Alleviating the physical discomfort in healthy individuals with Moringa seed extract: a randomized, double-blind, placebo-controlled, parallel-group trial

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ABSTRACT

Background: Moringa oleifera is well recognized for its rich nutritional content and diverse bioactive compounds. Moringa, abundant in various bioactive compounds such as flavonoids and alkaloids present across all plant parts including leaves, stems, and roots, is especially rich in glucomoringin within its seeds. The antioxidant activity of moringin has already been verified in numerous cellular and animal experiments.

Objective: The study investigates the effects of Moringa seed extract (MSE), abundant in glucomoringin, on fatigue-related physical discomfort and sleep quality, as indicators of quality of life (QOL) in healthy individuals.

Methods: A randomized, double-blind, placebo-controlled, parallel-group study was conducted, administering MSE containing 12 mg of glucomoringin or placebo to healthy adult males and females daily for four consecutive weeks. Quality of life (QOL) questionnaire on fatigue, physical discomfort, sleep, and motivation was evaluated using a visual analog scale (VAS) at the start of the study and the end of each subsequent week until week 4. The impact on QOL was assessed through subjective evaluations and oxidative stress markers.

Results: As a result, the efficacy of MSE intake in reducing stiff shoulder/neck pain, joint pain, and muscle pain was confirmed. Based on the oxidative stress markers, significant effects were observed among the participants with relatively high oxidative stress. A greater improvement in physical discomfort, reduced fatigue, and enhanced sleep quality were also noticed among female participants with MSE consumption.

Conclusion: MSE consumption has showed potential in lowering physical discomfort brought on by exhaustion, promoting overall QOL, and improving sleep quality. (UMIN000049070)

Keywords: moringa; moringin; glucomoringin; physical discomfort; sleep quality; fatigue; pain
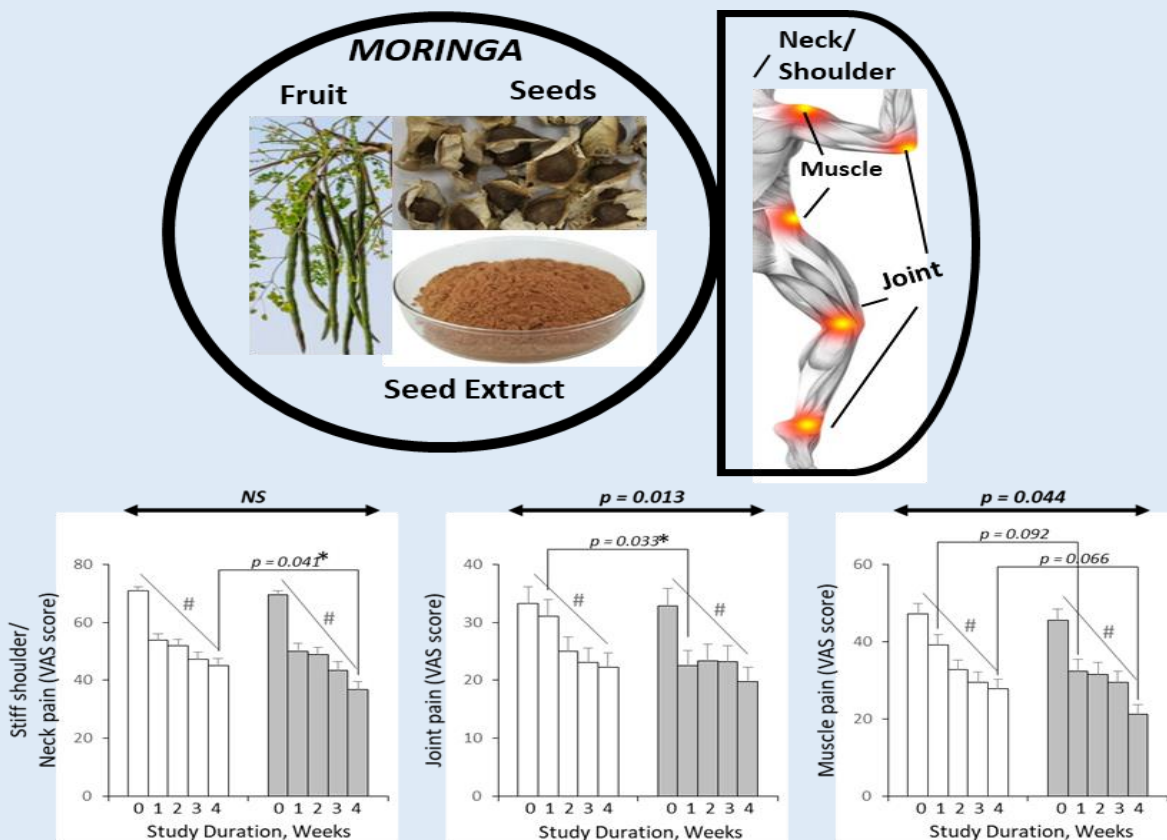
INTRODUCTION

Fatigue and associated physical discomfort are indicators that signal a perturbation in the body's homeostasis and are familiar problems for those of us living in today's stressful society. Since chronic fatigue and physical pain can greatly impair quality of life (QOL), reduce productivity [1,2], and may even elevate the risk of developing certain diseases [3], countermeasures against them are extremely important. It is important to reduce or eliminate stress and fatigue factors, but it is also considered effective to improve resistance by enhancing the body's innate defense capabilities. Since it has been suggested that oxidative stress is closely related to fatigue and physical discomfort [4–6], food ingredients

that reduce oxidative stress are also attracting attention as one of the countermeasures [7,8].

In this study, we examined Moringa seed extract (MSE) as a potential agent to enhance QOL. Moringa oleifera (Moringaceae), a plant widely cultivated in regions such as Africa, Asia, and Northern India, is not only rich in nutrients such as proteins, vitamins, and minerals but also contains various phytochemicals and antioxidants [9–11]. This has led to its unique classification as a 'superfood,' utilized not just as a food source but also for its several medicinal properties, which include antibacterial, anti-inflammatory, analgesic, anti-infectious, anti-lifestyle disease, and anticancer effects, etc [12–14]. Moringa, abundant in various bioactive compounds such as flavonoids and alkaloids present across all plant parts including leaves, stems, and roots, is especially rich in glucomoringin within its seeds [10,15]. Glucomoringin is metabolized in the body into moringin, a type of isothiocyanate [16]. Moringin has been shown to interact with the Keap1-Nrf2 system, an innate biological defense mechanism, thereby augmenting antioxidant, anti-inflammatory, and detoxification processes in the body [17]. The antioxidant activity of moringin has already been verified in numerous cellular and animal experiments [18–22]. In previous clinical trial, we have indicated that the consumption of MSE could potentially elicit anti-fatigue and anti-lower back pain effects [23]. Recently, it has been suggested that moringin may alleviate pain stimuli by acting on transient receptor potential ankyrin subtype 1 protein (TRPA1), a pain receptor, causing it to undergo desensitization [24]. Additionally, it has been newly discovered that MSE might contribute to sleep improvement by regulating neurotransmitters in the brain [25,26]. However, the analgesic and sleep improvement effects of MSE have not been thoroughly explored in human clinical trials. Therefore, in this study, we sought to evaluate the impact of MSE on QOL, especially fatigue-associated physical discomfort and sleep quality in healthy individuals using

a randomized, double-blind, placebo-controlled, and a parallel-group design.

MATERIALS AND METHODS

Study design: The study was designed as a randomized, double-blind, placebo-controlled, and parallel-group trial, with participants allocated in a 1: 1 ratio by a block randomization method. The study protocol was jointly prepared by Taiyo Kagaku Co., Ltd., Mie, Japan (TKC) and Taiyo International Inc. MN, USA (TI). The study was conducted by ethical principles based on the Helsinki Declaration (revised at the 2013 WMA Fortaleza General Assembly [Brazil]) and the Ethical Guidelines for Human Medical Research (Notification No. 1 of the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare, and Ministry of Economy, Trade and Industry, 2021). A thorough review was conducted to ensure the protection of human rights and safety assurance. The study protocol received approval from the institutional review board of Ueno Asagao Clinic (approval number: 2022-32; Date of approval: September 7, 2022), and was registered at the UMIN-CTR (Trial ID: UMIN000049070). The protocol remained unchanged from the final setup throughout the study period. The clinical study was carried out at the Ueno Asagao Clinic from September to December 2022.

Study food: Tablets of Moringa seed extract (MSE, Moringa Extract S®) containing glucomoringin used in this study were provided by TKC. Based on a prior clinical trial, wherein daily administration of 12 mg of glucomoringin resulted in improvements in anti-fatigue and lower back pain [23], the dosage of glucomoringin for the present trial was likewise set at 12 mg. The placebo tablets contained dextrin instead of MSE were also provided. The treatment and placebo tablets were identical in appearance and flavor to ensure the double-blind nature of the study.

Study participants: The inclusion criteria were healthy Japanese individuals, both male and female, aged

between 30 and 64 years. The exclusion criteria were: (1) chronic fatigue sufferers; (2) those not experiencing fatigue at all; (3) individuals under pharmacological treatment for an illness or those with a history of serious illness; (4) individuals with a body mass index (BMI) exceeding 30; (5) severe anemia sufferers; (6) individuals allergic to the test product, other foods, or medical products; (7) individuals consuming excessive amounts of alcohol (over 60 g/day); (8) individuals likely to experience lifestyle changes; (9) habitual consumers of health-promoting foods; (10) regular consumers of foods related to the test material, such as raw cruciferous vegetables (e.g., radish, cabbage, arugula, watercress, turnip, wasabi, mustard, broccoli sprouts, radish sprouts); (11) individuals who are or may be pregnant, or are lactating; (12) individuals who participated in other clinical studies within the past three months; (13) individuals or family members involved in functional food or cosmetic companies; and (14) any individuals deemed inappropriate for the study by the principal investigator.

Sample size: The required sample size of 140 participants, split evenly between the two intervention groups, was derived from a prior study detailing the effect size and standard deviation of fatigue as measured by a visual analogue scale (VAS) [27] after 4 weeks of MSE supplementation [23]. Given a significance level of $p \leq 0.05$, power of 0.80, an effect size of 7.7, and standard deviation of 19.8, we determined the requisite sample size to be 140 participants, ensuring sufficient statistical power for the study.

Selection, randomization, and blinding: All study procedures were managed by TES Holdings Co., Ltd. (TES, Tokyo, Japan) in the consignment from TKC. Initial screening involved an assessment of participating individual's lifestyle habits, physical condition, and QOL as per the VAS and the Profile of Mood States Short Form 2 (POMS-2, Japanese version) [28]. Of 260 individuals, 140 participants who met the predetermined inclusion

and exclusion criteria were recruited. These participants were randomly allocated to either the MSE or the placebo group via a block randomization design stratified by age, sex, and VAS score of fatigue and physical discomfort. An independent TES researcher, not directly involved in the study's planning, execution, or analysis, performed the allocation procedure. The allocation remained blind to all participants and investigators until the study's intervention and data analysis stages were completed.

Intervention and Outcomes: Recruited participants were randomly assigned to consume either MSE or placebo tablets daily for four consecutive weeks. Baseline and final-intervention assessments (at 4 weeks) were conducted at the clinic, which included a physical examination and QOL questionnaires, evaluated using a VAS and POMS-2. Participants also completed the weekly VAS questionnaire at home during weeks 1 and 3 of the study. Urine samples were collected at baseline and after intervention (4 weeks) to measure oxidative stress markers; urinary 8-hydroxydeoxyguanosine (8-OHdG) [29] and urinary 8-epimer of Prostaglandin F2α (8-iso-PGF2α) [30]. Participants were advised to maintain their regular lifestyle patterns, including diet, exercise, and sleep, and to avoid changes in alcohol consumption and smoking habits. Also, participants were asked to refrain from extreme exercise, overeating, drinking alcohol, smoking, and staying up late the day before the clinic visits, and advised to avoid overeating, morning exercise, and alcohol consumption on the day of their examination visit. The primary outcome was the QOL as rated on VAS, while the secondary outcomes included POMS-2 scores, urinary oxidative stress markers, and the safety of MSE formulation.

Evaluation of QOL: QOL questionnaire on fatigue, physical discomfort, sleep, and motivation was evaluated using a VAS [27] at the start of the study and the end of each subsequent week until week 4. Participants were

asked to rate their physical and mental conditions on the VAS, ranging from 0 (best) to 10 (worst). Fatigue and physical discomfort-related inquiries were conducted following the methodology proposed by the Japan Society for Fatigue Science and encompassed daily fatigue, stiff shoulder/neck pain, joint pain, muscle pain associated with daily activities, knee pain, headache, eye strain, and dry eye. Regarding sleep quality and motivation, the following items were evaluated: initiation of sleep, waking during sleep, fatigue on waking up, refreshment on waking up, daytime sleepiness, and motivation towards work and study. In addition, participants' mental conditions were evaluated using POMS-2 [28] at the beginning and end of the study. POMS-2 is a seven-point questionnaire with 65 questions describing seven different moods: "anger-hostility," "confusion–bewilderment," "depression–dejection," "fatigue–inertia," "tension–anxiety," "vigor–activity," and "friendliness.

Analysis of urinary oxidation biomarkers: Urinary samples were collected and stored at -80℃ until assay. Measurements of urinary 8-OHdG [29] and 8-iso-PGF2α [30] were conducted by the Japan Institute for the Control of Aging (JaICA), Nikken SEIL Co., Ltd., Shizuoka, Japan, using a competitive ELISA kit (8-OHdG: New 8-OHdG check, JaICA, Shizuoka, Japan, catalog no. KOG-200S/E; urinary 8-iso-PGF2α: 8-iso-PGF2α ELISA kit, Enzo Life Sciences Inc. NY, USA, catalog no. ADI-900-010) as per the manufacturer's protocol.

Statistical analysis: Data analysis was conducted using Microsoft Office Excel 2016 (Microsoft Corp.), SAS (SAS 9.4), and SPSS (Statistics 26). Basic statistics such as mean, standard deviation, standard error of mean, maximum, and minimum values were calculated for each categorical data point at each time point. Both the actual measured values and the changes from the baseline were used in the analysis. The significance level was set at 5% ($p \le 0.05$; two-sided) for all tests, and trends were considered at 10% ($p \le 0.10$). The data are presented in tables and graphs as mean ± standard error unless otherwise stated. Between-group comparisons were performed using an unpaired two tailed Student's t-test comparing the intervention and placebo groups at each examination. All data for the unpaired t-tests were confirmed to be equally distributed. Scores from sources other than the VAS questionnaire were treated as nonparametric data, and the Mann-Whitney U test was used for comparisons between groups. Normally distributed data were analyzed with a paired two tailed Student's t-test for within-group comparisons for each observation period before and after intake. Whereas, data from other than the physical VAS questionnaire was analyzed using a non-parametric Wilcoxon signed-rank test. In case of the significance difference between the urinary antioxidant markers, the subgroup analyses were performed by creating subgroups within baseline urinary antioxidant scores above and below the median value of the oxidative stress levels. Also, the subgroup analyses were performed for male and female participants for any gender differences in oxidative stress levels.

RESULTS

Participant characteristics: A schematic illustration of the CONSORT flow chart of the study is presented in Fig.1. Two hundred and sixty (n = 260) healthy individuals, both male and female, aged 30 to 64 were screened initially. Of 260, the 140 participants were selected based on the inclusion and exclusion criteria, and randomly assigned to either the MSE or placebo groups. All recruited participants (n = 140) who had consumed the test food at least once (MSE (n = 70), placebo (n = 70)), were included in the full analysis set (FAS) for safety endpoints analysis. During the study, four participants (MSE (n = 2), placebo (n = 2)) withdrew for personal reasons, leaving 136 participants who completed the study. Further, one participant (MSE (n = 1)) was excluded from the validity analysis due to multiple missing data points, thus 135 participants were

included in per protocol set (PPS), and analyzed for primary and secondary endpoints. The baseline characteristics of these 135 participants are outlined in Table 1. No significant difference was observed between

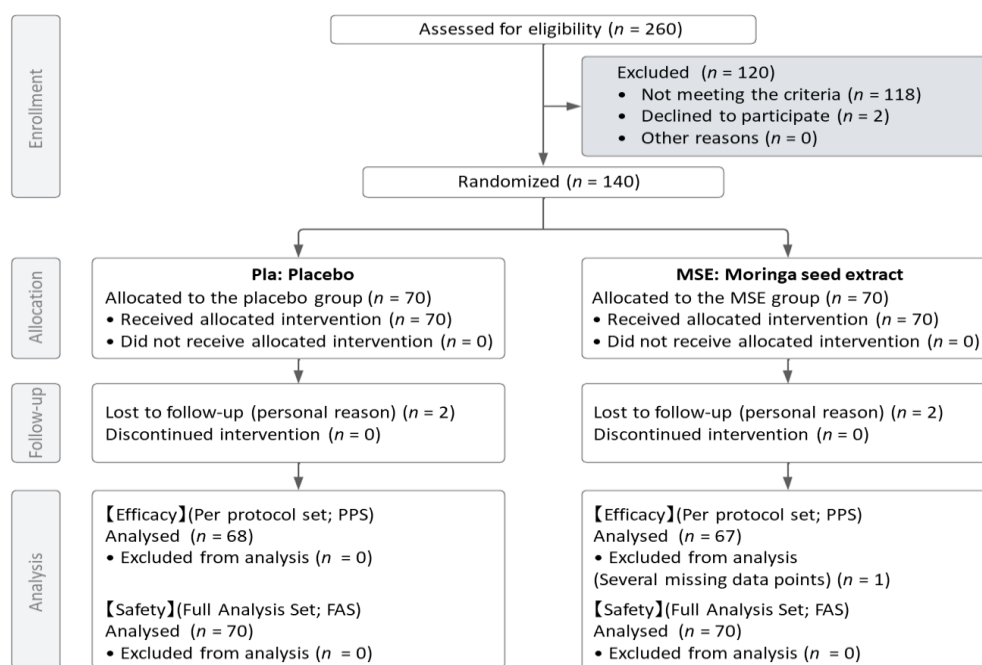groups for any parameter confirming the homogeneity among the groups.

Figure 1. Flow chart of study subjects

Table 1. Participants' demographics at baseline

	Pla group (n=68)	MSE group (n= 67)	*p*-value
Gender (Male/Female)	33/35	34/33	0.797
Age (years)	49.5 ± 6.2	50.0 ± 6.5	0.687
Height (cm)	166.2 ± 8.9	165.9 ± 8.2	0.791
Weight (kg)	62.3 ± 12.6	62.8 ± 11.8	0.812
Body fat (%)	25.3 ± 7.3	26.0 ± 6.7	0.597
Body Mass Index (kg/m^2)	22.4 ± 3.3	22.7 ± 3.1	0.587
Systolic blood pressure (mmHg)	113.0 ± 14.2	117.1 ± 13.7	0.092
Diastolic blood pressure (mmHg)	72.9 ± 10.7	74.7 ± 9.8	0.319
Working hours (h/day)	8.9 ± 1.5	8.8 ± 1.2	0.890
Sleep time (h/day)	6.8 ± 0.9	6.6 ± 0.7	0.178
Smoking (cigarettes) (number/day)	0.9 ± 3.3	1.5 ± 3.5	0.325
Alcohol (g/week)	2.6 ± 5.7	3.9 ± 5.9	0.217

Data are represented as Mean ± SD. Pla: Placebo group; MSE: Moringa seed extract group. No significant differences were detected between the Pla and MSE groups (Significance at p ≤ 0.05*).

VAS questionnaires scores of all participants: Figure 2 presents the VAS scores of fatigue, physical discomfort, sleep, and motivation for all participants. Significant improvements in scores on almost all evaluated items were noted in both the MSE and placebo groups, with the MSE group showing a greater improvement in stiff shoulder/neck pain, joint pain, and muscle pain. The scores for stiff shoulder/neck pain in the MSE group were 69.4±1.4 at baseline (0w) and 36.7±2.9 after 4 weeks of intake (4w), compared to 70.8±1.4 (0w) and 44.9±2.7 (4w), respectively, in the placebo group. Whereas, between-group comparisons revealed a significantly lower score in the MSE group at 4w (p=0.041*) (Supplementary Table S1). Regarding joint pain, between-group comparisons revealed a significantly lower score in the MSE group at 1w (p=0.033*). Also, the change in joint pain scores from the baseline after MSE consumption showed a significant difference (p=0.013*)

compared to the placebo group in the repeated measures two-way ANOVA (Supplementary Table S2). Concerning muscle pain related to daily movement, between-group comparisons revealed a trend of lower scores in the MSE group at 1w (p=0.092) and 4w (p=0.066) after intake. In addition, the change in muscle pain scores from baseline after MSE consumption differed significantly (p=0.044*) compared to the placebo group in the repeated measures two-way ANOVA (Supplementary Table S2). Further, the interventions significantly improved scores on most of the evaluated items of the POMS-2 questionnaire, but there were no significant differences between the MSE and placebo groups in the observed moods index scores (data not shown).
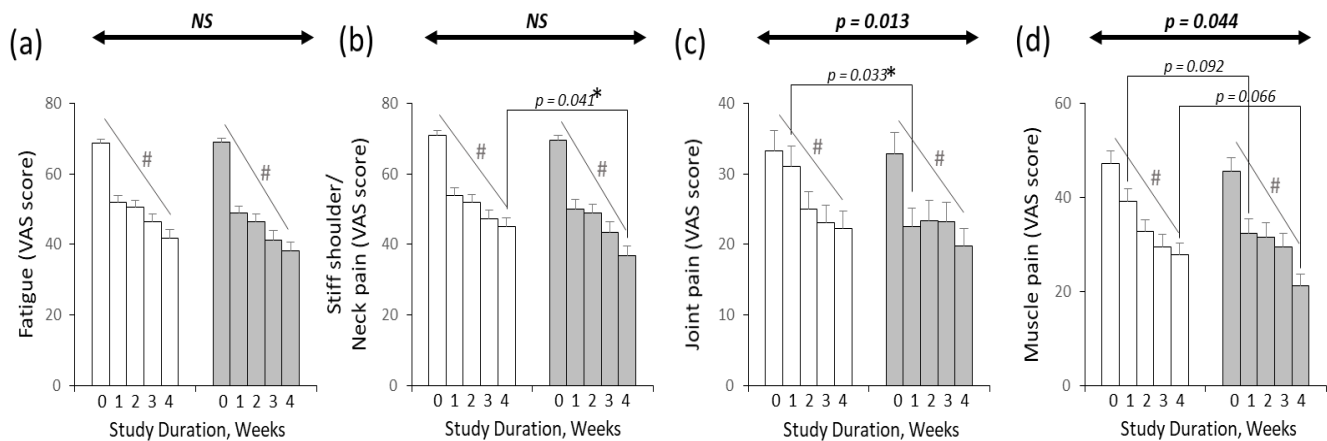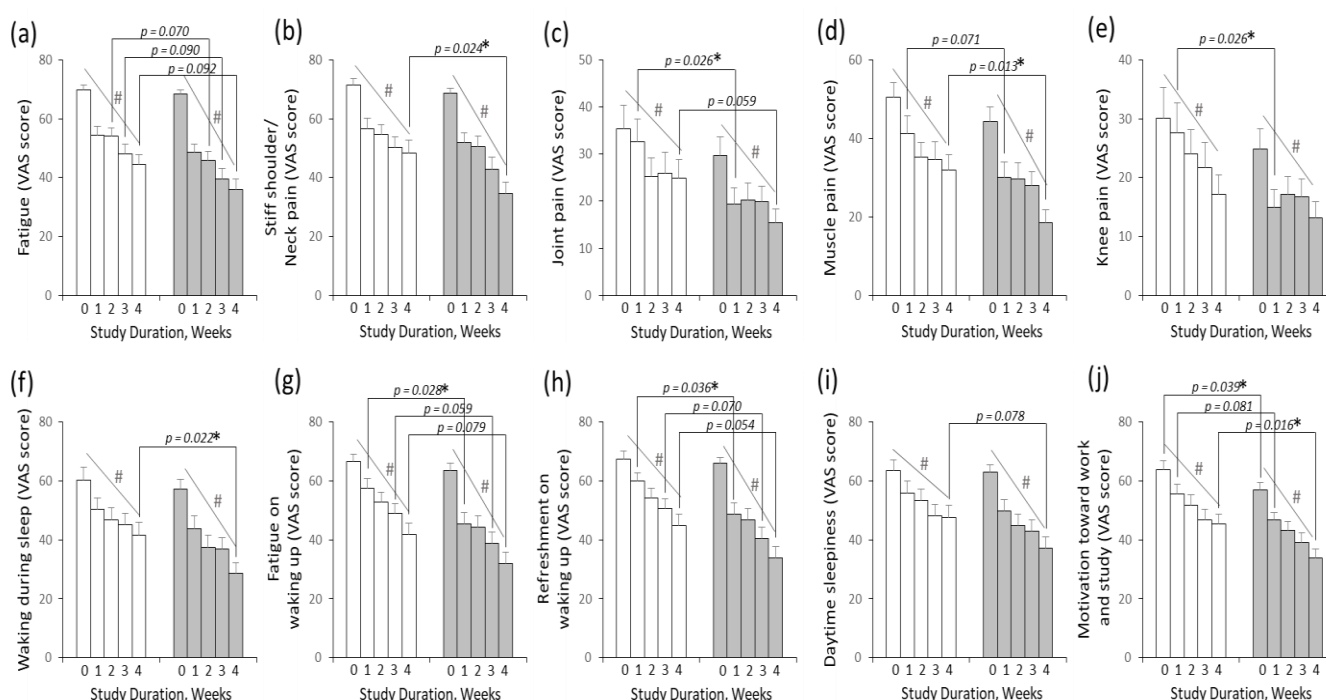

Figure 2. The profiles of subjective physiological conditions assessed by the VAS questionnaire among the all subjects. Data are represented as Mean ± SEM. VAS scale: 0 100 (best to worst). (a) Fatigue, (b) Stiff shoulder/Neck pain, (c) Joint pain, (d) Muscle pain. Bar (empty): Placebo (Pla) group (n = 68); Bar (filled): Moringa seed extract (MSE) group (n = 67). (Keys: NS: Non-significant; *: Significant at p ≤ 0.05 (Between-group comparison with placebo); #: Significant at p ≤ 0.05 (Within-group comparison with baseline); Lines with an arrow (Dark): Repeated measure two-way ANOVA Pre/ Post model (Difference); Between groups).

VAS questionnaires scores of subjects with urinary 8-iso-PGF2α above the median value*: While the baseline mean value of urinary 8-iso-PGF2α was significantly higher in the MSE group, we performed subgroup

analyses by dividing participants into subgroups on the basis of median value of their baseline urinary 8-iso-PGF2α levels. Among the participants with basal urinary 8-iso-PGF2α values above the median, the MSE group

exhibited improvement in several questionnaire items, a trend that was not observed in participants with lower basal urinary 8-iso-PGF2α levels. Figure 3 presents the VAS scores related to fatigue, physical discomfort, sleep, and motivation among participants with higher basal urinary 8-iso-PGF2α levels. The between-group comparisons revealed significantly lower or trending lower scores in the MSE group for the following physical discomfort-related items: stiff shoulder/neck pain at 4w (p=0.024*), joint pain at 1w (p=0.026*) and 4w (p=0.059), muscle pain at 1w (p=0.071) and 4w (p=0.013*), and knee

pain at 1w (p=0.026*). In addition, the MSE group showed significantly lower or trending lower scores for the following sleep quality and motivation-related items: waking during sleep at 4w (p=0.022*), fatigue upon waking at 1w (p=0.028*), 3w (p=0.059), and 4w (p=0.079), feeling refreshed upon waking at 1w (p=0.036*), 3w (p=0.070), and 4w (p=0.054), daytime sleepiness at 4w (p=0.078), and motivation towards work and study at 1w (p=0.039*), 2w (p=0.081), and 4w (p=0.016*).

Figure 3. The profiles of subjective physiological conditions assessed by the VAS questionnaire among the subjects with urinary 8-iso-PGF2t above the median value. Data are represented as Mean ± SEM. VAS scale: 0 100 (best to worst). (a) Fatigue, (b) Stiff shoulder/Neck pain, (c) Joint pain, (d) Muscle pain, (e) Knee pain, (f) Waking during sleep, (g) Fatigue on waking up, (h) Refreshment on waking up, (i) Daytime sleepiness, (j) Motivation toward work and study. Bar (empty): Placebo (Pla) group (n = 27); Bar (filled): Moringa seed extract (MSE) group (n = 41). (Keys: NS: Non-significant; *: Significant at p ≤ 0.05 (Between-group comparison with placebo); #: Significant at p ≤ 0.05 (Within-group comparison with baseline))
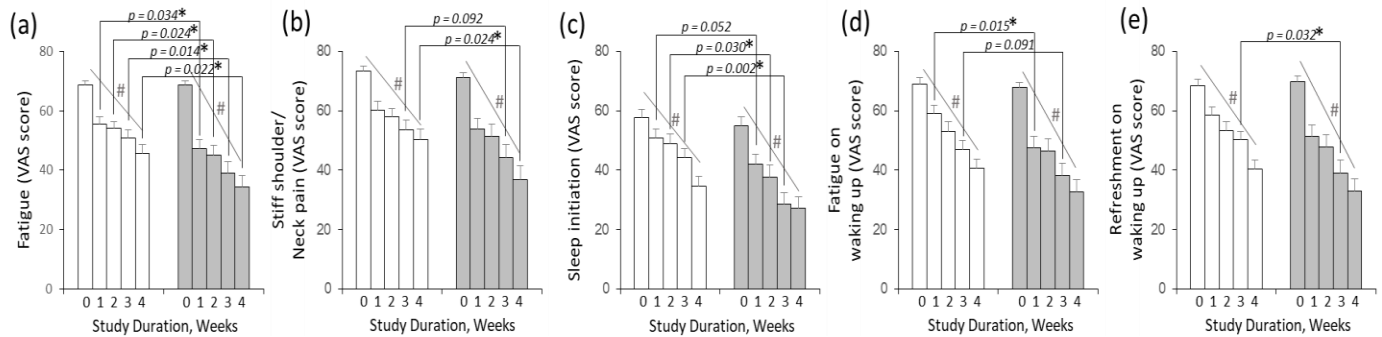
VAS questionnaires scores of female subjects: Given the reported gender differences in oxidative stress levels, the effectiveness of MSE was separately evaluated for male and female participants. A substantial placebo effect was observed in male participants resulting in no significant

differences in questionnaire scores between the placebo and MSE groups. Conversely, female participants in the MSE group showed improvement over the placebo in several questionnaire items. Figure 4 displays the VAS scores of fatigue, sleep, and motivation among female

subjects. In between-group comparisons, the MSE group's scores for the following items were significantly lower or tended to be lower than those of the placebo group: fatigue at 1w (p=0.034*), 2w (p=0.024*), 3w (p=0.014*), 4w (p=0.022*), stiff shoulder/neck pain at 3w

(p=0.092) and 4w (p=0.024*), sleep initiation at 1w (p=0.052), 2w (p=0.030*), 3w (p=0.002*), fatigue upon waking at 1w (p=0.015*) and 3w (p=0.091), and feeling refreshed upon waking at 3w (p=0.032*).

Figure 4. The profiles of subjective physiological conditions assessed by the VAS questionnaire among the female subjects. Data are represented as Mean ± SEM. VAS scale: 0 100 (best to worst). (a) Fatigue, (b) Stiff shoulder/Neck pain, (c) Sleep initiation, (d) Fatigue on waking up, (e) Refreshment on waking up. Bar (empty): Placebo (Pla) group (n = 33); Bar (filled): Moringa seed extract (MSE) group (n = 35). (Keys: NS: Non-significant; *: Significant at p ≤ 0.05 (Between-group comparison with placebo); #: Significant at p ≤ 0.05 (Within-group comparison with baseline)).

Urinary oxidation biomarkers: Table 2 lists the urinary level of 8-iso-PGF2α and 8-OHdG oxidative stress markers. It has been reported that the results for urinary 8-iso-PGF2α and urinary 8-OHdG can vary depending on the measurement method [38,39]. According to JaICA, the institution performing the analysis, the historical reference values for the ELISA kit used in this study for healthy adults were urinary 8-iso-PGF2α: 0.00 to 6.77 ng/mg Cre, and urinary 8-OHdG: 0.0 to 16.4 ng/mg Cre. Both baseline levels in the MSE and placebo groups were within the normal range and remained within this range after four weeks. However, the mean urinary 8-iso-PGF2α value was significantly higher in the MSE group (p=0.031*, 3.2±1.2 in MSE, and 2.8±0.9 in placebo group), indicating a significant difference between the groups.

Even after 4 weeks of consumption, the mean 8-iso-PGF2α value of both groups did not significantly change, and the level in the MSE group remained higher than that in the placebo group, while the significant difference between the groups dissipated (p=0.092, 3.3±1.7 in MSE, and 2.9±1.2 in placebo group). Relatively lower levels of 8-OHdG were noticed in both MSE and placebo groups after 4 weeks of consumption. The levels of 8-OHdG were 7.2±2.2 (0w) and 7.0±2.5 (4w), and 7.6±2.6 (0w) and 7.1±3.0 (4w) in the MSE and placebo groups, respectively. No significant differences were observed between the MSE and placebo groups. Additionally, no gender differences were identified for the two oxidative stress markers (data not shown).

Table 2. Oxidative stress markers in urine (8-iso-PGF2t and 8-OHdG)

		Baseline	Week 4
8-iso-PGF$_{2t}$(ng/mg Cre)	Pla (n =68)	2.8 ± 0.9	2.9 ± 1.2
	MSE (N=67)	3.2 ± 1.2	3.3 ± 1.7
	p-value	0.031*	0.092
8-OHdG (ng/mg Cre)	Pla (n=68)	7.6 ± 2.6	7.1 ± 3.0
	MSE (N=67)	7.2 ± 2.2	7.0 ± 2.5
	p-value	0.258	0.752

Data are represented as Mean ± SEM. Pla: Placebo group; MSE: Moringa seed extract group. *: Significant at p ≤ 0.05 (Between-group comparison with placebo); Cre: Creatinine

Safety Evaluation: No adverse events or side effects attributable to the study ingredient were observed in all participants. In overall analysis, systolic blood pressure (SBP) was significantly higher in the MSE group than in the placebo group after 4 weeks of consumption. However, this is possibly due to the influence of the baseline value because the significant difference was not observed in the net change of SBP, thus suggesting no cause for concern. Also, no significant differences in body weight and body fat percentage between the groups were observed throughout the study period.

DISCUSSION

Several therapeutic properties, including anticancer, antibacterial, antiproliferative, and anti-hypertensive effects, have been supported by various in-vitro and in-vivo studies on the bioactive compounds of Moringa roots, seeds, and leaves [12,14,31]. However, there is a scarcity of evidence regarding similar effects and activities in human studies. Particularly, the seeds of Moringa have been reported to help in treating hyperthyroidism, gout, cramp, Crohn's disease, rheumatism, epilepsy, sexually transmitted diseases, and can act as antimicrobial and anti-inflammatory agents [32,33]. In clinical trials, glucomoringin and isothiocyanates moringin extracted from Moringa seeds have demonstrated effective antioxidant and anti-

inflammatory activity [34]. Our previous clinical study identified the anti-fatigue effect and reduction in low back pain associated with MSE [23]. In this present nutritional intervention study, we aimed to examine the efficacy of MSE specifically for fatigue-associated physical discomfort and sleep quality, using a randomized, double-blind, placebo-controlled, parallel-group design. Healthy adult participants consumed either MSE or placebo once daily for four consecutive weeks. The primary outcomes, fatigue, physical discomfort, sleep, and motivation were assessed using the VAS on a QOL questionnaire. As a result, it was newly demonstrated that the consumption of MSE has a positive effect on improving stiff shoulder/neck pain, joint pain, and muscle pain. In addition, subgroup analysis showed that MSE intake improved other physical discomfort and improved sleep quality in subjects with relatively high oxidative stress markers and in female. As is well shown that evaluations of mood and sensation can be strongly influenced by placebo effects [35], this study also showed significant improvements in all items of the VAS questionnaire in both the placebo and MSE groups. However, the MSE group's scores tended to be better on almost all of the questions, with significant or nearly significant differences between groups for some items indicating MSE's effectiveness.

Additionally, it is worth noting that elevated oxidative stress markers have been observed in patients with chronic fatigue syndrome [36,37] and in exercise tolerance clinical trials [38,39], implying a significant association between oxidative stress and fatigue. In animal models, it has been demonstrated that Moringa intervention helps reduce oxidative stress markers such as 8-isoprostane and 8-OHdG [40]. In our study, we investigated the impact of MSE intervention on antioxidant stress status biomarkers as a secondary outcome. However, the results of our study did not indicate a significant effect of MSE consumption in reducing these oxidative stress markers. It is important to consider that due to the variable antioxidant status of the healthy subjects participating in our study, no definitive conclusions can be drawn regarding the antioxidant stress potential of MSE intervention. The heterogeneity of individual biomarker basal values may explain the absence of significant effects observed. Also, relatively low levels of baseline oxidative stress in the subjects could be another contributing factor for the inability to find an effect. The values of urinary 8-iso-PGF2α and urinary 8-OHdG antioxidant levels in the MSE and placebo groups at baseline and during intervention were within the normal range in this study.

In addition, we hypothesized that a population with uniformly high levels of oxidative stress biomarkers, particularly urinary 8-iso-PGF2α, could facilitate the detection of significant antioxidant stress effects. To test this hypothesis and correct for urinary 8-iso-PGF2α imbalance between groups, we conducted a stratified analysis by dividing the study participants based on the median value of their urinary 8-iso-PGF2α levels at baseline. This analysis confirmed the efficacy of MSE on several items of the QOL questionnaire in study participants with higher urinary 8-iso-PGF2α levels. Consequently, significant differences were observed in stiff shoulder/neck pain, joint pain, muscle pain, knee pain, waking during sleep, fatigue upon waking up, refreshment upon waking up, daytime sleepiness, and motivation towards work and study with MSE consumption compared to placebo after four weeks of intervention. In contrast, no significant differences were observed between the MSE and placebo groups in the scores of all QOL questionnaire items for study participants with low urinary 8-iso-PGF2α levels throughout the ingestion period. These findings confirm that MSE is highly effective in individuals with higher levels of oxidative stress, suggesting that MSE has antioxidant effects and the potential to ameliorate QOL.

It is noteworthy that several reports have indicated differences in oxidative stress levels between males and females [41,42]. Therefore, we conducted a stratified analysis for male and female participants in our study. While no differences in oxidative stress biomarker levels and QOL questionnaire items were observed at baseline for both male and female participants (data not shown), the efficacy of MSE was recognized in several items of the QOL questionnaire among female participants, whereas no efficacy was detected in male participants compared to placebo. Notably, a substantial placebo effect was observed in male participants, which might explain the lack of efficacy of MSE in this group, highlighting the influence of placebo effects as a confounding factor in our study. Hence, the findings indicate that the placebo effect hinders the benefits of MSE intervention on QOL, particularly in male participants. It is important to note that no such gender difference was observed in the previous clinical trial. Further investigation with a larger number of participants is necessary to explore this gender difference in more detail.

For a comprehensive understanding of the molecular mechanisms involved, multiple studies have established a strong correlation between oxidative stress and both chronic fatigue and physical pain. While the precise mechanisms are still not fully understood, existing literature suggests that oxidative stress may play a role in tissue damage [43], mitochondrial dysfunction [44], aberrant energy metabolism [45], immune cell

irregularities [46], and central inflammation [47]. Another plausible mechanism involves the generation of pain sensations through the action of a specific oxidative byproduct on pain receptors [48].

The MSE employed in this study contains glucomoringin, a bioactive compound, at a concentration of 10% or higher. Glucomoringin is converted to isothiocyanate moringin by myrosinase produced by intestinal bacteria in the body [16]. Isothiocyanates, including moringin, are electrophilic compounds that activate the Keap1-Nrf2 regulatory system, an in vivo defense mechanism against oxidative stress [17]. The Keap1-Nrf2 system is activated when Keap1, a stress sensor, interacts with a stressor, Nrf2, a transcription factor, escapes Keap1 regulation. Nrf2 then accumulates in the cell, migrates into the nucleus, and induces the expression of various biological defense factors, such as antioxidant, anti-inflammatory, and detoxification enzymes, by regulating downstream genes involved in counterbalancing oxidative stress [17,49]. It is important to note that the Keap1-Nrf2 system is activated not only by stressors but also by various exogenous and endogenous small molecules (inducers) to enhance the body's defense capabilities [50]. Other food components such as sulforaphane and curcumin have been shown to act on the Keap1-Nrf2 system [51,52], and the activating effect of moringin has been demonstrated to be comparable or even stronger than those compounds [19,53,54]. In vitro studies have confirmed that moringin activates Nrf2 and increases the expression of downstream antioxidant and detoxification enzymes [19]. Moreover, the potential of moringin to stimulate in vivo antioxidant and anti-inflammatory responses has been verified in murine models of Parkinson's disease [21] and obesity [22].

The ameliorative effect of MSE on physical discomfort can also be attributed to its analgesic properties. Moringin has been reported to act on TRPA1 receptors, which are involved in the perception of pain and temperature [24]. When TRPA1 receptors receive stimuli such as lipid peroxides and hydrogen peroxide, they transmit electrical signals to the brain, resulting in the sensation of pain [48]. Moringin acts as an agonist of TRPA1 receptors, desensitizing them and potentially alleviating pain stimuli [24]. Moringa, similar to curcumin and cinnamon, has been traditionally used as an analgesic, and TRPA1 is considered to be a mediator of the analgesic effects of these plants [55]. Furthermore, studies using rats and mice have demonstrated the peripheral analgesic activity of MSE [56–58]. Additionally, application of a cream containing moringin has been reported to alleviate neuropathic pain in a mouse model of multiple sclerosis by inhibiting inflammatory pathways, enhancing anti-inflammatory pathways, and blocking voltage-dependent ion channels [59].

Furthermore, the sleep-enhancing effects of MSE may involve the modulation of neurotransmitters. Recent experiments using mice have demonstrated that Moringa seed oil and Moringa seed ethanol extract activate the inhibitory GABAergic system in the hypothalamus while suppressing the excitatory glutamatergic system, thereby promoting sleep-inducing behaviors [25,26]. These findings suggest that MSE has the potential to regulate brain function by modulating neurotransmitters.

Overall, MSE and glucomoringin have the potential to enhance QOL through various mechanisms. However, a comprehensive understanding of these mechanisms is still limited, suggesting the need for further research and extensive exploration. Also, this study had limitations, such as the inability to assess the collective effects of MSE on oxidative stress markers and the influence of placebo effects on the subjective VAS questionnaire. Consequently, future investigations with larger sample sizes should address these factors for more robust conclusions.

In general, the functional foods are a category of foods that go beyond basic nutrition and can have a positive impact on health. Moringa, a nutrient-rich plant, is frequently associated with the functional foods due to its numerous potential health benefits [60, 61]. It is rich in vitamins, minerals (calcium, potassium, and iron), and antioxidants. Moringa leaves, seeds, and pods are used in a variety of culinary dishes and traditional medicine. Moringa extract is increasingly being incorporated into various functional food products, such as powder, capsules, teas, and energy bars. These products are marketed for their potential health benefits, including improved energy, better digestion, and enhanced general health [60-63].

CONCLUSION

In summary, we have demonstrated that the intervention of MSE was effective in physical discomforts improvement such as stiff shoulder/neck pain, joint pain, and muscle pain in healthy subjects. In addition, the sleep quality improvement effects of MSE were observed in participants with higher levels of oxidative stress, and female participants. The variability in oxidative stress levels and the influence of gender and placebo effects should be taken into account in future studies. The knowledge gained from these investigations will contribute to the efficient utilization of MSE in functional food and effective nutraceuticals.

Supplementary Materials: The following supporting information can be downloaded at FFHD homepage. Supplementary Table S1: The VAS scores of physiological conditions among all subjects; Supplementary Table S2: The changes of VAS scores of physiological conditions from the baseline among the all subjects; Supplementary Table S3. The VAS scores of physiological conditions among the subjects with urinary 8-iso-PGF2t above the median value; Supplementary Table S4: The changes of VAS scores of physiological conditions from the baseline among the subjects with urinary 8-iso-PGF2t above the median value; Supplementary Table S5: The VAS scores of physiological conditions among the female subjects; Supplementary Table S6: The changes of VAS scores of physiological conditions from the baseline among the female subjects.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Ueno Asagao Clinic (approval number: 2022-32; Date of approval: September 7, 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data are provided in the manuscript.

Conflicts of Interest: There were no particular conflicts of interest. However, referring to a potential conflict of interest, AA, MPK, SM, MO, and NS were employed by Taiyo Kagaku Co., Ltd.; DT was an employee of Taiyo International Inc., a subsidiary of Taiyo Kagaku Co., Ltd.

Sample Availability: Samples of MSE are available upon request.

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