50)	p
Age (average in years)	37.36 ± 8.25	38.28 ± 9.18	0.60
Sex (%)			
- Male	24.0	40.0	0.09
- Female	76.0	60.0	
Marital Status (%)			
- Single	22.0	28.0	0.14
- Married	56.0	48.0	
- Divorced/Separated	8.0	10.0	
- Widowed	14.0	14.0	
Occupation (%)			
- Housewife	50.0	52.0	0.20
- Farmer	6.0	16.0	
- Trader	16.0	4.0	
- Worker	18.0	18.0	
- Other (pupils, students, unemployed)	10.0	10.0	
Anthropometric data			
- BMI (kg/m²)	19.27 ± 3.70	19.27 ± 2.78	1.0
- MUAC (cm)	25 (23;26)	25 (23 ; 26)	0.78
WHO clinical stage (%)			
- Stade III	36 (72.0)	44 (88.0)	0.05
- Stade IV	14 (28.0)	6 (12.0)	
Biological data			
- Hemoglobin (g/dL)	10.37 ± 1.75	10.37 ± 1.97	1.0
- Albumin* (g/dL)	3.25 ± 0.76	3.59 ± 0.85	0.05
- Creatinemia* (mg/100mL)	1 (0.9 ; 1.2)	1 (0.9 ; 1.2)	0.56
- ALT (UI/L)*	36 (30;47)	29 (18;39.5)	0.03
- Amylasemia* (UI/L)	106 (75;128)	103 (82 ; 129.5)	0.77
- Gamma globulins* (g/dL)	2.56 ± 0.91	2.48 ± 1.00	0.70

*The number of participants supplemented with spirulina (S) and in the control group (C) are for Albumin (S = 47; C = 43); Creatinemia (S = 49; C = 47), ALT (S = 50; C = 47), Amylasemia (S = 49; C = 47) and Gamma globulins (S = 47; C = 42),

and withdrawal of consent were the primary reasons for the premature release of participants assigned to supplementation with spirulina (p = 0.02). The number of deaths recorded was greaterin the control group (8 cases, 16%) than in the intervention group (5 cases, 10%). However the difference in mortality incidence density ratios was not significant (Table 3).

Adherence to ARV treatment and spirulina supplementation

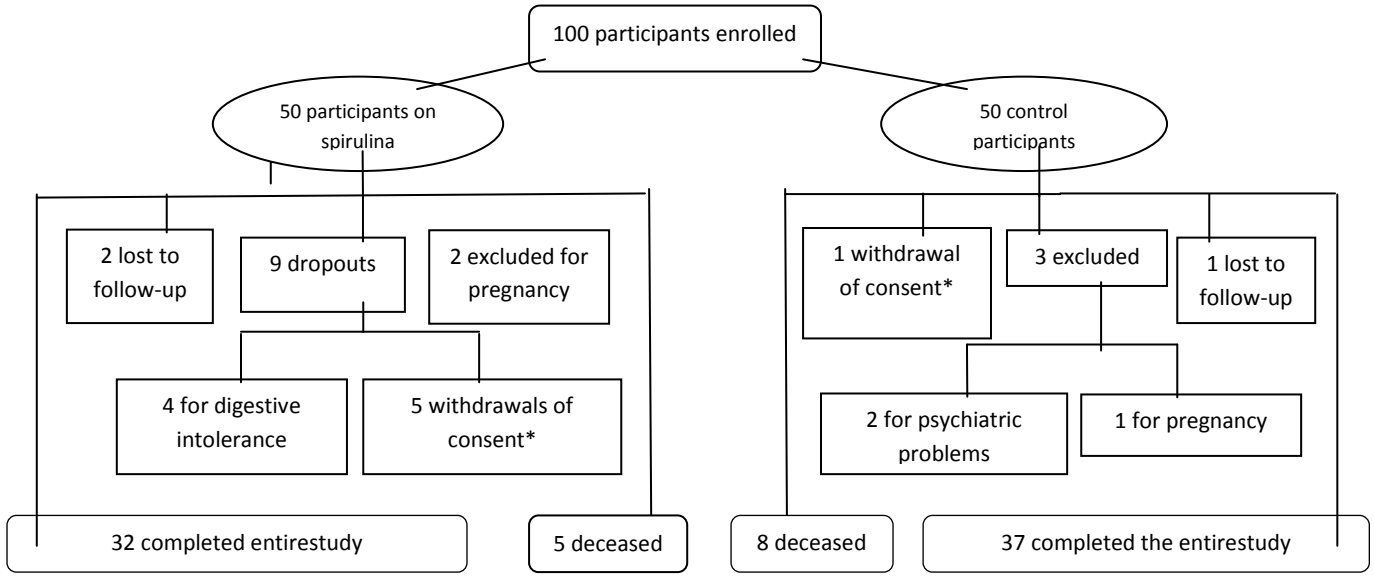
All participants who completed the study were compliant with ARV treatment. As for adherence to spirulina supplementation, 82% of participants in the intervention arm respected continuous daily doses in the first month of the study (M1), 77% in the third month (M3), 62% in the sixth month (M6), and 82% in the ninth month (M9). Non-compliance varied from 1 to 6 days per month and was primarily due to either digestive intolerance or difficulties consuming spirulina during meals.

Table 2: Evolution of anthropometric parameters throughout follow-up.

	Spirulina group	Control group	p
BMI (kg/m²)			
BMI M0	19.27 ± 3.70	19.27 ± 2.78	0.99
BMI M3	20.44 ± 3.62	20.12 ± 2.77	0.65
BMI M6	21.71 ± 3.05	20.90 ± 2.69	0.21
BMI M9	21.90 ± 3.56	21.08 ± 2.73	0.27
Gain in BMI (kg/m²)			
- Gain from M0 to M3	0.73 (-0.27 ; 1.81)	0.33 (-0.38 ; 1.31)	0.19
- Gain from M3 to M6	0.74 (0 ; 1.47)	0.08 (1 ; 1.78)	0.71
- Gain from M6 to M9	0 (-3.72 ; 0.55)	-0.16 (-0.54 ; 0.89)	0.94
MUAC (cm)			
MUAC M0	24.79 ± 2.73	24.49 ± 2.43	0.57
MUAC M3	26.05 ± 2.42	25.15 ± 1.93	0.07
MUAC M6	27.10 ± 2.35	26.18 ± 2.38	0.08
MUAC M9	27.44 ± 2.25	25.86 ± 2.43	0.007
Mean gain in MUAC (cm)			
- Gain from M0 to M3	1 (0 ; 2)	0 (-1 ; 1)	0.000
- Gain from M3 to M6	1 (0 ; 2)	1 (0 ; 2)	0.61
- Gain from M6 to M9	0 (0 ; 2)	0 (-1 ; 1)	0.60

Table 3: Mortality incidence density ratio of control vs supplemented participants.

Follow-up (months)	Control vs spirulina participants	*p*
M6	2.1 (0.5 ; 8.4)	*0.29*
M9	1.7 (0.5 ; 5.1)	*0.37*



* Withdrawal of consent: a patient enrolled in the study and later decides not to participate.

Figure 1: Participants' follow-up report.

Evolution of the patients' anthropometric parameters

Between M0 and M9, mean BMI increase was greater in those participants taking spirulina than for controls, though the difference was not significant (Table 2).

Over the course of follow-up, participants on spirulina had a greater increase in mean mid-upper arm circumference (MUAC) than controls. This increase was significant in the ninth month of the follow-up ($p = 0.007$). The mean gain in MUAC was also higher in the intervention group, with a significant difference in the first 3 months of follow-up ($p < 0.001$).

Evolution of biological parameters

Intervention group participants had a significantly greater increase in plasma albumin ($p = 0.04$) and hemoglobin levels ($p = 0.002$) as compared to those in the control group in the ninth month of follow-up (Figures 2a and 2b). Furthermore, the intervention group showed a significant decrease in serum gamma globulins in the sixth month of follow-up ($p = 0.04$) and in serum creatinin in the ninth month ($p = 0.01$) (Figure 2c and 2d). There was no significant difference between the two groups in mean ALT and amylase levels (Figures 2e and 2f).

DISCUSSION

HIV infection and malnutrition are cofactors in the weakening of the immune system, biological disorders, an increased susceptibility to infection, and weight loss among PLHIV. Our study on the benefits of spirulina supplementation for these individuals was not without limitations. Firstly, the study had a limited sample size. In addition, no biological tests were used to assess adherence to spirulina supplementation. Given that observance to the treatment regimen was established by counting the remaining packets of spirulina, it was not possible to confirm that the control group did not also take spirulina, a readily available product in Burkina Faso. Lastly, participants were not randomized to treatment arms. However, despite these limitations, several results seem important to report.

By the ninth month of follow-up of HIV-1 positive participants in WHO defined stages III or IV of the infection, results showed that spirulina supplementation significantly improved the participants' renal function as well as protein, anemic, and immune status as compared to those participants who did not take supplementations. However, supplementation had no significant effect on the participants' weight.

The significant decrease in creatinemia among supplemented participants suggests a nephroprotective action possibly due to the antioxidative properties of certain components of spirulina, such as phycocyanin (Romay et al., 2003). Our results therefore contradict those of Yamani et al. (2009) which noted a significant increasing of creatinemia in individuals taking spirulina. However, there continues to be debate surrounding the scientific arguments for the potential nephroprotective action of spirulina. For example, the fact that the algae is rich in amino and nucleic acids (4-6% of dry weight), substances inducing the production of uric acid which, in excess, can result in kidney stones and gout, contradicts arguments as to this protective potential (Falquet et Hurni, 2006; Charpy et al., 2008).

The mean value of albuminemia in the intervention group was significantly higher than that of the control group in the sixth and ninth months of follow-up and was associated with a significant improvement in the participants' anemia status, suggesting a favorable effect of spirulina on these parameters. These results show both a recovery of protein synthesis in supplemented participants as a result of the protein and amino acid content of spirulina and a recovery of red blood cell synthesis due to the algae's high iron content (Falquet et Hurni, 2006). A significant improvement in the protidemia of participants on spirulina was also reported among PLHIV in the Central African Republic on a daily 10 gram spirulina regimen

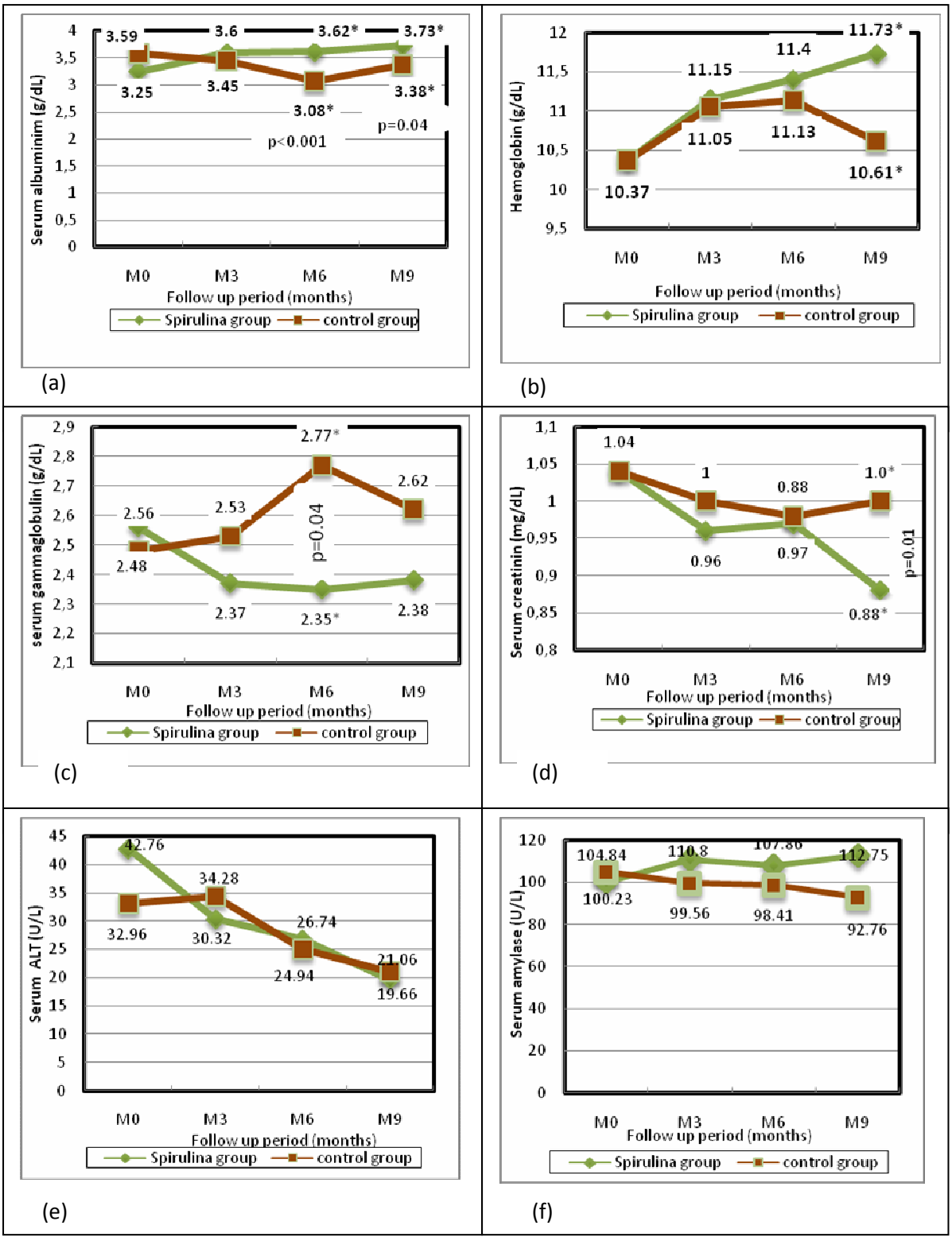


Figure 2 : Evolution of biological parameters (Albumin (a), Hemoglobin (b), Gammaglobulins (c) Creatinin (d), ALT (e), Amylasemia (f)), between the two groups of participants.

(Yamani et al., 2009). In addition, the progressive increase of hemoglobin levels, more prominent in the ninth month for the intervention group, may also be attributed to the high iron content of the algae (Selmi et al., 2011).

Supplementation with spirulina may have contributed to the decrease in gamma globulins seen in the intervention group at month six ($p = 0.04$). These gammaglobulins generally increase in cases of infectious diseases, inflammatory responses, and malnutrition (Li et al., 2007; Kalpana et al., 2011). A decrease in their synthesis could reflect an improvement of the immune system and nutritional status of individuals in the intervention group, thus making them less vulnerable to infection than the controls. Many authors have previously noted this effect of spirulina on the immune system which is linked to the specific sulfated polysaccharides of the algae (Calcium spirulan (Ca-Sp), sodium spirulan (Na-Sp)) and immulina which appears to possess immunostimulating properties (Liu et al., 2000; Remirez et al., 2002; Cherng et al., 2007). In addition, experiments on animals (notably chickens and rats) have shown spirulina to be capable of positively regulating the immune system by increasing the phagocytic function of macrophages, namely the activity of T cells and Natural Killer (NK) cells, by releasing pathogen inactivating interferon-gamma (IFN-γ) (Cherng et al., 2007; Selmi et al., 2011).

The absence of a significant effect of spirulina on the participants' weight can be attributed to the low dosage given (10 grams/day) in relation to the nutritional needs of PLHIV. These findings support those reported in the Central African Republic by Yamani E. et al. (2009) who found a 10 grams daily dose of spirulina to have no significant effect on the average weight of participants.

The acceptability of spirulina was considered to be low due to the high proportion of dropouts among the intervention group. This could be linked to the organoleptic properties of spirulina. Indeed, its salty taste, ''dried fish'' smell (Jordan, 2006), intensely green hue, and the degree to which it changes the color of food prevent it from being hidden and could render dishes unappetizing.

Conclusion

HIV infection is often accompanied by malnutrition and biological disorders affecting such vital organs such as the kidneys and the liver. Supplementation with spirulina, a protein and micronutrient rich edible algae, is a commonly used means of combating these disorders. However, the long term effects of spirulina on certain vital biological parameters have yet to be established. Although this study found spirulina to have no significant effects on anthropometric parameters, its effect on biochemical and hematological levels merit further study. Randomized clinical trials on large samples with longer follow-up would be needed to either confirm or disprove these trends and to better determine other potential nutritional benefits of spirulina.

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