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Passionflower extract improves diurnal quality of life in Japanese subjects with anxiety: A randomized, placebo-controlled, double-blind trial

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ABSTRACT

Background and objective: *Passiflora incarnata* (passionflower) has traditionally been used to treat insomnia and anxiety. We recently reported that an aqueous ethanol extract of passionflower (PFE) and its flavonoid glycosides, enhanced the expression of *Period* (*Per*) 2, a clock gene, in mouse liver and fibroblasts. However, the influence of PFE on daily activities or emotions has not been examined in humans.

Aim: This study conducted a clinical trial of PFE supplementation in healthy Japanese participants and investigate if PFE influences sleep and emotions.

Methods: This randomized, double-blind, placebo-controlled study examined the effects of PFE (200 mg daily) containing 3% flavonoid glycosides (6 mg daily). We enrolled 44 Japanese men and women who were reluctant to work, do house chores or engage in irregular shift work. All subjects were randomly allocated into either the PFE group (n=22) or the placebo group

(n=22) using a computerized random-number generator. Capsules containing either PFE or placebo were administered for 12 weeks between August 2017 and January 2018. Both emotional status and sleep quality were evaluated by using the Japanese version of Medical Outcomes Study Short-Form 36-Item Health (SF-36) questionnaire and the Oguri-Shirakawa-Azumi (OSA) sleep inventory score at 6- and 12-week of ingestion.

Results: The per protocol set comprised 20 subjects in the PFE group and 18 subjects (20 subjects for OSA and safety evaluation) in the placebo group. After intake of PFE (200 mg/day) for 6 weeks, some of the SF-36 domain scores were significantly improved compared with those of the placebo group, including the scores for role/social component summary, social functioning, and role-emotional. After 12 weeks, the scores for mental component summary and vitality showed significant improvement in the subjects taking PFE (200 mg/day) compared to those taking placebo. In contrast, none of the OSA sleep score parameters were significantly improved by PFE compared with placebo. Laboratory tests did not reveal any abnormalities suggesting adverse effects of PFE.

Conclusions: Intake of PFE (200 mg/day for 12 weeks) improved several emotional parameters related to daytime social and mental activities. PFE was suggested to be useful for improving anxiety.

Trial Registration: UMIN-CTR: UMIN000028622

Foundation: Oryza Oil & Fat Chemical Co., Ltd.

Keywords: SF-36 questionnaire; passionflower; *Passiflora incarnata*; flavonoid; emotion; Oguri-Shirakawa-Azumi sleep inventory

BACKGROUND

Passiflora (*P.*) *incarnata* is commonly known as passionflower and has been used as an herbal sedative and an anxiolytic. Clinically, an anxiolytic effect of passionflower has been reported in patients before undergoing surgery. For example, a single oral dose of *P. incarnata* (260 mg) suppressed anxiety in patients before tooth extraction, suggesting an anxiolytic effect [1]. In addition, passionflower improved the State Anxiety Inventory score (STAI-S), which indicates psychological states, just before spinal anesthesia [2]. Moreover, oral administration of *P. incarnata* (500 mg) improved the numerical rating scale (NRS) anxiety score in patients before surgery without having a sedative effect [3]. The effect of *P. incarnata* on anxiety was reported to be equivalent to that of oxazepam [4] or melatonin [5]. Passionflower also has been found to improve sleep disturbance. One example, Ngan *et al*. [6] reported that passionflower tea improves sleep in subjects with mild fluctuation of sleep quality. In regards to more severe symptoms, passionflower has been reported to promote the efficacy of clonidine in patients

with opioid dependence [7]. The use of *P. incarnata*, has been observed to improve anxiety and sleep patterns gives it a promising outlook for future use.

Preclinical studies of passionflower extract (PFE) have demonstrated psychotropic effects in mouse behavioral models. Administration of PFE (approximately 400 mg/kg) to mice increased momentum in the unfamiliar environment test [8] and the elevated plus maze test [9]. It has been suggested that GABA-sensitive neurons [10, 11] and an opioidergic mechanism [10] are involved in the anxiolytic effect of PFE, especially during long-term administration. On the other hand, we previously found that the same PFE material as this study enhances the expression of clock genes such as *period* (*Per*) 2 in mouse liver and fibroblasts [12]. Based on these reports, we considered that PFE might influence sleep and emotions in humans, thus we conducted a clinical trial in healthy Japanese adults to investigate this possibility.

MATERIALS AND METHODS

Participants

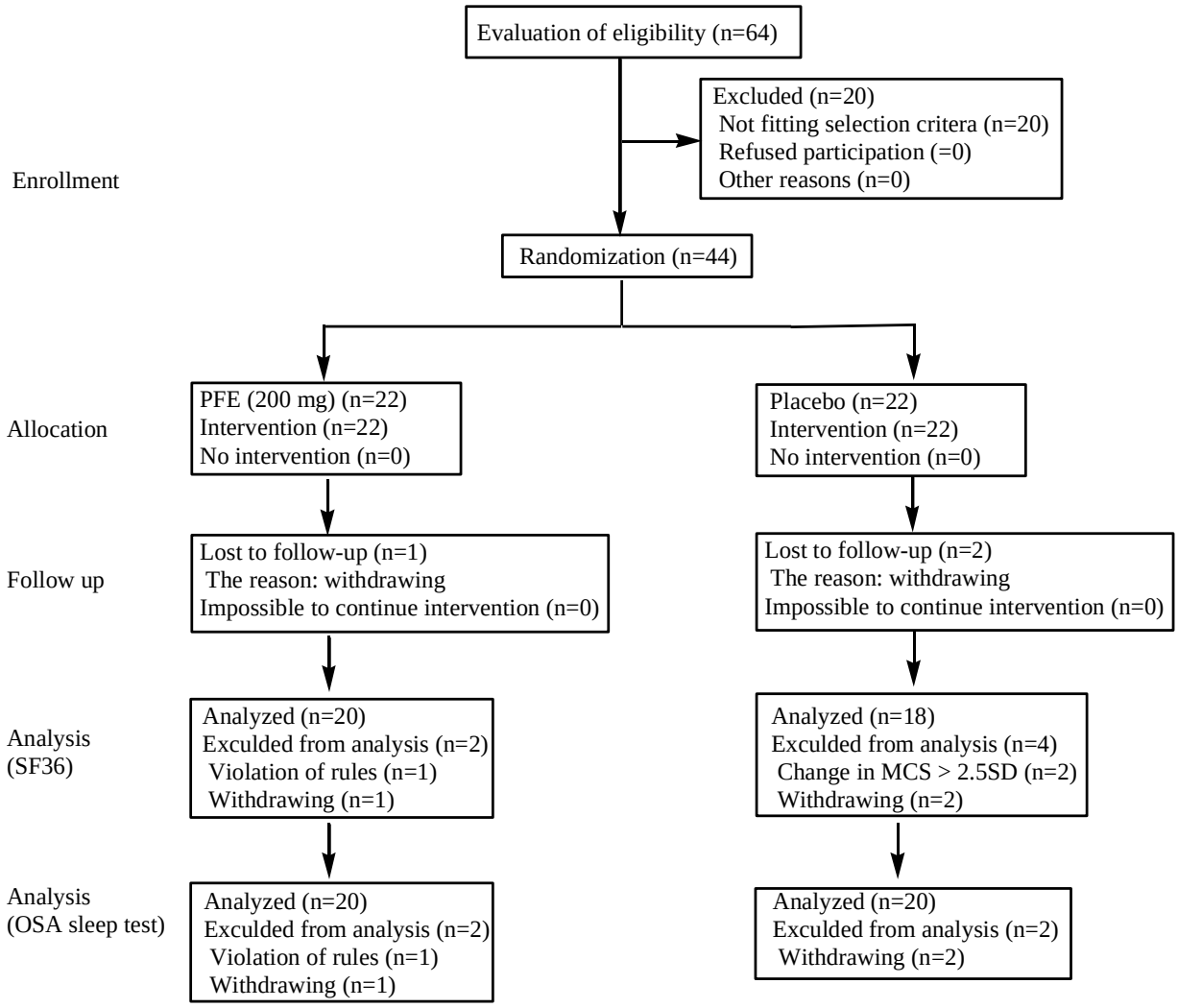
All subjects were recruited from August 8 to September 30, 2017 through the Go106 website (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan). The inclusion criteria were healthy Japanese adults (male or female) with disturbance of their life rhythm or lack of motivation when engaging in their job or housework. Exclusion criteria were as follows.

1) Current or previous cancer, heart failure, or myocardial infarction.
2) Current treatment for arrhythmia, hepatitis, nephritis, rheumatoid arthritis, cerebrovascular disease, diabetes, hyperlipidemia, hypertension, or other chronic diseases.
3) Current use of medications or dietary supplements.
4) Experienced allergic reaction to foods related to passionflower or medicines.
5) Persons with hay fever, house dust allergy, or asthma.
6) Pregnancy, lactation, or expected/planned pregnancy during the study period.
7) Subjects currently participating in another clinical trial or who had participated within the previous 3 months.
8) Subjects determined to be inappropriate for the study for other reasons by the attending physician.

Sixty-four subjects with relatively low mental component summary (MCS) scores in the Japanese version of Medical Outcomes Study Short-Form 36-Item (SF-36) Health Survey [13] were selected after they were confirmed to be suitable for the study by a physician (Fig. 1). Then, the allocation controller, who was not directly involved in this study, allocated 22 subjects into 2 groups, using add-in software for Microsoft Excel (StatLight #11, Yukms Co. Ltd., Kanagawa, Japan). The allocation was based on the SF-36 MCS score [mean and standard deviation (SD)], male/female ratio, and age in an approximately 1:1 ratio. The subjects were asked to avoid excessive eating and drinking and to maintain a regular lifestyle during the study period. One day before testing, subjects were required to avoid excessive drinking of alcohol

and hard exercise, including a 6 hour fast, with the exception of water, prior to blood collection.

Figure 1. Flowchart showing the disposition of the subjects



Preparation and allocation of test articles

The test samples (indistinguishable brown capsules containing either PFE or placebo) were provided by Oryza Oil & Fat Chemical Co., Ltd. as hard capsules. The PFE capsules contained 100 mg of Passionflower Extract-P (standardized passionflower extract powder with 3.0% total flavonoids, including 0.90% isovitexin, 0.27% isovitexin *O*-glucoside, 0.39% isoschaftoside, and 0.24% homoorientin) and 100 mg of dextrin. Passionflower Extract-P was consisting of 60% PFE, 38% dextrin, and 2% fine silicon dioxide. The placebo capsules contained 196 mg of dextrin and 4 mg of fine silicon dioxide. Information about the allocation was strictly protected by third-party study allocation controllers not directly involved in the study, and this information was not disclosed to any other party until the subjects for analysis were determined at a clinical conference after study completion.

Study protocol

This randomized, placebo-controlled, double-blind, parallel-group study was carried out at Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan), and statistical analysis was performed by ORTHOMEDICO Inc. The protocol was registered at the University Hospital

Medical Information Network Clinical Trials Registry (UMIN000028622). Subjects took two appropriate capsules (either placebo or PFE) daily within 1 hr before going to sleep for 12 weeks. All subjects recorded a daily report including capsule ingestion, lifestyle, urination, menstruation (only women), and implementation of questionnaire.

Following examination items were conducted baseline and 6 and 12 weeks after intake. The SF-36 Health Survey was used to assess the primary outcomes and was completed before treatment and after 6 and 12 weeks of intake. The SF-36 is shown in Table 1. Answer sheets were processed according to the manual, [14] and scores from 0 to 100 were calculated for the domains of physical functioning, role-physical, bodily pain, general health perception, vitality, social functioning, role-emotional, and mental health. Furthermore, these categories divided into physical component summary (PCS), mental component summary (MCS), and role/social component summary (RCS) and were calculated. The higher value indicates the better health condition.

Table 1. SF-36 (Ver. 2)

Question **Score**	**1**	**2**	**3**	**4**	**5**	**6**
1. In general, would you say your health is:	Excellent ○	Very good ○	Good ○	Fair ○	Poor ○	-
2. Compared to one year ago, how would you rate your health in general now?	Much better now than one year ago ○	Somewhat better now than one year ago ○	About the same ○	Somewhat worse now than one year ago ○	Much worse now than one year ago ○	-
3. The following items are about activities you might do during a typical day. Does your health limit you in these activities? If so, how much?	Yes, limited a lot	Yes, limited a little	No, not limited at all	-	-	-
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.	○	○	○	-	-	-
b. Moderate activities, such as moving a table, using a vacuum cleaner, bowling, or playing golf.	○	○	○	-	-	-
c. Lifting or carrying groceries.	○	○	○	-	-	-
d. Climbing several flights of stairs.	○	○	○	-	-	-
e. Climbing one flight of stairs.	○	○	○	-	-	-
f. Bending, kneeling, or stooping.	○	○	○	-	-	-
g. Walking more than a kilometer.	○	○	○	-	-	-
h. Walking several meters.	○	○	○	-	-	-
i. Walking one hundred meter.	○	○	○	-	-	-
j. Bathing or dressing yourself.	○	○	○	-	-	-
4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?	All of the time	Most of the time	Some of the time	A little of the time	None of the time	-
a. Cut down the amount of time you spent on work or other activities.	○	○	○	○	○	-
b. Accomplished less than you would like.	○	○	○	○	○	-
c. Were limited in the kind of work or other activities.	○	○	○	○	○	-
d. Had difficulty performing work or	○	○	○	○	○	-

Question **Score**	**1**	**2**	**3**	**4**	**5**	**6**
other activities (for example, it took extra effort).						
5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems?	All of the time	Most of the time	Some of the time	A little of the time	None of the time	-
a. Cut down the amount of time you spent on work or other activities.	○	○	○	○	○	-
b. Accomplished less than you would like.	○	○	○	○	○	-
c. Didn't do work or other activities as carefully as usual.	○	○	○	○	○	-
6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?	Not at all ○	Slightly ○	Moderately ○	Quite a lot ○	Extremely ○	-
7. How much bodily pain have you had during the past 4 weeks?	None ○	Very mild ○	Mild ○	Moderate ○	Severe ○	Very severe ○
8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?	Not at all ○	A little ○	Moderately ○	Quite a lot ○	Extremely ○	-
9. How much of the time during the past 4 weeks were you?	All of the time	Most of the time	Some of the time	A little of the time	None of the time	-
a. Full of pep	○	○	○	○	○	-
b. Very nervous	○	○	○	○	○	-
c. So down in the dumps that nothing could cheer you up	○	○	○	○	○	-
d. Calm and peaceful	○	○	○	○	○	-
e. Full of energy	○	○	○	○	○	-
f. Downhearted and blue	○	○	○	○	○	-
g. Worn out	○	○	○	○	○	-
h. A happy person	○	○	○	○	○	-
i. Tired	○	○	○	○	○	-
10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?	All of the time	Most of the time	Some of the time	A little of the time	None of the time	-
11. How true or false is each of the following statements for you?	Definitely true	Mostly true	Don't know	Mostly false	Definitely false	-
a. I seem to get sick a little easier than other people.	○	○	○	○	○	-
b. I am as healthy as anybody I know.	○	○	○	○	○	-
c. I expect my health to get worse.	○	○	○	○	○	-
d. My health is excellent.	○	○	○	○	○	-

The Oguri-Shirakawa-Azumi (OSA) sleep score was used to assess the sleep quality as secondary outcomes [15]. The questionnaire covers items such as remaining fatigue,

concentration ability, deepness of sleep, relaxing, dullness, appetite, dozing off, clearness of thought, nightmares, quality of sleep, feelings, dreaming, waking at night, answering ability, sleeping time, and deepness of sleep. The participant gave a score for each item, based on how if that statement was true or false. We evaluated the following 5 factors: sleepiness on rising (Factor I), initiation and maintenance of sleep (Factor II), frequent dreaming (Factor III), feeling of refreshment (Factor IV), and sleep length (Factor V).

Laboratory tests

Analysis of blood and urine was performed by LSI Medience Corporation (Tokyo, Japan). All examination items were conducted at a baseline and after 6 and 12 weeks of intake. A venous blood sample was collected from an arm vein and the following tests were performed for assessment of safety.

Hematology components were as follows: red blood cell count, leukocyte count, hemoglobin, hematocrit, platelet count, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC).

Biochemical components were the following: total protein, total bilirubin, urea nitrogen, creatinine, uric acid, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglyceride, hemoglobin (Hb) A1c, blood glucose, alkaline phosphatase (ALP), creatinine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GTP), amylase, lactate dehydrogenase (LDH), Na, K, Cl, Ca, Fe, and inorganic phosphorus (IP).

In addition, urine samples were collected for qualitative evaluation including protein, glucose, urobilinogen, bilirubin, ketone bodies, pH and occult blood.

Ethics, adherence, and compliance

This study was performed according to the Declaration of Helsinki (2013 revision), and was carried out in conformity with ethical considerations. This protocol was approved by the Ethics Committee of Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan) on August 7, 2017 (Approved ID: 1708-1703-OY01-01-TC), and substantial deviation from the protocol required authorization by the committee. All subjects received a full explanation about the protocol and purpose of the study before giving consent for participation. No subject was part of sponsor or funder companies.

Statistical analysis

Primary and secondary outcomes are reported as the median and interquartile range. The Mann-Whitney *U*-test was used for comparisons between the placebo group and the PFE group. The results of the physical examination and blood tests are reported as the mean and SD. The paired student's *t*-test was used for evaluation of the significance of differences between, before, and after ingestion of the test sample. The χ^2-test was used for urinalysis parameters, with normal and abnormal values being coded as "1" and "0", respectively. We set the significance level at 5% with no adjustment for multiple comparisons. SPSS (Ver. 23.0, Japan IBM) or Microsoft Excel 2013 was used for statistical evaluation.

RESULTS

Study performance

The study was performed from October 23, 2017 to January 20, 2018. During the study period, one subject in the PFE (200 mg) group and 2 subjects in the placebo group withdrew from the study for personal reasons. In addition, one subject was excluded from the analysis in the PFE (200 mg) group because of violation of compliance. Analysis of MCS data, showed that changes of the values exceeded 2.5 SD in 2 subjects from the placebo group. These subjects were excluded from analysis in the placebo group of SF-36. Accordingly, 20 subjects (5 male, 15 female, 44.5 ± 11.5 years) were available for analysis of both, SF-36 and the OSA sleep inventories in the PFE (200 mg) group. While, 18 (5 male, 13 female, 38.9 ± 10.8 years) and 20 subjects (5 male, 15 female, 38.5 ± 10.4 years) were respectively available for SF-36 and OSA sleep inventory analysis in the placebo group. Table 2 shows the physical profile of the subjects included in analysis.

Table 2. Physical profile of the subjects

	Baseline		12 W	
	PFE (200 mg)	Placebo	PFE (200 mg)	Placebo
Height (cm)	160.6 ± 7.5	162.3 ± 7.4	–	–
Body weight (kg)	56.0 ± 11.1	59.5 ± 12.4	56.8 ± 10.5	60.3 ± 12.1
BMI (kg/m^2)	21.6 ± 3.3	22.5 ± 3.8	21.9 ± 3.1	22.8 ± 3.7
Body fat ratio (%)	22.9 ± 6.2	24.5 ± 6.7	23.2 ± 6.1	23.9 ± 6.9

Data are represented as the mean ± SD (n = 20). There were no significant differences between the placebo group and the PFE (200 mg) group.

SF-36 parameters

Table 3 lists the outcome of SF-36. After 6 weeks, changes of RCS, social functioning, and role-emotional were significantly larger in the PFE (200 mg) group compared with the placebo group ($P = 0.008$, $P = 0.027$, $P < 0.050$, respectively). Similarly, changes of MCS and vitality after 12 weeks were significantly larger in the PFE (200 mg) group than the placebo group ($P = 0.048$, $P = 0.022$, respectively). There was also a significant difference in the absolute value of vitality ($P = 0.045$).

OSA sleep inventory

As shown in Table 4, the OSA sleep inventory scores did not demonstrate any significant differences between the two groups for any sleep parameters at any time of assessment.

Table 3. Changes of SF-36 parameters

	Baseline		6 W		12 W	
	PFE (200 mg)	Placebo	PFE (200 mg)	Placebo	PFE (200 mg)	Placebo
PCS	57.3 (51.2 - 60.4)	53.2 (50.1 - 62.4)	54.1 (51.2 - 58.1) -2.5 (-7.0 - 3.6)	56.5 (51.7 - 62.1) 0.5 (-2.6 - 5.0)	54.0 (51.3 - 58.5) -0.6 (-5.0 - 1.1)	55.2 (51.3 - 59.8) 0.6 (-4.3 -4.7)
MCS	39.8 (31.8 - 43.3)	40.9 (34.3 - 44.3)	44.9 (39.3 - 51.4) 7.4 (4.7 - 10.9)	46.4 (40.1 - 50.7) 7.9 (3.7 - 12.1)	51.0 (45.6 - 56.0) 10.0 (4.8 -16.6)*	46.2 (40.5 - 50.2) 5.4 (2.3 - 11.3)
RCS	40.5 (35.2 - 48.2)	44.7 (39.9 - 53.4)	52.3 (46.5 - 58.0) 8.3 (1.8 - 12.6)**	50.5 (35.7 - 53.8) -0.3 (-6.3 - 4.7)	51.5 (47.0 - 55.2) 5.9 (2.3 - 15.2)	54.8 (44.7 - 58.6) 2.9 (-0.7 -13.4)
Physical functioning	54.2 (46.1 - 57.8)	54.2 (47.0 - 57.8)	54.2 (54.2 - 57.8) 0.0 (0.0 - 7.2)	54.2 (50.6 - 57.8) 0.0 (0.0 - 3.6)	56.0 (53.3 - 57.8) 3.6 (0.0 - 4.5)	56.0 (47.9 - 57.8) 0.0 (0.0 - 3.6)
Role function	44.1 (41.6 - 55.7)	54.1 (36.6 - 55.7)	54.1 (45.8 - 55.7) 0.0 (0.0 - 7.5)	55.7 (43.3 - 55.7) 0.0 (0.0 - 5.8)	55.7 (44.9 - 55.7) 1.7 (0.0 - 13.3)	55.7 (52.4 - 55.7) 0.0 (0.0 - 6.6)
Bodily pain	49.7 (39.8 - 49.5)	47.0 (44.7 - 61.7)	52.3 (44.7 - 61.7) 2.0 (-4.4 - 9.9)	58.1 (50.1 - 61.7) 0.0 (0.0 - 12.3)	61.7 (48.8 - 61.7) 4.7 (0.0 - 11.8)	54.6 (51.2 - 61.7) 0.0 (0.0 - 15.2)
General health perception	44.2 (39.8 - 49.5)	44.2 (41.5 - 48.6)	49.5 (44.2 - 54.8) 3.7 (0.0 - 8.9)	45.5 (43.4 - 52.2) 2.7 (0.0 - 9.1)	52.2 (44.2 - 55.5) 7.2 (0.0 - 9.5)	46.9 (44.2 - 51.5) 0.0 (-0.8 - 9.6)
Vitality	37.0 (26.5 - 43.4)	37.0 (28.2 - 42.6)	45.0 (37.0 - 50.6) 9.6 (3.2 - 12.8)	45.0 (34.6 - 49.8) 6.4 (4.0 - 12.0)	53.0 (45.0 - 56.3) * 16.1 (9.6 - 19.3)*	46.6 (43.4 - 49.0) 8.0 (4.0 - 12.8)
Social role functioning	37.7 (36.1 - 45.7)	44.1 (37.7 - 55.4)	50.6 (44.1 - 57.0) 12.9 (0.0 - 14.5) *	47.4 (39.3 - 57.0) 0.0 (0.0 - 6.4)	57.0 (44.1 - 57.0) 12.9 (4.8 - 19.3)	57.0 (47.4 - 57.0) 6.4 (0.0 - 12.9)
Role-emotional	39.4 (31.1 - 51.9)	43.6 (35.3 - 54.0)	56.1 (43.6 - 56.1) 6.2 (0.0 - 13.5) *	49.8 (35.3 - 56.1) 0.0 (0.0 - 8.3)	56.1 (50.9 - 56.1) 10.4 (3.1 - 17.7)	56.1 (47.7 - 56.1) 6.2 (0.0 - 12.5)
Mental health	39.7 (37.1 - 46.5)	38.4 (33.0 - 49.1)	47.8 (41.1 - 57.2) 8.1 (2.0 - 11.4) *	46.5 (41.1 - 51.2) 8.1 (5.4 - 10.7)	51.8 (47.8 - 57.2) 10.7 (5.4 - 14.1)	49.1 (41.1 - 54.5) 6.7 (2.7 - 10.1)

Actual scores (upper lines) and changes of the scores (lower lines) are represented as the median and interquartile range (n = 18 for the PFE group and n = 20 for the placebo group). Asterisks indicate significant differences vs. placebo at *: $p < 0.05$, **: $p < 0.01$.

Table 4. Changes of OSA scores

	Baseline		6 W		12 W	
	PFE (200 mg)	Placebo	PFE (200 mg)	Placebo	PFE (200 mg)	Placebo
Factor I Sleepiness on rising	35.5 (33.0 - 39.8)	33.8 (30.8 - 37.6)	41.7 (38.3 - 46.0) 4.6 (1.8 - 9.5)	43.2 (37.8 - 45.8) 8.5 (5.3 - 11.4)	44.7 (41.3 - 49.3) 7.1 (4.2 - 11.0)	42.4 (37.2 - 47.8) 7.7 (3.9 - 12.4)
Factor II Initiation and maintenance of sleep	37.3 (33.5 - 39.5)	40.7 (35.5 - 44.9)	43.6 (39.8 - 48.8) 5.6 (2.6 - 11.1)	43.1 (40.9 - 51.4) 4.9 (2.2 - 6.7)	45.7 (43.3 - 49.1) 7.1 (4.6 - 13.6)	42.2 (40.6 - 56.0) 5.2 (-0.3 - 13.2)
Factor III Frequent dreaming	43.2 (38.8 - 49.7)	41.0 (34.5 - 51.2)	47.8 (40.6 - 52.6) 3.3 (-4.5 - 6.1)	51.1 (40.8 - 56.4) 2.5 (0.0 - 11.7)	44.0 (39.5 - 54.0) 0.0 (-3.3 -11.7)	48.5 (39.2 - 55.0) 4.5 (0.3 - 8.9)
Factor IV Refreshing	38.3 (34.3 - 41.1)	36.9 (28.7 - 40.9)	46.4 (42.0 - 49.2) 7.5 (3.4 - 13.3)	43.0 (39.5 - 46.7) 8.4 (3.8 - 10.5)	46.6 (42.9 - 50.2) 8.6 (2.0 - 14.4)	43.6 (41.3 - 48.8) 10.4 (3.2 - 13.9)
Factor V Sleep length	39.7 (35.9 - 42.3)	40.2 (34.7 - 43.1)	43.8 (40.0 - 51.2) 5.3 (2.1 - 10.6)	42.6 (39.7 - 48.9) 5.7 (-0.6 - 9.9)	45.3 (42.5 - 52.0) 7.9 (1.1 - 13.7)	45.0 (39.0 - 49.1) 5.0 (-0.1 - 10.1)

Actual scores (upper lines) and changes of the scores (lower lines) are represented as the median and interquartile range (n = 20 in both groups).

Table 5. Changes of the blood pressure, pulse rate, and hematology components

	Baseline	**6 W**	**12 W**	**Standard value**
Systolic pressure (mmHg)				
PFE (200 mg/day)	117.1 ± 20.6	117.2 ± 20.0	122.5 ± 23.8	< 125
Placebo	114.7 ± 12.4	114.2 ± 11.9	119.9 ± 13.6	
Diastolic pressure (mmHg)				
PFE (200 mg/day)	71.5 ± 12.7	73.3 ± 14.6	75.5 ± 15.1	< 85
Placebo	71.6 ± 10.7	73.9 ± 9.1	75.3 ± 8.9	
Pulse rate (beats/min)				
PFE (200 mg/day)	71.5 ± 11.8	72.8 ± 9.9	73.7 ± 11.0	
Placebo	72.3 ± 9.5	76.6 ± 11.9	75.0 ± 11.9	
Red blood cells ($\times 10^4$cells/μL)				
PFE (200 mg/day)	455 ± 31	453 ± 37	457 ± 31	Male 430 - 570
Placebo	460 ± 40	466 ± 38	468 ± 40	Female 380 - 500
Leukocytes (cells/μL)				
PFE (200 mg/day)	5435 ± 1625	5065 ± 1432	4785 ± 1215	3300 – 9000
Placebo	5515 ± 1565	5735 ± 1589	5695 ± 1686	
Hemoglobin (g/dL)				
PFE (200 mg/day)	13.7 ± 1.2	13.7 ± 1.1	13.8 ± 1.1	Male 13.5 - 17.5
Placebo	13.4 ± 1.7	13.5 ± 1.6	13.7 ± 1.7	Female 11.5 - 15.0
Hematocrit (%)				
PFE (200 mg/day)	43.0 ± 3.3	42.7 ± 3.4	43.6 ± 3.1	Male 39.7 - 52.4
Placebo	42.1 ± 4.0	42.5 ± 3.8	43.3 ± 3.9†	Female 34.8 - 45.0
Platelets ($\times 10^4$cells/μL)				
PFE (200 mg/day)	26.3 ± 4.4	26.8 ± 4.6	27.1 ± 5.6	14.0 - 34.0
Placebo	28.5 ± 4.1	29.3 ± 4.9	28.5 ± 4.9	
MCV (fL)				
PFE (200 mg/day)	94.6 ± 3.6	94.4 ± 3.5	95.5 ± 3.1	85 - 102
Placebo	91.6 ± 6.5	91.4 ± 6.9	92.8 ± 7.1††	
MCH (pg)				
PFE (200 mg/day)	30.2 ± 1.2	30.3 ± 1.0	30.3 ± 1.1	28.0 - 34.0
Placebo	29.2 ± 2.7	29.0 ± 2.7	29.3 ± 2.8	
MCHC (%)				
PFE (200 mg/day)	31.9 ± 0.7	32.1 ± 0.6	31.7 ± 0.8	30.2 - 34.0
Placebo	31.8 ± 1.4	31.7 ± 1.4	31.6 ± 1.5	

Data are represented as the mean ± SD (n = 20 in both groups). Daggers denote significant differences from before PFE or placebo ingestion at †: $p < 0.05$, ††: $p < 0.01$.

Laboratory data and adverse effects

The blood pressure, pulse rate, and hematological components are listed in Table 5. In the placebo group, the hematocrit and MCV were significantly increased at 12 weeks compared with the initial values, but there were no significant changes in the PFE group. With regard to biochemical components (Tables 6a and 6b), total protein and Ca decreased slightly within reference ranges after ingestion of PFE for 12 weeks, while there was a significant increase of LDL-cholesterol, HDL-cholesterol, blood glucose, and amylase. However, these changes were all within reference ranges. Urinalysis parameters showed no changes in either group (Table 7).

Table 6a. Changes of biochemical parameters

	Baseline	6 W	12 W	Standard value
Total protein (g/dL)				
PFE (200 mg/day)	7.10 ± 0.39	7.09 ± 0.42	6.95 ± 0.38†	6.7 - 8.3
Placebo	7.11 ± 0.38	7.08 ± 0.29	7.07 ± 0.30	
Total bilirubin (mg/dL)				
PFE (200 mg/day)	0.79 ± 0.32	0.83 ± 0.31	0.82 ± 0.30	0.2 - 1.2
Placebo	0.83 ± 0.31	0.88 ± 0.36	0.84 ± 0.35	
Urea N (mg/dL)				
PFE (200 mg/day)	12.1 ± 3.3	11.6 ± 3.1	11.5 ± 2.8	8 – 20
Placebo	11.3 ± 3.0	12.5 ± 3.8	11.3 ± 2.7	
Creatinine (mg/dL)				
PFE (200 mg/day)	0.66 ± 0.13	0.66 ± 0.13	0.64 ± 0.12	Male 0.61 - 1.04
Placebo	0.67 ± 0.09	0.65 ± 0.09	0.63 ± 0.10†	Female 0.47 - 0.79
Uric acid (mg/dL)				
PFE (200 mg/day)	4.7 ± 1.6	4.8 ± 1.6	4.6 ± 1.6	Male 3.8 - 7.0
Placebo	4.8 ± 1.2	4.9 ± 1.2	4.5 ± 1.2	Female 2.5 - 7.0
Total cholesterol (mg/dL)				
PFE (200 mg/day)	203 ± 35	206 ± 36	208 ± 37	120 – 219
Placebo	192 ± 37	195 ± 27	205 ± 30†	
LDL-cholesterol (mg/dL)				
PFE (200 mg/day)	110 ± 29	114 ± 32	118 ± 31†	65 - 139
Placebo	110 ± 34	114 ± 28	123 ± 32††	
HDL-cholesterol (mg/dL)				
PFE (200 mg/day)	72 ± 16	77 ± 16††	78 ± 16††	Male 40 - 85
Placebo	61 ± 14	62 ± 15	66 ± 16†	Female 40 - 95
Triglyceride (mg/dL)				
PFE (200 mg/day)	75 ± 43	74 ± 44	72 ± 34	30 - 149
Placebo	89 ± 62	91 ± 65	98 ± 53	
HbA1c (%)				
PFE (200 mg/day)	5.3 ± 0.3	5.3 ± 0.3	5.3 ± 0.3	4.6 - 6.2
Placebo	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.3	
Blood glucose (mg/dL)				
PFE (200 mg/day)	79 ± 10	83 ± 8	83 ± 8†	70 - 109
Placebo	80 ± 6	81 ± 7	84 ± 15	
ALP (U/L)				
PFE (200 mg/day)	175 ± 42	170 ± 41	174 ± 41	100 - 325
Placebo	170 ± 34	172 ± 38	173 ± 37	

Data are represented as the mean ± SD (n = 20 in both groups). Daggers denote significant differences from before PFE or placebo ingestion at †: $p<0.05$ and ††: $p<0.01$.

Table 6b. Changes of biochemical parameters.

	Baseline	6 W	12 W	Standard value
CK (U/L)				
PFE (200 mg/day)	102 ± 83	103 ± 77	115 ± 99	Male 60 – 270
Placebo	83 ± 52	101 ± 54	83 ± 43	Female 40 – 150
AST (U/L)				
PFE (200 mg/day)	21.9 ± 6.8	21.7 ± 6.3	23.1 ± 10.9	10 – 40
Placebo	20.1 ± 5.2	20.1 ± 4.9	19.7 ± 5.2	
ALT (U/L)				
PFE (200 mg/day)	19.3 ± 8.8	18.5 ± 7.7	19.1 ± 9.4	5 – 45
Placebo	18.3 ± 7.2	19.8 ± 8.5	18.6 ± 10.6	
γ-GTP (U/L)				
PFE (200 mg/day)	24.4 ± 17.8	23.6 ± 16.5	23.2 ± 16.2	Male ≤80
Placebo	32.0 ± 31.2	28.6 ± 21.4	28.0 ± 20.2	Female ≤30
Amylase (U/L)				
PFE (200 mg/day)	75.4 ± 20.9	79.0 ± 22.5	80.9 ± 21.8†	40 – 122
Placebo	72.6 ± 20.1	70.8 ± 18.6	76.1 ± 20.4†	
LDH (U/L)				
PFE (200 mg/day)	180 ± 25	177 ± 20	177 ± 27	120 – 240
Placebo	171 ± 29	164 ± 26	166 ± 23	
Na (mEq/L)				
PFE (200 mg/day)	141 ± 2	141 ± 1	140 ± 2	137 - 147
Placebo	141 ± 2	140 ± 2†	140 ± 2†	
K (mEq/L)				
PFE (200 mg/day)	4.0 ± 0.3	4.1 ± 0.4	4.1 ± 0.4	3.5 - 5.0
Placebo	4.0 ± 0.2	4.0 ± 0.2	3.9 ± 0.3	
Cl (mEq/L)				
PFE (200 mg/day)	102 ± 2	102 ± 2	101 ± 2	98 - 108
Placebo	102 ± 2	102 ± 1	101 ± 2	
Ca (mg/dL)				
PFE (200 mg/day)	9.2 ± 0.4	9.2 ± 0.3	9.0 ± 0.3†	8.4 - 10.4
Placebo	9.4 ± 0.3	9.2 ± 0.3	9.0 ± 0.3†	
Fe (μg/dL)				
PFE (200 mg/day)	104 ± 26	105 ± 43	115 ± 47	Male 50 - 200
Placebo	90 ± 35	90 ± 47	88 ± 42	Female 40 - 180
IP (mg/dL)				
PFE (200 mg/day)	3.5 ± 0.6	3.3 ± 0.5	3.4 ± 0.4	2.5 - 4.5
Placebo	3.6 ± 0.6	3.3 ± 0.5†	3.5 ± 0.5	

Data are represented as the mean ± SD (n = 20 in both groups). Daggers denote significant differences from before PFE or placebo ingestion at †: $p < 0.05$ and ††: $p < 0.01$.

Table 7. Changes of urinalysis parameters

	Week	PFE (200 mg/day)		Placebo		Standard value
		Standard value		Standard value		
		Within	Without	Within	Without	
Protein	0	18	2	15	5	(-)
	6	18	2	11	9	
	12	18	2	13	7	
Glucose	0	20	0	20	0	(-)
	6	19	1	20	0	
	12	20	0	20	0	
Urobilinogen	0	20	0	20	0	(±)
	6	20	0	20	0	
	12	20	0	20	0	
Bilirubin	0	20	0	20	0	(-)
	6	20	0	20	0	
	12	20	0	20	0	
pH	0	19	1	20	0	5.0-7.5
	6	20	0	19	1	
	12	17	3	20	0	
Occult blood	0	18	2	16	4	(-)
	6	15	5	17	3	
	12	16	4	13	7	
Ketone bodies	0	20	0	20	0	(-)
	6	20	0	19	1	
	12	20	0	20	0	

Data are represented as number of subjects within or without of standard values.

DISCUSSION

Previous clinical studies of passionflower have shown that it is effective for suppressing preoperative anxiety when a single dose was administered before surgery [1-3, 5]. While these studies evaluated the acute effect of passionflower in patients, the current study demonstrated that PFE improved MCS, RCS, social functioning, role-emotional, and vitality in healthy subjects performing shift work or with moderate anxiety related to their job/housework. These parameters were composed of mental factors [13]. PFE ameliorated anxiety due to unhealthy lifestyle or daily tasks. In addition, this effect of PFE was demonstrated to persist for several months. In a previous long-term intervention study, Gibbert *et al*. [16] administered PFE for 12 weeks and evaluated the effect on resistance to stress and QOL in patients suffering from

nervous restlessness. They found that PFE improved resilience and QOL, corresponding well with our results. Regarding the anxiolytic mechanism of passionflower, PFE has been reported to inhibit GABA uptake into rat cortical synaptosomes and binding of GABA analogs to the GABA receptor [17].

Flavonoids and an alkaloid (halmine), have been reported as promising active compounds in PFE [8]. The PFE used in our study had a standardized flavonoid content, including 6 flavonoid glycosides, and did not contain halmine. Therefore, flavonoids may play a major role in the anxiolytic effect of PFE.

Investigation of sleep quality showed that PFE did not affect the OSA sleep score, even though it was given orally just before sleep, and only improved diurnal psychological function. The target subjects of this study did not have sleep disorders. If PFE had been evaluated in subjects with sleep problems, it may have improved sleep quality, as reported by Ngan et al. [6].

CONCLUSIONS

This study demonstrated that intake of PFE (200 mg/day for 12 weeks) ameliorated mental anxiety in healthy subjects associated with irregular shift work and routine daily tasks. Therefore, PFE may be useful for improving the quality of life. Furthermore, the intake of PFE was found to be safe under the conditions of the study.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CK, creatinine kinase; GTP, glutamyltransferase; HDL, high density lipoprotein; Hb, hemoglobin; IP, inorganic phosphorus; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCS, mental component summary; MCV, mean cell volume; NRS, numerical rating scale; OSA, Oguri-Shirakawa-Azumi; PCS, physical component summary; *Per*, period; PFE, passionflower extract; RCS, role/social component summary; SD, standard deviation; SF-36, Short-Form 36-Item; STAI-S, state anxiety inventory score; TG, triglyceride

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Authors' Contributions: Dr. Takara conducted the study and performed the tests. Dr. Shimoda prepared test samples and Dr. Shimoda and Mrs. Hirano wrote the manuscript. Mr. Shimizu determined the contents of flavonoids in test sample. Mr. Yamamoto and Ms. Suzuki coordinated the study and analyzed the data.

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