Improvement in Quality of Sleep by the Intake of Saffron-derived Crocin and Safranal

—A Randomized, Double-blind, Placebo-controlled, Parallel-group Trial—

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ABSTRACT

Saffron, a spice, is the dried stigma of a perennial of the family Iridaceae, originating from South-western Asia. In addition to being used as a spice, is known for its sedative and analgesic effects and is used in herbal medicine in China and other countries. Due to these effects, it is known to induce relaxation and improve sleep. The pharmacologically active components are thought to be crocin and safranal. To investigate the effects of saffron extract on sleep, we conducted a placebo-controlled parallel-group trial with 52 individuals who consumed a dietary supplement containing saffron extract. The main evaluation items were the Oguri-Shirakawa-Azumi sleep inventory MA version（OSA-MA）and sleep analysis by electroencephalography（EEG）. Finally, a total of 45 individuals were analyzed. As for the OSA-MA, the group which consumed the saffron supplement showed significant improvement compared to the control group regarding sleepiness upon rising, initiation and maintenance of sleep, and frequent dreaming. Analysis of EEG recordings during sleep showed that the group that consumed the saffron supplement experienced significantly shorter sleep onset latency and significantly reduced nocturnal awakening. Furthermore, the time spent in non-REM sleep increased. Thus, saffron extract, containing crocin and safranal, encourages sleep onset, increases deep sleep, reduces nocturnal awakening, and improves sleep rhythm. The study was registered with the University Hospital Medical Information Network（UMIN）Clinical Trial Registry before implementation（UMIN000035882）.

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KEY WORDS　Saffron, Affron®, Safranal, Crocin, Sleep rhythm, Quality of sleep, Healthy adults

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INTRODUCTION

While the role of sleep is not completely understood, many studies have been conducted regarding the influence of the state and quality of sleep on physical and mental health. Recently, many studies have reported that sleep influences hypertension,[1] diabetes,[2] and depression. Contemporary society is often referred to as 'stressed' and it is known that stress during the day often induces insomnia.[3,4] About one in five Japanese individuals has a problem with their sleep and one in nine regularly use sleep-inducing drugs.[5] This suggests that many people suffer from a lack of sleep. Insomnia and sleep disorders increase the risks for lifestyle-related diseases, including type II diabetes, hypertension, hyperlipidemia, and obesity.[6] Furthermore, it has been reported that sleep disorders influence mental health by reducing concentration and motivation due to reduced brain activity.[7-9] It is impossible to solve these sleep problems solely by prescribing sleep-inducing drugs, as is done currently. Consequently, it is necessary to use food, beverages, and supplements to improve the quality of sleep.

For humans and other higher vertebrates, sleep is divided into non-REM and REM sleep. The major characteristics of these two kinds of sleep differ with respect to autonomic nerve activity and physiological functions（e.g., blood pressure, heart rate, breathing, and temperature）. These are stable and constant in non-REM sleep, but in REM sleep they show significant changes and are therefore unstable.[10] Furthermore, non-REM sleep is divided into four stages according to the depth of sleep. In particular, many delta waves（slow waves with high amplitude）appear in stages 3 and 4. This is referred to as slow wave sleep（SWS）, which is considered to represent deep sleep.[11] Delta waves contribute to the quality of sleep[12] and constantly appear.[13]

Saffron is derived from the flower of *Crocus sativis*, a perennial herb of the genus Crocus and family Iridaceae, and is known as the world's most expensive spice by weight. It has been used in medicine since ancient times, and in particular as a medicine for women due to its analgesic, sedative, and emmenagogic properties.[14] It is reported to show many additional pharmacological actions, including antioxidative,[15] anti-inflammatory,[16] and anti-tumor effects.[17]

The major components of saffron are crocin and safranal,[18] and research into the anti-anxiety and sleep-improving effects of extracts containing these elements is actively being pursued. So far, Nishide and colleagues have confirmed that tablets containing saffron extract improve the quality of sleep of healthy adults.[19]

We examined whether saffron extract contributes to the improvement of sleep quality using a dietary supplement containing affron®, a saffron extract containing both crocin and safranal, by conducting a randomized, double-blind, placebo-controlled, parallel-group trial. Safranal and crocin, components of affron® that have been found to contribute to improving sleep quality, were measured and standardized by HPLC-MS/ESI.[16] We evaluated participants using the Oguri-Shirakawa-Azumi sleep inventory MA version（OSA-MA）and their sleep status using EEG recordings during sleep.

MATERIALS AND METHODS

1　Participants

The participants of the study were healthy Japanese adults, aged 20 to 64 years old, who were dissatisfied with their quality of sleep. We conducted the Japanese version of the Pittsburgh Sleep Questionnaire（PSQI-J）,[20] drew blood for testing, and conducted medical interviews. All participants gave informed consent for participation in the study after being briefed on the study details. And their written informed consent before study began was obtained from each. Participants were selected based on a screening test using the following criteria: those who are mainly engaged with desk work; who are dissatisfied with their quality of sleep, often feel tired, and have difficulty falling asleep or are light sleepers; and who sleep at least four hours a day. We excluded those who a）suffer from sleep disorders such as insomnia, b）share the bed with one or more other persons, c）have irregular lengths and patterns of sleep due to shift work, d）have a BMI of 25 or above, e）get up to urinate frequently during the night, f）suffer from hay fever or asthma, g）drink an extremely large amount of alcohol, or h）were judged unfit for participation by the medical doctor in charge of the study.

2　Study design

The study protocol was ethically screened by the Kenshokai Ethics Committee（Approval Date. January 31, 2019 Approval No.）and after obtaining approval was implemented in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects（Japan Ministry of Health, Labour and Welfare, Japan Minis-

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try of Education.）. The study was registered with the University Hospital Medical Information Network（UMIN）Clinical Trial Registry before implementation（UMIN000035882）. The study was conducted at Ikiiki Clinic and was designed to be a randomized, double-blind, placebo-controlled, parallel-group trial. For power analysis, we conducted the PSQI-J and blood testing at the screening stage using SAS Power and Sample Size 14.1（SAS, Cary, NC）to select appropriate participants, noting that Alireza and colleagues[21] found significant differences in subjective indicators among diabetic patients. The selected participants were adjusted so as to eliminate any bias in regard to sex, BMI, or PSQI-J between groups. An independent key controller with no involvement in the study carried out the allocation using block randomization. Then, as a pre-intake study, the OSA-MA[22] and analysis of sleep EEG recordings were conducted. There were two weeks of pre-intake observation and four weeks of intake observation. Before the intake began and during the intake period, the quality of sleep using the OSA-MA and analysis of EEG recordings during sleep was subjectively evaluated.

3　Test compounds

The test compound used in the study was a dietary supplement, in tablet form, containing affron®（saffron extract; Pharmactive Biotech Products, Madrid, Spain）, and tablets which did not contain saffron extract but had exactly the same ingredients otherwise. Each tablet was 150 mg, and each participant was asked to take 1 tablet per day. The saffron extract tablet contained 6.6 μg of safranal and 0.66 mg of crocin. **Table 1** shows the composition of the supplements used in the study.

4　Subjective evaluation of the quality of sleep

For subjective evaluation of the quality of sleep, we used the OSA-MA. This questionnaire is subjective and standardized to evaluate the quality of sleep of middle-aged and older Japanese individuals.[20] The questionnaire was to be completed as soon as the participant woke up. It consists of 16 questions, and each question is measured by a 4-point scale. Points were converted to adjusted Zc scores and added up across 5 categories: sleepiness upon rising, initiation and maintenance of sleep, frequent dreaming, feeling refreshed, and sleep length. The higher the Zc and category scores, the better the quality of sleep. In this study, we used the mean Zc scores of each question item and category scores from OSA-MA completed on the mornings of workdays.

Table 1　Composition of the trial dietary supplement（per tablet of 150 mg）

Treatment / Shape	Saffron capsule	Placebo capsule
Safranal	6.6 μg	ND
Crocin	0.66 mg	ND
Calories	0.59 kcal	0.29 kcal
Protein	0.0006 g	0 g
Fat	0.004 g	0.0036 g
Carbohydrates	0.14 g	0.13 g
Salt equivalent	0.000035 g	0.000014 g

Dosage: 1 tablet/day
ND＝not detectable

5　Electroencephalography during sleep

EEG recordings during sleep were obtained using the ZA-9 electroencephalograph（Proassist Ltd., Osaka, Japan）and were analyzed using Sleep Sign-Lite®（KISSEI COMTEC, Nagano, Japan）. EEG recordings during sleep were recorded and measured at each individual's home using a mobile electroencephalograph with a sampling rate of 128 Hz and 2 channels（Brainwave Sensor ZA-9; Proassist, Osaka, Japan）.[23] Before going to bed, the participants attached electrodes coated with disposable Ag/AgCl to their forehead and mastoid processes to measure brain waves, and to below the outer corner of the right eye and above the outer corner of the left eye to measure eye movements. Raw signals were stored on an SD card and analyzed offline. EEG recordings during sleep were divided into 30 second intervals and brain waves were categorized as: delta（1.0-4.0 Hz）, theta（4.0-8.0 Hz）, alpha（8.0-12.0 Hz）, and beta（16.0-35.0 Hz）. The mean spectral density and standard deviation of each category were evaluated according to the participants' bands. Spectral data that were more than 3 standard deviations away from the mean and those observed when the participants were awake were excluded from the analysis as outliers. The bands of each domain were standardized by the mean of all participants' data. The analysis of brain waves led to the following classifications of sleep status: awake, REM sleep（stage R）, shallow non-REM sleep（stages N1 and N2）, and deep non-REM sleep（stage N3）. We also calculated sleep onset latency, defined as the length of time taken to reach deep non-REM sleep（N3）, and the length of nocturnal awakening.[24-26]

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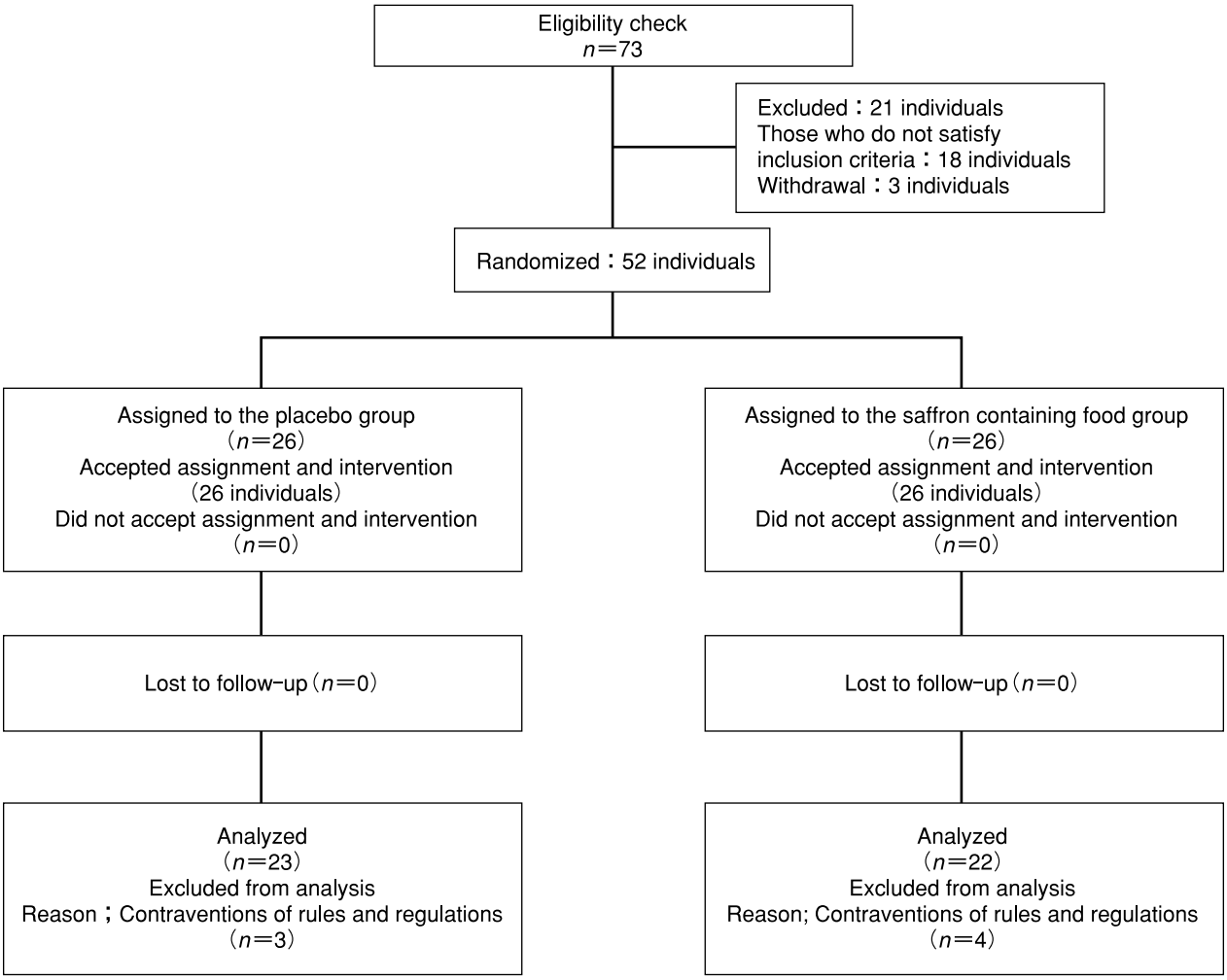


Fig. 1　Flowchart showing selection procedure for participants

Table 2　Participants' characteristics

	Saffron		Placebo		*P*-value
	n	Mean±SD	*n*	Mean±SD	
Age（year）	22	37.32±9.80	23	37.52±10.59	0.95
BMI（kg/m^2）	22	21.34±2.01	23	21.12±1.68	0.69
PSQIG（point）	22	8.50±1.71	23	8.48±1.62	0.97
Male: female ratio	22	7/15	23	6/17	0.75

6　Statistical analysis

JMP 13.0.0（SAS Institute Japan, Tokyo, Japan）was used for statistical analyses. Data are expressed as mean±standard deviation. In analyzing EEG recordings during sleep, the length osf each stage was evaluated as a proportion of each participant's sleep duration. To compare the two groups, Student's *t*-test was used.

RESULTS

1　Participants

Fig. 1 shows a flow chart outlining participant selection. Seventy-three individuals were screened and fifty-two were selected（twenty-six for the saffron group and twenty-six for the placebo group）. Seven participants who violated the study's rules and regulations during the study period were excluded from the analysis. **Table 2** shows the participants' characteristics.

2　OSA-MA

Table 3 shows the category scores of the OSA-MA. There were significant differences between the saffron group and the placebo group in reference to First factor; sleepiness upon rising, Second factor; initiation and maintenance of sleep, and Third factor; frequent dreaming. Significant differences between scores of

Table 3　OSA-MA results

	Pre-intake				After 4 weeks of intake			
	Saffron		Placebo		Saffron		Placebo	
Statistical measures	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD
First factor: Sleepiness upon rising	22	11.78±1.41	23	11.60±2.07	22	15.28±2.93	23	11.26±3.02
Second factor: Initiation and maintenance of sleep	22	13.33±2.14	23	13.71±1.45	22	15.40±2.81	23	11.77±2.32
Third factor: Frequent dreaming	22	21.78±3.55	23	21.90±5.73	22	23.72±5.49	23	16.70±7.40
Fourth factor: Feeling refreshed	22	13.07±1.97	23	13.01±2.05	22	12.85±4.41	23	11.43±3.37
Fifth factor: Sleep length	22	14.18±4.30	23	14.33±3.76	22	12.93±6.21	23	12.48±3.74

	Change				*P*-value between groups		
	Saffron		Placebo				
Statistical measures	*n*	Mean±SD	*n*	Mean±SD	Pre-intake	Four weeks later	Change
First factor: Sleepiness upon rising	22	3.50±2.51	23	−0.34±3.59	0.73	<0.01	<0.01
Second factor: Initiation and maintenance of sleep	22	2.07±2.38	23	−1.94±2.85	0.48	<0.01	<0.01
Third factor: Frequent dreaming	22	1.93±4.92	23	−5.21±6.41	0.93	<0.01	<0.01
Fourth factor: Feeling refreshed	22	−0.22±4.87	23	−1.58±3.80	0.92	0.23	0.30
Fifth factor: Sleep length	22	−1.25±5.20	23	−1.85±3.29	0.91	0.77	0.65

Table 4　Analysis of electroencephalography（EEG）recordings during sleep

	Pre-intake				After 4 weeks of intake			
	Saffron		Placebo		Saffron		Placebo	
Statistical measures	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD	*n*	Mean±SD
Sleep length（minutes）	22	407.15±24.06	23	397.43±29.50	22	410.87±23.69	23	397.78±29.41
Time to fall asleep（minutes）	22	24.20±19.23	23	24.24±25.68	22	12.07±10.11	23	21.50±19.52
Time to reach deep sleep（N3）（minutes）	22	21.02±14.57	23	20.69±12.10	22	11.44±11.59	23	17.92±9.90
Length of nocturnal awakening（%）	22	2.71±1.47	23	2.77±1.29	22	1.31±1.05	23	3.47±1.77
REM sleep（%）	22	21.73±2.46	23	22.00±2.65	22	20.63±2.23	23	22.02±2.48
Non-REM sleep（%）	22	70.34±4.01	23	69.46±3.85	22	74.32±3.41	23	67.90±4.05
Shallow sleep（N1+N2）（%）	22	46.86±5.51	23	47.08±3.83	22	49.32±5.15	23	46.22±3.97
Deep sleep（N3）（%）	22	23.47±4.42	23	22.38±3.86	22	25.00±4.27	23	21.68±3.79

	Change				*P*-value between groups		
	Saffron		Placebo				
Statistical measures	*n*	Mean±SD	*n*	Mean±SD	Pre-intake	Four weeks later	Change
Sleep length（minutes）	22	3.73±9.28	23	0.34±12.74	0.23	0.11	0.32
Time to fall asleep（minutes）	22	−12.13±12.41	23	−2.74±8.14	0.98	<0.05	<0.01
Time to reach deep sleep（N3）（minutes）	22	−9.59±9.35	23	−2.77±4.69	0.93	<0.05	<0.01
Length of nocturnal awakening（%）	22	−1.40±1.07	23	0.70±1.07	0.88	<0.01	<0.01
REM sleep（%）	22	−1.10±1.02	23	0.02±0.93	0.72	0.06	<0.01
Non-REM sleep（%）	22	3.98±1.41	23	−1.56±1.84	0.46	<0.01	<0.01
Shallow sleep（N1+N2）（%）	22	2.46±1.26	23	−0.86±1.39	0.88	<0.05	<0.01
Deep sleep（N3）（%）	22	1.52±0.76	23	−0.70±1.23	0.38	<0.01	<0.01

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the saffron group and the placebo group were noted for Q2 of the OSA-MS on concentration（saffron group: pre-intake 14.05±3.67, four weeks later 18.95±4.90; placebo group: pre-intake 18.95±4.90, four weeks later 12.72±3.94, difference between groups: $P<0.0001$）, Q3 on soundness of sleep（saffron group: pre-intake 14.36±4.07, four weeks later 17.82±3.57; placebo group: pre-intake 14.04±3.55, four weeks later 13.61±3.94, difference between groups: $P=0.0005$）, Q5 on feelings of physical fatigue（saffron group: pre-intake 13.23±2.84, four weeks later 15.35±4.44; placebo group: pre-intake 13.11±3.24, four weeks later 12.72±3.99, difference between groups: $P=0.0418$）, Q7 on sleepiness until falling asleep（saffron group: pre-intake 10.95±5.43, four weeks later 12.55±5.51; placebo group: pre-intake 12.04±2.75, four weeks later 8.04±5.68, difference between groups: $P=0.01$）, Q8 on the level of alertness（saffron group: pre-intake 11.14±2.14, four weeks later 16.25±4.3; placebo group: pre-intake 10.91±3.45, four weeks later 11.30±5.43, difference between groups: $P=0.0016$）, Q9 on having nightmares during sleep（saffron group: pre-intake 24.57±5.12, four weeks later 24.70±6.18; placebo group: pre-intake 22.46±8.26, four weeks later 18.35±9.17, difference between the groups: $P=0.0095$）, Q10 on ease of falling asleep（saffron group: pre-intake 12.36±3.26, four weeks later 15.77±4.03; placebo group: pre-intake 12.91±3.23, four weeks later 13.08±4.60, difference between groups: $P=0.0435$）, and Q12 on dreaming while asleep（saffron group: pre-intake 19.00±3.84, four weeks later 22.59±7.32; placebo group: pre-intake 21.04±5.97, four weeks later 15.04±7.18, difference between groups: $P=0.0011$）.

3　Electroencephalography during sleep

Table 4 shows the results of EEG analysis during sleep. The length of time taken to fall asleep and time of nocturnal awakening were significantly shorter in the saffron group compared to the placebo group. The length of time taken to reach deep sleep（N3）was significantly shorter in the saffron group compared to the placebo group. Furthermore, there were significant increases in the time spent in non-REM sleep, shallow sleep（N1＋N2）, and deep sleep（N3）in the saffron group compared to the placebo group.

4　Adverse events

There were three adverse events, including influenza, during the study period. However, each case was mild and no causal relationship with the intake of the study supplement was noted.

DISCUSSION

There are several natural substances which are effective in improving sleep and their active ingredients have been identified. Saffron has been reported to increase non-REM sleep in mice in a dose-dependent manner.[16] Studies in animals have shown that the components of saffron concurrently induce the activation of sleep-encouraging neurons and block wakefulness-encouraging neurons in animals.[27,28] The specific mechanism of action has been described as a promotion of re-absorption of serotonin[29,30] by safranal and inhibition of the re-absorption of dopamine and norepinephrine by crocin, thus adjusting the balance of both serotonin and dopamine.[31] As is the case for tryptamine, depletion of the serotonergic system can lead to mental instability.[32] These actions can reduce anxiety and sleep disruption brought about by stress and improve depression. There have been a small number of studies on the effects of saffron extract on sleep. In this study, we analyzed EEG during sleep to examine this in detail, and have confirmed that saffron extract increases non-REM sleep, encourages falling asleep, and reduces nocturnal awakening. Subjective indicators showed that sleepiness at the time of rising was reduced and the level of alertness was increased. This means that the discomfort felt at the time of rising due to a disturbed sleep rhythm was improved. We concluded that taking saffron extract adjusts serotonin and dopamine levels, which reduces the time taken to fall asleep and the time taken to reach deep sleep. This encouraged the improvement of sleep rhythm, which led to improvement in the subjective indicators.

CONCLUSION

We conducted a randomized, double-blind, placebo-controlled, parallel-group trial of adults with sleep problems using a dietary supplement containing saffron extract. Significant improvements were seen in the group taking the saffron supplement compared to the placebo group in these categories of the OSA-MA: sleepiness upon rising, initiation and maintenance of sleep, and frequent dreaming. Analysis of EEG recordings during sleep showed a reduction in the length of time taken to fall asleep and duration of nocturnal awakening, and an increase in the proportion of non-REM sleep in the saffron group. These results

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show that saffron extract containing crocin and safranal is effective in improving the quality of sleep and sleep rhythm.

【**Author contributions**】 conceptualization, R. T. and A. S.; project administration, R. T and A. Y.; food design, M. H., M. T. and J. K.; formal analysis, R. T., A. Y. and K. Y.; supervision, S. K.

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