Sleep Enhancement by Saffron Extract affron® in Randomized Control Trial

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ABSTRACT

Background A clinical trial was conducted using 21 healthy adults randomly assigned to a saffron extract group or placebo group in order to confirm the increase of sleep quality by taking saffron extract.
Objective To determine the effect of saffron extract on the sleep quality of 21 healthy adults.
Trial design A clinical parallel, double-blind, randomized control trial（RCT）was conducted. Subgroups were defined by good sleeper or poor sleeper using a PSQI test for analysis.
Method The participants, who gave self-report information, were under a randomized controlled trial（RCT）study designed for all participants receiving the saffron extract（crocin: 0.6 mg/day）or placebo.
Result There was a significant reduction in score for the Pittsburgh sleep quality index（PSQI）only in the group treated with the saffron extract. In addition, a significant saffron extract effect on daytime dysfunction over 4 weeks was apparent between the extract group and the placebo group in the subjects with poor sleeper at baseline.
Conclusion Overall, the effect of saffron extract on sleep quality in healthy adults was observed.（UMIN000026112）
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KEY WORDS Crocus sativus, Saffron, Sleep quality, Pittsburgh sleep quality index, Clinical trial

INTRODUCTION

According to an epidemiological survey in 2012, approximately twenty percent of Japanese people suffered with chronic symptomatic sleep disturbances, and it suggested that the appropriate interventions for better sleep might be needed for better health and quality of life.[1)] Moreover, it is well known that sleep disorder negatively affects work efficiency and increases risk in driving among other things. Some natural products have been used to enhance sleep for many centuries, although most have not been chemically or pharmacologically characterized. In fact Sansoninto and Saikokaryukotsuboreito and/or Kei-

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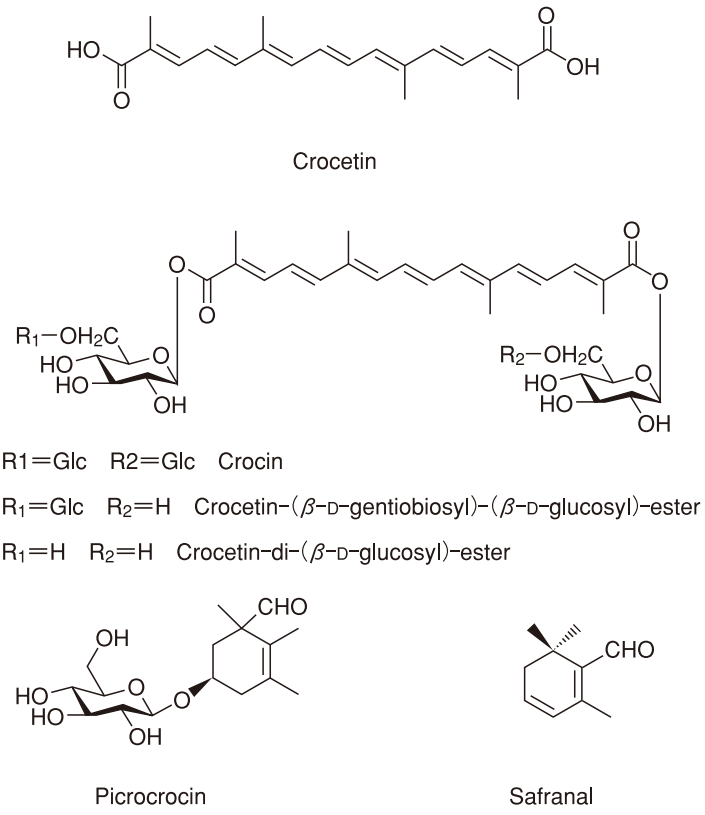

Fig. 1　Constituents of saffron

shikaryukotuboreito in Japanese Kampo medicine prescriptions have been listed as the sleep enhancer in Jin Gui Yao Lue and Shang Hang Lun respectively, from approximately two thousand years ago.[2)]

Saffron, *Crocus sativus* L., which belongs to the Iridaceae family, is a perennial herb with corms that have been cultivated for 3500 years, and now found mainly in Iran, and other countries such as Spain, Greece and Morocco. The plant blooms only once a year, and the manual harvest of stigmas should be performed within a very short time after. This is time-consuming with approximately 90000-170000 flowers yielding 5 kg of fresh stigmas or about 1 kg of dried stigmas. This contributes greatly to its high price.

Saffron is used in medicine, and also as a flavoring and a coloring agent. It has three main components: crocins bright yellow colouring carotenoids, picrocrocin bitter taste and safranal spicy aroma.

The carotenoid pigments consist of crocetin-di-(β-D-glucosyl)-ester, crocetin-(β-D-gentiobiosyl)-(β-D-glucosyl)-ester and crocetin-di-(β-D-gentiobiosyl)-ester（crocin）.[2,3)] Picrocrocin[4)] is a precursor of spicy aroma, safranal which is contained as a mono glycoside as shown in **Fig. 1** Furthermore saffron contains several minor components like 7-*O*-sophoroside of kaempferol,[5)] cyclohexanone derivative's glycosides[6)] and an anthraquinone derivative from tissue culture.[7)]

Considering the historical background of the pharmacological activity of saffron, De Materia Medica indicated that the fresher the saffron is, the more active it is. In addition, it promotes a bright facial skin tone and affects diuretic activity and inflammation.[8)] The Chinese Compendium of Materia Medica[9)] showed that saffron can be used successfully to treat depression. In the last 20 years it has become evident that saffron and its major constituent, crocin（**Fig. 1.**）has a wide range of uses including as an antioxidant,[10,11)] as a hypolipidemic agent[10,12)] as well as its anti-inflammatory properties.[13)] It is also used to treat Alzheimer's,[14)] and depression.[15)] Using various experimental animal models of brain disorders, such as cerebral ischemia[16)] and memory impairment, the neuroprotective activities of crocin in saffron were also investigated.[17-20)] Its anti-cancer activities *in vitro* and *in vivo*[21)] have been investigated extensively. Using mice, the anti-proliferation effects on human colorectal cancer cells,[22)] anti-skin cancer activity of crocin in saffron[23,24)] and anti-colon cancer[25)] were also determined. From the above indicated wide phar-

macological activities of saffron and/or its major constituent, crocin saffron can be considered a multifunctional herb medicine.

As previously indicated, Sansoninto and Saikokaryukotsuboreito and/or Keishikaryukotuboreito in Japanese Kampo medicine prescriptions have been used to promote sleep. In fact in a clinical trial for mental health patients suffering from sleep disorder,[26] the combination of Keishikaryukotsuboreito, Saikokaryukotuboreito and Sansoninto, and saffron enhanced sleep. From this evidence it is easily suggested that saffron might be related to sleep enhancement.

The aim of this study is to determine the effects of saffron extract on sleep quality. Secondly, a subgroup analysis of sleeping behaviour is conducted. Further details from the subgroup analysis are reported.

SUBJECTS AND METHODS

1　Test article

Saffron extract affron® was provided by SBS Company, Lim-ited, in the form of tablets formulated from a pressed dried saffron extract, standardized to contain saffron extract. This included 0.6 mg of crocin, silicon dioxide fine powder, microcrystalline cellulose, starch decom-position product, calcium stearate, coating agents, taken daily. Placebo were provided in the form of tab-lets containing the same ingredients as test tablets excluding saffron extract, with the same color, shape and size. The same amount of starch decomposition product was added to the placebo instead of the saf-fron extract which includes 0.6 mg/day of crocin. Two tablets were taken once a day.

2　Subjects and study design

Healthy adults（11 males, 10 females）were recruited from Western Japan. The key exclusion criteria were defined as individuals（1）who were continuously taking confounding nutritional supplements, quasi-medicine or/and medicine having the same activity with saffron product tested;（2）whose healthy diet had begun, by at least 4 weeks before the trial;（3）who were night or shift workers;（4）who were having or needed medical treatment（hormone replacement therapy, pharmacotherapy, kinesiology, dietary therapy）;（5）who currently had a disease or history of a diseases related to carbohydrate metabolism, or lipid metabolism, liver or kidney function, the circulatory system, respiratory, endocrine, immune, or nervous systems;（6）who had a history of alcohol and/or drug abuse;（7）who had a food allergy;（8）who were pregnant and/or lactating;（9）who were participating in another clinical trial（related to food, medicine, quasi-medicine, medical equipment）within 4 weeks in the 4 weeks prior to the trial or during this study. No participant withdrew. The twenty one participants were randomized to the saffron extract or placebo groups, stratified by self-reporting age. All data were collected at User Life Science Company, Limited.

A clinical parallel, double-blind, placebo-controlled study was conducted. The study was carried out in accordance with the principals of the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects（notification No. 3, 2014）. This study was also registered at University Hospital Medical Information Network（UMIN）Clinical Trial Registry, http://www.umin.ac.jp（UMIN000026112）. All subjects gave their written informed consent before the start of the study.（Kinki University, IRB NO. 201802; 1st March, 2018）

3　Assessment schedule

Participants initially attended a screening visit during which inclusion and exclusion criteria were checked with date of birth, sleep duration, wake up time and sleeping time, using the questionnaire of the Pittsburgh sleep quality index（PSQI）.[27] All participants received the saffron extracts（crocin: 0.6 mg/day）or placebo for 4 weeks. The PSQI questionnaire and also the questionnaires of the positive affect and negative affect schedule（PANAS）,[28] the Depression anxiety and stress scale（DASS-21）,[29] the profile of mood states（POMS）[30] for pre-intervention and post-intervention were collected. During the 4-week intervention period, participants self-completed a questionnaire of their compliance in terms of the investigational products or placebo consumption. All data were processed and analyzed by User Life Science Company, Limited.

4　Outcome measures

The Pittsburgh sleep quality index（PSQI）[27] was designed to measure sleep quality. PSQI consists of nineteen self-rated questions, which are grouped into scores with the seven component scores of subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. These component scores were combined to produce a global sleep

Table 1　Results for PSQI score

Survey item	Group	Baseline	*P*-value for placebo vs. saffron (Baseline)	4 wk	*P*-value for interaction	*P*-value for baseline vs. 4 wk
PSQIG point (0-21)	Placebo (n=11)	6.27±0.57	0.54	5.82±0.80	0.13	0.40
	Saffron (n=10)	6.90±0.91		4.90±0.74		0.04
Sleep quality	Placebo (n=11)	1.64±0.20	0.61	1.36±0.24	0.70	0.19
	Saffron (n=10)	1.50±0.17		1.10±0.23		0.17
Sleep latency	Placebo (n=11)	1.18±0.33	0.97	1.00±0.33	0.50	0.34
	Saffron (n=10)	1.20±0.36		0.80±0.33		0.17
Sleep duration	Placebo (n=11)	1.27±0.27	0.74	1.36±0.24	0.16	0.59
	Saffron (n=10)	1.40±0.27		1.00±0.33		0.22
Habitual sleep effiency	Placebo (n=11)	0.00±0.00	0.34	0.18±0.12	0.09	0.17
	Saffron (n=10)	0.10±0.10		0.00±0.00		0.34
Sleep disturbances	Placebo (n=11)	1.09±0.16	0.97	0.73±0.14	0.52	0.04
	Saffron (n=10)	1.10±0.18		0.90±0.18		0.34
Use of sleeping medication	Placebo (n=11)	0.36±0.28	0.88	0.36±0.28	—	—
	Saffron (n=10)	0.30±0.30		0.30±0.30		—
Daytime dysfunction	Placebo (n=11)	0.73±0.14	0.14	0.82±0.18	0.07	0.68
	Saffron (n=10)	1.30±0.33		0.80±0.25		0.05

ITT analysis
Values represent the mean±SE

quality index score, which is indicated in **Table 1**. In this paper, the significant results of PSQI are reported, although PANAS, DASS-21, POMS as primary outcomes were also measured.

5　Randomization

The randomization sequence was computer generated by an independent operator using simple randomized design. The sequence was saved to a password-protected spreadsheet, and groups were coded A and B. The randomization allocation was managed by recruiters, in order to ensure that the research assistants were blind to participants' group allocations. In addition, the randomization schedule and coding of group allocations were not accessible to the research assistants conducting the trial.

6　Blinding

First, participants were provided with only partial information on the study. All participants attended appointments in the same location and with the same format, as well as similar duration and frequency. All communication between participants and research staff during the period of intervention was done directly between participants and their respective recruiters. Research assistants did not have direct contact with participants for the duration of the intervention. Final assessments were organized by the recruiters, and research assistants remained blind to condition for the final assessment of outcomes. Prior to assessment, participants were reminded not to reveal the group to which they had been assigned. Statistical analyses were conducted by external statistic analyzers, who were blind to group allocation prior to analysis.

7　Statistical analysis

Data were analyzed with the SPSS statistical software. Analyses were performed on an intention-to-treat (ITT) basis. *T*-test and chi-squared test were used to determine statistical significance of participants sample characteristics for all outcomes. The means and standard errors against the individual evaluation were calculated. The Group comparison method, regarding individual evaluation average and comparison between before treatment and after treatment for all outcomes, was analyzed by repeated ANOVA following Bonferroni post hoc test. The tendency of variation between before and after treatment was observed by the reciprocal action (group comparison×before-and-after

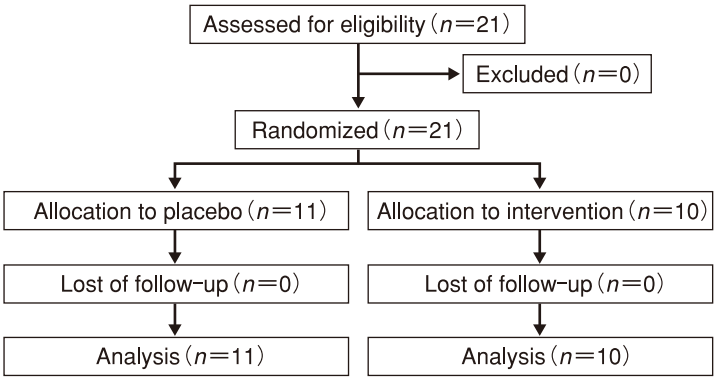

Fig. 2　Flow diagram of the progress through the phases of a parallel randomized trial of two groups

test）. Further analyses were conducted for comparisons of sleep quality outcomes, which were conducted with subjects defined by baseline sleep quality; good sleeper or poor sleeper, as good sleeper might have already achieved a high sleep quality, of which an increase could not be measured. The simple main effect was also analyzed. Statistical significance was set at the $P=0.05$ level.

RESULTS

Regarding the age of participants, a total of 21 healthy adults（over 21 years）aged 37.73±4.98 years（placebo: 11 participants）and 37.70±5.45 years（saffron extract group: 10 participants）were recruited. No subjects were withdrawn（**Fig. 2**）. All participants received the saffron extracts（crocin: 0.6 mg/day）or placebo on 14th February, 2017. The questionnaire for pre-intervention was collected on 16th February, 2017 and for post-intervention on 23rd March, 2017. No adverse events were observed. There was no significant difference when questionnaires between the saffron extract group and placebo group were compared; the two groups' characteristics were approximately equivalent. Analyses were performed on an intention-to-treat（ITT）and subgroup analysis with the subjects selected as good or poor sleepers.

Although the clinical trial for PANAS, DASS-21 and POMS as primary outcomes was performed, the focus was only on sleep quality because no significant differences relating to them were observed（**Appendix 1-3**）.

The results of the PSQI score are shown in **Table 1**. A significant lowering in the PSQI score after treatment was observed only in the saffron extract group（$P=0.042$）. In addition, a tendency towards a reduced level of daytime dysfunction after treatment was detected only in the saffron extract group（$P=0.052$）. Moreover, there was a tendency towards significant interaction over 4 weeks in daytime dysfunction between the extract group and placebo group（$P=0.070$）. However, a significant reduction in sleep disturbances was observed only in the placebo group（$P=0.038$）.

A remarkable difference in the saffron extract effect was shown in the subgroup analyses defined by baseline sleep quality; good sleeper or poor sleeper（**Table 2**）. A significant saffron extract effect on daytime dysfunction over 4 weeks was apparent between the extract group and placebo group in the subjects with poor sleeper at baseline（$P=0.047$）, whereas no significant differences were found in good sleepers. In addition, there were tendencies towards significant interactions over 4 weeks in PSQI score（$P=0.083$）and habitual sleep efficiency with poor sleeper（$P=0.091$）. Significant reductions in sleep latency（$P=0.047$）and daytime dysfunction（$P=0.047$）were observed only in the saffron extract group with poor sleeper. A tendency towards a reduced level of PSQI score after treatment was detected only in the saffron extract group（$P=0.050$）. There was no significant difference in the placebo group, but a tendency towards a reduction of sleep latency was observed in the placebo group with poor sleeper.

DISCUSSION

This study revealed an increase in waking at midday when taking saffron extracts, as it showed that a tendency towards significant interaction over 4 weeks in daytime dysfunction was apparent between the extract group and placebo group. In addition, a significant saffron extract effect on daytime dysfunction over 4 weeks was also apparent in both groups in the subjects

Table 2　Results for PSQI score; good sleeper and poor sleeper

	Good sleeper（*n*=7）					
	Placebo（*n*=4）			Saffron（*n*=3）		
Survey item	Baseline	4 wk	*P*−value	Baseline	4 wk	*P*−value
PSQIG point	4.25±0.25	3.75±1.25	0.72	3.67±0.67	3.33±0.33	0.42
Sleep quality	1.00±0.00	0.75±0.25	0.39	1.33±0.33	1.00±0.00	0.42
Sleep latency	0.25±0.25	0.50±0.50	0.39	0.33±0.33	0.67±0.67	0.42
Sleep duration	1.25±0.48	1.25±0.25	1.00	1.00±0.00	0.67±0.67	0.42
Habitual sleep effiency	0.00±0.00	0.00±0.00	—	0.00±0.10	0.00±0.00	—
Sleep disturbances	0.75±0.25	0.25±0.25	0.18	0.67±0.33	0.67±0.33	—
Use of sleeping medication	0.25±0.25	0.25±0.25	1.00	0.00±0.00	0.00±0.00	—
Daytime dysfunction	0.75±0.25	0.75±0.25	—			

	Poor sleeper（*n*=14）					
	Placebo（*n*=7）			Saffron（*n*=7）		
Survey item	Baseline	4 wk	*P*−value	Baseline	4 wk	*P*−value
PSQIG point	7.43±0.48	7.00±0.76	0.41	8.29±0.81	5.57±0.95	0.05
Sleep quality	2.00±0.22	1.71±0.29	0.36	1.57±0.20	1.14±0.34	0.29
Sleep latency	1.71±0.36	1.29±0.42	0.08	1.57±0.42	1.14±0.40	0.05
Sleep duration	1.29±0.36	1.43±0.37	0.36	1.57±0.30	1.14±0.40	0.36
Habitual sleep effiency	0.00±0.00	0.29±0.18	0.17	0.14±0.14	0.00±0.00	0.36
Sleep disturbances	1.29±0.18	1.00±0.00	0.17	1.29±0.18	1.00±0.22	
Use of sleeping medication	0.43±0.43	0.43±0.43	—	0.43±0.43	0.43±0.43	
Daytime dysfunction	0.71±0.18	0.86±0.26	0.60	1.71±0.36	1.00±0.31	0.05

PPS analysis
Values represent the mean±SE

with poor sleeper at baseline. The increases in sleep quality and sleep efficacy using saffron extracts were also considered, as were the tendencies towards significant interactions over 4 weeks in PSQIG score（PSQI global score）and habitual sleep efficiency in subjects with poor sleeper.

Matsuhashi reported that in a clinical trial on mental health patients suffering from sleep disorder, their sleep quality was enhanced by taking Japanese Kampo medicine prescription, such as Keishikaryukotuboreito, Saikokaryukotuboreito and Sansoninto which contain saffron extract.[26] These patients had some tolerance to medicines due to taking many kinds of medicine, not only these Japanese Kampo medicine, but also modern medicines for mental illnesses, medicines such as perphenazine, trihexyphenidyl hydrochloride, haloperidol and/or flunitrazepam. Therefore, these patients seemed to need alternative treatment to reduce drug dosage. Previously, by feeding crocin, a major component of saffron, to mice, the occurrence of non−REM sleep was observed and as a result also that of REM sleep.[31] This study suggested that crocin accumulated and remained in the body for 40 days; as a result of this, sleeping behavior improvement was noted, of which a mechanism might be that crocin works to modulate the histaminergic or cholinergic arousal systems, which induces non−REM sleep. The concentration of crocin in saffron is known to be extremely small, and this might not be enough to promote sleeping quality.[26] It was confirmed that the concentration of crocin in commercial saffron is 1.5 to 2.0% because crocin is unstable, depending on the conjugated polyene structure and also the cleavage of glycoside linkage in crocin is occurred by glycoside that is linked to Cronin due to an endogenous β−glucosidase with moisture in saffron. This is the reason why saffron should be stored under dry and cold conditions.[31] Therefore, it is strongly suggested from the study that saffron extract supplementation which provides high concentrate crocin could be helpful for those with mental health problems and sleep disorder.

This study has several limitations. Generalizability of results may be limited, as the participants of the medical trial study might have a tendency to be health conscious and be highly knowledgeable about health. However, we believe that our eligibility criteria are

also applicable to the Japanese population as a whole. For the subgroup analysis, each stratum included insufficient subjects, thus analysis of more subjects is needed for an accurate analysis.

Further study with more subjects is warranted to determine the effects of saffron extract on quality of sleep quality.

In summary, it was concluded that the saffron extract containing about 0.6 mg of crocin（approximately 0.016 mg/kg/day）enhanced sleep in the healthy population. Although there were limitations, statistical analysis showed a significant increase of daytime dysfunction in subjects with poor sleeper. In addition, the tendencies to significant interactions over 4 weeks in PSQIG score and habitual sleep efficiency were also found. Previously we confirmed that crocin antagonizes ethanol inhibition of NMDA receptor-mediated responses related to long-term potentiation.[17] Recently Tatsuki et al.[32] found the inflow of calcium via NMDA receptor into the brain, resulting in the enhancement of sleep. In addition, a study using various gene-manipulated mice, such as knock-out（KO）mice of receptors for adenosine, histamine, or dopamine, found that crocin-induced sleep was reduced in histamine H1 receptor KO mice.[33] Another study described the mechanism of an antagonistic effect by the binding of saffron extract and crocetin to the NMDA receptor, which might block the channel pore of the NMDA receptor system.[34] From this evidence, it can be suggested that crocin might stimulate sleeping behavior through the NMDA receptor.

【Conflict of Interest】 This study was conducted by a contract research organization, User Life Science Company, Limited（Izuka, Fukuoka, Japan）and was funded by Kurume Research Park Company, Limited（Kurume, Fukuoka, Japan）. Tablets（saffron extracts and placebo）were provided by the SBS Company, Limited（Tenjin, Fukuoka, Japan）.

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Appendix 1　Results for DASS–21 score

Survey item	Group	Baseline	4 wk	*P*–value for interaction	*P*–value for baseline vs. 4 w
Depression	Placebo（*n*＝11）	4.82±1.54	3.73±1.55	0.30	0.51
	Saffron（*n*＝10）	6.30±1.83	2.70±1.01		0.048
Anxiety	Placebo（*n*＝11）	3.36±0.77	1.73±0.69	0.33	0.14
	Saffron（*n*＝10）	5.30±1.66	2.10±0.56		0.01
Stress	Placebo（*n*＝11）	6.73±1.81	4.18±1.56	0.38	0.14
	Saffron（*n*＝10）	8.70±1.84	4.00±1.01		0.01

Values represent the mean±SE.

Appendix 2　Results for PANAS score

Survey item	Group	Baseline	4 wk	*P*–value for interaction	*P*–value for baseline vs. 4 w
Positive affect	Placebo（*n*＝11）	20.27±1.76	22.73±1.12	0.20	0.30
	Saffron（*n*＝10）	26.60±2.34	24.60±2.32		0.42
Negative affect	Placebo（*n*＝11）	20.91±1.52	18.91±2.46	0.33	0.51
	Saffron（*n*＝10）	24.60±2.92	18.30±2.26		0.06

Values represent the mean±SE.

Appendix 3　Results for POMS score

Survey item	Group	Baseline	4 wk	*P*−value for interaction	*P*−value for baseline vs. 4 w
Tension−Anxiety	Placebo（*n*＝11）	52.09±2.90	47.27±2.74	0.50	0.18
	Saffron（*n*＝10）	51.80±3.31	50.50±3.73		0.72
Depression−Dejection	Placebo（*n*＝11）	51.27±2.67	51.73±3.42	0.67	0.90
	Saffron（*n*＝10）	54.20±3.80	52.40±3.94		0.63
Anger−Hostility	Placebo（*n*＝11）	54.91±4.33	50.00±3.77	0.25	0.11
	Saffron（*n*＝10）	55.50±3.75	55.70±3.57		0.95
Vigor	Placebo（*n*＝11）	39.36±2.32	40.82±2.53	0.29	0.58
	Saffron（*n*＝10）	44.50±2.59	50.00±3.33		0.06
Fatigue	Placebo（*n*＝11）	54.55±4.02	51.64±3.66	0.85	0.27
	Saffron（*n*＝10）	58.30±2.78	54.70±3.35		0.19
Confusion	Placebo（*n*＝11）	58.45±3.71	54.82±3.48	0.41	0.14
	Saffron（*n*＝10）	58.00±4.09	57.30±3.35		0.78

Values represent the mean±SE.

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