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Anti-fatigue effect of OM-X, fermented plant extract with lactic acid bacteria and bifidobacteria: A randomized, placebo-controlled, double-blind, comparative study

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ABSTRACT

The objective of this study is to elucidate how the OM-X made from many kinds of plants fermented using lactic acid bacteria (LAB) and bifidobacteria contributes to anti-fatigue. A randomized, placebo-controlled, double-blind, comparative study design was adopted. We investigated the effects of 12-week ingestion of the test food or placebo. Visual analogue scale (VAS) and Chalder fatigue scale (CFS) were examined to evaluate the feeling of fatigue, and diacron-reactive oxygen metabolites (d-ROMs) and Biological Antioxidant Potential (BAP) were measured to appraise comprehensive antioxidative ability. We also evaluated the safety of the food. As a result, significant differences between the two groups were detected in VAS and CFS. No safety-related matter occurred. It turned out that the ingestion of OM-X improved feelings of fatigue for healthy people with temporary fatigue. Additionally, there was no adverse effect by the ingestion of the food.

1. Introduction

We often feel fatigued in our daily lives. Fatigue indicates conditions of lack of energy, low vitality, decreased motivation, etc., so that it affects the amount of daily activity considerably. On the other hand, the constitution of the WHO defines it as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (World Health Organization, 1948). In other words, feeling tired means not to be sick, but far from being healthy. Fatigue is known to be caused by oxidation (Lee et al., 2018) and decreased liver function (Swain, 2006), and functional food ingredients are attracting attention for prevention and treatment. In recent studies, traditional plants (Lee et al., 2015), green tea (Murase et al., 2005), astaxanthin (Ikeuchi, Koyama, Takahashi, & Yazawa, 2006), and fucoidan (Chen et al., 2015) have demonstrated beneficial effects in the suppression of fatigue accumulation.

Furthermore, in recent years, the beneficial effects of fermented foods on health have been attracting scientific attention. The compounds produced by the bacteria responsible for fermentation are well known to have many health benefits, including antioxidant and anti-inflammatory effects (Şanlier, Gökcen, & Sezgin, 2019). OM-X is a fermented dietary supplement that was developed by Dr. Iichiroh Ohhira, which has been sold not only in Japan but also in Southeast Asia, Europe, and the United States for more than 30 years. It is made from many kinds of vegetables, fruits, seaweeds, and mushrooms fermented using 12 strains of LAB and bifidobacteria. Nutritionally, OM-X provides carbohydrates (oligosaccharides and dietary fiber), fats (short-chain fatty acids), proteins, amino acids, some vitamins, minerals, and polyphenols. In human clinical studies, OM-X has shown beneficial effects on bone health (Kawakami et al., 2003), oral ulcerations (Hashim, Rahman, & Philip, 1999), and athletic performance (Kawakami et al., 1998). Therefore, in the current study, we report on a 12-week long-term intake, a randomized, placebo-controlled, double-blind, comparative study to examine how OM-X affects people's fatigue.

Abbreviations: LAB, lactic acid bacteria; CFS, chalder fatigue scale; VAS, visual analogue scale; d-ROMs, diacron-reactive oxygen metabolites; BAP, biological antioxidant potential; TH10, *Enterococcus faecalis* TH10; FOSHU, food for specified health uses; ATP, adenosine triphosphate; ROS, reactive oxygen species.

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2. Materials and methods

2.1. Study design

A randomized, placebo-controlled, double-blind paralleled design was implemented from August to November 2021 at Japan Clinical Trial Association; JACTA, Tokyo. This trial was registered at UMIN Clinical Trial Registry as UMIN000045191.

2.2. Participants

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who enrolled in the monitor bank of InCROM Inc. (Nishiwaki, Hyogo), recruited from July through August 2021.

The inclusion criteria were as follows: (1) Healthy Japanese males and females from 40 to 59 years of their age; (2) Subjects aware of temporary fatigue. The exclusion criteria were as follows: (1) Subjects with chronic fatigue or diagnosed chronic fatigue syndrome; (2) Subjects with serious cerebrovascular disease, heart disease, liver disease, renal disease, gastrointestinal disease, psychiatric disease, or designated infectious diseases; (3) Subjects receiving treatment (hormone replacement therapy, drug therapy, exercise therapy, diet therapy, etc.) or those who are judged to be need of treatment at a time of receipt of informed consent. (4) Subjects with continuous usage of supplements and/or functional foods affecting fatigue, including food for specified health uses (FOSHU); (5) Subjects with drugs or food allergies; (6) Subjects with a history of alcohol dependence or drug dependence; (7) Smoker[1]; (8) Pregnant, or those who plan to become pregnant during the study, or with breastfeeding; (9) Subjects who have participated in other clinical trials 1 month prior to receipt of informed consent, or those who plan to enroll in other clinical trials during this trial; (10) Subjects who plan to vaccination for COVID-19 within 7 days before the visit; (11) Subjects who are judged as unsuitable for the study by the principal investigator.

2.3. Randomization

According to inclusion/ exclusion criteria, 40 subjects were selected and sequentially allocated to two groups by 20 each using a random number table. Among those, 2 of the Group A declined to participate in the study, and remained 38 subjects launched allocated intervention. In the process of assignment by the person in charge, background factors such as gender and age were taken into consideration to avoid biased distribution. The allocation list was sealed and strictly controlled until the end of the study. Subjects in Group A received the test sample (Active) and subjects in the Group B received Placebo. As shown in Fig. 1.

2.4. Test foods

The test food, OM-X is made of the fermented extract, which consists of several kinds of Japanese plants with 12 kinds of LAB and bifidobacteria such as TH10. Fulvic acid, D-amino acids, L-amino acids, melanoidin, polyphenols, organic acids, fatty acids, vitamins, minerals, dietary fibers, oligosaccharides, LAB, and bifidobacteria are included in the Active. The placebo was made chiefly from safflower oil. To evaluate the effectiveness of OM-X as a whole, we did not add neither fermented extracts nor LAB as base ingredients for OM-X. The amount of daily intake was 3 softgel capsules (1 capsule weighs 580 mg). Both Active and placebo, which were prepared by BIOBANK Co., ltd., were indistinguishable in shape, color, or taste, and were managed by identification marks, thus all involved were blinded. Table 1-1 and Table 1-2 show the ingredients contained in OM-X production and the composition of organic acids and carbohydrates in OM-X.

2.5. Experimental procedures

Subjects in Group A ingested Active and those in the other Group B took in Placebo, both had 3 softgel capsules once a day for 12 weeks. All subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyle and habits; to avoid excessive amounts of food, drink, or alcohol; to avoid any medicine or FOSHU (food for specified health uses) that aimed at anti-fatigue; to maintain a daily record of their physical condition, sleeping, appetite, and exercise; and to send the diary once a week to the study coordinator.

2.6. Outcome

The objective of this study was to elucidate the improvement of a feeling of fatigue by the ingestion of the food made of fermented plant extract with LAB and bifidobacteria. To evaluate this objective, VAS (Visual analog scale), Chalder fatigue scale (CFS) were measured as the primary outcome. Furthermore, as the secondary outcomes, d-ROMs and BAP test by blood, and safety of the food were determined. These measurement outcomes except safety evaluation were conducted upon pre and post-intervention.

2.6.1. VAS

After visiting examination room, the subject sat in a chair and indicated the degree of fatigue in these 7 days by a x-mark on the horizontal line with a length of 10 cm written on the VAS paper on the desk, which line meant that the left end was “the best feeling without feeling tired at all” and the right end was “the worst tired enough to do nothing”. The distance from the left end to the x-mark (unit: cm, up to 5/100) was measured and evaluated as a VAS score. In each VAS exam, we adopted an independent entry method that marked without looking at the previous record.

2.6.2. CFS

Fatigue level was evaluated using the Chalder fatigue scale (CFS) (Chalder et al., 1993) Japanese version. This scale consists of 14 factors of physical fatigue and mental fatigue, and adopted Likert-scale from none (1 point) to very high (4 points), 56 points of full points.[2] The higher the score, the higher fatigue level.

2.6.3. d-ROMs and BAP

The d-ROMs (diacron-reactive oxygen metabolites) and the BAP (Biological Antioxidant Potential) were measured from subject’s blood. The d-ROMs can evaluate oxidative stress inclusively in the living body by assaying the concentration of hydroperoxide (ROOH) in the blood (Seki, 2009). The BAP evaluates antioxidant capacity by assaying the reducing ability, that antioxidants in plasma donate electrons to active oxygen to prevent the oxidation reaction (Dohi et al., 2005). Both d-ROMs and BAP were measured using an active oxygen/ free radical automatic analyzer (free radical analytical system: FRAS, Wismerll Company Limited). In addition, the modified BAP/ d-ROMs ratio was calculated to appraise antioxidative ability of the living body comprehensively (Nagata et al., 2008) [15]. The modified BAP/ d-ROMs ratio represents the balance of the antioxidation, which is BAP divided by d-ROMs and further divided by coefficient of 7.541. The higher this value, the more defensive from oxidative stress (Nagata et al., 2008).

2.6.4. Safety

The safety of the test food was evaluated utilizing a written questionnaire during the study. Any untoward medical occurrence during the study period in participants has defined an adverse event; it does not

[1] Those who have smoked in the last year.

[2] The original CFS adopts 0–3 point of Likert-scale, 42 points of full points.

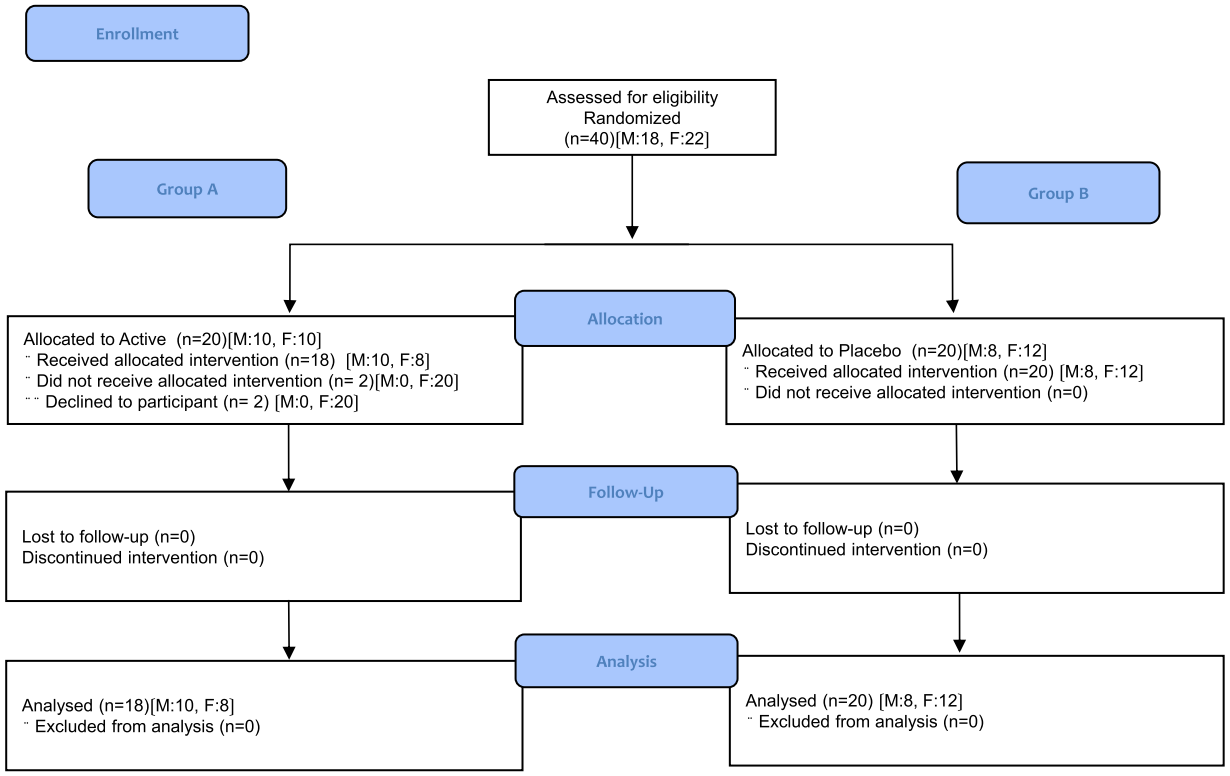

Fig. 1. Flow diagram of subject disposition.

Table 1-1
Composition of food materials of making OM-X.

	%
Fruits; Prune. fig, Chinese bayberry, Chinese matrimony, Blueberry, Yuzu (lomon)	28.6
Vegetables; Japanese mugwort, Komatsuna	8.6
Mushrooms; Shiitake mushroom, Maitake (dancing mushroom), Agaricus mushroom	4.1
Seaweeds; Wakame (brown seaweed), Hijiki seaweed, Konbu (Kelp)	2.7
Sugar	55.4
LAB; *Bifidobacterium breve ss. Breve, Bifidobacterium infantis ss. Infantis, Bifidobacterium longum, Enterococcus faecalis, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei ss. casei, Lactobacillus fermentum, Lactobacillus helvericus ss. jugurti, Lactobacillus plantarum, Streptococcus thermophillus*	0.6

Table 1-2
Composition of organic acids and carbohydrates in OM-X.

	g/100 g
Citric acid	0.18
Lactic acid	0.11
Malic acid	0.10
Formic acid	0.06
Acetic acid	0.05
Succinic acid	0.02
Propionic acid	N.D.
Butyric acid	N.D.
Glucose	8.87
Fructose	6.46
Galactose	0.52
Mannose	0.38
Isomaltose	0.21
Panose	0.11

necessarily have to have a causal relationship with the test food. And the principal investigator determined the relations between cause and consequence of the adverse events.

2.7. Data analysis

An ITT analysis was adopted and no sample size was used. All statistics were expressed as mean ± SD. Chi-square test and Student's *t*-test were used to compare the subject's backgrounds between groups. As for measured value, the paired *t*-test was used for intragroup analysis, and Student's *t*-test was used for intergroup comparisons of changes from the baseline (⊿0-12w). Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. Statistical analyses were performed using Excel Tokei 3.23 (BellCurve) and Statcel 4 (Yanai, 2015). The results were considered significant at a < 5 % level in the two-sided test.

3. Results

3.1. Participant demographics

The 38 subjects made a start with assigned ingestion. None of the subjects in both groups dropped out, and all of 38 completed the study. The test food intake rates were 100 % and 100 %, respectively. Thus, data obtained from 38 subjects (Group A; 18, Group B; 20) were used for efficacy analysis (Fig. 1). The subjects were 41–58 y.o., with an average of 50.6 ± 5.0 years. There were no significant differences in gender ratio, age, and CFS at baseline among groups (Table 2).

3.2. Test results

Table 3 indicates the results of measurement. As for VAS, the intragroup analysis in Active showed significant differences, though the Placebo depicted no significant difference. Further, there was a

Table 2
Subject demographics.

Item	Unit	Group A	Group B
Subjects*	number	18	20
Male: Female*	number	10:8	8:12
Age*	year	49.4 ± 5.0	51.7 ± 4.8
CFS*	point	15.8 ± 9.0	16.3 ± 4.8

Values are expressed as the mean ± SD.
* no significant difference.

Table 3
Test results.

outcome	Unit	amount of change (⊿0-12w)[1]		p-value [2]
		Active (n = 18)	Placebo (n = 20)	
VAS	score	−2.41 ± 2.45 **	0.10 ± 2.58	0.004 ##
CFS	point	−2.2 ± 2.5 **	−0.2 ± 3.2	0.043 #
d-ROMs	U.CARR [3]	−20.9 ± 109.1	5.3 ± 125.8	0.499
BAP	μmol/l	103.3 ± 417.1	−230.5 ± 970.3	0.185
modified BAP/ d-ROMs ratio	ratio	4.25 ± 15.23	0.15 ± 5.14	0.264

Values are expressed as the mean ± SD.
[1] ** $p < 0.01$ against pre-ingestion.
[2] # $p < 0.05$, ## $p < 0.01$ between-group difference.
[3] an arbitrary unit (1U.CARR = 0.08 mg/ 100mlH_2O_2).

significant difference between the two groups. CFS indicated significant differences within Active, and in intergroup analysis among Active and Placebo. There were no significant differences in both intra and intergroup comparisons of the d-ROMs, the BAP, and the modified BAP/ d-ROMs ratio.

3.3. Adverse event

No adverse events associated with the test food were observed in the course of the reporting.

4. Discussion

We conducted a randomized, placebo-controlled, double-blind, comparative study for examining the effect of the OM-X made from many kinds of plants fermented using LAB and bifidobacteria on feelings of fatigue. The ingestion of 3 softgel capsules of the OM-X improved the VAS and CFS significantly. Additionally, the comprehensive antioxidative ability (modified BAP/ d-ROMs ratio) increased, although there was no significant difference between the groups. Furthermore, no safety-related matter occurred during the test period.

The Japanese society of fatigue science defines “fatigue as a state of decreased physical activity with peculiar discomfort and desire for rest, caused by excessive physical and mental activity, or illness” (Japanese society of fatigue science, 2011). In other words, fatigue means a decrease in activity ability caused by overloading on mind and body, and that indicates symptoms such as decreased thinking ability, reduced response to stimuli, impaired attention, distraction, slow movement, decreased activity, blurred vision, headache, stiff shoulders, and low back pain. Fatigue is divided into physical fatigue (means peripheral fatigue) and fatigue felt mainly by the brain (means central fatigue). Both fatigues are caused by the imbalance of the endocrine system such as nervous system, immune system, and hormones in the body, which imbalance is due to mental stress (i.e., human relationship, working relationship), bodily stress (i.e., overwork), physical stress (i.e., ultraviolet rays), chemical stress (i.e., chemical agent, residual agricultural chemicals), biological stress (i.e., viruses, bacteria) (Kuratsune, 2006; Kuratsune, 2010). Recent studies have elucidated that the cause of fatigue in modern people is radical oxygen in the autonomic center system (Kajimoto, 2015). Humans take in oxygen by breathing, produce adenosine triphosphate (ATP), and have the energy needed for life activities. In these processes, a large amount of reactive oxygen species (ROS) is produced (Nagata, 1997; Sato & Inoue, 2002). The ROS harms cells and intracellular protein components, which damages become signals to increase immune system cytokines, thus abnormalities occur in the metabolism/ signal transduction system. It is considered through that repairing damaged cell components requires more energy and substances than is required for normal cell activity, cell repair does not proceed due to a lack of available energy and substances under fatigue condition, metabolism/ signal transduction system worsen more and more, and these lead to over-exhaustion or chronic fatigue (Tanaka, 2009). Besides, focusing on the changes in the brain when a person feels fatigued, it was found that the hypothalamus and anterior cingulate gyrus region, which are the centers of the autonomic nerves of the brain, are tired strongly. In this area, sympathetic and parasympathetic nerves are balanced such as the following physical condition management (ex., elevating heart rate, accelerating breath, thermoregulation), adjustment of mental stress and relaxedness. That is to say, it is this area of the brain that becomes tired, whether people use their own brain or body (Kajimoto, 2015).

Since the increased radical oxygen inside of the body leads to cancer, aging, and lifestyle diseases (Nagata, 1997; Seishima et al., 2015), it is important to remove the radical oxygen for health. Suppressing oxidation by radical oxygen is called antioxidant. For antioxidation, antioxidative substances or enzymes that decompose radical oxygen are significant. There are mentioned vitamins A, C, E, CoQ10, flavonoid polyphenols, non-flavonoid polyphenols, sulfur compounds, and carotenoids as typical antioxidative substances (Japan foundation for aging and health, 2022; Nakamura, 2013). In particular, polyphenols contained in plants have high antioxidant ability, and this ability is proportional to the amounts of polyphenols subsisted in plants (Kimura, Yamagishi, Suzuki, & Oita, 2005). We verified anti-fatigue effect of OM-X on humans in a randomized, placebo-controlled, double-blind, parallel-group trial this time. Since OM-X is produced by fermenting plant materials with LAB over several years, numerous metabolites such as antioxidants are produced during the fermentation period. Metabolomic analysis has confirmed that OM-X extract contains hundreds of low-molecular-weight compounds, including proteins, peptides, organic acids, short-chain fatty acids, dietary fiber, vitamins (except VC), and polyphenols (data not shown). Previous studies have shown that OM-X extract regulates metabolic enzymes in the livers (Wakame et al., 2017), and improve muscular endurance in fatigue mice for significantly reducing triglyceride levels in blood and increasing mRNA levels of carbamoyl phosphate synthetase 1 (Cps1) and arginase 1 (Arg1) in the urea cycle (Itoh et al., 2017). Furthermore, administration of OM-X extract to mice with deficient vitamin C synthesis (SMP30/GNL knockout mice), which is essential for antioxidant activity in the mouse body, has been shown to improve antioxidant capacity and significantly improve transaminase (AST, ALT) levels, which indicate deterioration of liver function (Wakame et al., 2020). Furthermore, it is known that LAB has an immunostimulatory activity (Perdigon et al., 1995; Morimoto et al., 2005) and anti-allergic property (Majamaa & Isolauri, 1997), and also has a probiotic effect of keeping the balance of intestinal bacteria and regulating the function of intestine (Hara et al., 1993). In recent years, studies on the relationship between LAB and anti-fatigue are making progress, so that the anti-fatigue effects of LAB have been revealed (Muto et al., 2021; Makino et al., 2018). The mechanism has deemed the action by improving the balance of the intestinal flora (Matsuo, 2015), and the brain-gut-microbiota action (Wei et al., 2021) which LAB themselves and substances produced by LAB stimulate the intestinal canal and affect the brain (Suganya & Koo, 2020; Watanabe, 2022). OM-X extract has been shown to inhibit inflammatory reactions

in the intestinal tract in mice with induced intestinal inflammation and to promote the formation of tight junctions and intestinal epithelium (Takahata et al., 2014).

Bifidobacteria contained in OM-X representing probiotics along with LAB has revealed many clinical effects related to immune function. For instance, *B. longum* BB536 is known to have an anti-hay fever effect (Xiao et al., 2007), an anti-influenza effect (Namba et al., 2010), and ulcerative colitis improving effect (Takeda et al., 2009). It is also thought that bifidobacteria suppress immune responses such as Th1, Th2, and Th17, and induce regulatory T cells (Tregs) involved in the induction of immune tolerance (Kwon et al., 2010). In addition, it is reported that acetic acid, which is a metabolite of bifidobacteria, has a preventive effect on O-157 in mice (Fukuda et al., 2011). Ameliorating effect of *B. bifidum* BGN4 and *B. longum* BORI on cognitive function in mice (Kim et al., 2021), and improvement effect of a mixture of LAB (*L. fermentum* LF16, *L. rhamnosus* LR06, *L. plantarum* LP01) and bifidobacteria (*B. longum* BL04) on human depression and quality of sleep (Marotta et al., 2019) were reported, thus the effects of bifidobacteria on the brain have also been suggested. The following is an analogy that the various antioxidants, LAB, and bifidobacteria contained in OM-X may have suppressed oxidation caused by active oxygen, improved the balance of the intestinal environment, and suppressed inflammation, thereby improving the balance of the autonomic nervous system and producing an anti-fatigue effect.

In this study, VAS and CFS were used as scales of fatigue. The Japanese society of fatigue science previously mentioned has developed the guideline for clinical evaluation of anti-fatigue, and cited VAS and CFS as subjective evaluation indexes for effectiveness trial (Japanese society of fatigue science, 2011). VAS was formulated mainly in the field of anesthesiology to objectively evaluate subjective symptoms which are difficult to measure (i.e., pain sensation), and the availability of the scale has been established (Ministry of Health, Labour and Welfare, 2020). Recently, VAS has also been used as a scale of health-related QOL and fatigue, and has been employed in many studies such as food functionality evaluation (Tsuda et al., 2019; Najima & Miyata, 2018; Shimizu, Fukuda, & Yamamoto, 2009; Kim et al., 2013). CFS is a scale reported by Chalder et. al. (Chalder et al., 1993), and the reliability and validity of the Japanese version have also been admitted (Tanaka et al., 2008). As with VAS, CFS is widely used in domestic and international study (Holton et al., 2020; Kushima et al., 2021; Pöttgen et al., 2018; Shimizu et al., 2009). Additionally, the Japanese society of fatigue science lists d-ROM (oxidative stress) (Seki, 2009) and BAP (antioxidative ability) (Dohi et al., 2005) as scales of oxidative stress (Japanese society of fatigue science, 2011). Prof. Nojima (2011) reported as the oxidative stress value (d-ROMs value) increased, the antioxidative ability (BAP value) to control that oxidative stress decreased, and the long-increasing oxidative stress level strongly related to the pathological formation of chronic fatigue (Nojima, 2011). In late years, the modified BAP / d-ROMs ratio has been used as a scale of comprehensive antioxidant ability (Imai, Kobayashi, & Uenishi, 2021; Morimoto et al., 2020). As a result of our study, it is thought that the considerable elevation of modified BAP / d-ROMs ratio in active indicates the anti-fatigue effect of OM-X. On the other hand, although the d-ROM, BAP, and modified BAP/ d-ROMs ratios inactive greatly improved all, no significant difference came out between the groups. It is presumed that the dispersion in the individual subjects was high. Since this study targeted healthy adults with temporary fatigue, there could be a low difference in before- and-after compared to sick. An objective evaluation method of fatigue for healthy people remains to be seen. In addition, our primary goal was to evaluate the effect of OM-X itself this time, thus we dared to study the effect of each individual ingredient (fermented extract, LAB, or bifidobacteria) in a separate study to be followed.

5. Conclusion

In conclusion, we found out that the 12-week ingestion of OM-X made of fermented plant extract with LAB and bifidobacteria might ameliorated the feeling of fatigue for healthy people with temporary fatigue. In addition, no adverse event was triggered by the test food.

Ethics statements

The authors ensure that the current clinical trial has been carried out in accordance with the ethical principles of the declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms, Tokyo, and was conducted in compliance with the protocol. Written informed consent was obtained from all subjects prior to enrolment.

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CRediT authorship contribution statement

Takeshi Kaneko: Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Akinobu Miyata:** Methodology, Validation, Investigation, Data curation, Writing – review & editing, Supervision. **Muneaki Takahata:** Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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