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Evaluation of Glycaemia, Creatininaemia and Uraemia in Rabbits (Oryctolagus Cuniculus) Fed with Spirulina

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Abstract

Spirulina is a micro-alga which has been consumed worldwide for centuries as a nutritional supplement due to its richness in nutrients. We set ourselves the objective of evaluating the impact of consuming this micro-alga on certain biochemical parameters.

In this study, rabbits of *Oryctolagus cuniculus* species were used. Different doses of spirulina were administered intraperitoneally to the rabbits while the control lot received physiological water. Analyses were performed on days D_0, D_7, D_{14} and D_{21}.

On day D_{21}, the blood glucose levels recorded ranged from 0.99 ± 0.07 to 1.37 ± 0.08 g/L; that of the control lot was 0.94 ± 0.28 g/L. Uraemias ranged from 0.22 ± 0.03 to 0.28 ± 0.28 g/L; that of the control lot was 0.25 ± 0.06 g/L. Creatininaemias ranged from 10.00 ± 0.00 g/L to 12.66 ± 1.15 g/L; that of the control lot was 11.66 ± 0.57 g/L.

Since no significant variation of the analyzed parameters was recorded, spirulina consumption does not cause deleterious effects on kidney and glucose metabolism.

Keywords: Glycaemia, creatininaemia, uraemia, rabbit, *Oryctolagus cuniculus*, Spirulina.

1.0 INTRODUCTION

The use of plants in therapy (phytotherapy) is very old and is currently experiencing renewed interest among the public. It is possible to use the whole plants or the extracts they provide. (Mark, 2001).

Spirulina is a powder derived from a microscopic alga, *Spirulina platensis* from the cyanobacteria family. This seaweed originating from Kanem (Chad) (Clément, 1978) is cultivated and consumed throughout the world as a nutritional supplement since hundreds of years for its therapeutic and nutritional virtues. It is a traditional food of certain Mexican and African populations and can contain up to 70% proteins; it is rich in mineral salts, antioxidants, trace elements and numerous vitamins (B1, B2, B12, E, etc.) (Sall *et al*., 1999).

The nutritional properties of spirulina make it a food source that deserves special attention in our developing countries where there is an acute problem of food availability (Sall *et al*., 1999).

The research of some authors (Clément, 1978; Sall *et al.;* Kambou *et al.;* 2018), showed that spirulina is a source of proteins, carbohydrates, macro-elements, trace elements, vitamins and Fatty acids. Moreover,

according to Kambou *et al.* (2015a), spirulina restores hemoglobin and the number of red blood cells in experimentally anemic rabbits. This author also demonstrated that spirulina has immunostimulatory properties in rabbits (Kambou *et al.,* 2015b).

With the promotion of natural or "Bio" products, consumption of food supplements is increasingly observed. This practice, which certainly can show some interests, does not always remain without danger. Indeed, unlike drugs, the marketing of food supplements does not require a marketing authorization.

Since recent years, this seaweed cultivated and consumed in Côte d'Ivoire as a nutritional supplement has not been the subject of several studies. The WHO recommends assessment of safety and efficacy of herbal medicines in order to standardize their use and to integrate them into conventional healthcare systems (WHO, 2000).

To ensure the safety of consuming population, we proposed to evaluate the biotolerance of spirulina produced in Côte d'Ivoire. To do so, in this study, the objective was to study the impact of spirulina on glycaemia and markers of kidney function.

2.0 MATERIALS

Plant material

The plant material consisted of the dry biomass of *Spirulina platensis* whose powder (spirulina) was supplied by the SAP (Société Agro-Piscicole) of Lamé in the Department of Adzopé, in the South-East of Côte d'Ivoire where the production unit is located.

Animal material

Young adult rabbits (2 to 3 months old) of both sexes, belonging to the *Oryctolagus cuniculus* species (Burstein, 1970) of Leporidae family (Trivedi, 1978) were used in this study. They were not under any drug treatment and acclimatized for one week in the animal house of Training and Research Unit of Pharmaceutical and Biological Sciences of the Félix Houphouët-Boigny University. The rabbits had a mean body mass of 1.58 ± 0.16 kg and were fed with pellets supplied by Ivograin® and watered with tap water.

METHODS

Spirulina administration

Four (4) groups of 3 rabbits, including a control group were used. After acclimatization, the administration of the various products to animals was made intraperitoneally every two days for three weeks using a 5cc syringe and the injected volume was 1 mL. The control group received physiological water while the other three (3) groups were subjected to 100 mg/kg of bw, 250 mg/kg of bw and 700 mg/kg of bw of *Spirulina platensis* powder prepared with physiological water (NaCl 0.9%) as solvent.

Blood samples and analysis

Before the treatment (D_0) and on days D_7, D_{14} and D_{21}, blood was taken from fasting rabbits on the marginal vein of the ear. Samples were collected in dry tubes and centrifuged at 3000 rpm for 5 minutes. The sera obtained were aliquoted and stored at -20°C in the freezer for biochemical investigations. Biochemical markers such as serum glucose, uraemia and creatininaemia were determinated.

Serum glucose was performed by colorimetric analysis while, hexokinase was determined by enzymatic, end-point method (Bergmeyer *et al*., 1970). The determination of uraemia was carried out by a urease enzymatic and kinetic method (Tietz, 1987). The determination of creatininaemia was by the colorimetric and kinetic method methods (Tietz, 1999).

Statistical analysis of data

The computer program Graph Pad Prism 5.01 (San Diego California, USA) was used to carry out the statistical analyses of the data. The results were expressed as means ± standard deviation. Variations in the parameters were observed by performing tests of comparison of means by analysis of variances (ANOVA ONE WAY), using a Tukey post hoc test. A probability threshold of $P < 0.05$ was chosen for the significance of all analyses.

3.0 RESULTS

Glycaemia

Mean blood glucose values ranged from 0.96 ± 0.09 to 1.37 ± 0.08 g/L before the treatments. No statistically significant difference (P > 0.05) between the blood glucose values in all groups of rabbits was observed on day D_0, before the administration of the different doses of spirulina (Table 1). As with the physiological water used as a control, no dose of spirulina resulted in a statistically significant change in blood glucose (P > 0.05) during the treatments. The mean blood glucose values obtained after the treatments with the different doses of spirulina were in the range of 0.99 ± 0.07 to 1.37 ± 0.08 g/L (Table 1).

Table 1: Changes of glycaemia (g/L) during treatments

		Days				
Groups (n=3)	**Treatments**	**D_0**	**D_7**	**D_{14}**	**D_{21}**	**P^1**
Group 1	**NaCl (0.9%)**	0.96 ± 0.09[a]	1.11 ± 0.02[a]	1.18 ± 0.21[a]	0.94 ± 0.28[a]	> 0.05
Group 2	**Spirulina (100 mg/kg)**	0.97 ± 0.07[a]	1.02 ± 0.19[a]	0.98 ± 0.05[a]	0.99 ± 0.07[a]	> 0.05
Group 3	**Spirulina (250 mg/kg)**	1.03 ± 0.05[a]	1.10 ± 0.09[a]	1.17 ± 0.01[a]	1.08 ± 0.05[a]	> 0.05
Group 4	**Spirulina (700 mg/kg)**	0.99 ± 0.04[a]	1.06 ± 0.04[a]	1.24 ± 0.03[a]	1.37 ± 0.08[a]	> 0.05
	P^2	> 0.05	> 0.05	> 0.05	> 0.05	

Means followed by the same letters in the same column are not statistically different at the 5% level. P^1: Within treatment comparison; P^2: Between treatment comparison

Uraemia

Mean urea values ranged from 0.19 ± 0.01 to 0.25 ± 0.02 g/L before treatments. No statistically significant difference (P > 0.05) between the urea values in all groups of rabbits was recorded at day D_0, before the administration of different doses of spirulina (Table 2).

All doses of product used including physiological water did not result in a statistically significant variation (P > 0.05˜) in urea values during the treatments. The mean values of urea obtained after the treatments with the different doses of spirulina were between 0.22 ± 0.03 and 0.28 ± 0.07 g/L (Table 2).

Table 2: Changes of uraemia (g/L) during treatments

		Days				
Groups (n=3)	**Treatments**	**D_0**	**D_7**	**D_{14}**	**D_{21}**	**P^1**
Group 1	**Nacl (0.9%)**	0.21± 0.02[a]	0.24 ± 0.09[a]	0.20 ± 0.02[a]	0.25 ± 0.06[a]	> 0.05
Group 2	**Spirulina (100 mg/kg)**	0.19 ± 0.01[a]	0.19 ± 001[a]	0.24 ± 0.06[a]	0.22 ± 0.03[a]	> 0.05
Group3	**Spirulina (250 mg/kg)**	0.23 ± 0.07[a]	0.20 ± 0.08[a]	0.18 ± 0.01[a]	0.26 ± 0.05[a]	> 0.05
Group 4	**Spirulina (700 mg/kg)**	0.25 ± 0.02[a]	0.29 ± 0.07[a]	0.24 ± 0.03[a]	0.28 ± 0.07[a]	> 0.05
	P^2	> 0.05	> 0.05	> 0.05	> 0.05	

Means followed by the same letters in the same column are not statistically different at the 5% level. P^1: Within treatment comparison; P^2: Between treatment comparison.

Creatininaemia

Mean creatinine values ranged from 9.33 ± 1.15 to 13.33 ± 0.57 mg/L before treatments. No statistically significant difference (P > 0.05) between the creatinine values in all groups of rabbits was observed on day

D_0, before the administration of the different doses of spirulina. (Table 3). Similarly to physiological water, no dose of spirulina resulted in a statistically significant ($P > 0.05$) change in creatinine during the treatments. The mean creatinine values obtained after the treatments with the different spirulina doses ranged from 10.00 ± 0.00 to 12.66 ± 1.15 mg/L (Table 3).

Table 3: Changes of creatininaemia (mg/L) during treatments

		Days				
Groups (n=3)	**Treatments**	**D_0**	**D_7**	**D_{14}**	**D_{21}**	**P^1**
Group 1	**NaCl (0.9%)**	11.66 ± 057^a	13.33 ± 0.33^a	9.66 ± 1.15^a	11.66 ± 0.57^a	> 0.05
Group 2	**Spirulina (100 mg/kg)**	9.33 ± 1.15^a	10.00 ± 1.73^a	12.33 ± 0.57^a	10.00 ± 1.00^a	> 0.05
Group3	**Spirulina (250 mg/kg)**	10.33 ± 3.51^a	11.33 ± 3.21^a	10.66 ± 1.52^a	11.00 ± 1.00^a	> 0.05
Group 4	**Spirulina (700 mg/kg)**	13.33 ± 0.57^a	13.00 ± 3.00^a	10.00 ± 1.00^a	12.66 ± 1.15^a	> 0.05
	P^2	> 0,05	> 0,05	< 0,05	> 0,05	

Means followed by the same letters in the same column are not statistically different at the 5% level. P^1: Within treatment comparison; P^2: Between treatment comparison

4.0 DISCUSSION

When we embarked on this study, the problematic was to know if the daily intake of spirulina, given its nutritional richness, could not lead to hyperglycaemia which could lead to diabetes mellitus and/or kidney disease. The results obtained indicated no significant difference in the different values of glycaemia, uraemia and creatininaemia in all groups of rabbits on day D_0, before the experiments. These results showed that there is a homogeneity in the biochemical parameters of rabbits (Luciane *et al.,* 2008). In addition, the mean values of some metabolites obtained on day D_0, before the experimentations, are similar to those determined by Boucher and Mouaille (1996), Sakandé *et al.* (2003) and Coulibaly (2007).Indeed, Sakandé *et al* (2003) determined mean values for glucaemia, uraemia and creatininaemia of 0.70 ± 0.21 g/L, 0.29 ± 0.03 g/L and 9.62 ± 2.82 mg/L, respectively. Coulibaly (2007) obtained mean values of 0.79 ± 0.18 g/L, 0.42 ± 0.10 g/L and 6.88 ± 1.66 mg/L for these same parameters, respectively.

Biological intolerance after parenteral administration or oral administration of a drug substance could be manifested by metabolic disorders of the parameters investigated, indicating dysfunction of the related organs (Sundberg *et al.*, 1994). Our results did not indicate statistically significant variations in the values of the biochemical parameters investigated during the treatments of rabbits with spirulina. This could therefore be an indication of the proper functioning of kidney and pancreas.

The non-significant variation of glycaemia indicates that the carbohydrates contained in spirulina are normally metabolised. Indeed, these results confirm the low content of assimilable carbohydrates in this alga (Falquet and Hurni, 2006; Quillet, 1975; Shekharam et al., 1987).

Therefore, prolonged intake of this nutritional supplement could not induce hyperglycaemia that could lead to diabetes. The carbohydrates contained in spirulina are essentially polysaccharides easily assimilated by the organism with a minimum of intervention of insulin without tiring pancreas and without precipitating hypoglycaemia (Vidalo, 2012).

Renal function is assessed by measuring serum creatinine and serum urea concentrations (Davis and Berdt, 1994; Finco, 1997; Correges *et al*., 1998). Uremia and creatinemia did not vary significantly during our experimentations. The results obtained with these biomarkers of kidney indicate that kidney does not experience deterioration caused by the daily administration of spirulina, which reveals that the doses of spirulina used should not have a pernicious action on the metabolism of urea and creatinine. Indeed, the serum creatininaemia is usually measured to determine acute or chronic renal failure (Luber, 1988). Furthermore, urea, which is a molecule formed in liver cells during ureogenesis, is capable of undergoing partial reabsorption. This phenomenon, which takes place in renal tubules into the general bloodstream, occurs after the excretion of urea from glomeruli into the urine according to Routh *et al* (1978) and Vander

et al. (1989). In addition, the kidney plays an important role in physiological glucose homeostasis by regulating blood glucose levels through the phenomenon of gluconeogenesis**,** allowing for the production of glucose to be released into blood and reabsorption of glucose from the glomerular filtrate (Ibrahim *et al.,* 2015).

5.0 CONCLUSION

The objective of this study was to investigate the impact of spirulina on blood glucose and renal function markers. At the end of the study, it was found that in rabbits treated with spirulina for three (3) weeks, no significant variation outside the reference values was recorded in blood glucose, uraemia and creatininaemia. These results indicate that spirulina consumption does not have deleterious effects on glucose, urea and creatinine metabolism. They also showed that daily intake of spirulina does not affect the functioning of certain organs such as pancreas and kidney.

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