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Immune and allergenic effects of the microalga *Coccomyxa* sp. strain KJ in healthy humans: A pilot study

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Abstract

Background. The *Coccomyxa* sp. strain KJ (*Coccomyxa* KJ), a microalga found in Japan, has a potential function in controlling viral infections. Recently, its dry powder has been marketed as a health food product.

Objectives. This pilot study investigated the effects of *Coccomyxa* KJ powder tablet intake on allergic reactions and immune functions in healthy participants.

Materials and methods. Nine healthy volunteers (4 males and 5 females) who expressed interest in foods containing *Coccomyxa* KJ, and were willing to undergo blood tests, were recruited. Each individual was asked to take 2 *Coccomyxa* KJ powder tablets (0.3 g) before breakfast once a day for 4 weeks. The salivary immunoglobulin A (IgA) level and blood parameters (white blood cell (WBC) count, eosinophil and lymphocyte counts and percentages, natural killer (NK) cell activity, interleukin (IL)-6 level, and T helper (Th)1/Th2 cell ratio) were evaluated at baseline and weeks 2 and 4.

Results. The 4-week intake of *Coccomyxa* KJ did not affect salivary IgA levels, WBC count, eosinophil and lymphocyte counts and percentages, or the Th1/Th2 ratio. There were significant differences in the NK cell activity after 4 weeks, with an average increase of 11.78 (95% confidence interval (95% CI): 6.80–16.76). None of the patients experienced adverse reactions during or after the study.

Conclusions. Long-term *Coccomyxa* KJ intake improved NK cell activity without causing adverse effects on the indicators of local immunity, systemic inflammation and immune response balance. This study suggests that *Coccomyxa* KJ powder tablets can induce beneficial immune modifications without causing any adverse effects.

Key words: allergic reactions, immune functions, *Coccomyxa* sp. KJ

Background

Typical representatives of the genus *Coccomyxa* are 6–14 μm × 3–6 μm in size and are irregularly oval or spherical. Moreover, *Coccomyxa* species are characterized by a parietal chloroplast shape without a pyrenoid and the absence of flagella.[1,2] *Coccomyxa*-like organisms of the Trebouxiophyceae class are classified into 3 genera according to their morphology. Only species with massive and partially stratified mucus belong to the genus *Coccomyxa*.[3] In the class Trebouxiophyceae, the genus *Choricystis* represents a unique phylogenetic lineage; however, whether *Coccomyxa* and *Pseudokococcomyxa* are 2 distinct genera remains unresolved.[4] According to Jaag (1933), many strains are available in public culture collections.[1]

The genus *Coccomyxa* belongs to the green algae class Trebouxiophyceae and can be subdivided into the Chlorella, Oocystis and Trebouxia lineages using molecular approaches.[5,6] All known strains of *Coccomyxa* belong to the Elliptochloris clade.[7,8] *Coccomyxa* was the first terrestrial green alga to have a fully sequenced genome[7] and be classified by The National Center for Biotechnology Information (NCBI). The following species have been identified, named and registered: *C. melkonianii*, *Coccomyxa cf. olivacea 078*, *C. onubensis*, *C. parasitica*, *C. polymorpha*, *Coccomyxa cf.*, *C. vinatzeri*, and *C. viridis*. Moreover, many unclassified *Coccomyxa* are registered. *Coccomyxa* sp. strain KJ (IPOD FERM BP-22254) (hereafter referred to as "*Coccomyxa* KJ") is also registered in the NCBI.

The nucleotides, proteins, identical protein groups, and taxonomy of *Coccomyxa* KJ have been analyzed. The unicellular algae, belonging to the class Trebouxiophyceae, were found to be a different species from the previously identified *Coccomyxa*. *Coccomyxa* KJ, a microalga found in Japan, has been studied for the extraction and utilization of its intrinsic lipid components as a bioenergy source.[9–11] *Coccomyxa* KJ were isolated by Prof. Hideaki Miyashita in a Rural Biomass Research Project funded by the Ministry of Agriculture, Forestry and Fisheries of Japan.[12] *Coccomyxa* is a genus of algae, approx. 5 μm in size, that inhabits ponds and hot springs. *Coccomyxa* were cultivated in open ponds at a pH between 3.0 and 4.0 to minimize the chance of contamination with other phototrophs and protozoa. *Coccomyxa* KJ can store up to 30% oil and more than 50% protein when the culture conditions are controlled.[9] They can grow rapidly in minimal mineral media and accumulate triacylglycerols with lipid bodies at levels >60% of their dry weight (w/w) at the time of nitrogen decrease. Furthermore, *Coccomyxa* KJ has a high hydrocarbon production capacity and is capable of producing hydrocarbons when the ratio of nitrogen to dry weight is less than 2 wt%. Therefore, the hydrocarbon content per w/w unit of *Coccomyxa* KJ can be increased. It has been proposed to use the produced hydrocarbons as an alternative to fossil fuels (e.g., biodiesel fuel). However, replacing fossil fuels with hydrocarbons from microalgae requires lowering the cost of producing hydrocarbons. For this purpose, microalgae with higher hydrocarbon production capacity are required. *Coccomyxa* KJ is a unicellular green alga with very high triacylglycerol (TAG) productivity isolated from hot spring water (Japanese Patent Application Laid-Open No. 2015-015918), and can be cultured in an open-system culture (Japanese Patent Application Laid-Open No. 2014-117202).

The virucidal action of *Coccomyxa* KJ has been reported.[13–16] Supplementation of *Coccomyxa* KJ in the diet of mice facilitated the induction of neutralizing antibodies against the influenza virus and maintained the antibody titer.[13] Monogalactosyl diacylglyceride (MGDG) isolated from *Coccomyxa* KJ was able to inactivate clinical isolates of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a time- and concentration-dependent manner. Experimental results showed that *Coccomyxa* KJ helped to advance a potent virus-destructive action factor against SARS-CoV-2, which is an enveloped virus, by causing envelope damage that resulted in the loss of viral host-cell-binding ability. Of the total fatty acids in MGDG, α-linolenic acid (C18:3) accounted for about 72% and 7,10,13-hexadecatrienoic acid (C16:3) for 23%.[14] Moreover, MGDG from *Coccomyxa* KJ showed virucidal activity against herpes simplex virus type 2 (HSV-2), the pathogen that causes genital herpes. Physical changes in the shape of HSV-2 were observed after treatment with MGDG, and electron microscope evaluation revealed a decrease in particle size and possible damage to the viral envelope. Similar to the morphological findings, the viral particles lost their ability to bind to host cells. The HSV-2 treated with high concentrations of MGDG was not pathogenic in animal models, indicating that MGDG exhibits irreversible virus-killing activity against HSV-2 particles. In an animal model of genital herpes caused by HSV-2, mice treated intravaginally with MGDG exhibited prophylactic effects by suppressing viral yield and herpes lesion formation in the genital cavity, and had higher survival rates than solvent-treated control mice. Thus, *Coccomyxa* KJ provides a new prophylactic option against HSV infection. In addition, *Coccomyxa* KJ exhibits an inhibitory effect in viruses, such as norovirus, which have capsids on the external surface. In an animal model, *Coccomyxa* KJ-treated HSV-2 displayed envelope damage and no pathogenicity.

The viral suppression effects of *Coccomyxa* KJ on non-envelope viruses, such as feline calicivirus and murine norovirus, were demonstrated using animal models.[15] Studies in piglets also suggest that *Coccomyxa* may reduce viral infection.[16] The infiltration of chronic diseases that reduce productivity has become an issue in pig farming in Japan, and countermeasures against chronic diseases are being taken, such as improving sanitary environments, thorough cleaning and disinfection, and administration of vaccines and antimicrobial agents. On the other hand, research is being conducted to improve productivity in this area and promote pig farming that does not depend on antimicrobial

agents. Yamada et al. demonstrated the effects of intranasal administration of a polysaccharide solution of microalgae *Coccomyxa* extract (*Coccomyxa* solution) on antibody titers involved in respiratory disease in piglets.[16] Piglets treated with *Coccomyxa* solution showed a trend toward higher body weight ($p < 0.1$) at the 6th week (77–78 days old) and higher average daily body gain from 4 to 6 weeks compared to the non-treated group (control group). Antibody titers for mycoplasma pneumonia (MPS) tended to be higher in the control group than in the treated group ($p < 0.1$), and the positive rate was also higher, suggesting that intranasal administration of *Coccomyxa* solution may reduce MPS infection in piglets. Moreover, *Coccomyxa* sp. enhances antiviral activity, antitumor effects and immune function in animals.[14–18]

Coccomyxa KJ has recently gained recognition for inducing neuroprotective effects,[19] enhancing learning and memory,[20] and inhibiting benign prostate hyperplasia.[21] In addition, its ability to regulate the immune system has been shown. The rough polysaccharide isolated from *Coccomyxa* KJ regulated an immune response in chickens[21] and inhibited an inflammatory reaction in RAW 264.7 macrophages after the lipopolysaccharide stimulation. In a study using human leukocytes, a *Coccomyxa* KJ-immunostimulation mechanism was elucidated.[22] *Coccomyxa* KJ coordinated the differentiation of T cells into effector, memory and anergic T cells in response to *Staphylococcus aureus* superantigen infection. The effect of *Coccomyxa* KJ on superantigen-triggered immune responses was investigated. The results revealed that *Coccomyxa* KJ stimulated human peripheral blood-derived mononuclear cells in toxic shock syndrome 1 and moderately decreased the number of activated T cells. Furthermore, the inflammatory cytokine levels remained unchanged; however, the secretions of interleukin (IL)-1β, IL-17, IL-4, and IL-13 increased, while IL-2, tumor necrosis factor alpha (TNF-α), IL-18 and IL-10 decreased. When an immune response was not inhibited by Treg cells, *Coccomyxa* KJ reinforced the expression of the stem cells of T memory cell markers. Therefore, *Coccomyxa* KJ may improve the excessive activation and immunological inhibition of T cells in response to a superantigen by modulating the fate of T cells. According to the currently understood immune mechanism, *Coccomyxa* KJ may act on immune functions to suppress the growth of viruses and bacteria and some cases of cancer.

The demand for food supplements to improve health is increasing.[23] A study conducted by Food Supplements Europe demonstrated that their use has the potential to reduce the incidence of disease-related events and healthcare expenditures.[24] *Coccomyxa* KJ grows in a straightforward manner and has high nutritional value and a positive effect on the immune system. However, no data regarding the reaction of healthy individuals to *Coccomyxa* KJ administration exist. Furthermore, details concerning *Coccomyxa* KJ ingredients are still unknown.

Objectives

Recently, *Coccomyxa* KJ dry powder has been marketed as a health food product. Therefore, this pilot study aimed to evaluate the potential health hazards and effects of regular *Coccomyxa* KJ consumption over 4 weeks on the immune system of healthy individuals. In addition, the components of *Coccomyxa* KJ were analyzed.

Materials and methods

Participants

A poster recruiting volunteers for this study was published and could be accessed via the website of Tokai University Hospital (Isehara, Japan) from June to October 2020. We recruited healthy volunteers in their 30s and 40s who were willing to consume food containing *Coccomyxa* KJ powder tablets. The study involved 9 healthy Japanese adult volunteers (4 males and 5 females with a mean age of 38.2 years, an age range of 37–47 years, and a body mass index (BMI) of 20–25 kg/m^2) who were living healthy lifestyles. The exclusion criteria were as follows: smoking, use of antibiotics or pro/prebiotics (as a dietary supplement) within 6 weeks before the study, use of drugs that modify the composition of the gut microbiota (e.g., antidiabetic drugs, cholesterol-lowering drugs and proton pump inhibitors), use of laxatives within 4 weeks of the study, the presence of chronic or intestinal diseases, pregnancy, psychiatric problems, following a special diet (e.g., vegetarian, high-fiber or high-protein diets), and excessive alcohol consumption (over 20 g/day).

Ethics

The study design was explained to all healthy volunteers before participation in the study using a consent explanation form, and written consent was obtained. To protect the privacy and personal information of the volunteers, all data related to this study were anonymized; each volunteer was assigned a number in the order of their application for participation. The sex and age of the participants were recorded. No personally identifiable information was obtained, and numbers that could be linked to their names or identities were not used. The review board of Tokai University approved the study (approval No. 20R051), which followed the tenets of the Declaration of Helsinki.

Intervention

We asked the participants to take 2 *Coccomyxa* KJ tablets (0.15 mg/tablet) every day (before breakfast) for 4 consecutive weeks. The dosage was determined based on the quantity administered to mice in a previous study.[15] During

the intake period, the participants were asked to avoid dieting or overeating, i.e., deviating substantially from their lifestyle before participating in the study. Volunteers using foof supplements regularly were requested to stop taking them a month before taking the *Coccomyxa* KJ tablets.

Outcome measures

Saliva and blood tests were performed at 3 timepoints: before the start of the intake and at 2 and 4 weeks after the intake. Laboratory tests were performed, including general peripheral blood tests, white blood cell (WBC) counts, and eosinophil and lymphocyte counts, using the XE-2100™ Automated Hematology System (Sysmex, Kobe, Japan), as eosinophils reportedly reflect allergic changes.[25] To assess immune function and mucosal immunity, salivary immunoglobulin A (IgA) level was examined using immunonephelometry (JCA-BM8000 series; JEOL Ltd., Tokyo, Japan). In addition, the natural killer (NK) cell activity in the peripheral blood was determined using the ^{51}Cr release method in a Gamma Counter (PerkinElmer Inc., Waltham, USA). The T helper (Th)1/Th2 (interferon-gamma (IFN-γ)/IL-4/CD4) cell counts were determined with flow cytometry (FACSCanto II™; BD Biosciences, Franklin Lakes, USA). The IL-6 level was measured using an electrochemiluminescence immunoassay system (Lumipulse G1200; Fujirebio Co., Ltd., Tokyo, Japan) to determine if induction of inflammation is a potential adverse effect of the tablet.[26–30]

The participants were questioned every 7 days about their health condition (presence or absence of adverse effects and allergic reactions, including skin rash, anorexia, vomiting, diarrhea, and unpleasantness) to reveal any adverse reactions or health hazards associated with long-term *Coccomyxa* KJ tablet consumption. If adverse effects were observed, the participants were asked to immediately stop taking the tablet and inform us if they felt unwell or developed a rash during intake.

Statistical analyses

We investigated whether the measured values for each individual exhibited any changes before the consumption of the *Coccomyxa* KJ tablets and 2 and 4 weeks after their intake. The mean and standard deviation (M ±SD) were calculated by setting the difference in value before the intake and 2 weeks after the intake as Δ2w, before the intake and 4 weeks after the intake as Δ4w, and 2 and 4 weeks after the intake as Δ4–2w. We determined whether these values differed from 0 using a two-sided paired t-test with a significance level of 5%. In addition, the 95% confidence interval (95% CI) was calculated for these differences. When the Shapiro–Wilk test rejected the normality of data distribution, the Wilcoxon signed-rank test was used with a significance level of $\alpha = 0.05$ and a multiplicity Bonferroni correction.

Ingredient analysis of *Coccomyxa* KJ

Coccomyxa KJ ingredients were analyzed by Japan Food Research Laboratories (JFRL; Tokyo, Japan).

Results

Table 1 summarizes the salivary IgA level, WBC count, eosinophil count and percentage, lymphocyte count and percentage, NK cell activity, IL-6 level, and Th1/Th2 ratio before the start of the consumption of food containing *Coccomyxa* KJ, and at weeks 2 and 4 of its intake. Table 2 shows the results of the statistical analysis for Δ2w, Δ4w and Δ4–2w. Additionally, we determined the 95% CIs for the mean of these differences. Only data for which normality was rejected by the Shapiro–Wilk test are shown with medians and 1st and 3rd quartiles.

No significant difference was observed between the salivary IgA level (reference range: 110–410 mg/dL), WBC count (reference range: males: 3900–9800/μL; females: 3500–9100/μL), eosinophil count (70–440/μL) and percentage (0.0–6.0%), or lymphocyte count (630–5782/μL) and percentage (18.9–59.0%) before the start of intake and at weeks 2 and 4 of *Coccomyxa* KJ tablet intake.

No significant change was found in the NK cell activity at baseline (reference range: 18–40%) and at week 2 of *Coccomyxa* KJ tablet intake. However, significant differences were observed in NK cell activity at Δ4w ($p < 0.0167$), with a mean increase of 11.78 (95% CI: 6.80–16.76). Thus, the Δ4w NK cell activity was significantly upregulated, as determined using the paired t-test (Fig. 1). For the NK cell activity, normality was not rejected when analyzed with the Shapiro–Wilk test for the difference between paired data. However, normality was rejected for some differences in the paired data, such as lymphocyte percentage at Δ2w. Therefore, Wilcoxon's signed-rank test assessed all differences. Nonetheless, only Δ4w NK cell activity was significantly different, as shown by the paired t-test. Additionally, we determined the 95% CIs for the average of these differences.

No significant change in the IL-6 level (reference value: <7.0 pg/mL) was observed in the course of this study. Similarly, no significant changes were found in the Th0 IFN$^-$ IL-4$^-$ or TH0 IFN$^+$ IL-4$^+$ cell populations, or in the Th1/Th2 ratio before or during the 4-week intake of *Coccomyxa* KJ tablets. Moreover, none of the patients complained of illnesses during the intake period, and no adverse reactions, including allergic reactions, were observed. The results of the component analysis are shown in Table 3.

Discussion

The effects of *Coccomyxa* KJ consumption were examined in 9 healthy volunteers. The results of the blood tests at week 4

Table 1. Comparison between baseline and weeks 2 or 4 and between weeks 2 and 4 of *Coccomyxa* intake

Intake	WBC [/μL]	Eosinophil count [/μL]	Eosinophil percentage [%]	Lymphocyte count [/μL]	Lymphocyte percentage [%]	Salivary IgA level [mg/dL]	NK cell activity [%]	IL-6 [pg/mL]	Th1 cell count	Th2 cell count	Th1/Th2 ratio [%]	Th0 ($INFγ^-$, $IL4^-$)	Th0 ($INFγ^+$, $IL4^+$)
Before	5955.56 ±860.38	137.11 ±107.78	2.22 ±1.47	2220.56 ±359.30	37.56 ±5.70	14.79 ±5.70	34.44 ±6.83	0.67 ±0.28	20.17 ±7.03	2.57 ±0.67	8.37 ±3.51	75.03 ±7.77	2.21 ±0.93
Week 2	5755.56 ±1088.44	146.78 ±134.76	2.33 ±1.89	2119.22 ±329.45	37.44 ±5.14	13.84 ±5.07	36.78 ±8.47	0.63 ±0.18	21.21 ±8.08	3.67 ±1.22	6.13 ±2.29	72.04 ±9.97	3.07 ±1.63
Week 4	5822.22 ±1388.67	139.56 ±175.05	2.33 ±2.98	2093.78 ±333.88	37.67 ±8.86	15.93 ±4.67	46.22 ±9.43	0.66 ±0.31	21.16 ±8.67	3.31 ±1.30	7.24 ±3.43	72.43 ±10.57	3.11 ±1.42

Data are reported as mean ± standard deviation (M ±SD) (n = 9). WBC – white blood cell count; NK – natural killer; Th – T helper cell; INF – interferon; IL – interleukin; IgA – immunoglobulin A.

Table 2. Differences in the measured value for each individual between before the intake of *Coccomyxa* and 2 (Δ2w) and 4 weeks after the intake (Δ4w)

Interval	Summary of statistics	WBC [/μL]	Eosinophil count [/μL]	Eosinophil percentage [%]	Lymphocyte count [/μL]	Lymphocyte percentage [%]	Salivary IgA [mg/dL]	NK cell activity [%]	IL-6 [pg/mL]	Th1 cell count	Th2 cell count	Th1/Th2 ratio [%]	Th0 ($INFγ^-$, $IL4^-$)	Th0 ($INFγ^+$, $IL4^+$)
Δ2w	M ±SD	−200.00 ±487.62	9.67 ±111.88	0.11 ±1.79	−101.33 ±353.16	−0.11 ±5.88	−0.94 ±3.88	2.33 ±10.28	−0.03 ±0.20	1.04 ±1.67	1.10 ±1.24	−2.23 ±3.01	−2.99 ±2.65	0.86 ±0.91
	95% CI	(−597.56, 197.56)	(−81.55, 100.88)	(−1.35, 1.57)	(−389.26, 186.59)	(−4.90, 4.68)	(−4.11, 2.22)	(−6.05, 10.72)	(−0.20, 0.13)	(−0.32, 2.41)	(0.09, 2.11)	(−4.68, 0.22)	(−5.15, −0.83)	(0.11, 1.60)
	median	–	–	–	–	4.0	0.0	–	–	–	–	–	–	–
	1^{st} and 3^{rd} quartile	–	–	–	–	−6.0, 5.0	−0.2, 1.1	–	–	–	–	–	–	–
Δ4w	M ±SD	−133.33 ±985.45	2.44 ±197.41	0.11 ±3.41	−126.78 ±289.86	0.11 ±8.12	1.14 ±4.23	11.78 ±6.11*	−0.01 ±0.30	0.99 ±2.08	0.74 ±1.25	−1.12 ±3.88	−2.60 ±3.56	0.90 ±0.99
	95% CI	(−936.77, 670.10)	(−158.50, 163.39)	(−2.67, 2.89)	(−363.10, 109.54)	(−6.51, 6.73)	(−2.30, 4.59)	(6.80, 16.76)	(−0.25, 0.23)	(−0.70, 2.68)	(−0.27, 1.76)	(−4.28, 2.04)	(−5.50, 0.30)	(0.09, 1.71)
Δ4–2w	M ±SD	66.67 ±742.37	−7.22 ±119.46	0.00 ±2.21	−25.44 ±237.23	0.22 ±6.68	2.09 ±3.47	9.44 ±10.08	0.02 ±0.26	−0.06 ±2.21	−0.36 ±0.66	1.11 ±2.66	0.39 ±1.92	0.04 ±0.93
	95% CI	(−538.58, 671.92)	(−104.61, 90.17)	(−1.80, 1.80)	(−218.86, 167.97)	(−5.22, 5.67)	(−0.74, 4.92)	(1.23, 17.66)	(−0.19, 0.23)	(−1.86, 1.75)	(−0.89, 0.18)	(−1.06, 3.28)	(−1.17, 1.95)	(−0.71, 0.80)
	median	–	–	–	–	–	–	–	0.0	0.9	–	–	0.0	–
	1^{st} and 3^{rd} quartile	–	–	–	–	–	–	–	−0.1, 0.0	−0.8, 1.6	–	–	−0.7, 0.2	–

* $p < 0.05$ (significance $\alpha = 0.05$ using a paired t-test). Each test was conducted in triplicate; Bonferroni revision ($p < 0.0167$) was adopted. WBC – white blood cell; NK – natural killer; IL – interleukin; Th – T helper cell; 95% CI – 95% confidence interval M ±SD – mean ± standard deviation. Values with significant differences confirmed using the paired t-test and Wilcoxon's signed-rank test are indicated by an asterisk beside the M ±SD.

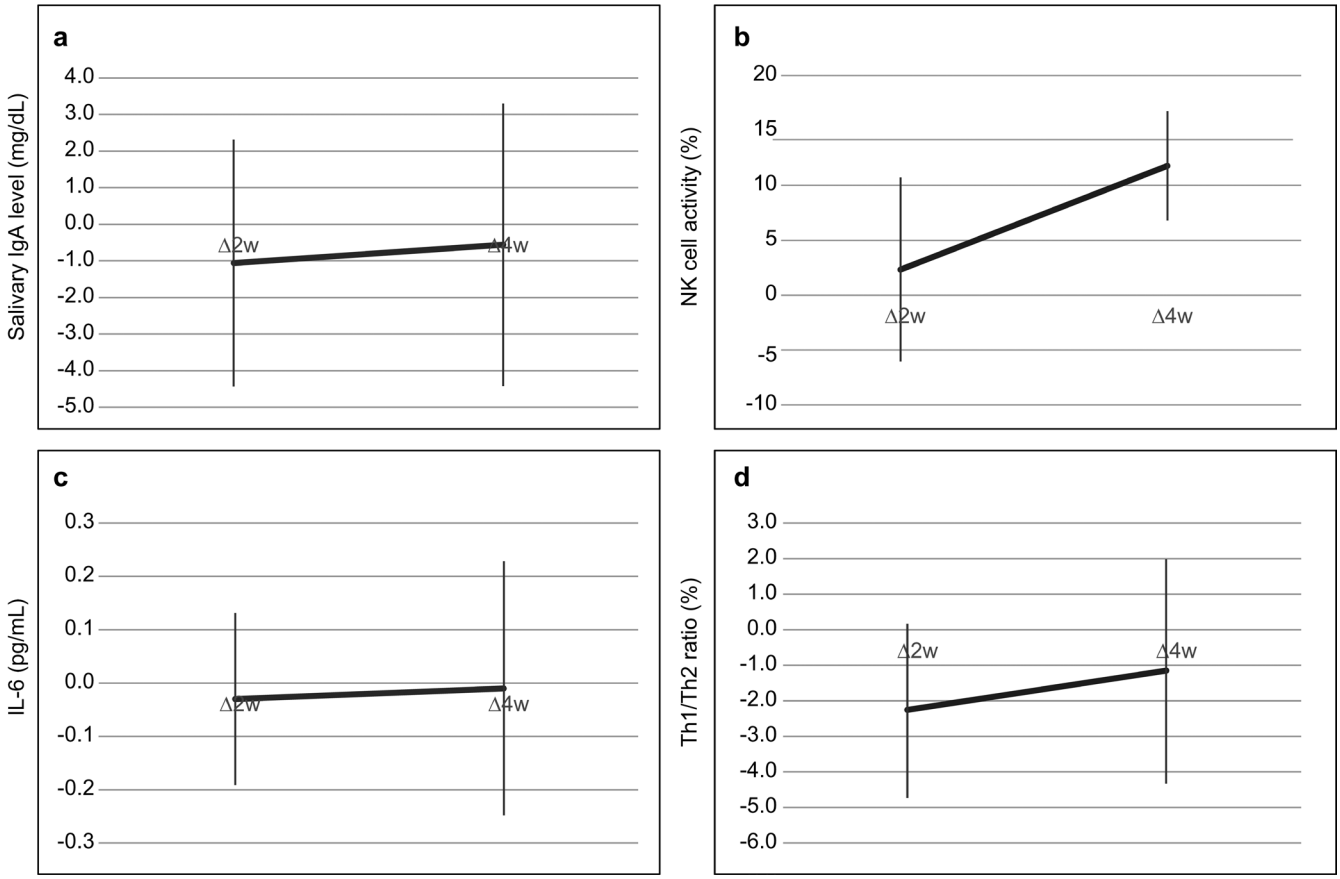

Fig. 1. Plot of 95% confidence interval (95% CI) of the mean for the difference between the 2 timepoints of *Coccomyxa* KJ intake. The 95% CI of the mean for the differences in the salivary immunoglobulin A (IgA) level (A), natural killer (NK) cell activity (B), interleukin (IL)-6 level (C), and T helper cell (Th)1/Th2 ratio (D) were shown. Only NK cell activity after 4 weeks (Δ4w) showed a significant increase in *Coccomyxa* KJ intake

showed upregulated NK cell activity, indicating an improved immune function, and increased attacking cancer cells and virus-infected cells.[26] Natural killer cells play an essential role in immunological surveillance, infection prevention and immune function regulation, and are therefore regarded as immune function indicators.[29] Thus, our findings suggest that long-term *Coccomyxa* KJ intake may help prevent viral and bacterial infections as well as cancer. In contrast, the WBC count, eosinophil count and percentage, lymphocyte count and percentage, Th1/Th2 ratio, and salivary IgA level remained unchanged throughout the 4 weeks of *Coccomyxa* KJ tablet intake. No allergic changes were suspected from the long-term intake. Thus, the results of the salivary IgA and IL-6 analysis implied no adverse effects on mucosal immunity or the systemic inflammatory response.[25,29]

The Th1 cells are responsible for cellular immunity via T-cell activation and enhancement of cytotoxic activity, whereas Th2 cells are involved in B-cell activation and humoral immunity. The immune function is normally regulated via the Th1/Th2 balance in living organisms.[30] Disruption of this balance may cause various diseases, such as cancer and allergies, due to the decrease in antitumor activity and excessive IgE production. Therefore, by measuring the Th1/Th2 balance, the quality of the body's immune response can be estimated in the context of these conditions. Our results suggest that long-term intake of *Coccomyxa* KJ may improve NK cell activity without affecting local immunity, causing systemic inflammation or damaging the immune response balance. *Coccomyxa* KJ exerts antiviral and anti-inflammatory effects during viral infections.[13–16] However, the *Coccomyxa* KJ components responsible for these beneficial effects have not yet been indentified and require further investigation. Nonetheless, according to the component analysis, *Coccomyxa* KJ contains many nutrients and may be suitable for human consumption with many advantages when used as a food.

Limitations

This pilot study was conducted before initiating a large-scale study. The number of healthy volunteers was small, and the search for indicators of allergic reactions and immune function was limited. In addition, healthy volunteers were only adults in their 30s and 40s to minimize potential bias. This age range was selected as most adults in their 20s are not particularly concerned about their health, and only a few show interest in healthy food.[31] Also, many people in their 50s and above regularly use medications or supplements, which could interfere with the results. Therefore, adults in their 30s and 40s with no underlying diseases and an interest in health maintenance were targeted.

Table 3. Component analysis of *Coccomyxa* sp. strain KJ

Main substances	Nutrition composition	Value
Basic ingredients (6)	water (g/100 g)	2.9
	protein (g/100 g)	54.9
	fat (g/100 g)	15.7
	ash (g/100 g)	5.2
	carbohydrate (g/100 g)	21.3
	sodium (mg/100 g)	47.3
Amino acids (20)	threonine (g/100 g)	2.24
	lysine (g/100 g)	2.56
	histidine (g/100 g)	1.03
	phenylalanine (g/100 g)	2.38
	tryptophan (g/100 g)	0.87
	leucine (g/100 g)	3.92
	isoleucine (g/100 g)	1.96
	methionine (g/100 g)	0.9
	valine (g/100 g)	2.66
	alanine (g/100 g)	3.6
	glycine (g/100 g)	2.62
	proline (g/100 g)	2.18
	glutamic acid (g/100 g)	5.4
	serine (g/100 g)	2.02
	tyrosine (g/100 g)	1.65
	aspartic acid (g/100 g)	4.56
	cystine (g/100 g)	0.5
	arginine (g/100 g)	3.65
	hydroxyproline (mg/100 g)	90
	GABA gamma-aminobutyric acid (mg/100 g)	795
Minerals (9)	phosphorus (mg/100 g)	472
	iron (mg/100 g)	128
	calcium (mg/100 g)	71.2
	potassium (mg/100 g)	1450
	magnesium (mg/100 g)	149
	copper (mg/100 g)	2.05
	zinc (mg/100 g)	9.27
	manganese (mg/100 g)	3.75
	selenium (µg/100 g)	8
Vitamins (13)	vitamins A (mg/100 g)	4.84
	vitamins B1 (mg/100 g)	0.54
	vitamins B2 (mg/100 g)	3.59
	vitamins B6 (mg/100 g)	1.46
	vitamins B12 (µg/100 g)	2.7
	vitamins C (mg/100 g)	216
	vitamins E (mg/100 g)	20.5
	vitamins K1 (mg/100 g)	0.163
	folic acid (mg/100 g)	4.1
	pantothenic acid (mg/100 g)	1.41
	biotin (mg/100 g)	0.154
	inositol (mg/100 g)	71
	niacin (mg/100 g)	23.5
Unsaturated fatty acids (10)	myristoleic acid	qualitative analysis
	palmitoleic acid	qualitative analysis
	oleic acid (g/100 g)	0.92
	eicosenoic acid (g/100 g)	0.16
	hexadecadienoic acid (g/100 g)	0.14
	linoleic acid (g/100 g)	1.02
	eicosadienoic acid (g/100 g)	0.01
	hexadecatrienoic acid (g/100 g)	1.49
	linolenic acid (g/100 g)	3.53
	11,14,17-eicosatrienoic acid (g/100 g)	0.01
Others (9)	polyphenols (g/100 g)	0.42
	spermidine (mg/100 g)	17
	α-carotene (mg/100 g)	5.8
	β-carotene (mg/100 g)	55.2
	lutein (mg/100 g)	178
	zeaxanthin (mg/100 g)	36
	stigmasterol	qualitative analysis
	campesterol	qualitative analysis
	β-sitosterol	qualitative analysis

Conclusions

Coccomyxa KJ intake significantly upregulated NK cell activity, thereby improving immune function. No adverse reactions occurred during the intake period. To identify the specific effects of *Coccomyxa* KJ, a comparative study between placebo and intake groups with a larger number of healthy volunteers from different age groups is required.

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