



## Measuring Serum Toxicity Markers to Evaluate the Safety of Commercially Available Spirulina Products in Mice

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### Abstract

Spirulina a multicellular, blue-green alga has drawn attention as a viable food supplement due to its suitable nutrient composition, however, there is a dearth of information regarding its safety. This study aimed to measure the heavy metal concentrations in commercially available Spirulina products and evaluate the safety using the *in vivo* method. A total of 54 mice were randomly divided into three groups; Group 1 (n = 18) served as a control and received a basal diet. Group 2 (n = 20) served as a test and received Spirulina powder (15%) blended with a basal diet. Group 3 (n = 16) serves as a standard and received a basal diet supplemented with nutritional supplements. The findings showed that the concentration of serum aspartate aminotransferase, alanine aminotransferase, cystatin C, and troponin I after consuming the experimental diets was not statistically different between groups ( $p > 0.05$ ). The concentrations of mercury (0.000036 mg/kg), lead (0.0047 mg/kg), cadmium (0.00048 mg/kg), and arsenic (0.0046 mg/kg) were very little to cause toxic effect and the levels were below the European Communities Commission (EC) recommended maximum heavy metal levels in food stuffs. Therefore, consumption of Spirulina at a proportion of 15% does not exert any hepatic, renal, and cardiac toxicities in the mice. However, evaluating the safety of higher doses (> 15%) is required.



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### Introduction

Spirulina is a multicellular, photosynthetic, blue-green alga that grows naturally in the marine and

fresh water environment.<sup>1</sup> Spirulina is almost a complete functional food and dietary supplement, it contains almost all vital nutrients that are required

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for the healthy functioning of the body.<sup>2</sup> It has a high content of polyphenols, phytosterols, carotenoids, polysaccharides, lectins, mycosporine-like amino acids, halogenated compounds, polyketides, protein, polyunsaturated fatty acids, minerals, vitamins, and various other bioactive compounds.<sup>3-6</sup> Spirulina can be considered as one of the few sources of dietary polyunsaturated fatty acid  $\gamma$ -linolenic acid (GLA) after human milk; the unsaturated fatty acids, oleic and linoleic acids as well as the saturated fatty acids palmitic acids are other major fatty acids that Spirulina can offer to the human diet.<sup>4</sup> Due to its suitable nutrient composition, Spirulina is recently drawing attention to nutraceuticals, pharmaceuticals, food, and feed productions.<sup>7</sup>

Despite its suitable nutritional value, there are few safety concerns related to Spirulina consumption. Exposures during cultivation, processing, and packaging may cause contamination in the final Spirulina product.<sup>8</sup> In agricultural areas, heavy metal contamination is common due to the usage of certain pesticides and fertilizers that contain toxic metals.<sup>9</sup> Moreover, it is noted that spirulina can bind heavy metal ions from the water and embed them in the cell vacuole.<sup>10</sup> Pollutant and pesticide residues in the water may also be the cause of contamination.<sup>8</sup> The Dietary Supplements Information Expert Committee (DSI-EC) reviewed and analyzed recent regulatory and pharmacopeial sources, human clinical trials, and animal studies to assess the potential adverse effect of Spirulina. After reviewing this information, The DSI-EC concluded that the available evidence does not indicate the adverse effect of Spirulina on human health and other public health concerns.<sup>11</sup> However, there is still a limitation of information from animal and human studies concerning the adverse effects and safety of Spirulina consumption.<sup>12</sup> Hence, the aim of the present study was to measure the heavy metal concentrations in commercially available Spirulina products and evaluate the safety of Spirulina by using the *in vivo* animal model.

### Materials and Methods

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) Health Research Ethics Committee-KNCHREC approved

the protocols used in this study (approval number: KNCHREC00026).

### Spirulina Samples

Spirulina samples used in this study were obtained from local producers which are located at the shores of Lake Victoria, Kisumu County, Kenya, and Healthy U store. Spirulina was cultivated in a pond covered with a greenhouse. After the harvest, the biomass of Spirulina was dried and grounded to obtain powdered products.

### Experimental Animals

A total of 54 mice between the age group of 5-8 weeks and a bodyweight of 21-38 g were used in the study. Animals were caged individually at room temperature with 12 h of light and dark cycle. All mice had *ad libitum* access to feed and water. The mice were habituated to the experiment and housing condition (feeding and handling) for 3 days before starting the experiment.

### Feeding and Treatment

The mice were randomly divided into three experimental groups. Group 1 (n = 18) served as a control group and received a basal diet (a commercial layers feed composed of maize, wheat bran, fishmeal, groundnut cake) once a day for four weeks. Group 2 (n = 20) served as a test group and received Spirulina powder (15%) blended with a basal diet. Group 3 (n = 16) served as a standard group and received a basal diet supplemented with appropriate quantities of casein protein, calcium carbonate, iron sulfate, zinc sulfate, phosphate, retinol, folic acid, and cyanocobalamin to receive nutrient levels equivalent to test diet. The nutrient requirements of experimental mice and toxicities due to an overdose of some nutrients were considered when preparing the experimental diets.<sup>13-15</sup> Daily intake of feed was recorded during the experiment and the mice were weighed before and after the feeding experiment.

### Blood Sample Collection

The safety of consuming the experimental diets was evaluated based on serum level toxicity markers. Serum concentrations of toxicity markers were measured after the mice had been fed the experimental diets for four weeks. At the end of the experimental period, mice were anesthetized using

chloroform, and blood was collected by cardiac puncture using a 23G needle and placed in heparin tubes. The collected blood was allowed to clot in an upright position for 60 minutes at room temperature and centrifuged at 2500 rpm for 15 minutes. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), cystatin C, and troponin I was analyzed by ELISA (Elabscience, Houston, TX) assay kit as per the instructions. The blood level of toxicity markers was analyzed at the AFYAMAX polyclinic laboratory.

### Heavy Metal Analysis

The concentration of heavy metals in *Spirulina* was analyzed by following the previously published method.<sup>16</sup> For this, 10 mL of nitric acid was added to 10 g of powdered samples. The mixture was heated for 10 minutes using a block digester (Avishkar Int., India). After cooling, 5 mL nitric acid was added, heated again for 30 minutes, and the solution left as such for 10 min to cool. Then 2 mL distilled water, 3 mL hydrogen peroxide, and 2 mL hydrochloric acid were added and the mixture heated again for another 10 minutes. The solution was filtered using Whatman filter paper 1 and diluted to 100 mL using distilled water. Standard stock solutions were prepared by dissolving pure metals into solvents at the concentration of 1000 mg/L, working standard solutions were prepared by diluting the stock solution into five concentrations using solvents. Thereafter,

samples and standard solutions were injected into the atomic absorption spectrophotometer (Rayleigh WFX-210, China). Readings on atomic absorption spectrophotometer were done at different wavelengths (193.7, 228.8, 253.7, and 283.3 nm) for arsenic, cadmium, mercury, and lead respectively.

### Statistical Analysis

Data were analyzed using the statistical software IBM SPSS (23). Normal distribution was tested with the Kolmogorov–Smirnov test. One-way ANOVA (between-group variation) was used to evaluate statistically significant differences between groups at a significance level of 0.05.

### Results and Discussions

#### Bodyweight

The bodyweight of control, test, and standard group mice before the experiment was  $28.4 \pm 4.98$ ,  $26.6 \pm 4.37$ , and  $29.6 \pm 4.10$  respectively. At the end of the feeding experiment, the weight of control, test, and standard group mice was  $37.3 \pm 5.41$ ,  $36.3 \pm 5.03$ , and  $38.3 \pm 5.12$  respectively.

The weight of the mice as measured at the start and end of the experiment (Figure 1) was not varying significantly between the different dietary treatments group ( $p > 0.05$ ). However, at the end of the experiment, all mice had significant weight increments ( $p < 0.01$ ).

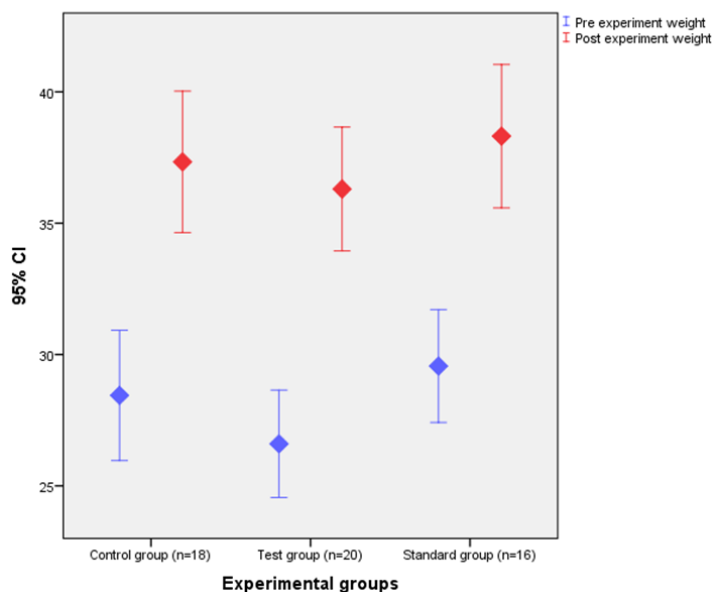
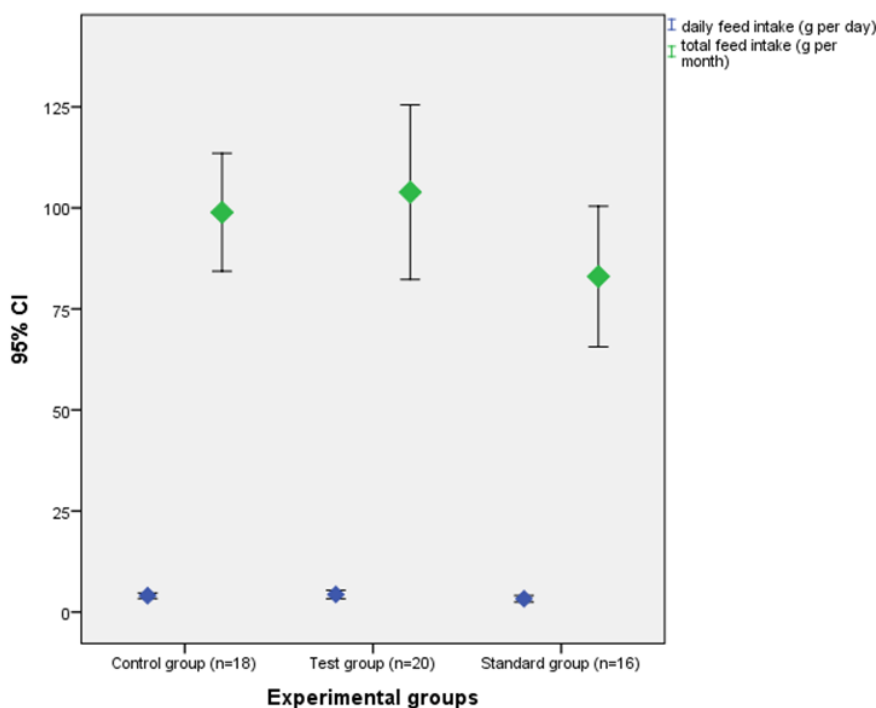


Fig. 1: Before and after experiment weight of control, test, and standard group mice

**Feed Intake**

The daily feed intake of control, test, and standard group mice was 4.00 + 1.4, 4.30 + 2.2, and 3.90 + 1.5 g respectively, which was not significantly different

(p > 0.05). Further, the total feed intake of control, test, and standard group mice during the experiment was 99 + 29, 104 + 46, and 83 + 32 g respectively, also not affected statistically (p > 0.05).



**Fig. 2: The daily and total feed intake of control, test, and standard group mice during the experiment**

**Table 1: Serum concentrations of AST, ALT, Cystatin C, and Troponin I from control, test, and standard group mice**

Toxicity marker	Control	Test	Standard	aNormalrange
<b>Mean (SD)</b>				
AST (U/L)	36 (0.70)	36 (0.11)	36.5 (0.14)	35-140
ALT (U/L)	12 (0.12)	12.1 (0.70)	12 (0.42)	10-35
Cystatin C (mg/L)	0.61 (0.28)	0.65 (0.21)	0.61 (0.11)	0.6-1
Troponin I (ng/mL)	0.004 (0.0028)	0.004 (0.004)	0.005 (0.004)	0-0.03

aLegacy laboratory services, 2020

**Serum Toxicity Markers**

After the mice had been fed for four weeks, a blood sample was taken and analyzed for toxicity markers to evaluate the toxic effect of consuming the experimental diets on the internal body organs

of the mice (Table 1). Statistical analysis showed that the serumAspartate aminotransferase (AST), Alanine aminotransferase (ALT), Cystatin C, and Troponin I concentrations was not significantly different between groups (p > 0.05). This indicated

that the consumption of the test diet does not exert any different effect on the internal organs of the mice than control and standard diets.

Although there is a lack of standardized values among laboratories to determine the elevated level of toxicity markers, commonly defined normal ranges such as 35-140 U/L for aspartate aminotransferase, 10-35 U/L for alanine aminotransferase, 0.6-1 mg/L for cystatin C, and 0-0.3 ng/mL for troponin I were used as a comparison.<sup>17</sup> The results showed that the concentration of serum toxicity markers measured in this current study was in agreement with the normal range. Further, the concentration of alanine aminotransferase found in this study was lower than 16.05 U/L and 16.7 U/L reported by<sup>18</sup> from the low and high dose of Spirulina consumption in rats respectively. Moreover,<sup>19</sup> evaluated the toxic effect of phycocyanin, a natural colorant from Spirulina consumption in rats, and reported no induce symptoms of toxicity nor mortality in rats.

#### Heavy Metal Concentrations

The safety issues regarding Spirulina consumption are mainly associated with its chemical composition. In this study, heavy metals concentration in Spirulina was measured as one of the safety parameters. Table 2 summarized the heavy metal concentrations in Spirulina and the recommended maximum heavy metal levels in foodstuff.

The findings showed that concentrations of mercury (0.000036 mg/kg), lead (0.0047 mg/kg), cadmium (0.00048 mg/kg), and arsenic (0.0046 mg/kg) measured in this study was below the European Communities Commission (EC) recommended maximum heavy metal levels of mercury (0.1 mg/kg), lead (0.1 mg/kg), cadmium (0.05 mg/kg), and arsenic (0.1 mg/kg) in foodstuff.<sup>20</sup> Spirulina samples used in this study were produced under a condition (green houses), this could explain the lower heavy metal concentrations in the analyzed Spirulina samples.<sup>16</sup> also reported little concentration of mercury (0.008 mg/kg), lead (0.109 mg/kg), cadmium (0.031 mg/kg), and arsenic (0.002 mg/kg) from Saudi Arabian Spirulina. Also<sup>9</sup> reported little concentration of arsenic (0.006-0.578 mg/kg), lead (0.1-1.206 mg/kg), cadmium (0.003-0.069 mg/kg) and mercury (0.002-0.028 mg/kg) was also reported from commercial Spirulina products. Furthermore, in the United States, the FDA (Food and Drug Administration) classified Spirulina as GRAS (generally recognized as safe). Moreover, according to the classification of the Center for Food Safety and Applied Nutrition (CFSAN), Spirulina biomass is classified as 'other dietary supplement without toxicological effects'. In addition to the United States FDA, Health Canada, Food Standards Australia New Zealand (FSANZ), and Brazilian Health Regulatory Agency (ANVISA) have granted permission for using Spirulina products as a novel food ingredient.<sup>21,22</sup>

**Table 2: Heavy metal concentrations in Spirulina and recommended maximum heavy metal levels in foodstuff**

Metal	mean (SD)	a Maximum level
	(mg/kg)	
Mercury	0.000036 (0.000008)	0.1
Lead	0.0047 (0.01)	0.1
Cadmium	0.00048 (0.0025)	0.05
Arsenic	0.0046 (0.002)	0.1

aSource: European Communities Commission (EC), 2006

#### Conclusion

The findings revealed that the concentration of heavy metals in the commercially available Spirulina products is very little to cause toxic effects on the consumers. The results showed that the concentration of serum aspartate aminotransferase,

alanine aminotransferase, cystatin C, and troponin I was not statistically different between control, test, and standard groups; this indicated that consumption of Spirulina does not exert any different effect on internal organs of the mice than other experimental diets. Further, based on toxicity markers analysis,

consumption of Spirulina at a proportion of 15% does not cause any hepatic, renal, and cardiac toxicities in the mice.

### Recommendation

Further studies evaluating the safety of high dose (> 15%) Spirulina consumption is required.

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### Conflict of Interest

The authors do not have any conflict of interest.

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