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[原著]

Safety evaluation of *Chlorogonium capillatum* (*Haematococcaceae*) as a potential food for 28 days in rats.

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Summary

The safety of the alga *Chlorogonium capillatum* as a human food source was evaluated in a 28-day oral subacute toxicity study using rats. The freeze-dried powder of *C. capillatum* was orally administer to male and female rats in dosage of 1000 mg/kg/day for a period of 28 days. Neither mortality nor changes in general condition was observed in either the treatment group or the control group throughout the 28-day administration period. For males of the *C. capillatum*-administered group, there was significantly decrease in weight at 14-day and 28-day. However, these changes were within the range of normality. In the hematological tests and serum biochemical tests performed at the time of completion of the end of the administration period, no influences of *C. capillatum* were observed. At autopsy, macroscopic observation of organs and tissues, and organ weight measurement at the end of the experimental period revealed no significant influences of *C. capillatum* feeding. In conclusion, considering the absence of adverse effects of *C. capillatum* in this 28-day oral subacute toxicity study, the non-observed adverse effect level (NOAEL) was estimated more than 1000 mg/kg/day of *C. capillatum* for both of male and female rats.

Keywords: *Chlorogonium capillatum*, human food, subacute toxicity

Introduction

The unicellular green alga *Chlorogonium* is an organism that has been successfully used as prey for cultivating various protists (Sakaguchi et al. 2002) and small copepod crustaceans (Kumar and Rao 1999). Recently, it was reported that *C. capillatum* was the suitable food for brine shrimp larvae (Nishida et al. 2023). The DMSO (Dimethyl sulfoxide) extract of *C. capillatum* enhanced NGF (nerve growth factor) and BDNF (brain-derived neurotrophic factor) secretion significantly in 3T3-L1 fibroblasts (not published). NGF and BDNF are

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important for the survival, maintenance, and regeneration of specific neuronal populations in the nervous system (Liao et al. 2015, Yanez et al. 2017, Zagrebelsky et al. 2018). Depletion of these neurotrophic factors has been linked with disease such as not only neurodegenerative diseases but also diabetic peripheral neuropathy (Sun et al. 2018). So, *C. capillatum* might be a candidate for preventive and/or therapeutic activities for neurodegenerative disease that could be of potential clinical interest.

The safety of *C. capillatum* was evaluated in an acute toxicity study with mice (not published). The LD50 (median lethal dose) value of *C. capillatum* was found to be a greater than 2000 mg/kg for combined male and female mice.

This report concerns a 28-day oral subacute toxicity study with rats, using *C. capillatum* powder as a dietary supplement.

Materials and Methods

Alga

Chlorogonium capillatum (NIES 3374) was obtained from Dr. Toshinobu Suzaki (Kobe University). Cells were grown in 0.1% KW21 (Daiichi Seimo Co. Ltd.) solution in 200L photo bio-reactor (Φ50cm) at a temperature of 25 ± 2 °C and continuous illumination of 200 µmol/ m^2/s.

The cells were harvested by centrifugation and immediately heat-treated at 90 °C for 7 minutes to inactivate chlorophyllase, and then dehydrated by freeze-drying. The algal powder was heat-treated at 90 °C for 12 hours. The content of existing pheophorbide and total pheophorbide in the dried alga was 84.4 mg% and 96.5 mg%, respectively (Furukawa et al. 2018). The composition of the dried alga was 42.7% protein, 8.9% lipids, 11.4% carbohydrate and 31.4% ash.

Animal experiments

Five-week-old male and female rats of the Sprague-Dawley strain (Jcl:SD) were purchased from CLEA Japan, Inc. and kept in our laboratory for one week before use. The animals were housed in an animal room with controlled temperature (23.0 – 25.9 °C) and relative humidity (48.5 – 67.5%) and were fed a normal diet (CE-2, FEED ONE Co. Ltd.).

Otsuka distilled water (Otsuka Pharmaceutical Factory Inc.) was used as the vehicle for the preparation of test article suspension. Weighed out, and diluted to 10% *C. capillatum* suspension was administered by oral gavage at a rate of 10 mL/kg body weight each day. Leftover test suspension was discarded.

The animals were divided into two groups of six males and six females. Test articles were administered by oral gavage at dosage level of 1000 mg/kg. The test continued for 28 days. Otsuka distilled water was given instead of the test compound as a control.

During the experimental period, each animal was observed daily, as a rule, for general condition and the occurrence of death. The body weight of each animal was measured weekly. The feed consumption during a three-day period was measured.

At the termination of the administration period, a blood sample was collected from each animal after fasting for one day. The blood specimens were analyzed for red blood cell count, hematocrit value, hemoglobin, platelet count and white blood cell count.

A portion of the blood sample was allowed to clot and was centrifuged to separate the serum. The serum thus obtained was subjected to the following measurements and tests (AST, ALT,

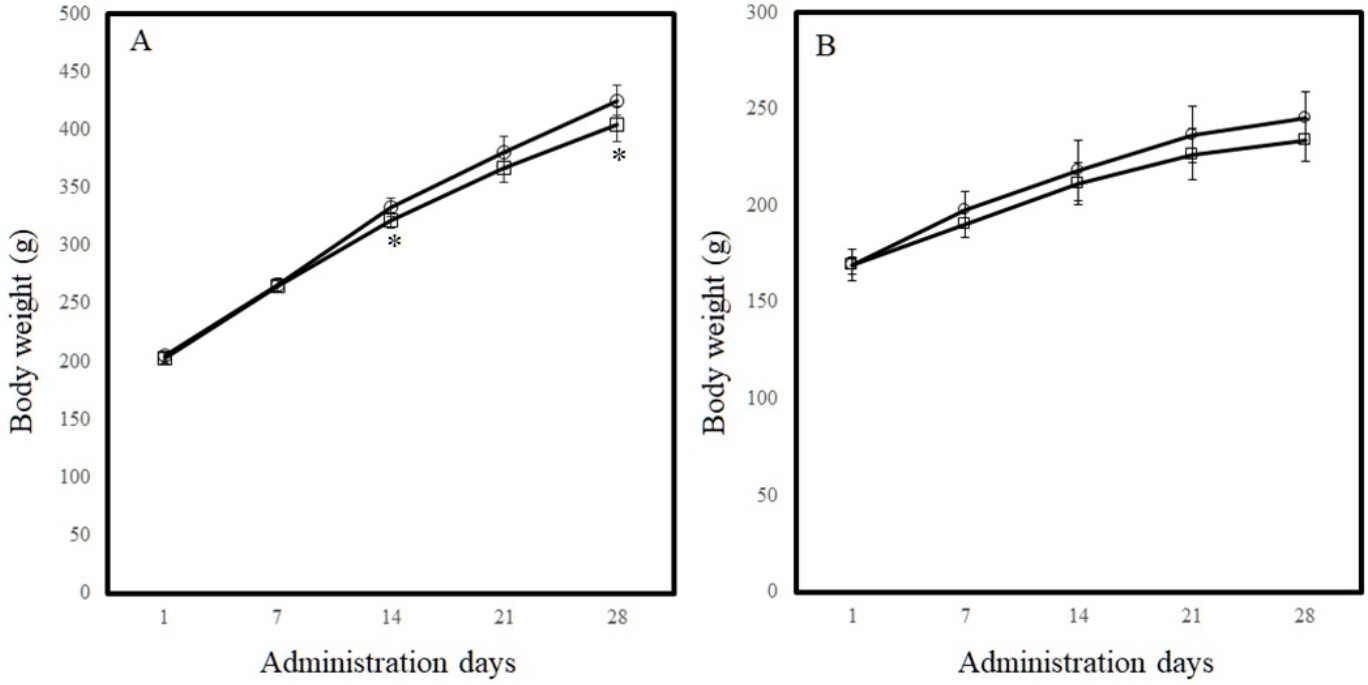

Figure 1 Body weight changes of male rats (A) and female rats (B) administered *C. capillatum* powder orally for 28 days.
Each point represents the mean ± SD of six rats.
○: control, □: *C. capillatum* treatment
*; P<0.05

ALP, γ-GTP, glucose, total cholesterol, triglycerides, total protein, A/G ratio, albumin, total bilirubin, blood urea nitrogen and creatinine.

One day after the termination of the administration period, the animals were autopsied. The following organs were isolated and weighed: heart, lungs, liver, spleen, kidneys, adrenals, testis and ovaries. The weight of each organ relative to the total body weight was calculated.

After the F-test, the T-test was used in the case of equal variance and Welch test was used in the case of unequal variance. P values < 0.05 were considered significant.

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Hokudo.

Results and Discussion

During the experimental period, no environmental factors other than the stated variables were thought to have affected the results of this study. No deaths occurred in males or females in either the control or the *C. capillatum*-administered group. The general condition of the animals in both groups and sexes was normal too. No symptoms of photosensitivity such as dermatitis were observed in any of the animals. The changes in the mean body weight are shown in Fig.1. For males of the *C. capillatum*-administered group, there was significantly decrease in weight at 14-day and 28-day. However, these changes were within the range of normality. For males receiving *C. capillatum*-administered group, there was significantly decrease in feed consumption between 17 and 20-day (data not shown). This change was within the range of normality. And it is conceivable that this decrease in feed

Table 1 Hematorogical parameters of rats administered *C. capillatum* powder orally for 28 days

Group	RBC (10^4/μL)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (%)	Platelet (10^4/μL)	WBC (10^3/μL)
Males								
Control	776 ± 24	15.3 ± 0.3	47.3 ± 1.3	61 ± 2	19.7 ± 0.5	32.3 ± 0.6	72.4 ± 7.3	7.10 ± 1.27
C. capillatum	762 ± 22	15.3 ± 0.2	47.2 ± 0.6	62 ± 2	20.1 ± 0.4	32.5 ± 0.5	74.5 ± 6.6	5.85 ± 0.58
Females								
Control	760 ± 28	14.9 ± 0.1	46.2 ± 1.3	61 ± 3	19.7 ± 0.6	32.4 ± 1.0	75.4 ± 15.4	4.31 ± 1.57
C. Capillatum	758 ± 30	14.8 ± 0.3	48.4 ± 0.9*	64 ± 2	19.6 ± 0.5	30.7 ± 0.5*	87.4 ± 6.0	4.21 ± 1.34

RBC : red blood cell, Hb : hemoglobin, Ht : hematocrit value, MCV : mean corpuscular volume, MCH : mean corpuscular hemoglobin, MCHC : mean corpuscular hemoglobin concentration, WBC : white blood cell
Values are mean ± SD (N = 6)
*; $P<0.05$

consumption may have affected weight change.

Table 1 shows the results of hematological tests for the males and females. For females receiving *C. capillatum*, hematocrit vale (48.4 ± 0.9 %) was significantly higher and MCHC (30.7 ± 0.5 %) was significantly lower. However, these parameters were within the range of normality (hematocrit vale; 46.2 – 51.6 %, MCHC; 30.1 – 33.4 %). So, it is thought that these changes were not an expression of toxicity due to *C. capillatum*, because these changes were not shown in the males. There were no significant differences in other hematological parameters between the control group and test group.

Table 2 shows the results of serum biochemical tests for the males and females. For males receiving *C. capillatum*, triglyceride (24 ± 8 mg/dL) and total protein (5.3 ± 0.2 mg/dL) were significantly lower, but these were within the range of normality (triglyceride; 17–90 mg/dL, total protein; 5.1 – 5.8 mg/dL). For females receiving *C. capillatum*, the ratio of A/G (0.84 ± 0.04) was significantly higher, but this parameter was within the range of normality (0.70 – 0.87).

Autopsy found no abnormalities in either males or females in any of the animal groups. The weight of each organ relative to the total body weight is summarized in Table 3. Increases in the relative weight of kidneys were significant in both of males (800 ± 57 mg/100g BW) and females (810 ± 48 mg/100g BW) receiving *C. capillatum*. However, it is thought that these changes were accidental phenomena, because these were within the range of normality (males; 657 – 871 mg/100g BW, females; 640 – 848 mg/100g BW). Over-all, these results do not appear to indicate any major toxicological effects in the animals fed *C. capillatum*.

The total profile was not completed in the present study and the acute toxicity study, and therefore human consumption of the alga should be approached with caution. Nonetheless, considering the large amount of *C. capillatum* consumed by the experimental rats, the non-observed adverse effect level (NOAEL) was estimated more than 1000 mg/kg/day of *C. capillatum*, and the absence of adverse effects in these animals, this 28-day oral subacute toxicity study may be indicative of the safety of *C. capillatum* for human consumption.

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Table 2 Serum biochemical parameters of rats administered *C. capillatum* powder orally for 28 days.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	γ-GTP (U/L)	Glucose (mg/dL)	T-Chol (mg/dL)	TG (mg/dL)	TP (g/dL)	A/G ratio	Albumin (g/dL)	T-Bil (mg/dL)	UN (mg/dL)	Creatine (mg/dL)
Males													
Control	182 ± 47	24 ± 5	169 ± 26	> 1	100 ± 16	54 ± 6	39 ± 12	5.5 ± 0.2	0.75 ± 0.03	2.3 ± 0.1	0.1 ± 0.0	18.2 ± 2.4	0.30 ± 0.01
C. Capillatum	177 ± 50	23 ± 3	150 ± 28	> 1	102 ± 20	52 ± 15	24 ± 8*	5.3 ± 0.2*	0.77 ± 0.02	2.3 ± 0.0	0.1 ± 0.0	17.3 ± 2.9	0.27 ± 0.04
Females													
Control	178 ± 20	23 ± 4	88 ± 27	> 1	83 ± 8	59 ± 10	10 ± 3	5.8 ± 0.2	0.77 ± 0.03	2.5 ± 0.2	0.1 ± 0.0	16.0 ± 3.7	0.29 ± 0.04
C. Capillatum	168 ± 21	18 ± 5	79 ± 14	> 1	87 ± 18	65 ± 7	12 ± 5	5.7 ± 0.1	0.84 ± 0.04*	2.6 ± 0.1	0.1 ± 0.0	15.6 ± 2.0	0.30 ± 0.02

TG : triglyceride, TP : total protein, T-Bil : total bilirubin, UN : urea nitrogen
Values are mean ± SD (N = 6)
*; P<0.05

Table 3 Relative organ weights (mg/100g BW) in rats administered *C. capillatum* powder orally for 28 days

	Heart	Lung	Liver	Spleen	Kidneys	Adrenals	Testes / Ovaries
Group							
Males							
Control	307 ± 13	382 ± 21	2936 ± 210	220 ± 20	718 ± 27	13.4 ± 1.5	972 ± 100
C. Capillatum	319 ± 13	394 ± 27	2964 ± 80	202 ± 14	800 ± 57*	14.1 ± 1.8	967 ± 90
Females							
Control	337 ± 17	489 ± 24	2945 ± 42	237 ± 26	749 ± 45	27.8 ± 5.6	44.1 ± 3.4
C. Capillatum	326 ± 15	502 ± 16	3011 ± 120	231 ± 25	810 ± 48*	30.9 ± 3.2	47.3 ± 7.6

Values are mean ± SD (N = 6)
*; P<0.05

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Chlorogonium capillatum (クロロゴニウム) のラットへの 28 日間連続投与による安全性評価

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要旨

緑藻 *Chlorogonium capillatum* の食用としての安全性について，ラットを使用した 28 日間の経口亜急性毒性試験にて評価した。 *C. capillatu*m の凍結乾燥粉末を雌雄のラットに 1000 mg/kg/day の用量で 28 日間連続経口投与した。 28 日間の投与期間を通じて，投与群，対照群ともに死亡例や全身状態の変化は観察されなかった。 *C. capillatum* を投与した雄グループでは，14 日目と 28 日目に体重が有意に減少した。しかし，これらの変化は正常の範囲内であった。 投与期間終了時に実施した血液学的検査および血清生化学検査では，*C. capillatum* の影響は認められなかった。 剖検時、器官および組織の肉眼的観察，および実験期間終了時の器官重量測定により，*C. capillatum* の投与による重大な影響は認められなかった。 以上，28 日間経口亜急性毒性試験において *C. capillatum* の有害作用がなかったことから，雌雄ラットにおける *C. capillatum* の無毒性量 (NOAEL) は 1000 mg/kg/day 以上と推察された。

キーワード：クロロゴニウム、食用、亜急性毒性試験