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Assessment of potential toxicological aspects of dietary exposure to silicon-rich spirulina in rats

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A B S T R A C T

Silicon has beneficial effects especially on bones and skin and is important in cardiovascular pathophysiology. Furthermore, in spontaneously hypertensive rats, it reduces hypertension and increases antihypertensive and antiatherogenic gene expressions in the aorta. Thus, incorporating silicon into spirulina could be a way to produce a bioavailable food supplement.

The potential toxic effects of silicon-rich spirulina (SES) through haematological and biochemical parameters and inflammatory and oxidative status were evaluated in rats' blood and liver tissue. The study consisted in a 90-day experiment on female and male rats supplemented with three doses (28.5, 57 and 285 mg/kg BW/day) of SES. No mortality, abnormal clinical signs, behavioural changes or macroscopic findings were observed whatever the groups. Haematological parameters were not modified in SES treated-groups. No marked change was recorded in biochemical parameters The liver endogenous antioxidant enzymes (SOD, GPx, catalase) activities were not modified whatever the gender and the dose, just as markers of oxidative stress ($O_2^{\circ-}$, TBARS, thiols) and inflammation such as IL-6 and TNF-alpha.

Our findings indicate that dietary supplementation of silicon-rich spirulina on rats has no harmful side nor toxic effects and could be beneficial especially in the case of suspicion or installation of pathologies due to oxidative stress.

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1. Introduction

Silicon is a very important element in the living world (Exley, 1998). It is soluble essentially in the form of orthosilicic acid $Si(OH)_4$ in the surface waters. But beyond 2 mM and at neutral pH, this acid polymerizes into various forms of silica (colloidal solid) (Refitt et al., 2003) little or no soluble. At biological pH, silicon chemistry in solution mainly relates orthosilicic acid (Knight and Kinrade, 2001) which can form stable complexes with molecules containing hydroxyl groups (Ingri, 1978). Silicon has been identified associated with various biomolecules including proteins and carbon hydrates (Bond and McAuliffe, 2003). Silicon is indispensable for cell growth and trophic tissues in humans as in animals, and as such it is an essential element. Its digestive absorption is a function of its chemical presentation. Naturally, silicon is

essentially in insoluble form, so poorly available (mineral silicon). On the other hand, even in a soluble form, silicon is slightly stable because it tends to polymerize rapidly at high concentration, which is the case of orthosilicic acid $Si(OH)_4$ which is the more soluble form of silicon and which has the better bioavailability (Calomme and Vanden Berghe, 1997; Jugdaohsingh et al., 2002), although it remains in a mineral form.

The silicon absorption in humans is largely performed in the form of orthosilicic acid $Si(OH)_4$ found in the drinking water or formed after food hydrolysis through the digestive tract (Refitt et al., 1999). The bulk of the silicon being in the form of colloidal silica is poorly absorbed by the body. In humans, daily intake is about 20–50 mg and the body needs 5–6 mg/day (Anonymous, 2004).

Biological importance of silicon has to be considered in the context of its body distribution. The highest concentrations are found in bone and connective tissues such as aorta, trachea, tendons and skin, where silicon appears as an actor in turnover processes through collagen and elastin synthesis (Seaborn and Nielsen, 1993). Silicon supplementation has been reported to have beneficial effects on these tissues and especially bones (Jugdaohsingh, 2007) and skin (Calomme and Vanden Berghe, 1997). Silicon deficiency, in contrast, has been associated to detrimental effects on bone mineralization and growth or skin elasticity and healing (Carlisle,

1976). The importance of silicon has also been demonstrated in cardiovascular pathophysiology and especially in the prevention of atherosclerosis (Loeper et al., 1979). An inverse relationship between the ingestion of silicon and the development of atherosclerosis has been reported (Loeper et al., 1988), and in spontaneously hypertensive rats, silicon supplementation reduces hypertension and increases antihypertensive and antiatherogenic gene expressions in the aorta (Maehira et al., 2011). Thus, a supplementation may be justified in preventing these effects, especially in the case of suspicion or installation of pathologies.

A fundamental step is to transform silicon in an organic form that is much more assimilated by the body than mineral forms. This is what we have achieved by incorporating it into spirulina.

The cyanobacteria Spirulina platensis is commercially available for human consumption. Spirulina are a good source of vitamins and minerals and are used as nutraceutical food supplements with no toxic side effects (Chamorro et al., 1988). They are particularly suitable for the production of specific bioactive compounds that they are able to accumulate in an organically bio-transformed form. Thus, new kind of food supplements has been developed and could serve as a rich source of trace elements (Mazo et al., 2007). Silicon is one of those trace elements essential in human nutrition.

Incorporating silicon into spirulina is a way to produce a bioavailable food supplement. Thus, given the beneficial effects of silicon and the antioxidant, hypolipidaemic and anti-inflammatory properties of spirulina (Deng and Chow, 2010) a food supplement was prepared. It has been shown that spirulina had no toxic effects *in vivo* and *in vitro* and did not release toxins (Heussner et al., 2012; Yang et al., 2011).

Whereas Buesen et al. (2014) reported that silicon dioxide nanomaterials neither cause local nor systemic effects upon subacute oral administration in rats and Horie et al. (2014) demonstrated that silicon dioxide nanoparticles do not exert potent cytotoxic effects on cells in culture, others have shown that (i) silicon dioxide increased enzymatic activities in the plasma, indicating an endothelial lung damage (Deb et al., 2012), (ii) silica nanoparticles exposure triggered a pro-inflammatory status in human bronchial epithelial BEAS-2B cells (Skuland et al., 2014), (iii) silicon in the form of silicon carbide induced no cytotoxicity but triggered pro-oxidative and pro-inflammatory responses of variable intensity on RAW 264.7 macrophages (Boudard et al., 2014).

Thus, we aimed to investigate here for the first time the safety and potential toxicity of repeated doses administration of silicon-rich spirulina (for 90 days) on rats. The maximum dose administered corresponds to 5 times the daily human recommended dose by the European Food Safety Authority.

2. Materials and methods

2.1. Materials

Spirulina (produced at PhycoBiotech, Lunel, France) grown in 25 m^3 pools under greenhouse with natural light, and temperature is controlled at 33 °C, in a Zarouk's medium in the presence of sodium metasilicate (Na$_2$O$_3$Si). This medium contained NaHCO$_3$, 16.8 g/L; K$_2$HPO$_4$, 0.5 g/L; NaNO$_3$, 2.5 g/L; K$_2$SO$_4$, 1.0 g/L; NaCl, 1.0 g/L; MgSO$_4$.7H$_2$0, 0.2 g/L; CaCl$_3$, 0.04 g/L; FeSO$_4$.7H$_2$0, 0.01 g/L; EDTA, 0.08 g/L; H$_3$BO$_3$, 2.86 mg/L; MnCl$_2$.4H$_2$O, 220 mg/L; CuSO$_4$. 5H$_2$O, 79 mg/L; MoO$_3$, 15 mg/L; and Na$_2$MoO$_4$, 21 mg/L and was supplied with light aeration (30 L/min) and the addition of 0.03% CO$_2$. The pool is continuously stirred with pumps. At the end of the growth, the biomass was recovered and filtered trough a 40 mm membrane, thoroughly washed with distilled water, frozen and lyophilized. Resultant spirulina contained 1% silicon, as indicated by the manufacturer.

2.2. Animals and experimental design

Thirty male and thirty female Sprague-Dawley rats (Janvier-Labs, Le Genest-St-Isle, France) weighing ~100 g were housed at 23 ± 1 °C and were randomly divided in five groups of six animals for each sex, then subjected to a 12 h light/dark cycle with free access to both food and water. They were handled in compliance with European Union rules and according to the guidelines of the NIH (National Research Council, 1985) and the Committee for Animal Care at the University of Montpellier (France).

They were fed *ad libitum* on a standard diet supplied by SAFE (Scientific Animal Food and Engineering, Augy, France) consisting of 236 g/kg casein, 3.5 g/kg L-methionine, 300 g/kg corn starch, 30 g/kg maltodextrin 10, 290.5 g/kg sucrose, 50 g/kg cellulose, 45 g/kg vegetable oil (10.3% energy of the diet), 35 g/kg mineral mix and 10 mg/kg vitamin mix. Vitamin and mineral mixes were formulated according to AIN-93 guidelines (Reeves et al., 1993). Rats and uneaten food were weighed every day. For each sex, rats of each group received daily by gavage either tap water (Control, CTR) or an aqueous suspension of crude spirulina (SP) at a dose of 285 mg/kg BW, or silicon-rich spirulina (SES) at a dose of 28.5 mg/kg BW (SES1 group) or SES at a dose of 57 mg/kg BW (SES2 group), or SES at a dose of 285 mg/kg BW (SES3) for 90 days. All animals were observed twice daily for general appearance, behaviour, signs of morbidity and mortality (once before treatment and once after). The SES2 dose (57 mg/kg BW/day) corresponds to the EFSA recommendations adapted to the rat (EFSA, 2004). The SES1 dose (28.5 mg/kg BW/d) is a two-fold lower dose, while the SES3 dose is fivefold times higher than SES2.

2.3. Sampling

At the end of the experimental period, rats were deprived of food overnight and blood samples were collected under anaesthesia by cardiac puncture. The liver was perfused with 0.15 M NaCl to remove residual blood, rapidly excised, weighed, sectioned for analyses, and stored at –80 °C. Heart, spleen and kidneys were rapidly excised, blotted dry and weighed.

2.4. Haematological and biochemical plasma parameters

All samples were collected early in the working day to reduce biological variation. Test tubes containing EDTA, as an anticoagulant, were used for haematology. Haematological parameters (red blood cells (RBCs), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBCs), neutrophils, eosinophils, basophils, lymphocytes, monocytes, platelets) were determined with an ABX Pentra DX 120 whole blood automated analyser (Horiba ABX, Montpellier, France).

Heparinized tubes were used for blood biochemistry. All parameters (urea, creatinine, cholesterol (CH), triglyceride (TG), chloride (Cl), calcium (Ca), phosphorus (P), bilirubin, aspartate transaminase (ASAT), alanine transaminase (ALAT) and gamma-glutamyl transferase (GGT)) were determined in plasma samples, after centrifugation (2500 rpm, 15 min), with a Cobas® 6000 automated analyser (Roche, Meylan, France).

2.5. Oxidative status

Liver superoxide anion (O_2°$^-$) production by NADPH oxidase were evaluated by the intensity of lucigenin-enhanced chemiluminescence (10 μM lucigenin), measured with a luminometer (Perkin Elmer Wallac, Victor, Turku, Finland) on tissue homogenates, as previously described (Romain et al., 2012). Results were expressed as relative light units (RLU/mg protein).

The index of lipid peroxidation was determined from the liver homogenate by measuring thiobarbituric acid reactive species (TBARS) according to Sunderman et al. (1985). After thiobarbituric acid had been reacted with malondialdehyde, the reaction product was measured spectrometrically at 532 nm. Optical imperfections were corrected according to Allen (1950) at 508 and 556 nm. Results were expressed as nanomoles of TBARS per gram of tissue.

Total thiols (SH) were determined according to Faure and Lafond (1995). The method is based on the reaction of 5,5′-dithiobis (2-nitrobenzoic) (DTNB) with the samples producing a yellow product, the thionitrobenzoic acid (TNB), measured spectrometrically at 412 nm. Results were expressed as nanomoles of SH per milligram of protein.

Protein content was determined by using a commercial protein assay (Sigma, Saint Quentin Fallavier, France) according to the method of Smith et al. (1985) and using bovine serum albumin as standard.

Liver tissue was homogenized in 50 mM phosphate buffer (pH 7.0) and spun at 3500 g for 15 min at 4 °C. The superoxide dismutase activity (SOD) was assayed in the supernatant using the method of Marklund and Marklund (1974) and Marklund (1976), based on a competition between the oxidation reaction of pyrogallol by O_2°$^-$ and the dismutation of O_2°$^-$ by SOD. The reaction product was measured spectrometrically at 320 nm. Results were expressed as unity activity per milligram of protein. Glutathione peroxidase activity (GPx) was evaluated using the method of Flohé and Günzler (1984), and activity was expressed as milli-units per milligram of protein. The activity of catalase (CAT) was measured according to Beers and Sizer (1952). The enzyme catalyses the decomposition of hydrogen peroxide into water molecule. Activity was expressed as units per milligram of protein.

2.6. Inflammatory factors evaluation

For pro-inflammatory cytokines determination, liver tissue was homogenized in 10 mM Tris buffer (pH 7.4) containing 2M NaCl, 1 mM EDTA, 0.01% Tween 80, 1 mM phenylmethylsulfonyl fluoride, and centrifuged at 9000 g for 30 min at 4 °C. The

resultant supernatant was used for cytokines determination. Liver tumour-necrosis-factor-alpha (TNF day α) and interleukin-6 (IL-6) levels were quantified by ELISA using commercial kits from R&D Systems (Lille, France).

2.7. Statistical analyses

Data are shown as means ± SEM. Statistical analysis of the data was carried out using StatView IV software (Abacus Concept, Berkeley, CA) by one-way ANOVA followed by Student's t-test. Differences were considered significant at p < 0.05.

3. Results and discussion

3.1. Repeated dose (90 days) toxicological evaluation

All animals survived the 90-day treatment period. No abnormal clinical signs, behavioural changes or macroscopic findings were observed. No significant differences in food (Table 1) and water consumption were noted among groups. As shown in Table 1, there are no significant differences in body weight gain. As far as the major organs' weight, there was no effect of the treatment, except in female heart weight SES2 which was however to the limit of significance (p = 0.0476).

3.2. Haematological and biochemical parameters

Some haematological parameters differed between control and some treated groups (Table 2). In female rats, a 10% decrease in red blood cells number appeared just as a significant basophils per cent increase in SES2 group compared to CTR group. There are no differences in male groups. Moreover, all values were within usual limits (Bailly and Duprat, 1990; Fallon, 1996).

As shown in Table 3, biochemical parameters were not affected whatever the group except in female rats, who showed 29% (p = 0.0028), 21% (p = 0.0223) and 38% (p = 0.0002) reduced ALAT activity in SP, SES2 and SES3 groups respectively compared to controls. In male rats, no parameter changed regardless of the dose supplied. Female rats also exhibited reduced triglyceride levels whereas males did not. However, the data were within normal ranges according to Moraillon et al. (2010).

3.3. Liver oxidative status

$O_2^{\circ-}$ is the main free radical and we measured its production in the liver *via* the NADPH oxidase enzyme activity. TBARS level was analysed as a marker of lipid peroxidation, and total thiols level was measured to evaluate the extent of oxidative damage proteins. For these three parameters of the hepatic oxidative stress, there is no difference between the groups, with the exception of the female group SES3 (Table 4). Only TBARS level for SES3 female group was increased by 97.5% compared to CTR group and it is likely due to two animals in the group that responded differently, leading to high average and SEM values. However, the activity of NADPH oxidase, a pro-oxidative enzyme, measured through the $O_2^{\circ-}$ production, just as thiols content were not modified regardless of the SES dose. These findings suggest that SES treatment did not induce any oxidative stress. Moreover, females showed in particular a smaller $O_2^{\circ-}$ production and a lower thiols rate than males, that could be explained by an oestrogenic action since it is known to protect against oxidation (Chakrabarti et al., 2014; Shwaery et al., 1998).

Liver antioxidant enzyme activities SOD, GPx and CAT were not modified by SES treatment (Table 5). As seen above for $O_2^{\circ-}$ production and thiols, higher SOD and GPx activities appeared in females due to oestrogenic hormones (Borrás et al., 2010; Wu et al., 2014).

3.4. Liver inflammatory status

The IL-6 and TNF-α levels were measured as pro-inflammatory markers. As shown in Table 6, both levels were not significantly different among groups. This suggests that SES treatment does not trigger an inflammatory status, which reinforces the values obtained on oxidative stress status. In fact, oxidative stress and inflammation are closely linked. The pathways that generate mediators of inflammation such as interleukins are closely linked to oxidative stress (Reuter et al., 2010).

4. Conclusions

This study represents the first standard toxicological data on silicon-rich spirulina. No haematological nor biochemical anomalies were noted. SES treatment did not reveal any changes in food

Table 1
Body and organ weights in control rats (CTR) and after a 90-day treatment with crude spirulina (SP) at 285 mg/kg/BW/day, or silicon-rich spirulina at 28.5 (SES1), 57 (SES2) and 285 mg/kg BW/day (SES3).[a]

	Female				
	CTR	SP	SES1	SES2	SES3
Food intake (g/day)	21.3 ± 2.2	19.6 ± 3.6	21.6 ± 2.8	21.0 ± 1.9	21.9 ± 2.3
Body weight gain (g)	216 ± 17	184 ± 8	193 ± 6	186 ± 10	213 ± 12
Organ weight (% BW)					
Liver	3.00 ± 0.14	3.27 ± 0.12	3.03 ± 0.14	3.09 ± 0.08	2.85 ± 0.20
Heart	0.34 ± 0.01	0.37 ± 0.01	0.37 ± 0.02	0.38 ± 0.01*	0.35 ± 0.01
Rate	0.17 ± 0.01	0.20 ± 0.15	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
Kidneys	0.61 ± 0.01	0.67 ± 0.17	0.67 ± 0.03	0.66 ± 0.01	0.67 ± 0.03
	Male				
	CTR	SP	SES1	SES2	SES3
Food intake (g/day)	30.8 ± 3.9	29.7 ± 3.2	30.8 ± 3.0	29.9 ± 4.0	30.3 ± 3.7
Body weight gain (g)	469 ± 19	476 ± 37	513 ± 23	476 ± 24	458 ± 18
Organ weight (% BW)					
Liver	2.50 ± 0.13	2.72 ± 0.13	2.65 ± 0.06	2.50 ± 0.13	2.55 ± 0.12
Heart	0.28 ± 0.01	0.32 ± 0.03	0.28 ± 0.01	0.29 ± 0.01	0.29 ± 0.01
Rate	0.23 ± 0.01	0.26 ± 0.02	0.21 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
Kidneys	0.52 ± 0.01	0.53 ± 0.01	0.51 ± 0.02	0.54 ± 0.01	0.55 ± 0.02

[a] Data are expressed as mean + SEM.
* P < 0.05 compared to untreated rats (CTR group).

Table 2
Haematological parameters in control rats (CTR) and after a 90-day treatment with crude spirulina (SP) at 285 mg/kg/BW/day, or silicon-rich spirulina at 28.5 (SES1), 57 (SES2) and 285 mg/kg BW/day (SES3).[a]

	Female				
	CTR	SP	SES1	SES2	SES3
RBC ($\times 10^4$/mm^3)	720 ± 17	707 ± 16	729 ± 26	647 ± 38*	703 ± 28
Haemoglobin (g/dL)	14.40 ± 0.40	14.18 ± 0.48	13.38 ± 0.48	13.40 ± 0.20	14.27 ± 0.59
Haematocrit (%)	38.90 ± 0.93	39.70 ± 1.25	39.80 ± 1.53	37.30 ± 0.40	39.40 ± 1.25
MCV (fl)	53.80 ± 0.80	54.30 ± 0.76	54.50 ± 0.56	54.30 ± 0.33	54.20 ± 0.75
MCH (pg)	20.0 ± 0.40	20.10 ± 0.29	19.00 ± 0.50	19.60 ± 0.31	20.30 ± 0.30
MCHC (g/dL)	36.90 ± 0.28	35.80 ± 0.63	35.90 ± 0.97	35.90 ± 0.58	36.20 ± 0.40
WBC ($\times 10^2$/mm^3)	27.0 ± 4.0	24.0 ± 1.4	28.0 ± 2.7	25.0 ± 4.8	28.0 ± 3.4
Neutrophils (%)	12.00 ± 1.74	13.50 ± 1.15	13.20 ± 1.03	12.40 ± 0.72	13.00 ± 0.80
Eosinophils (%)	0.60 ± 0.18	0.60 ± 0.25	0.50 ± 0.16	0.90 ± 0.16	0.70 ± 0.24
Basophils (%)	0.17 ± 0.03	0.23 ± 0.03	0.28 ± 0.03	0.32 ± 0.06*	0.23 ± 0.04
Lymphocytes (%)	86.3 ± 1.9	84.4 ± 1.1	85.0 ± 1.2	85.8 ± 0.7	85.0 ± 0.9
Monocytes (%)	0.9 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	0.6 ± 0.0*	1.0 ± 0.1
Platelets ($\times 10^3$/mm^3)	697 ± 82	698 ± 76	710 ± 63	638 ± 112	597 ± 43
	Male				
	CTR	SP	SES1	SES2	SES3
RBC ($\times 10^4$/mm^3)	821 ± 40	791 ± 47	757 ± 77	823 ± 20	732 ± 38
Haemoglobin (g/dL)	14.82 ± 0.63	14.95 ± 0.99	13.70 ± 1.29	14.98 ± 0.21	13.86 ± 0.72
Haematocrit (%)	41.60 ± 1.81	42.80 ± 2.35	40.80 ± 3.86	42.20 ± 0.66	39.50 ± 1.86
MCV (fl)	50.80 ± 0.86	51.30 ± 0.33	51.80 ± 0.60	51.50 ± 0.86	52.20 ± 0.54
MCH (pg)	18.10 ± 0.37	18.90 ± 0.59	18.30 ± 0.21	18.30 ± 0.28	18.60 ± 0.20
MCHC (g/dL)	35.70 ± 0.37	36.70 ± 1.01	35.30 ± 0.29	35.50 ± 0.13	35.60 ± 0.21
WBC ($\times 10^2$/mm^3)	68.0 ± 8.7	66.0 ± 1.1	54.0 ± 7.4	58.0 ± 6.6	60.0 ± 1.1
Neutrophils (%)	10.20 ± 1.55	11.00 ± 1.37	10.20 ± 0.61	11.20 ± 1.91	7.90 ± 0.46
Eosinophils (%)	0.40 ± 0.18	0.60 ± 0.16	0.40 ± 0.14	0.30 ± 0.03	0.40 ± 0.08
Basophils (%)	0.10 ± 0.03	0.13 ± 0.04	0.20 ± 0.09	0.13 ± 0.05	0.12 ± 0.03
Lymphocytes (%)	87.2 ± 1.7	86.4 ± 1.6	87.5 ± 1.0	86.6 ± 1.9	98.5 ± 0.5
Monocytes (%)	2.0 ± 0.4	1.9 ± 0.3	1.7 ± 0.4	1.8 ± 0.1	2.1 ± 0.6
Platelets ($\times 10^3$/mm^3)	646 ± 71	602 ± 74	528 ± 74	590 ± 782	561 ± 97

[a] Data are expressed as mean + SEM.
* P < 0.05 compared to untreated rats (CTR group).

Table 3
Blood biochemical parameters in control rats (CTR) and after a 90-day treatment with crude spirulina (SP) at 285 mg/kg/BW/day, or silicon-rich spirulina at 28.5 (SES1), 57 (SES2) and 285 mg/kg BW/day (SES3).[a]

	Female				
	CTR	SP	SES1	SES2	SES3
Urea (mmol/L)	5.04 ± 0.43	4.79 ± 0.46	5.93 ± 0.36	5.51 ± 0.47	4.72 ± 0.31
Creatinine (μmol/L)	32.28 ± 2.16	32.77 ± 3.11	34.82 ± 1.31	36.00 ± 4.20	30.68 ± 1.38
CH (mmol/L)	2.74 ± 0.32	2.51 ± 0.40	2.38 ± 0.27	1.97 ± 0.08	2.01 ± 0.12
TG (mmol/L)	2.21 ± 0.53	0.80 ± 0.13*	0.90 ± 0.17*	0.64 ± 0.06*	1.14 ± 0.11
Cl (mmol/L)	97.30 ± 0.84	98.20 ± 0.70	98.50 ± 0.85	98.30 ± 0.33	98.00 ± 0.45
Ca (mmol/L)	2.72 ± 0.04	2.70 ± 0.05	2.66 ± 0.06	2.65 ± 0.02	2.70 ± 0.03
P (mmol/L)	1.71 ± 0.10	1.87 ± 0.15	1.49 ± 0.08*	1.85 ± 0.07	2.00 ± 0.15
Bilirubin (μmol/L)	2.50 ± 0.22	2.33 ± 0.21	2.00 ± 0.00	2.33 ± 0.21	2.33 ± 0.21
ASAT (U/L)[b]	85.60 ± 4.21	86.20 ± 6.17	82.40 ± 7.84	86.40 ± 3.98	75.83 ± 3.14
ALAT (U/L)[b]	37.2 ± 2.35	26.20 ± 2.92*	31.00 ± 2.70	29.50 ± 1.65*	23.00 ± 1.63*
GGT (UI/L)[b]	3.33 ± 0.66	3.80 ± 0.37	4.00 ± 0.68	3.33 ± 0.42	4.50 ± 0.50
	Male				
	CTR	SP	SES1	SES2	SES3
Urea (mmol/L)	5.73 ± 0.15	6.04 ± 0.31	6.57 ± 0.44	6.54 ± 0.22	6.51 ± 0.32
Creatinine (μmol/L)	38.80 ± 1.03	40.28 ± 0.99	39.08 ± 1.73	39.23 ± 1.57	37.63 ± 2.78
CH (mmol/L)	1.71 ± 0.09	1.57 ± 0.03	1.71 ± 0.08	1.51 ± 0.09	1.50 ± 0.06
TG (mmol/L)	1.03 ± 0.08	1.04 ± 0.15	1.31 ± 0.17	1.14 ± 0.07	0.98 ± 0.14
Cl (mmol/L)	98.50 ± 1.15	97.50 ± 0.56	97.20 ± 0.31	98.00 ± 0.63	98.00 ± 0.84
Ca (mmol/L)	2.68 ± 0.06	2.62 ± 0.02	2.68 ± 0.02	2.68 ± 0.02	2.65 ± 0.05
P (mmol/L)	2.28 ± 0.08	2.25 ± 0.05	2.41 ± 0.07	2.27 ± 0.06	2.46 ± 0.05
Bilirubin (μmol/L)	2.17 ± 0.17	2.00 ± 0.00	2.00 ± 0.00	2.17 ± 0.17	2.00 ± 0.00
ASAT (U/L)[b]	106.60 ± 9.13	97.00 ± 2.70	90.60 ± 6.30	93.80 ± 5.72	96.33 ± 4.46
ALAT (U/L)[b]	46.80 ± 1.99	43.80 ± 2.76	44.80 ± 3.20	41.80 ± 3.66	44.60 ± 3.96
GGT (UI/L)[b]	0.83 ± 0.17	1.00 ± 0.00	1.33 ± 0.33	1.00 ± 0.00	0.83 ± 0.17

[a] Data are expressed as mean + SEM. * P < 0.05 compared to untreated rats (CTR group). [b] ASAT: aspartate transaminase; ALAT: alanine transaminase; GGT: gamma-glutamyl transferase.

Table 4
Oxidative stress markers in control rats (CTR) and after a 90-day treatment with crude spirulina (SP) at 285 mg/kg/BW/day, or silicon-rich spirulina at 28.5 (SES1), 57 (SES2) and 285 mg/kg BW/day (SES3).[a]

	Female				
	CTR	SP	SES1	SES2	SES3
$O_2^{\circ-}$ (RLU/mg prot)	1526 ± 229	1581 ± 222	1292 ± 134	1407 ± 234	1241 ± 178
TBARS (nmol/g)	292.2 ± 65.0	323.3 ± 88.4	389.9 ± 79.9	370.6 ± 88.6	577.3 ± 162.7*
SH (nmol/mg prot)	98.0 ± 23.9	77.1 ± 24.7	81.3 ± 29.5	79.4 ± 17.0	75.0 ± 18.1
	Male				
	CTR	SP	SES1	SES2	SES3
$O_2^{\circ-}$ (RLU/mg prot)	2432 ± 278	2593 ± 311	2312 ± 360	2115 ± 274	1947 ± 249
TBARS (nmol/g)	193.8 ± 64.4	191.3 ± 32.1	208.7 ± 36.6	227.9 ± 45.0	219.9 ± 45.3
SH (nmol/mg prot)	87.4 ± 27.3	107.7 ± 13.4	102.2 ± 13.4	105.8 ± 10.1	101.2 ± 10.4

[a] Data are expressed as mean + SEM.
* $P < 0.05$ compared to untreated rats (CTR group).

Table 5
Liver antioxidant enzyme activities in control rats (CTR) and after a 90-day treatment with crude spirulina (SP) at 285 mg/kg/BW/day, or silicon-rich spirulina at 28.5 (SES1), 57 (SES2) and 285 mg/kg BW/day (SES3).[a]

	Female				
	CTR	SP	SES1	SES2	SES3
SOD (U/mg prot)	20.3 ± 1.8	20.9 ± 2.9	20.9 ± 3.3	20.7 ± 3.7	21.6 ± 2.5
GPx (mU/mg prot)	6865 ± 202	6674 ± 571	7091 ± 892	6694.4 ± 384	7421 ± 636
CAT (U/mg prot)	702 ± 89	691 ± 112	676 ± 175	628 ± 82	667 ± 98
	Male				
	CTR	SP	SES1	SES2	SES3
SOD (U/mg prot)	17.5 ± 1.8	17.7 ± 3.1	18.2 ± 2.1	18.3 ± 2.6	17.2 ± 1.6
GPx (mU/mg prot)	3338 ± 562	4203 ± 886	3674 ± 496	3884 ± 420	3556 ± 533
CAT (U/mg prot)	728 ± 96	772 ± 134	711 ± 42	787 ± 108	749 ± 119

[a] Data are expressed as mean + SEM.

Table 6
Liver pro-inflammatory cytokine levels in control rats (CTR) and after a 90-day treatment with crude spirulina (SP) at 285 mg/kg/BW/day, or silicon-rich spirulina at 28.5 (SES1), 57 (SES2) and 285 mg/kg BW/day (SES3).[a]

	Female				
	CTR	SP	SES1	SES2	SES3
IL-6 (pg/mg prot)	726.8 ± 149.9	719.2 ± 217.8	611.9 ± 209.5	849.7 ± 274.5	649.41 ± 125.1
TNF-α (pg/mg prot)	417.6 ± 99.1	299.0 ± 99.0	457.8 ± 142.3	471.6 ± 73.5	418.1 ± 141.6
	Male				
	CTR	SP	SES1	SES2	SES3
IL-6 (pg/mg prot)	1083.5 ± 476.1	1098.7 ± 411.3	1061.7 ± 449.8	873.4 ± 120.0	1063.7 ± 280.2
TNF-α (pg/mg prot)	764.9 ± 244.1	634.0 ± 195.3	883.7 ± 373.1	837.8 ± 171.5	742.7 ± 260.0

[a] Data are expressed as mean + SEM.

and water (not shown) consumption and behaviours. Based on these findings, SES was well tolerated at daily doses of 28.5, 57 and 285 mg/kg BW/day for 90 days in the rats and there is no evidence for systemic and liver adverse effects and toxicity. Furthermore, there were no signs of oxidative stress or inflammation in the liver, even with the maximum dose of SES used, demonstrating the safety of this dietary supplement.

These findings suggest that silicon-rich spirulina does not induce adverse physiological or biochemical nor pathological changes and could have potential applications in several situations in which oxidative stress and/or inflammation are increased, such as cardiovascular diseases for example. This is in accordance with Yang et al. (2011) and Heussner et al. (2012) relative to spirulina, and corroborates the findings of Buesen et al. (2014) and Horie et al. (2014) although silicon was in the form of silicon dioxide nanoparticles in these two last studies. Elsewhere, further investigations are needed to understand by which mechanisms SES could protect the antioxidant system.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Aknowledgements

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