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Evaluation of the toxicity of *Arthrospira* (*Spirulina*) *platensis* extract

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Abstract In this study, the methanol extract of *Arthrospira* (*Spirulina*) *platensis* was examined for acute and subchronic toxicities. The extract did not produce any sign of toxicity within 7 days after feeding it at a single high dose of 6 g kg^{-1} body weight to female and male Swiss mice. For the subchronic toxicity test, the extract at doses of 6, 12, and 24 mg kg^{-1} body weight was orally administered to six male and six female Wistar rats daily for 12 weeks. Throughout the study period, we did not observe any abnormalities on behavior, food and water intakes, and health status among the treated animals. The hematology and clinical chemistry parameters of treated groups did not significantly differ from those of the controls in both sexes. Postmortem examination of the test groups also showed no abnormalities in both gross and histological findings. These results thus suggest that the methanol extract of *A. platensis* did not cause acute or subchronic toxicity in our experimental animals.

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Keywords Acute toxicity · *Arthrospira platensis* · Blue-green algae · Methanol extract · *Spirulina* · Subchronic toxicity

Introduction

Spirulina, now named *Arthrospira*, is an edible blue-green microalga (cyanobacterium) characterized by multicellular cylindrical trichomes in an open left-hand helix along the entire length (Tomaselli 1997). Due to its high content of good quality protein as well as being rich in vitamins, minerals, and other components beneficial to health such as essential fatty acids and antioxidant pigments like carotenoids, chlorophyll, and phycocyanin, this cyanobacterium has received much attention as a most promising and nutritious food source (Dillion et al. 1995). Also, its diverse biological and pharmacological properties (Belay 2002; Becker 2003; Khan et al. 2005; Mani et al. 2008) have promoted *Arthrospira* (*Spirulina*) as being a functional food, and thus, consumption of this microorganism as a nutritional therapeutic supplement gains popularity. Not only the "whole" *Arthrospira* (*Spirulina*) but also a wide range of the alga components primarily arisen from the two species, *Arthrospira platensis* and *Arthrospira maxima*, are now sold in health food markets worldwide. Commercialization of *Arthrospira* (*Spirulina*) for food and special feeds has occurred since the 1970s (Sánchez et al. 2003). This dietary microalga, however, has been utilized for many years without any reports of undesirable effect in living organisms, and its safety evaluations for human consumption have been extensively performed. Despite a number of toxicological reports having concluded that *Arthrospira* per se is not toxic (Krishnakumari et al. 1981; Chamorro et al. 1985, 1988, 1996, 1997; Chamorro and Salazar 1988; Salazar and Chamorro 1990; Salazar et al.

1996, 1998; Hutadilok-Towatana et al. 2008), there is little information as yet on the systemic toxicity of *Arthrospira* extracts which are also commercially distributed for dietary and nutraceutical purposes.

Previously, we have shown that either single or long-term intake of the whole *A. platensis*, up to high feeding levels, does not produce any toxicity in rodents (Hutadilok-Towatana et al. 2008). In this continuing study, *A. platensis* extract was then evaluated in both acute and subchronic toxicity tests in order to increase database on toxicology as well as confidence in *Arthrospira* (*Spirulina*)'s safety for consumption.

Materials and methods

Fresh *Arthrospira* (*Spirulina*) *platensis* (Nordstedt) Geitler, (Phormidiaceae) was supplied by Yord Thong 2001 Pty. Ltd. (Songkhla, Thailand). It was identified at the Songkhla Provincial Fisheries Office where a voucher herbarium specimen (specimen no. 134.1 19 16 01) is kept at the Faculty of Pharmaceutical Sciences, Prince of Songkla University.

Preparation of the extract

Arthrospira platensis was dried at 50°C and ground to powder using a grinder and a sieve no. 45. The dried powder (0.5 kg) was successively extracted by maceration with methanol (3 L×3 times). The methanol extract was filtered and evaporated to dryness in vacuo. Dried extract was then stored at 4°C in sterilized sealed plastic containers and kept away from light until use. The extract was prepared from the same batch of *A. platensis* throughout this study.

Experimental animals

Male and female Swiss mice with weights ranging from 30 to 36 g were obtained from the Animal House Facility Unit, Faculty of Science, Prince of Songkla University. They were then used for the acute toxicity tests. In the subchronic toxicity study, male and female Wistar rats at about 6 weeks of age and weighing between 300 and 450 g were supplied from the breeding colony of the Animal House Facility Unit, Faculty of Science, Prince of Songkla University. All procedures concerning animal treatments and experimentation in this study were reviewed and approved by the Institutional Committee for Ethical Use of Experimental Animals at Prince of Songkla University (approval no. 1210/713).

Acute toxicity study

The 50% lethal dose (LD_{50}) of *A. platensis* extract in mice was estimated by the up-and-down method (Bruce 1985). Doses were adjusted up or down by a constant multiplicative factor depending on the previous outcome. In this study, two groups of ten animals (control and test groups), each containing equal numbers of male and female animals, were used. A dose limit at 6 g kg^{-1} of the extract dissolved in co-solvent containing 4:4:1 (*v*/*v*/*v*) of propylene glycol/water/Tween 80 was administered orally (5 mL kg^{-1} body weight) to the test group. The control mice received co-solvent (5 mL kg^{-1} body weight) only. Following administration, they were closely observed for 7 days, for toxic signs and symptoms, and death. At the end of the period, all survivors were killed to examine vital organ gross changes.

Subchronic toxicity study

Male and female rats were randomly divided into four groups of six. Each group was housed separately in an identical wire-mesh-bottomed stainless steel cage and maintained in an air-conditioned room at 25±2°C, 50–60% relative humidity, and artificial illumination between 0600 and 1800 hours. Commercial chow diets (C.P. Mice Feed®, Charoen Phokphand Group, Bangkok, Thailand) and freshly filtered water were provided ad libitum. The dried extract was dissolved in co-solvent containing 4:4:1 (*v*/*v*/*v*) of propylene glycol/water/Tween 80 at various concentrations. During the 12 weeks of experimental period, *A. platensis* extract at doses of 6, 12, and 24 mg kg^{-1} body weight was orally administered (5 mL kg^{-1} body weight) to the animals in each treatment group daily, while co-solvent only was given to all control rats (5 mL kg^{-1}). The animals were observed daily for signs and behavioral changes. They were weighed initially and then twice a week until termination. Food and water intakes were also measured daily.

Once every 4 weeks, heparinized blood was collected by ocular bed puncture for hematology and biochemical analyses following overnight fast. Packed red cell volume measurements and counts of total and differential leukocytes were performed. Plasma was also separated from the collected blood for assays of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total and direct bilirubins, glucose, creatinine, urea nitrogen (BUN), uric acid, albumin, total protein, and Na^+, K^+, Cl^-, and HCO_3^- levels using a Lab Automation Model Synchron CX3 Delta (Beckman Coulter, USA). Diagnostic kits (CPT Diagnostics, Spain) based on enzymatic methods were used for total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride assays in these plasma samples.

At the end of the study, the rats were anesthetized with ether for blood collection and then killed by cervical dislocation. An autopsy was performed during which any macroscopic abnormalities were noted. The heart, liver, spleens, and kidneys were weighed immediately after removal. Samples of these organs were fixed in 10%

neutral buffered formalin and kept in that solution for further histopathological examination.

Statistical analysis

All data are presented as mean ± SEM. Statistical evaluations were performed by one-way analysis of variance and post hoc least significance difference test at the 95% confidence level using an SPSS program for Windows 11. Significance was judged at $p<0.05$.

Results

In order to obtain both polar and non-polar components of *Arthrospira* (*Spirulina*), the methanol extract of *A. platensis* was prepared and then used as the source for our investigation. A yield of 28.5% (*w*/*w*) was obtained.

Acute toxicity in mice

In this study, no toxic symptoms or death were observed in any of the animals within 7 days after treatment. The gross examination of their internal organs at the end of experimental period also revealed no pathological abnormalities. Therefore, LD_{50} of the methanol extract of *A. platensis* was higher than 6 g kg^{-1} of body weight upon one-time dispensing to mice.

Subchronic toxicity in rats

Consumption of *A. platensis* extract at any dose tested caused no abnormal appearances or clinical signs in the rats. In all cases, their feces were dried and darkly colored. Average daily food and water intakes among the groups were also similar throughout this study (data not shown). Measurements of the body weight over the whole experimental period found no differences among the four female groups ($p>0.05$). Their weights gradually increased with time at the same rate (Fig. 1). In the male rats, however, some differences were observed. The extract-receiving animals hardly gained weights (Fig. 1). At the 12th week, average weight gain of the control males was 60±4.30 g, whereas those of low-dose (6 mg kg^{-1}), middle-dose (12 mg kg^{-1}), and high-dose (24 mg kg^{-1}) groups were comparatively lower ($p<0.05$) (3.51±6.86, 22.07±6.85, and 16.33±12.98 g, respectively).

There were no consistent significant differences in the clinical chemistry results between our treated and control animals ($p<0.05$) as shown in Table 1. The blood electrolyte levels were not increased in any treated animals throughout the 12 weeks of investigation. All measured values of Na^+, K^+, Cl^-, and HCO_3^- were within the same ranges of 150–154, 5–7, 105–112, and 18–22 mmol L^{-1}, respectively, indicating that continuous intake of the methanol extract of *A. platensis* did not cause any deficiency in renal excretion since there was no accumulation of the electrolytes in the rat's circulation.

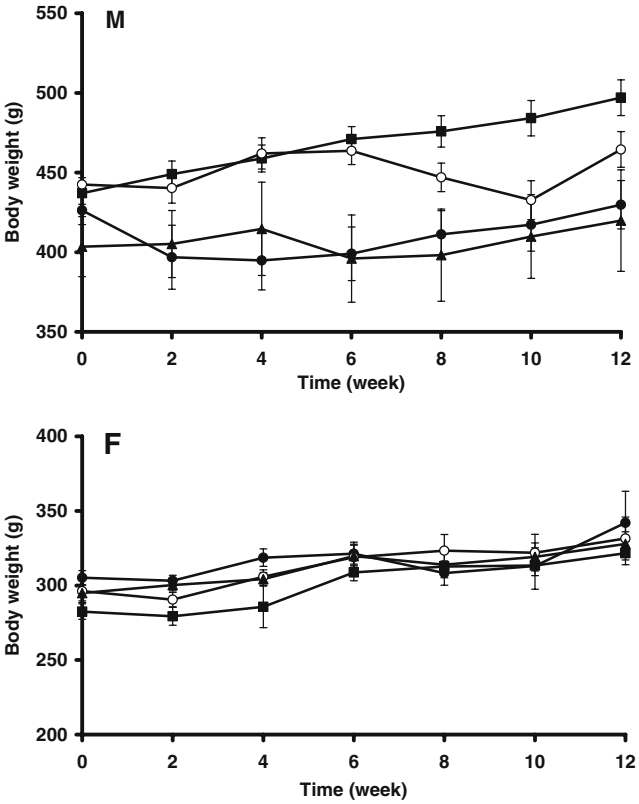

Fig. 1 Average body weights of male (*M*) and female (*F*) rats fed daily throughout 12 weeks with the methanol extract of *A. platensis* at different doses: 0 mg kg^{-1} (square), 6 mg kg^{-1} (*filled circle*), 12 mg kg^{-1} (*empty circle*), and 24 mg kg^{-1} (*triangle*). Values are mean ± SEM; $n=6$

To determine if *A. platensis* extract had any effects on blood cells and bone marrow activity of rats, hematological examinations were performed. As presented in Table 2, the average hematocrit values in all male groups were similar. Although some decreases ($p<0.05$) in this hematological parameter were detected in female rats after being fed with the extract at 6 and 12 mg kg^{-1} for 12 weeks, they were slight and not dose-related. In addition, normal blood smears were observed in all of the animals. At the end of the experiment, lymphocyte counts became relatively higher than the beginning values, except those of control and 6 mg kg^{-1} treated male groups. This increase in lymphocytes, however, was not associated with a comparable change in their total WBC numbers.

At autopsy, macroscopic observation of the internal organs did not show any abnormality in their gross appearances and weights due to the consumption of the extract (Table 3). In addition, we did not detect any damages in their gastrointestinal tracts, the potential and direct target for toxic effects of

Table 1 Mean blood chemistry values in rats treated with *A. platensis* extract for 12 weeks

Parameter	Dose (mg kg^{-1})			
	0	6	12	24
Males				
AST (IU L^{-1})	106.71±15.82 (110.14±7.55)	105.80±12.30 (80.71±3.78*)	139.50±27.16 (87.29±7.96)	109.00±12.05 (104.50±14.35)
ALT (IU L^{-1})	72.71±11.65 (39.71±1.90)	68.00±4.81 (39.14±1.29)	83.83±10.11 (47.14±3.17)	65.00±3.26 (48.00±2.58)
ALP (IU L^{-1})	59.14±3.77 (70.14±7.91)	94.40±6.99 (57.00±1.13)	134.33±51.56 (67.00±17.07)	88.00±19.30 (51.88±6.24)
Direct bilirubin (mg dL^{-1})	0.39±0.06 (0.49±0.09)	0.55±0.09 (0.59±0.10)	0.49±0.10 (0.46±0.08)	0.53±0.14 (0.60±0.10)
Total bilirubin (mg dL^{-1})	0.70±0.05 (0.93±0.06)	0.85±0.06 (1.00±0.10)	0.73±0.14 (0.78±0.08)	0.96±0.23 (1.03±0.12)
Total protein (g dL^{-1})	5.90±0.13 (5.93±0.16)	6.41±0.18** (5.95±0.06)	6.39±0.16** (6.51±0.80)	6.13±0.16 (5.85±0.26)
Albumin (g dL^{-1})	3.45±0.14 (3.60±0.04)	3.72±0.26 (3.58±0.06)	3.20±0.10 (3.60±0.10)	3.47±0.15 (3.52±0.04)
Cholesterol (mg dL^{-1})	70.06±2.61 (66.07±1.26)	65.81±5.16 (63.39±3.72)	61.29±2.77 (62.50±3.05)	65.81±4.28 (65.63±3.54)
TG (mg dL^{-1})	27.47±2.29 (27.27±2.80)	23.08±2.43 (25.97±2.37)	26.92±3.29 (25.97±1.30)	27.69±7.13 (25.00±1.49)
HDL-C (mg dL^{-1})	55.30±3.94 (48.74±2.11)	47.74±2.58 (47.06±2.57)	43.01±2.72** (38.66±2.17*)	42.58±2.58** (40.44±2.06*)
LDL-C (mg dL^{-1})	9.28±2.17 (11.88±2.76)	13.45±4.07 (11.14±1.75)	12.90±1.65 (18.65±3.02)	17.69±2.91** (19.96±3.27*)
Glucose (mg dL^{-1})	114.29±8.96 (103.30±4.40)	112.00±8.60 (116.49±7.20)	121.67±11.38 (112.09±6.01)	108.00±6.63 (104.90±8.41)
BUN (mg dL^{-1})	13.79±0.53 (14.10±0.66)	14.65±0.59 (13.18±0.34)	15.89±1.26 (12.25±0.48)	14.19±0.68 (12.67±0.48)
Creatinine (mg dL^{-1})	0.56±0.04 (0.60±0.03)	0.60±0.05 (0.51±0.03)	0.58±0.03 (0.61±0.03)	0.54±0.02 (0.61±0.04)
Uric acid (mg dL^{-1})	0.26±0.04 (0.30±0.04)	0.42±0.05** (0.26±0.06)	0.27±0.04 (0.31±0.04)	0.18±0.02 (0.53±0.08*)
Females				
AST (IU L^{-1})	116.00±19.63 (102.50±23.42)	160.67±18.78 (72.17±3.74)	135.00±23.81 (79.00±9.43)	103.33±14.46 (65.00±2.07)
ALT (IU L^{-1})	42.67±3.18 (36.00±2.32)	64.00±12.06 (32.17±1.44)	73.25±7.42** (29.57±2.11*)	53.50±3.06 (28.86±1.70*)
ALP (IU L^{-1})	31.00±2.64 (39.50±5.65)	58.67±16.75 (31.00±2.08)	93.75±14.08** (33.71±1.86)	50.00±10.27 (38.00±2.56)
Direct bilirubin (mg dL^{-1})	0.41±0.09 (0.58±0.09)	0.64±0.15 (0.55±0.09)	0.45±0.12 (0.58±0.08)	0.62±0.11 (0.61±0.06)
Total bilirubin (mg dL^{-1})	0.68±0.14 (1.03±0.15)	1.13±0.15 (0.90±0.11)	0.77±0.12 (0.96±0.10)	0.98±0.12 (0.94±0.08)
Total protein (g dL^{-1})	5.95±0.26 (5.32±0.30)	5.83±0.12 (5.72±0.31)	6.26±0.15 (6.20±0.29)	6.33±0.12 (6.25±0.18)
Albumin (g dL^{-1})	3.40±0.09 (3.58±0.13)	3.13±0.17 (3.53±0.18)	3.28±0.24 (3.72±0.08)	3.52±0.12 (3.87±0.11)
Cholesterol (mg dL^{-1})	73.12±9.38 (70.83±4.17)	62.37±2.15 (77.08±4.17)	74.20±8.12 (74.11±3.46)	88.17±2.72 (87.50±6.40*)
TG (mg dL^{-1})	25.64±6.78 (22.73±2.03)	20.51±2.57 (25.76±2.79)	26.93±2.22 (20.78±1.68)	29.49±4.62 (22.08±2.70)
HDL-C (mg dL^{-1})	58.06±11.18 (54.90±2.91)	49.46±2.15 (53.92±3.53)	59.68±6.10 (53.78±2.99)	64.52±4.41 (63.03±3.04)
LDL-C (mg dL^{-1})	9.93±2.74 (11.39±3.49)	8.80±0.51 (18.01±2.18)	9.14±2.98 (16.17±3.27)	17.76±1.75** (20.06±3.96)
Glucose (mg dL^{-1})	106.67±12.02 (101.28±6.10)	110.00±5.77 (96.16±4.76)	117.50±4.78 (102.20±4.66)	105.00±10.88 (98.90±6.80)
BUN (mg dL^{-1})	11.24±0.39 (14.29±0.58)	16.28±1.34** (11.47±0.52*)	12.50±0.56 (13.36±1.35)	16.67±0.83** (12.62±0.24)
Creatinine (mg dL^{-1})	0.57±0.03 (0.68±0.03)	0.70±0.06** (0.62±0.02)	0.60±0.00 (0.61±0.03)	0.53±0.02 (0.59±0.03*)
Uric acid (mg dL^{-1})	0.23±0.13 (0.35±0.07)	0.67±0.23 (0.32±0.03)	0.60±0.16 (0.29±0.03)	0.60±0.08 (0.24±0.04)

Values are mean ± SEM; $n=6$

Each corresponding initial value (week 0) is shown in parentheses

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *TG* triglyceride, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *BUN* blood urea nitrogen

*$p<0.05$ (vs control at week 0), **$p<0.05$ (vs control at week 12)

Table 2 Mean hematological values in rats treated with *A. platensis* extract for 12 weeks

Dose (mg kg^{-1})	Hct (%)	WBC (×10^3 μL^{-1})	Differential leukocytes (%)		
			(N)	(L)	(M)
Males					
0	46.29±0.18	6.53±0.34	11.67±2.85	73.33±6.01	15.00±3.46
	(43.00±0.38)	(6.47±0.59)	(0.57±0.37)	(91.86±1.26)	(7.57±1.09)
6	47.60±1.08	6.81±0.24	3.50±1.50	84.50±1.50	12.00±3.00
	(41.00±0.69)	(6.22±0.25)	(0.71±0.29)	(90.86±1.66)	(11.29±2.63)
12	46.33±0.84	7.13±1.61	3.00±2.00	91.00±1.00**	8.00±1.00
	(40.71±0.97)	(6.28±1.26)	(7.00±2.49*)	(72.57±5.57*)	(18.00±3.72*)
24	44.80±3.46	5.84±0.82	3.67±0.88	91.00±2.00**	5.33±1.20**
	(42.25±1.28)	(6.41±1.11)	(9.38±2.13*)	(65.25±4.14*)	(24.13±2.08*)
Females					
0	46.00±1.53	5.28±0.73	4.00±2.00	92.50±3.50	3.50±1.50
	(40.83±1.17)	(6.36±0.74)	(8.33±1.02)	(71.00±2.58)	(20.67±2.58)
6	40.67±0.88**	4.72±1.13	0	97.67±1.45	2.33±1.45
	(41.67±0.66)	(5.35±0.52)	(11.50±2.17)	(71.00±2.53)	(17.67±1.60)
12	39.75±2.17**	3.42±0.52**	1.33±0.33	89.33±2.67	9.33±2.85
	(40.14±1.64)	(4.41±1.61)	(9.57±2.27)	(71.43±3.03)	(19.00±1.90)
24	46.67±0.76	3.73±0.23**	3.33±1.67	91.33±0.88	5.33±0.88
	(43.57±0.43)	(3.92±0.45*)	(9.00±1.54)	(74.86±2.87)	(18.00±1.90)

Values are mean ± SEM; n=6

Each corresponding initial value (week 0) is shown in parentheses

Hct hematocrit, *WBC* total white blood cells, *N* neutrophil, *L* lymphocyte, *M* monocyte

*p<0.05 (vs control at week 0), **p<0.05 (vs control at week 12)

ingested foods. The results from gross examination were also confirmed by histopathological assessment of the internal organs. The extract did not produce any significant changes in heart, spleen, and kidney tissues of all the animals. Fatty change and degeneration of pericentral vein hepatocytes, however, appeared in two control male livers (not shown). Such incidence was arisen from an unknown cause and accounted for only 4.2% of the total samples.

Discussion

In this study, the methanol extract of *Arthrospira* (*Spirulina*) which is known to be rich in antioxidants such as phenolic acids, α-tocopherol, and β-carotene (Miranda et al. 1998) was prepared. The presence of antioxidants in our extract was visualized as many bright spots on purple background of TLC plates when sprayed with DPPH reagent (Takao et al. 1994).

Table 3 Mean organ weights in rats treated with *A. platensis* extract for 12 weeks

Dose (mg kg^{-1})	Organ weight (g)			
	Liver	Kidney	Spleen	Heart
Males				
0	15.10±0.96	3.54±0.37	0.91±0.11	1.68±0.32
6	14.49±1.85	3.30±0.64	0.82±0.05	1.50±0.02
12	15.50±1.07	3.43±0.30	0.92±0.00	1.48±0.08
24	14.04±2.18	3.01±0.01	0.99±0.07	1.28±0.01
Females				
0	10.48±0.33	2.55±0.11	0.69±0.01	1.18±0.04
6	10.61±0.77	2.48±0.17	0.79±0.18	1.27±0.25
12	11.71±0.41	2.50±0.05	0.88±0.05	1.10±0.00
24	10.91±0.47	2.67±0.16	0.83±0.01	1.06±0.05

Values are mean ± SEM; n=6

No significant differences were observed between the different treatments (p>0.05)

Results obtained in mice clearly demonstrated the safety of *A. platensis* extract. At a single dose up to 6 g kg^{-1} body weight (BW), it did not cause any toxicity. In comparison to our study, previous acute toxicity studies of whole *A. platensis* have revealed that the dried form of this microalga up to 3.5 g kg^{-1} BW, 800 mg kg^{-1} BW, and 10 g kg^{-1} BW is non-toxic to domestic fowl (Krishnakumari and Venkataraman 1981 as cited by Krishnakumari et al. 1981), rats (Krishnakumari et al. 1981), and mice (Hutadilok-Towatana et al. 2008), respectively.

During the 12-week subchronic toxicity test, the *A. platensis* extract suppressed the increase in body weight of male rats without affecting their water and food consumption. This effect has not been found before either in mice fed *Arthrospira maxima* for 13 weeks (Salazar et al. 1998) or in rats fed *A. platensis* for 12 weeks (Hutadilok-Towatana et al. 2008) and might not be specifically related to treatment since it was not dose-dependent. In spite of decreased body weight gain, however, their livers, kidneys, hearts, and spleens were not different in both final weights and morphology from the controls. Therefore, such decrease of body weight gain in male rats is unlikely to be resulted from the reduction of these four major internal organs.

Throughout the experimental period, all blood values obtained were within the normal ranges of rats (Casey and King 1980). Similar to previous subchronic studies (Salazar et al. 1998; Hutadilok-Towatana et al. 2008), our different results among groups were minor and, most importantly, were not dose-related. As a consequence, they were interpreted as biological variability normally found in rats rather than any treatment effects.

To date, hypocholesterolemic actions of *A. platensis* have been well-documented (Devi and Venkataraman 1983; Kato et al. 1984; Iwata et al. 1987; Nagaya et al. 1988; Ramamoorthy and Premakumari 1996; Colla et al. 2008). This inhibitory effect of *Arthrospira* (*Spirulina*) offers health benefit because of the connection between high blood cholesterol concentration and the incidence of cardiovascular disease (Grundy et al. 2004). It has been postulated that the blood cholesterol-lowering activity of *A. platensis* might be attributed to increased fecal excretion of cholesterol via inhibition of both jejunal cholesterol absorption and ileal bile acid reabsorption (Nagaoka et al. 2005). The major phycobiliprotein, C-phycocyanin, has recently been identified to play a crucial role in this action (Nagaoka et al. 2005). In our study, however, we did not observe any significant reductions of this blood lipid in *A. platensis* extract-treated rats relative to the control animals (Table 1). This discrepancy is still unknown but probably due to different experimental conditions. Obviously, the pronounced cholesterol reduction and related effects of this alga have been evident in subjects with high blood lipid levels (Devi and Venkataraman 1983; Kato et al. 1984; Iwata et al. 1987; Nagaya et al. 1988; Ramamoorthy and Premakumari 1996; Colla et al. 2008), and thus, hyperlipidemia may be a more sensitive experimental model in this regard. Although, *A. platensis* reduces hypercholesterolemia in cholesterol-fed rats, its non-polar components in either water-insoluble fraction or acetone extract does not elicit the same action (Hosoyamada et al. 1991; Nagaoka et al. 2005). Therefore, our negative results might be attributed to the high amount of these inactive compounds in the extract (not determined).

Throughout this study, the total WBC numbers were normal and lymphocytes were the major WBC populations as reported in literature (Matsuda et al. 2000). Peripheral blood lymphocytes of adult rats are predominantly of $CD4^+$ T cells with a high capacity for initiating antigen-specific immune responses (Tompkins et al. 1998). At the end of the treatment period, however, lymphocytes were increased in a similar manner among six experimental groups of both sexes, concurrently with decreased neutrophil and monocyte counts. The reason for such increase in lymphocyte values is still unknown but is unlikely to be age-related change in rats since the effect was not found in control and low-dose treated male groups. An elevation of this type of WBC normally occurs as a feature of viral infections (Miale 1982). In the present study, neither signs of infection nor spleen enlargement was observed in our experimental rats.

In conclusion, we have investigated both acute and subchronic toxicities of the methanol extract of *A. platensis*. Our results suggest that this extract at the doses tested did not cause any toxic effects to the experimental animals when administered orally. Therefore, the long history use of *Arthrospira* (*Spirulina*) without toxicity reports seems to be additionally supported by the data shown here.

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References

Becker W (2003) Microalgae in human and animal nutrition. In: Richmond A (ed) Handbook of microalgal culture, biotechnology and applied phycology. Blackwell, Oxford, pp 312–352

Belay A (2002) The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. J Am Nutraceut Assoc 5:27–48

Bruce RD (1985) An up- and down procedure for acute toxicity testing. Fundam Appl Toxicol 5:151–157

Casey JD, King DJ (1980) Clinical chemical values for some common laboratory animals. Clin Chem 26:1877–1879

Chamorro GA, Salazar M (1988) Dominant lethal assay of *Spirulina maxima* in male CD-1 mice after short-term and prolong-term feeding. J Food Prot 52:125–127

Chamorro G, Salazar M, Izquierdo E, Salazar S, Ulloa V (1985) Multi-generation study on reproduction and lactation in rats fed *Spirulina*. Arch Hydrobiol Beih 20:165–171

Chamorro GA, Herrera G, Salazar M, Salazar S, Ulloa V (1988) Short-term toxicity study of *Spirulina* in F3b generation rats. J Toxicol Clin Exp 8:163–167

Chamorro G, Salazar M, Pages N (1996) Dominant lethal study of *Spirulina maxima* in male and female rats after short-term feeding. Phytother Res 10:28–32

Chamorro G, Salazar S, Favila-Castillo L, Steele C, Salazar M (1997) Reproduction and peri- and postnatal evaluation of *Spirulina maxima* in mice. J Appl Phycol 9:107–112

Colla LM, Muccillo-Baisch AL, Costa JAV (2008) *Spirulina platensis* effects on the levels of total cholesterol, HDL and triacylglycerols in rabbits fed with a hypercholesterolemic diet. Braz Arch Biol Technol 51:405–411

Devi MA, Venkataraman IV (1983) Hypocholesterolemic effect of blue green algae Spirulina platensis in albino rats. Nutr Rep Int 28:519–530

Dillion JC, Phuc AP, Dubacq JP (1995) Nutritional value of the alga *Spirulina*. World Rev Nutr Diet 77:32–46

Grundy SM, Cleeman JI, Merz CN, Brewer HB, Clark LT, Hunninghake DB, Pasternak RC, Smith SC, Stone NJ (2004) Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment. Panel III Guidelines. J Am Coll Cardiol 44:720–732

Hosoyamada Y, Takai T, Kato T (1991) Effects of water-soluble and insoluble fractions of *Spirulina* on serum lipid components and glucose tolerance in rats. J Jap Soc Nutr Food Sci 44:273–277

Hutadilok-Towatana N, Reanmongkol W, Satitit S, Panichayupakaranant P, Ritthisunthorn P (2008) A subchronic toxicity study of *Spirulina platensis*. Food Sci Technol Res 14:351–358

Iwata K, Inayama T, Kato T (1987) Effects of *Spirulina platensis* on fructose-induced hyperlipidemia in rats. J Jpn Soc Nutr Food Sci 40:463–1467

Kato T, Takemoto K, Katayama H, Kuwabara Y (1984) Effects of *Spirulina* (*Spirulina platensis*) on dietary hypercholesterolemia in rats. J Jpn Soc Nutr Food Sci 37:323–332

Khan Z, Bhadouria P, Bisen PS (2005) Nutritional and therapeutic potential of *Spirulina*. Curr Pharm Biotechnol 6:373–379

Krishnakumari MK, Ramesh HP, Venkataraman LV (1981) Food safety evaluation: acute oral and dermal effects of the algae *Scenedesmus acutus* and *Spirulina platensis* on albino rats. J Food Prot 44:934–935

Mani UV, Iyer UM, Dhruv SA, Mani IU, Sharma KS (2008) Therapeutic utility of *Spirulina*. In: Gershwin ME, Belay A (eds) *Spirulina* in human nutrition and health. CRC Press, Boca Raton, pp 71–100

Matsuda H, Tanaka A, Itakura A (2000) Immunology and hematology. In: Krinke GJ (ed) The laboratory rat. Academic Press, London, pp 437–446

Miale JB (1982) Laboratory medicine: hematology. CV Mosby, St. Louis, pp 709–711

Miranda MS, Cintra RG, Barro SB, Manchini Fiho J (1998) Antioxidant activity of the microalga *Spirulina maxima*. Braz J Med Biol Res 31:1075–1079

Nagaoka S, Shimizu K, Kaneko H, Shibayama F, Morikawa K, Kanamaru Y, Otsuka A, Hirahashi T, Kato T (2005) A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of *Spirulina platensis* concentrate in rats. J Nutr 135:2425–2430

Nagaya N, Homma Y, Goto Y (1988) Cholesterol lowering effect of *Spirulina*. Nutr Rep Int 37:1329–1337

Ramamoorthy A, Premakumari S (1996) Effect of supplementation of *Spirulina* on hypercholesterolemic patients. J Food Sci Technol 33:124–128

Salazar M, Chamorro G (1990) Study of lethal dominant of *Spirulina maxima* in male rats. Sci Aliments 10:713–718

Salazar M, Chamorro GA, Salazar S, Steele CE (1996) Effect of *Spirulina maxima* consumption on reproduction and peri- and postnatal development in rats. Food Chem Toxicol 34:353–359

Salazar M, Martinez E, Madrigal L, Ruiz LE, Chamorro GA (1998) Subchronic toxicity study in mice fed *Spirulina maxima*. J Ethnopharmacol 62:235–241

Sánchez M, Bernal-Castillo J, Rozo C, Rodríguez I (2003) *Spirulina* (*Arthrospira*): an edible microorganism. A review. Univ Sci 8:7–24

Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K (1994) A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem 51:1780–1783

Tomaselli L (1997) Morphology, ultrastructure and taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira* (*Spirulina*) *platensis*. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. Taylor and Francis, London, pp 1–16

Tompkins AB, Hutchinson P, de Kretser DM, Hedger MP (1998) Characterization of lymphocytes in the adult rat testis by flow cytometry: effects of activin and transforming growth factor beta on lymphocyte subsets *in vitro*. Biol Reprod 58:943–951