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Research Article

Antianaemic effect of spirulina in rabbits (*Oryctolagus cuniculus*), a made and used food supplement in Côte d'Ivoire.

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Abstract: The powder commonly called SPIRULINA is made of *Spirulina platensis*, a microalgae cultivated for centuries and used in a preventive and curative purposes. Studies conducted on this blue-green algae cultivated in some countries of the world have shown many nutritional and pharmacological properties. This is what justifies its use in all the continents as nutritional supplement. With the scarcity of works done on SPIRULINA produced in Côte d'Ivoire, we set ourselves the objective to evaluate the anti-anaemic activity of this algae in the rabbits of *Oryctolagus cuniculus* species.In this studies we evaluated the anti-anaemic effect by administering SPIRULINA by oral route at different doses to different groups of phenylhydrazine hydrochloride anaemia rabbits(*Oryctolagus cuniculus)* and compared its effect with a reference antianaemic (Ranferon®-12). Hemograms by days: 2, 5, 12 and 19 showed a very highly significant increase in red blood cell count, hemoglobin and hematocrit at used doses. However, these increases were greater in treated groups at doses of 2000 mg / kg-bw and 4000 mg / kg-bw. Therefore, increases of 153.53% and 124% were observed respectively for red blood cell count and hematocrit at 2000 mg / kg-bw. For the dose of 4000 mg / kg-bw, we noticed an increase of hemoglobin at a rate of 77.74%. However, no significant difference was obtained between haematological parameters of treated groups of animals by both SPIRULINA and Ranferon®-12 at the end of treatments.To sum up, our study showed that SPIRULINA has an anti-anaemic effect in rabbits.
Keywords: Anti-anaemic, rabbit, *Oryctolagus cuniculus*, SPIRULINA, Food supplement, Côte d'Ivoire

INTRODUCTION

Anaemia is a public health issue that affects all categories of the world population according to World Health Organization [1-3]. According to this organization, two billion people worldwide are affected with a high rate in developing countries with prevalences of about 60% in pregnant women, 50% among children under 4 years and 45% among school-age children [4]. Several factors explain anaemia outbreak and the main reason is inherent to food. Nutritional anaemia is widespread throughout the world and particularly in sub-Saharan Africa where pregnant women, breast feeding babies and young children are most concerned [5,6].

Results of studies conducted in Côte d'Ivoire indicated that prevalence of anaemia were 47% in children aged from 5 to 18 years, 64% in non-pregnant and childbearing age women, 86% in pregnant women, 77% among pregnant teenagers, 42.7% in non pregnant teenagers, 72% of HIV-positive pregnant women and 67.5% among non-pregnant women with HIV [7-14]. Furthermore, higher prevalence of anaemia observed in Côte d'Ivoire was due to nutritional deficiencies (iron deficiency) and infectious diseases (HIV infection) [15-18].

Regarding the above observations, it therefore seems appropriate for us to investigate on how to do away with nutritional deficiencies in order to lessen anaemia prevalence in Côte d'Ivoire.

According to several studies, *Spirulina platensis* (Oscillatoriaceae), a microscopic "blue-green algae" a traditional food for some Mexican and African people, contains up to 70% proteins; rich in minerals, trace elements and vitamins [19].

In addition, studies showed that, this cyanobacterium has many biological activities such as inhibition of viral replication, prevention of liver diseases, hypoglycaemic effects, antihypertensive... [20]. *Spirulina platensis* exerts immunostimulatory effects in tilapia species *Oreochromis niloticus* [21].

According to the studies of Yapi *et al*. [22], supplementation of SPIRULINA improves nutritional profile proteins in burnt people.

Although several studies have been conducted on the nutritional and pharmacological effects of several species of SPIRULINA, the anti-anaemic properties of *Spirulina platensis* were not assessed. Therefore, our investigation is to evaluate the anti-anaemic effect of *Spirulina platensis* in rabbits and better inform people on its use.

Specifically, this is

- To determine the influence of different doses of *Spirulina platensis* on erythrocyte parameters,
- To evaluate and monitor the rate of hemoglobin and anaemia prevalence in anaemic rabbits treated with *Spirulina platensis* powder.
- To compare the evolution of hemoglobin rate and anaemia prevalence between anaemic rabbits and treated with different doses of *Spirulina platensis* and those treated with a reference anti-anaemic molecule.

MATERIALS AND METHODS

Plant material

The plant material was made up of dry powder of *Spirulina platensis* provided by SAP (Société Agro-Piscicole) of Lamé in the Department of Adzopé (Côte d'Ivoire) where the production unit is settled.

Animal material

Young-adult rabbits (aged from 2 to 3 months) of both sexes, belonging to *Oryctolagus cuniculus* species [23] from leporidae family [24], with a mean weight of 1.58 ± 0.16 kg, were fed with pellets provided by Ivograin® with free access to tap water.

Animal did not receive any medication and were acclimatized for a week in the Faculty of Pharmaceutical and Biological Sciences pet room of Felix Houphouet Boigny University (Côte d'Ivoire).

Induction of anaemia

Prior to animal treatments, blood samples were collected by the marginal vein from all rabbits in order to evaluate the erythrocyte parameters. Anaemia was induced in all rabbits by intraperitoneal administration of 40 mg / kg-bw of phenylhydrazine hydrochloride as described by Naughton *et al*. [25] for two days (Day 0 and Day 1).

Animal Treatments

In this study, the rabbits were divided into five groups, each consisting of three animals. A negative control group, a positive control group and three groups treated by different doses of *Spirulina platensis* powder prepared with distilled water as solvent. The negative control group received normal saline, the positive control group received the reference anti-anaemic (Ranferon®-12) and the three other groups respectively received *Spirulina platensis* powder at doses of 1000 mg / kg-bw, 2000 mg / kg-bw and 4000 mg / kg-bw of *Spirulina platensis* powder, for 14 days. Products administration was done by oral route.

Experimental procedures and protocols used in this study were approved by ethical committee of University Félix Houphouët Boigny. These guidelines were in accordance with the internationally accepted principles for laboratory use and care.

Blood samples collection and haematological analysis

Blood samples were performed every morning on fasting rabbits with 5 cc syringes, at the marginal ear vein in EDTA tubes containing an anticoagulant by days 0 (D0), 2 (D2), 5 (D5), 12 (D12) and 19 (D19).

Haematological analysis was performed using an automatic counter analyzer (Sysmex KX21-N) at the immuno-hematology laboratory of Cocody University Hospital.

The determined parameters were: red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.

Statistical analyses

Statistical analyses of data and figures were made using the software Graph Pad Prism 5.01 (San Diego California, USA).

The results were expressed as mean ± standard deviation and as proportions. The haematological parameters were analyzed by one way analysis of variance (ANOVA) and Tukey post hoc test. Comparison of parameters rates were performed using loglikelihood ratio test (G test) with the STATISTICA software "R" version Windows 2.0.1 [26]. Differences between groups were considered significant at $P < 0.05$.

RESULTS

Effect of the anaemic reference substance

The mean values of erythrocyte parameters by day 0 before administration of the anaemic reference substance, phenylhydrazine hydrochloride are shown in Figure 1.

In all groups of rabbits, our results indicated normal mean values of erythrocyte parameters which were: 4.56 ± 0.62 x10^6 / mm^3, 10.12 ± 0.76 g / dl, 35.01 ± 2.91%, 77.94 ± 5.42 fl, 21.89 ± 5.57 pg, 26.93 ± 3.06 g / dl, respectively for red blood cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

No statistically significant difference (P > 0.05) between similar erythrocyte parameters in all groups of rabbits was observed.

Variations in erythrocyte parameters obtained after administration of phenylhydrazine hydrochloride are indicated in Table 1.

Two days after administration of phenylhydrazine hydrochloride (D2), a significant decrease of some erythrocyte parameters in all groups of rabbits was observed. Thus, the mean values obtained were: 2.94 ± 0.68 x10^6 / mm^3, 6.35 g / dl ± 1.28 g / dl, 21.6 ± 3.62 pg, 72.56 ± 5.35 fl, respectively, for red blood cells count, hemoglobin, hematocrit and MCV. The observed decreases were significant for hemoglobin (P = 0.003) and red blood cell count (P = 0.03).

As far as the hematocrit and MCV were concerned, decreases were not significant (P = 0.05 and P = 0.65 respectively).

However for MCH and MCHC, after administration of phenylhydrazine hydrochloride, we observed quantitative increases which were not statistically significant (P = 0.8 and P = 0.13 respectively).

Variations of erythrocyte parameters during treatments

The evolution of erythrocyte parameters during treatments is summarized in Figure 1. By day 5 (D5), it indicated a quantitative increase of erythrocyte parameters for all groups of treated rabbits except for MCHC of the groups that received normal saline (Nacl 0.9%), Ranferon®-12, doses of 1000 mg / kg-bw and 2000 mg / kg-bw of SPIRULINA.

From day 5 (D5), we observed fluctuations of these parameters; the values obtained by day 19 (D19) still remained higher than that of day 2 (D2), except for groups treated with normal saline, Ranferon®-12, SPIRULINA at doses of 1000 mg / kg-bw and 2000 mg / kg-bw. By Day 19 (D19), we recorded a very highly significant (P < 0.0001) rate of red blood cell count, hemoglobin and hematocrit for treated groups at doses of 1000 mg / kg-bw, 2000 mg / kg-bw and 4000 mg / kg-bw. For the latter, the increase of red blood cell count was very significant (P < 0.001).

For group treated with Ranferon®-12, we observed a non-significant decrease of red blood cells count, a very highly significant (P < 0.0001) and highly significant (P < 0.001) respectively for hemoglobin and hematocrit.

However, we did not record any significant variation (P > 0.05) in the above-mentioned haematological parameters for the group treated with normal saline. The same observation was made with MCV, MCH and MCHC.

At the end of treatments, the highest mean value of red blood cell count was 5.02 ± 0.77 x10^6 / mm^3 at the dose of 2000 mg / kg-bw for SPIRULINA. As for hemoglobin and hematocrit, the higher mean rates were obtained with the doses of 4000 mg / kg-bw and 2000 mg / kg-bw for SPIRULINA. These rates were respectively 11.06 ± 0.11g / dl and 38.26 ± 4.93%.

The lower mean values of erythrocyte parameters after treatments were recorded with the dose of 1000 mg / kg-bw for SPIRULINA. They values were 4.28 ± 0.14 x10^6 / mm^3, 10.33 ± 0.15 g / dl and 35 ± 1.21% respectively for red blood cell count, hemoglobin and hematocrit.

Evolution of anaemia prevalence during treatments

The prevalence of anaemia after administration of phenylhydrazine hydrochloride during the treatment is confined in Table 2.

By taking into account the mean values of hemoglobin in rabbit which range from 9.4 to 17.4 g / dl, we recorded by day 2 (D2) and day 5 (D5), 100% of anaemia prevalence for all groups of rabbits. No significant difference was shown (P = 1).

This prevalence gradually fell as treatments were administered to different groups, except for the prevalence of the group that received normal saline which remained at 100% (P = 1).

By day 19 (D19), we did not notice any anaemia prevalence in groups treated with Ranferon®-12, SPIRULINA at doses of 1000 mg / kg-bw and 2000 mg / kg-bw and 4000 mg / kg mc (P < 2.10-16). However, the prevalence of anaemia for the negative control group remained stable at 100%.

Variations of erythrocyte parameters deviations during treatments

Variations of erythrocyte parameters values deviations caused by the administration of phenylhydrazine hydrochloride are illustrated in Figure 2. Concerning red blood cells, the results showed by day 19 (D19) the following rates of increase : 4.33% 20.78%, 62.73%, 153% and 56.38%, respectively for groups treated with normal saline, Ranferon®-12, SPIRULINA at doses of 1000mg / kg-bw of 2000 mg / kg-bw and 4000 mg / kg-bw.

Hemoglobin rate increased to 10.5%, 54.34%, 75.08%, 75.16% and 77.74%, respectively for treated groups with normal saline, Ranferon®-12, doses of 1000mg / kg-bw, 2000 mg / kg-bw and 4000 mg / kg-bw.

As for the hematocrit, we noticed an increase of 24%, 54.81%, 69.37%, and 68.32% respectively for treated groups with normal saline, Ranferon®-12, SPIRULINA at doses of 1000mg / kg-bw of 2000 mg / kg-bw and 4000 mg / kg-bw.

Regarding MCV and MCH of all groups, the increases were low. High rates were respectively observed for both parameters at 31.65% and 10.72%. Whereas, MCHC presented low decreases in all rabbits treated groups. The highest decrease rate was 20.73%, for normal saline treated group.

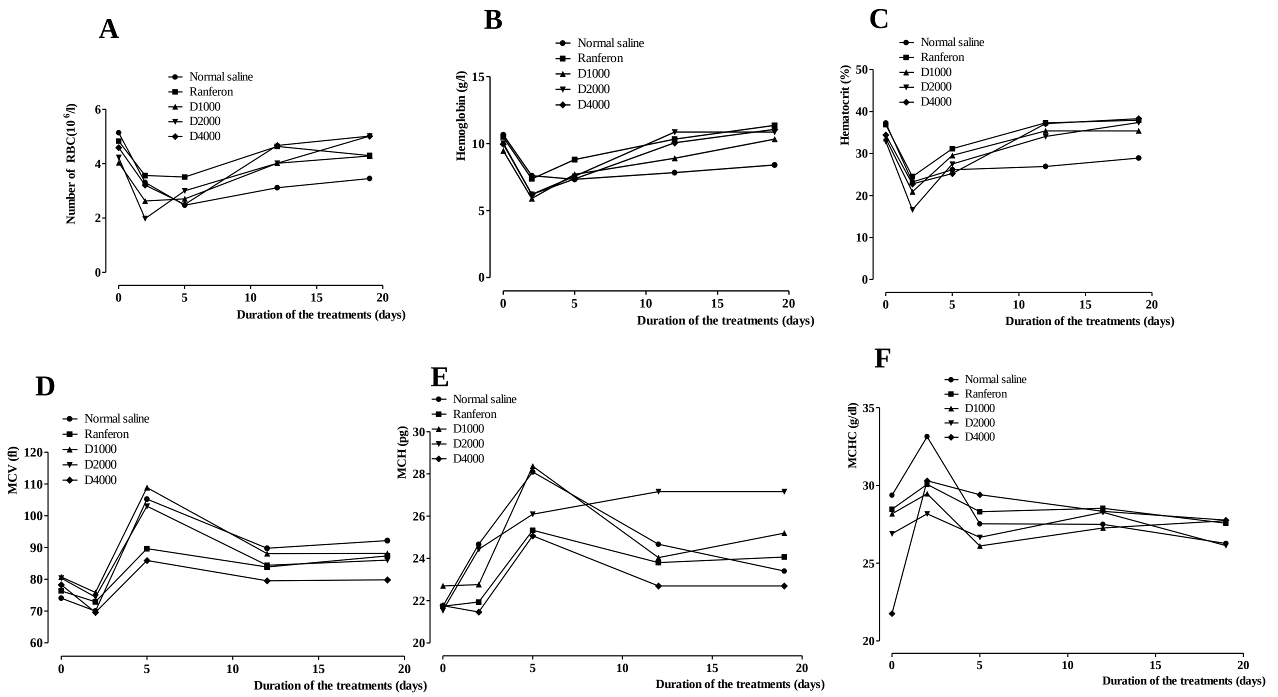

Figure 1: Evolution of erythrocyte parameters during the treatments
A:Red blood cell count, B: Hemoglobin, C: Hematocrit, D: Mean corpuscular volume (MCV), E: Mean corpuscular hemoglobin(MCH), F: Mean corpuscular hemoglobin concentration (MCHC), D1000: Dose of 1000 mg/kg of body weight, D2000: Dose of 2000 mg/kg of body weight, D4000: Dose of 4000 mg/kg of body weight

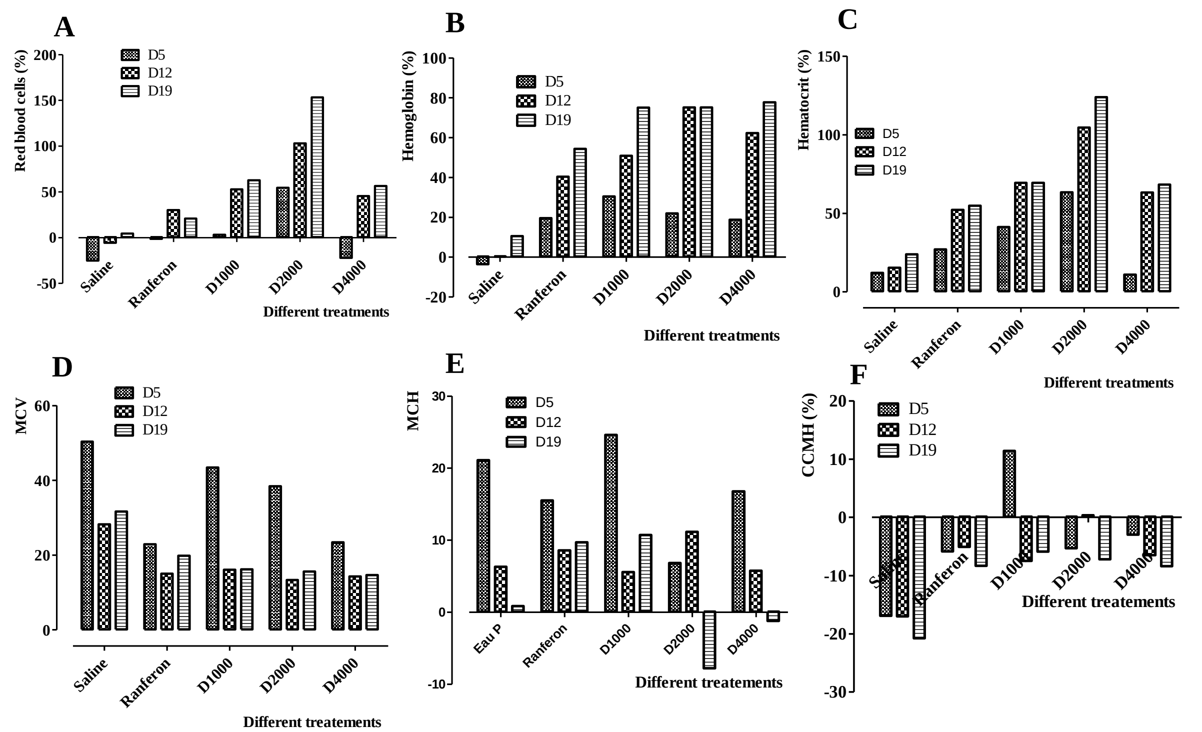

Figure 2: Evolution of erythrocyte parameters deviations during treatments

A: Red blood cells, B: Hemoglobin, C: Hematocrit, D: Mean corpuscular volume(MCV), E: Mean corpuscular hemoglobin(MCH), F: Mean corpuscular hemoglobin concentration (MCHC), D5: Day 5, D12: Day 12, D19: Day 19

Table-1: Variations of erythrocyte parameters after administration of the reference anaemic substance

N.	RBC	P^1	HB	P^1	HTE	P^1	MCV	P^1	MCH	P^1	MCHC	P^1
Saline	-35.6	0.005	-28.7	0.03	-37.38	0.003	-5.44	0.70	6.76	0.63	12.84	0.35
D1000	-34.73	0.007	-37.63	0.003	-37.79	0.003	-6.06	0.66	0.26	0.99	4.61	0.74
D2000	-53.19	9.10^{-6}	-53.14	9.10^{-6}	-50.88	2.10^{-5}	-7.37	0.60	13.46	0.32	4.68	0.74
D4000	-30.06	0.02	-37.75	0.003	-33.98	0.008	-11.03	0.42	-1.37	0.92	39.35	0.002
P^2	0.03	P^2	0.003	P^2	0.05	P^2	0.68	P^2	0.68	P^2	0.13	

RBC: Red blood cells, HB: Hemoglobin, HTE: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, P^1: Intra-treatment comparison, P^2: Inter- treatment comparison , N. Saline: Normal saline.

Table-2: Distribution of anaemia prevalence after treatments

	D2	P	D5	P	D12	P	D19	P
N. saline	100	**1**	100	**1**	100	**1**	100	**1**
Ranferon	100	**1**	100	**1**	0	**< 2.10^{-16}**	0	**< 2.10^{-16}**
D1000	100	**1**	100	**1**	33.33	**0,0008**	0	**< 2.10^{-16}**
D2000	100	**1**	100	**1**	0	**< 2.10^{-16}**	0	**< 2.10^{-16}**
D4000	100	**1**	100	**1**	0	**< 2.10^{-16}**	0	**< 2.10^{-16}**

DISCUSSION

The mean values of red blood cells, hemoglobin, hematocrit, MCV, MCH, and MCHC were similar to those obtained by Bléyéré *et al.* [27] and Ouedraogo *et al.* [28]. However, red blood cell count obtained by Aboh *et al.* [29] was 7.30 x10^6/mm^3 and statistically different from our value (4.56 ± 0.62 x10^6/mm^3). These results showed that blood cells may vary within a population of rabbits, or, from one area to another while respecting the reference values. According to Follet [30], red blood cell count ranges from 3.8 x10^6 / mm^3 to 7.9 x10^6 / mm^3.

The administration of phenylhydrazine hydrochloride caused by day 2 (D2), a statistically significant and very highly significant decrease in red blood cell count, respectively in treated groups with normal saline, SPIRULINA at doses of 2000 mg / kg-bw and 4000 mg / kg-bw. These results were in agreement with other studies [31-33], which showed that phenylhydrazine hydrochloride, is used in haemolytic anaemia induction and the study of its mechanism in some animal species such as rabbits, rats, mice, calf, duck, fowl...

As for MCV and MCH, they were no statistically significant variation by day 2 (D2). This could be explained by the fact that MCV and MCH respectively constitute the ratio between the hematocrit value and erythrocyte and between the rate of total hemoglobin and red blood cell count in a liter, these parameters varied in the same proportions. This is the case of normocytic normochromic anaemia [34].

Comparing the hemoglobin rate by day 2 (D2) after administration of phenylhydrazine hydrochloride to reference values indicated by Follet [30] and Quinton [35], we obtained 100% of anaemia prevalence.

Spirulina platensis powder administered at a dose of 1000 mg / kg-bw to the group of rabbits by day 2 (D2) resulted in a significant decline and doses of 2000 mg / kg-bw and 4000 mg / kg-bw, provoked a highly considerable decrease of anaemia prevalence by days 12 (D12) and (D19) with no anaemia, except for the negative control group with a stabilized prevalence at 100%.

We still got a non-significant increase of red blood cell count and the hemoglobin rate in rabbits treated with normal saline. This reversibility of anaemia is linked to food (pellets) made of protein, minerals, fats and vitamins consumed by rabbits during experimentation.

By day 19 (D19), we obtained a total recovery of anaemia for groups treated with Ranferon®-12, SPIRULINA at doses of 1000 mg / kg-bw, 2000 mg / kg-bw and 4000 mg / kg-bw. The highest hemoglobin rate (11.06 ± 0.11 g / dl) was obtained with the dose of 4000 mg / kg-bw, which represents a recovery rate of 77.74% with reference to the day 2 (D2). However, no significant difference was reported between the values of these hematological parameters of groups treated with different doses of SPIRULINA and Ranferon®-12 at the end of treatments. We conclude that *Spirulina platensis* treats efficiently anaemia as well as the reference product, Ranferon®-12.

SPIRULINA at the dose of 2000 mg / kg-bw which improves both red blood cell count and hemoglobin level is considered as the optimal dose. This property of SPIRULINA to reduce anaemia could be justified by its wide range of vitamins, minerals and trace elements essential for hematopoiesis [36].

The variation of deviations during treatments showed an increase of red blood cell count by day 19 (D19) of 20.78%, 62.73%, 153% and 56.38% compared to the day 2 (D2) respectively for the treated groups with Ranferon®-12, SPIRULINA at doses of 1000 mg / kg-bw 2000 mg / kg-bw and 4000 mg / kg-bw. As for the group treated with normal saline we noted a non-significant increase of 4.53%. This variation of deviations also revealed a highly significant evolution of hemoglobin which was 54.34%, 75.08%, 75.16% and 77.74%, respectively for the groups treated with Ranferon®-12, SPIRULINA at doses of 1000 mg / kg-bw 2000 mg / kg-bw and 4000 mg / kg-bw and not significant for the group treated with normal saline. Likewise, this increase was highly significant for hematocrit differences for which, changes of deviations by day 19 (D19) showed an evolution of 54.81%, 69.37%, 124% and 68.32%, respectively for groups treated with Ranferon®-12, SPIRULINA at doses of 1000 mg / kg-bw, 2000 mg / kg-bw and 4000 mg / kg-bw, with a low rate (24%) for group treated with normal saline.

As for MCV, the non significant decrease obtained by day 2 (D2) caused deviations with a non significant evolutions until day 19 (D19).

These results confirm on the one hand, a clear correlation between erythrocyts, hemoglobin, hematocrit and MCV and on the other hand, the efficiency of *Spirulina platensis* in the treatment of anaemia. For MCH and MCHC, we obtained by day 2 (D2) an increase of these parameters, then a fluctuation of their deviations up to day 19 (D19). However, the values obtained were not significant compared to day 0 (D0). These results showed that haemolytic anaemia induced by the administration of phenylhydrazine hydrochloride did not have any quantitative impact on MCH and MCHC. Therefore, it is normocytic-normochromic anaemia.

The resorption of anaemia with the reference antianaemic (Ranferon®-12) was also due to its composition in folic acid, iron, vitamin B12, ascorbic acid and zinc. Likewise, according to Falquet and Hurni [36], Helen [37], Sebastian [38] and Jean-Louis [39], in addition to these components, SPIRULINA contains other nutrients. All these nutrients were the cause of its relative efficiency in anaemia treatment. Indeed, iron is involved in heme molecule (porphyrin + iron) formation, one of the hemoglobin components. Vitamin B12, folate, vitamin B6, vitamin B2 and vitamin C are also necessary for erythropoiesis [40].

CONCLUSION

This study that aimed to evaluate the anti-anaemic effect of *Spirulina platensis* in order to optimize its use by mankind showed that the pharmacological effects of this microscopic algae actually gave it proven antianaemic properties. Thus, this nutritional supplement may be recommended in anemia treatment. However, further work could help discovering mechanisms involved in its action for the treatment of anemia. We also plan to carry out clinical research to define and validate it therapeutic properties in medicine and dietetic, this could be an alternative to public health issue caused by nutritional deficiency anaemia in the world and particularly in developing countries.

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