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Original Research Article

# Effect of saffron extract supplementation on mood in healthy adults with subclinical symptoms of depression: a randomized, double-blind placebo-controlled study

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A B S T R A C T

**Background:** Subclinical depressive symptoms, including low mood, fatigue and anxiety, refer to clinically relevant depressive manifestations that do not meet the criteria for major depressive disorder. These symptoms affect quality of life and can lead to chronic mental health issues. Nutritional interventions, such as saffron extract supplementation, may help modulate mood and inflammation, potentially alleviating these symptoms.

**Objectives:** This study evaluated the efficacy of a 6-wk saffron extract supplementation on mood in healthy individuals with subclinical neuropsychiatric symptoms and explored the underlying mechanisms.

**Methods:** This randomized, double-blind, placebo-controlled study involved 51 adult healthy individuals who received oral administration of either saffron extract or a placebo for 6 wk. The primary outcome was a composite *z*-score averaging standardized scores of depression (Beck Depression Inventory-II), anxiety (State-Trait Anxiety Inventory-YA), and fatigue (Multidimensional Fatigue Inventory 20). Secondary outcomes included neuropsychiatric scores, quality of life, inflammatory markers, and hypothalamic-pituitary-adrenal axis reactivity. Amino acid derivatives were analyzed in blood samples.

**Results:** Saffron extract did not significantly affect the primary outcome of combined depressive, anxiety, and fatigue symptoms (*z*-score) nor individual symptoms. However, it improved autoperceived mental health, as reflected in increased mental health scores over time on the Medical Outcome Study Short-Form 12 questionnaire, compared with placebo (mean at 6 wk: 53.8 ± 12.7 vs 44.6 ± 11.4 for placebo and saffron group, respectively; time × treatment, $P = 0.04$). There were no significant effects on inflammatory parameters or hypothalamic-pituitary-adrenal axis reactivity. Metabolomic analysis revealed that saffron extract significantly modulated N-acetyl-phenylalanine.

**Conclusions:** Saffron extract supplementation do not affect subclinical depressive symptoms, either as a composite score or individual symptom categories. A potential effect on improved mental health outcomes cannot be excluded but requires further replication in future well-powered trials. This trial (Saffromfood study) is registered at clinicaltrial.gov as NCT05690126 (https://clinicaltrials.gov/study/NCT05690126?term=NCT05690126&rank=1).

*Keywords*: subclinical depressive symptoms, saffron, dietary supplement, mood alteration, quality of life, inflammation, hypothalamic-pituitary-adrenal axis reactivity, metabolomic

## Introduction

Subclinical symptoms of depression are highly prevalent in the general population [1,2]. These subtle manifestations, encompassing persistent low mood, mild anhedonia, fatigue, and generalized worry, often go unrecognized and untreated. Although milder than clinical syndromes, they still significantly affect mental health and quality of life, increasing health care use and economic costs [3,4]. Moreover, epidemiological studies have shown that mild, subclinical, and/or

*Abbreviations:* AA, amino acid; BCAA, branched-chain amino acid; BDI, Beck Depression Inventory; CAR, cortisol awakening response; CRP, C-reactive protein; HPA, hypothalamic-pituitary-adrenal; MFI, Multidimensional Fatigue Inventory; MOS SF, Medical Outcome Study Short-Form; NRS, neurotoxicity rating scale; PLS-DA, partial least squares discriminant analysis; STAI, State-Trait Anxiety Inventory; VIP, variable importance in projection.

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chronic depressive symptoms predisposes to major depressive disorders [2,5,6]. Accordingly, initiating early intervention may represent an effective strategy to prevent the onset of clinically relevant depressive symptoms and potentially reduce the need for pharmacological interventions.

In recent years, natural compounds have drawn growing interest for managing emotional well-being and mood disorders [7–10]. Among these, saffron, derived from the stigmas of the *Crocus sativus*, has garnered attention due to its high content of bioactive apocarotenoids (≤8% on dry weight) such as crocetin, crocins, picrocrocin, and safranal. These compounds are believed to contribute to its therapeutic effects [10]. Several clinical studies have shown beneficial effects of saffron on mood, particularly in patients with major depressive disorder [11–13]. Recently, a translational study demonstrated the promotivational effect of saffron in a rat model of stress-induced anhedonia and in patients diagnosed with mild unipolar or bipolar depression [14]. Although mechanisms remain poorly understood, saffron modulates several biological processes involved in the regulation of mood and behavior [13,15,16], notably through its anti-inflammatory and antioxidant properties [17–19]. Elevated inflammatory markers such as IL-6 and C-reactive protein (CRP) are commonly found in major depression [20–22], and inflammation is known to alter monoamine transmission and hypothalamic-pituitary-adrenal (HPA) axis activity [21,23,24], both of which may contribute to depression [25,26]. Interestingly, saffron and its compounds have been shown to modulate these biological pathways in preclinical studies [13]. For example, we and others have recently reported in rodents that administration of a saffron extract improves serotoninergic and dopaminergic neurotransmission, while reducing depressive-like behaviors [10,27,28]. Additionally, clinical and preclinical studies have demonstrated that saffron also attenuates stress-induced HPA axis activation and related psychological stress response [29,30]. Numerous studies also emphasized the role of amino acids (AA) and their metabolites in mood regulation and mental health, given their involvement in neurotransmitter synthesis and other metabolic processes also crucial for brain function [31–34]. Disruptions in pathways such as tryptophan, tyrosine, and glutamine have been increasingly associated with psychiatric disorders [32,35]. Moreover, metabolites produced in AA catabolism, such as kynurenine and its derivatives, modulate neuroinflammation and oxidative stress, processes closely linked to neuropsychiatric conditions [36–38]. However, few studies have yet explored the effect of saffron on AA metabolism [27,28]. This knowledge could provide new insights into the mechanisms underlying the beneficial effects of saffron on mood. Furthermore, existing studies on saffron have largely focused on clinical depression and have not examined its impact on symptom dimensions, such as emotional and neurovegetative symptoms, nor delved deeply into its mechanisms of action [13]. Therefore, this study aimed to investigate whether saffron extract can improve emotional well-being and alleviate both emotional and neurovegetative symptoms in individuals with subclinical symptoms. We also determined the effect of saffron on inflammation, HPA axis activation, and AA metabolism, due to their key role in the control of well-being and mood.

## Methods

### Study participants

In total, 51 healthy adult volunteers (aged 18–50 y) with subjective complaints of anxiety and/or stress and low mood were recruited between December 2022 and May 2023 from an existing database available at a contract research organization (CRO, CEN Nutriment) based in Dijon, France. Participants were included if they were experiencing subclinical symptoms of depressed mood, anxiety, fatigue, and/or stress, as defined as having ≥6 symptoms of moderate or greater intensity among 18 symptoms on the neurotoxicity rating scale (NRS), a 5-point Likert scale ranging from absent to very severe assessing the intensity of general behavioral and neurovegetative symptom [39]. All participants had to be willing and able to comply with the study protocol, provide written informed consent, and be affiliated with a national health insurance system. Exclusion criteria were the following: BMI (in kg/m$^2$) <18.5 or >30 , psychiatric or a history of psychiatric illness; diagnosis of cognitive pathology; metabolic diseases; untreated or unstabilized hypertension or thyroid disease; severe chronic diseases (e.g., cancer, severe chronic pain, and HIV) or chronic inflammatory conditions (e.g., Crohn disease and rheumatoid arthritis); pathologies likely to affect the HPA axis; current anti-inflammatory, immune-modulator treatment; recent (last 6 mo) or current psychotropic treatment, intake of food supplements for anxiety, depression or insomnia or for improving/maintaining cognitive function; regular intake of corticosteroids; consumption of illegal psychotropic substances; elevated alcohol and/or cigarette consumption (>20 cigarettes/d); uncorrected visual impairment; food allergies/insensitivities; an hormonal status likely to induce an unstable/fluctuating emotional state (e.g., menopausal transition); presence of life event likely to induce unstable/fluctuating emotional state (e.g., change of professional function/situation, death of a family member, divorce, and surgery); female who were pregnant or lactating or seeking to become pregnant; worked night shifts; engaged in high levels of physical activity (to avoid bias in the salivary cortisol measures); consumed >500 mg caffeine per day; and dietary supplement use 2 wk before enrolment.

### Procedure

Each subject was randomly assigned to daily intake of either 30 mg saffron extract (the saffron group) or maltodextrin (the placebo group) using 4 permuted blocks randomization. The randomization was performed using Sealed Envelope Ltd, 2022, to create a blocked randomization list and by a person not involved in the study in order to ensure the double-blind conditions.

Eligible participants were initially screened by phone to verify suitability for study inclusion (Supplemental Figure 1). Those who met inclusion criteria were invited for a more detailed screening (V0 visit). During this visit, inclusion/exclusion criteria were confirmed, and eligible participants provided written informed consent, after reading a complete description of the study. Lifestyle and demographic data (age, sex, marital status, and educational level), heart rate, blood pressure, and a medical history were collected. At the end of this V0 visit, participants were provided with saliva collection containers and instructions for saliva collection. Within 7 days of the screening visit, participants returned to the laboratory for the first test visit (V1) where they received their pillbox. A second visit (V2) was programmed after 6 wk of supplementation (Supplemental Figure 1).

The study protocol was approved by the Institutional Committee of Protection of Persons (registration number 2022-A01556-37) and was registered in the clinicaltrials.gov registry (clinicaltrials.gov identifier: NCT05690126).

### Treatment and dosing schedule

Saffron extract (Activ'Inside) contained >2.5% of crocines (High performance liquid chromatography [HPLC] method) and >1.5% of safranal (UV Spectrometric method; ISO3632). Each product (saffron

extract or placebo) was presented in 2 hard-shell capsules with identical appearance (color and size), with each containing 370 mg powder, including 15 mg saffron extract for the saffron group or maltodextrin for the placebo group. A daily dose of 30 mg saffron (15 mg twice daily) was selected based on previous clinical trials demonstrating preliminary support for efficacy and safety in the treatment of mild-to-moderate depression and anxiety [40–42]. This dosage falls within the established safe range, as saffron is generally well tolerated at doses ≤1.5 g/d, with toxicity reported only >5 g/d [42]. Subjects received a pillbox containing 90 capsules at V1 and were instructed to take 2 capsules orally with water each day (the first one during breakfast and the second one during dinner) for 6 wk.

Compliance was assessed by counting the capsules that were returned by subjects. Participants with a compliance of <80% were considered to be noncompliant.

## Primary outcome

The primary outcome was a *z*-score combining the depression, anxiety, and fatigue scores. Depression was measured using the Beck Depression Inventory, Second edition (BDI-II) [43], anxiety with the State-Trait Anxiety Inventory (STAI) form YA [44], and fatigue with the Multidimensional Fatigue Inventory (MFI)-20 [45]. The composite score was the average of 3 *z*-scores [$z = (x - \mu)/\sigma$, where x = value for the individual, μ = mean for the total population (placebo + saffron), and σ = SD of the total population (placebo + saffron)].

## Secondary outcomes

### *Neuropsychiatric symptoms*

At baseline (V1) and at the end of the study (V2), depressive symptoms (BDI), anxiety (STAI), stress, fatigue (MFI), and neurovegetative symptoms, as well as sleep quality and quality of life, were assessed using validated self-reported questionnaires (French versions). General and neurobehavioral symptoms, sleep quality, and quality of life were assessed by self-reports, including the NRS, the Pittsburgh Sleep Quality Index [46], and the Medical Outcome Study Short-Form (MOS SF)-12 [47], respectively. The NRS, a 39-item self-report instrument, was used to assess a broad range of physical and psychological symptoms that were shown to relate to inflammatory conditions [40]. Symptoms were rated from 0 (not present) to 4 (extremely severe), with total scores ranging from 0 to 148. Previous studies have demonstrated the scale's sensitivity to symptoms of fatigue, mood disturbances, and cognitive difficulties in both clinical populations (e.g., patients receiving interferon-α therapy) and individuals with subclinical or inflammation-related conditions such as obesity with psychiatric comorbidity [48,49]. As previously described, NRS symptoms were grouped into 4 specific symptom dimensions for data analysis, including the dimensions of sickness (tiredness, fever, sick feeling, body aches, joint/muscle pain, and headaches), cognitive symptoms (indecisiveness, distractibility, episodes of confusion, word-finding problems, and memory impairment), anxiety (anxiety, tension, irritability, agitation, and worries about health), and altered sleep (difficulty getting to sleep or staying asleep and sleeping too much) [49]. The MOS SF-12 evaluates 8 domains as follows: physical functioning, role-physical, bodily pain, general health, energy/vitality, social functioning, role-emotional, and mental health. It also provides 2 summary scores: the Physical Component Summary and the Mental Component Summary. Subjective perception of stress was assessed by a visual analog scale (VAS) for stress, ranging from 1 (not stressed) to 10 (very stressed).

### *Blood parameters*

Fasting blood samples were collected at V1 and V2. For plasma preparation, EDTA tubes were centrifuged at +4 °C for 20 min at 2800 g, while serum was obtained by centrifuging dry tubes at +4 °C for 10 min at 1500 g. The resulting plasma and serum samples were then frozen at −80 °C in a biobank. Plasma concentrations of IL-6 was determined using the human IL-6 DuoSet ELISA kits (R&D systems), following manufacturer's instructions. The assay sensitivity and intra-/interassay variability were respectively 56.72 pg/mL, ±4.1%, and ±3.9%, respectively, and the limit of quantification was 0.031 pg/mL. Our data were distributed between 0.19 and 9.95 pg/mL. Serum concentrations of high-sensitivity (hs)CRP were assessed at the Automated Medical Biology Platform (PABiM) from Bordeaux University Hospital by quantitative immunoturbidimetry using the MULTIGENT CRP Vario assay on ARCHITECT cSystems analyzers (C8200 and C16000), according to manufacturer's instructions. All samples were analyzed in duplicates.

### *Salivary cortisol*

The HPA axis reactivity was examined by measuring the cortisol awakening response (CAR) in saliva. Saliva samples were collected by passive drooling into saliva sampling devices (Sarstedt Ltd). Samples were requested to be collected immediately upon waking and 30 min after, following recommendations provided in the literature [50]. Participants were advised to refrain from eating, brushing their teeth, smoking, or drinking anything aside from water until the collection ended for the day. After collecting the 2 samples, participants were asked to store the samples in a provided refrigerated bag until the test visit. Samples were then frozen at −80 °C until analysis. After thawing, the saliva samples were centrifuged at 1000 × *g* for 2 min. Salivary cortisol concentrations were determined by ELISA assay, according to the manufacturer's specifications (ENZO Life Sciences; ADI-900-071). All samples were analyzed in duplicates. The assay sensitivity and intra-/interassay variability were respectively 0.20 ng/mL, ±6.6%, and ±7.8%, respectively, and the limit of quantification was 0.04 pg/mL. Our data were distributed between 0.51 and 29.26 pg/mL.

## Exploratory analysis

### *Targeted metabolomics*

The target quantitation of essential AA, branched-chain AA (BCAA) and their catabolites generated by host and gut microbiota in plasma [trimethylamine, trimethylamine N-oxide, L-valine, L-methionine, histidine, L-isoleucine, L-leucine, L-phenylalanine, 3-methoxy-p-tyramine, L-tyrosine, serotonin, hippuric acid, kynurenic acid, 5-hydroxyindole-3-acetic acid, indole-3-acetic acid, indole-3-carboxaldehyde, indole-3-propionic acid, indole-3-acetic acid methyl ester,bindole-3-butyric acid, indole-3-lactic acid, L-tryptophan, xanthurenic acid, L-kynurenine, indoxyl sulfate, 3-(4-hydroxyphenyl)lactic acid, phenylacetylglutamine, N-acetylphenylalanine, p-cresol sulfate and p-cresol glucuronide] was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [51]. Briefly, plasma was thawed on ice and 25 µL were loaded on OSTRO plates (Water) together with 25 µL of

deuterated internal standards in methanol and 75 µL of ice-cold acetonitrile containing 1% formic acid for protein precipitation and lipid removal. Plates were shaken for 5 min at 500 rpm and filtered with a positive pressure manifold with nitrogen at 4 psi. Extraction was repeated, eluate was dried under nitrogen at 37 °C, and then resuspended in 200 µL of water:acetonitrile 95:5 (vol:vol) 0.1% formic acid. Two µl aliquots were analyzed by LC-MS/MS on ABSciex 6500+ (ABSciex) operated in the multiple reaction monitoring mode [51].

### *Satisfaction and tolerance questionnaire*

At the V2 visit, participants were asked to complete an internally designed questionnaire assessing product satisfaction and tolerance. In addition to evaluating perceived positive effects on mood and general well-being, the questionnaire specifically addressed tolerance by asking whether participants had experienced any adverse physical symptoms, including gastrointestinal discomfort (e.g., bloating, gas, and abdominal pain), nausea, or headaches. Participants could also report any other undesirable effects through an open-ended response. Additionally, it asked whether they found the product easy to use and well tolerated.

## Statistical analysis

This study was designed to evaluate the efficacy of saffron on emotional well-being. As no prior studies have assessed the effect of saffron on the composite z-score (our primary outcome, combining the BDI-II, STAI, and MFI), the sample size calculation was based on the BDI-II, the most extensively studied scale in this context. Sample size calculation to detect a difference of 4.1 points ($\sigma = 5.2$; effect size of 0.75) between the placebo group and the saffron group on the BDI-II score, with a 2-tailed significance level of 5% and 80% power, indicated that a total sample size of 50 participants was sufficient [52].

Statistical analyses were performed using RStudio 2024.12.0 (MetaboAnalystR package) and GraphPad Prism 10.0. Data were presented as mean ± SD. Normality was assessed by the Shapiro–Wilk test. According to data distribution, Mann–Whitney *U* test or *t* test were performed to compare the baseline characteristics of placebo and saffron groups.

The effect of saffron supplementation on psychological symptoms and biological parameters was studied using a linear mixed model with time and treatment as fixed effects and subject as random effect. The between-group difference in scores at 6 wk was analyzed and corrected using false discovery rate method of Benjamini and Hochberg. One participant in the saffron group exhibited an abnormally high hsCRP concentration (>30 mg/L) at baseline (V1), which was attributed to an acute inflammatory response due to tendinitis. This participant was excluded from the analysis of inflammatory markers. Similarly, 1 participant in the placebo group reported flu-like symptoms at V2 and had an elevated hsCRP concentration (14.2 mg/L) at that time point. This individual was also excluded from the inflammatory marker analysis. Given the very limited number of missing values, no imputation was performed. The models were fitted using restricted maximum likelihood to take into account data from participants with random missing values in a single response variable.

Multivariate analyses, including independent component analysis for visual assessment of group clustering and partial least squares discriminant analysis (PLS-DA) for group discrimination (PLS-DA), were performed on metabolomic data. For the PLS-DA metabolite was considered discriminant if its variable importance in projection (VIP) score was >1. Then, univariate analyses were conducted using Mann–Whitney Wilcoxon test to compare the selected metabolites (VIP score >1) between saffron and placebo. All analyses were conducted in the intention to treat population (Figure 1). There was no multiple testing correction for the analyses of the various secondary outcomes nor exploratory analyses (metabolomics). For all tests, level of significance was set at $P < 0.05$.

# Results

## Study population

Sixty-two subjects were screened for participation at the selection visit V0, and 54 meeting inclusion criteria were enrolled (Figure 1). Among them, 51 subjects performed the first test visit (V1), with 24 in the placebo group and 27 in the saffron group. During the 6 wk of supplementation, 1 participant from the saffron group was lost of follow-up and did not complete the second test visit (V2) (Figure 1). Sociodemographic and clinical data in the 2 groups are presented in Table 1. The mean age of participants was 32 y in the placebo group and 36 y in the saffron group. Female comprised 75% of the placebo group and 70% of the saffron group. The mean BMI was 23 (Table 1). There were no significant between-group differences on any baseline demographic measures (all $P > 0.05$). Participants had a mean score of 37.2 ± 13.0 on the NRS and of 7.9 ± 3.7 on the BDI scale, indicating moderate depressive symptomatology (>7). The mean anxiety and fatigue scores were 45.6 ± 8.8 and 59.3 ± 12.2, respectively. No significant between-group difference was observed at baseline on neuropsychiatric scores.

Compliance with study treatment was not significantly different between the placebo and saffron groups (96% vs 95%; $P = 0.71$). Saffron extract supplementation was well tolerated by participants. There was no difference between groups on the satisfaction questionnaire (data not shown), indicating that the subjects who received saffron found the product as tolerable as the placebo. In addition, no serious adverse reactions attributable to the product were reported in either group. No difference in the mild and moderate adverse events reported by the placebo ($n = 6$) and the saffron ($n = 9$) groups. Three adverse events potentially related to the treatment were reported in the saffron group (2 cases of headache and 1 of cystitis), and 1 event in the placebo group (mood alteration). All events were resolved spontaneously without requiring discontinuation of supplementation.

## Effect of saffron extract supplementation on emotional well-being

There was no significant difference between the saffron and placebo groups on the primary outcome, both groups showing a significant reduction in the *z*-score (combining BDI, MFI, and STAI state scores) over time (Figure 2A). However, a significant treatment × time interaction was measured for the mental health subscale of the MOS SF-12 quality of life questionnaire ($P = 0.045$) (Figure 2B). Pairwise comparisons revealed that participants receiving saffron had a higher mental health score than placebo after 6 wk of supplementation ($P = 0.017$) (Figure 2B). The other domains of the MOS SF-12 questionnaire, improved similarly in both groups, with no significant differences between them (Table 2).

Both groups showed significant reductions in depression (BDI) and anxiety (STAI) scores over time (Table 2). Additionally, fatigue

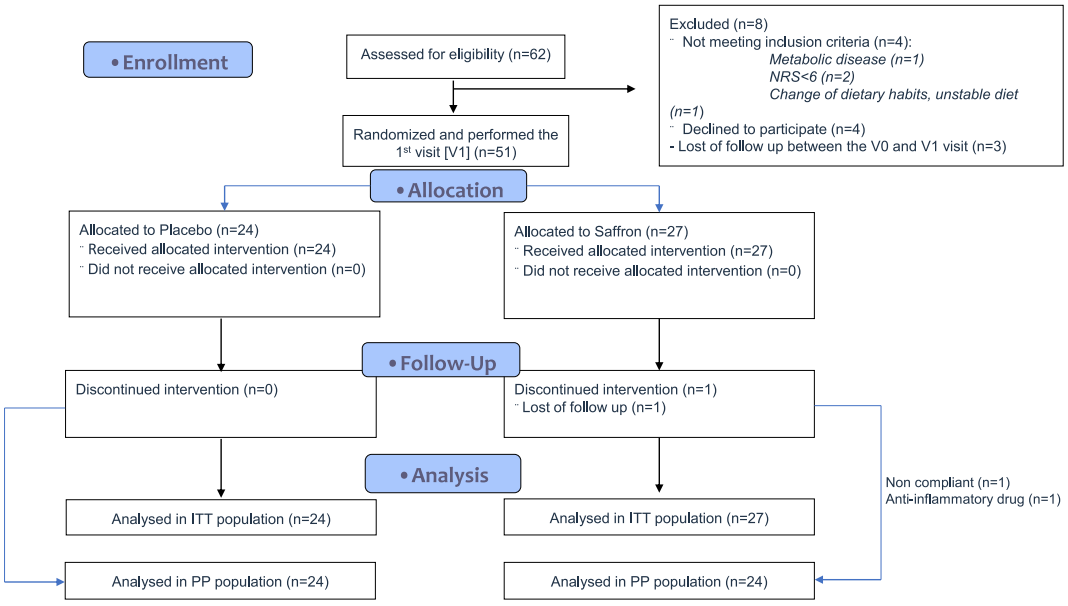


**FIGURE 1.** Flow chart of the SAFFROMFOOD study. ITT, intent-to-treat; PP, per-protocol.

**TABLE 1**
Baseline characteristics of study participants ($N = 51$).

| | Placebo ($n = 24$) | Saffron ($n = 27$) |
|---|---|---|
| Sociodemographic characteristics | | |
| Age (y) | 32.5 ± 8.9 | 36.6 ± 8.6 |
| Sex | | |
| Male | 6 (25.0) | 8 (29.6) |
| Female | 18 (75.0) | 19 (70.4) |
| Marital status | | |
| Couple/married | 15 (62.5) | 16 (59.3) |
| Single | 8 (33.3) | 9 (33.3) |
| Separated/divorced | 1 (4.2) | 2 (7.4) |
| Educational level | | |
| Primary | 0 (0.00) | 0 (0.0) |
| Secondary | 3 (12.5) | 5 (18.5) |
| Superior | 21 (87.5) | 22 (81.5) |
| No. of children | 1 ± 1 | 1 ± 1 |
| Clinical examination | | |
| Weight (kg) | 65.3 ± 12.5 | 65.9 ± 9.2 |
| BMI (kg/m$^2$) | 22.9 ± 3.2 | 23.5 ± 2.2 |
| SBP (mm Hg) | 114.3 ± 12.7 | 115.9 ± 14.2 |
| DBP (mm Hg) | 79.8 ± 9.1 | 79.5 ± 13.4 |
| Heart frequency (beat/min) | 71.4 ± 10.5 | 74.0 ± 10.6 |
| Smoking | | |
| Never | 18 (75.0) | 23 (85.2) |
| Occasional | 2 (8.3) | 3 (11.1) |
| <20/da | 4 (16.7) | 1 (3.70) |
| IL-6 (pg/mL) | 0.99 ± 0.86 | 1.24 ± 1.22 |
| hsCRP (mg/L) | 1.66 ± 2.49 | 2.08 ± 2.83 |
| CAR | 2.00 ± 4.66 | 5.13 ± 7.57 |
| Neuropsychiatric symptoms | | |
| NRS total score | 39.29 ± 10.36 | 35.44 ± 14.93 |
| Depression | 8.2 ± 3.7 | 7.6 ± 3.6 |
| Fatigue | 58.5 ± 11.9 | 60.0 ± 12.6 |
| Anxiety | 45.2 ± 8.4 | 46.0 ± 9.3 |

Values are means ± SD. $N = 51$. For the IL-6 and the hsCRP, $n = 26$ in placebo group (extreme value for hsCRP > 30 mg/L).
CAR, cortisol awakening response; DBP, diastolic blood pressure; hsCRP, high-sensitivity C-reactive protein; NRS, neurotoxicity rating scale; SBP, systolic blood pressure.

(MFI), stress (visual analog scale stress), sleep disturbances (Pittsburgh Sleep Quality Index), and neurovegetative symptoms (NRS) significantly decreased in both groups, with no statistical difference in the magnitude of the decreases between groups (Table 2).

## Effect of saffron extract supplementation on inflammation, HPA reactivity, and metabolome

We then assessed the impact of saffron extract supplementation on inflammatory status and HPA axis reactivity**.** Baseline IL-6 and hsCRP concentrations were comparable between placebo and saffron groups at baseline, with no evidence of inflammation in both groups excepted for 3 individuals in placebo group and 5 in the saffron group who displayed low-grade inflammation (hsCRP > 3) (Table 1, Figure 3B). No significant changes were found in these markers between V1 and V2 or between the saffron and placebo groups (Figure 3A). Salivary cortisol concentrations immediately after waking (T0), 30 min later (T+30), and CAR did not change within or between groups (Figure 3B).

The impact of the supplementation on pathways related to L-tryptophan, L-phenylalanine, L-tyrosine, and BCAAs was then explored. The unsupervised independent component analysis model of the plasma metabolomic profiles between placebo and saffron groups at V1 and V2 is shown in Figure 4A. The analysis revealed considerable overlap between groups and visits. The PLS-DA indicated modest separation, with some metabolites showing, however, elevated VIP score (>1), namely 5-hydroxyindole-3-acetic acid, N-acetylphenylalanine, trimethylamine, trimethylamine N-oxide, xanthurenic acid, and gut-microbial derived metabolites 3-3-hydroxyphenyl-3-hydroxypropionic acid, indole-3-lactic acid, and indole-3-butyric acid (Figure 4B, C). Notably, the concentrations N-acetylphenylalanine (an acetylated derivative of the phenylalanine) was significantly reduced after 6 wk of saffron supplementation (Figure 4D). The other metabolites showed no significant differences between the saffron and placebo groups, despite their high VIP scores.

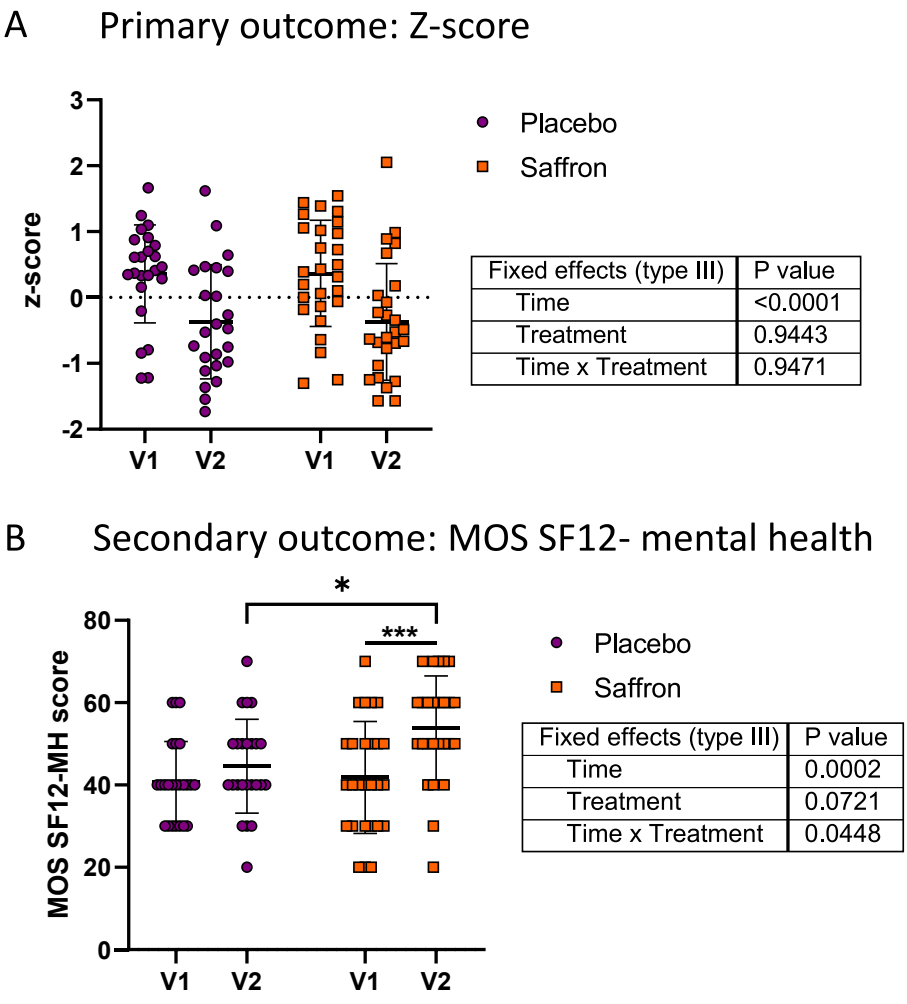


**FIGURE 2.** Effect of saffron extract supplementation on the *z*-score (primary outcome; $N = 51$). The *z*-score combined the BDI total score, the MFI total score, and the STAI state score. (A, B) Linear mixed model with time and treatment as fixed effects and subject as random effect. $^{*}P < 0.05$; $^{***}P < 0.001$. $n = 24$ in placebo group; $n = 26$ in the saffron group. One missing value in the saffron group at V2 (lost to follow-up). MOS SF, Medical Outcome Study Short-Form.

Interestingly, N-acetylphenylalanine concentrations at V2 correlated negatively with the mental health score of the MOS SF-12 questionnaire, indicating that the higher were the concentrations of N-acetylphenylalanine, the more severe were the emotional symptoms. Concentrations of this metabolite correlated positively with the severity of mood alterations on the NRS scale (Supplemental Figure 2A, B).

## Discussion

The present randomized, double-blind, placebo-controlled study investigated the potential benefits of 6-wk saffron extract supplementation on global emotional well-being and individual neuropsychiatric symptoms in healthy individuals with subclinical depressive, anxiety, and fatigue symptoms. To our knowledge, this study is the first to examine the effects of saffron extract on specific symptom dimensions. Furthermore, we explored the potential mechanisms of action of saffron by assessing its effects not only on inflammation and HPA axis reactivity but also, for the first time, on metabolome alterations. The primary outcome analysis revealed no significant differences between saffron and placebo groups in the composite *z*-score encompassing depression, anxiety, and fatigue measures. However, long-term saffron supplementation notably improved mental health scores on the SF-12 questionnaire compared with placebo, suggesting potential beneficial effects on overall mental health, which should be confirmed in future studies. Interestingly, we also reported a decrease in circulating concentrations of N-acetylphenylalanine, which was associated with better mood scores in the population under study.

The lack of significant effects on individual neuropsychiatric symptoms contrasts with earlier clinical trials demonstrating saffron's efficacy in reducing depression and anxiety scores, especially in patients with major depressive disorder [13,41,42]. This discrepancy could be attributed to the subclinical nature of symptoms in the present study sample. Indeed, saffron may exert more pronounced benefits in clinical populations with clinically relevant neuropsychiatric manifestations, in line with its impact on the underlying pathophysiological processes. Nonetheless, the improvement in mental health scores

**TABLE 2**
Effect of saffron extract supplementation on neuropsychiatric symptoms and quality of life ($N = 51$).

| | Placebo ($n = 24$) | | | | Saffron ($n = 27$) | | | | Linear mixed model | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | V1 | | V2 | | V1 | | V2 | | | | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | *P* time | *P* treatment | *P* time × treatment |
| Emotional *z*-score | 0.36 | 0.74 | −0.37 | 0.87 | 0.37 | 0.80 | −0.37 | 0.88 | <0.001 | 0.94 | 0.95 |
| Depression (BDI) | 8.3 | 3.9 | 5.0 | 3.4 | 7.6 | 3.6 | 4.5 | 4.8 | <0.001 | 0.60 | 0.74 |
| Anxiety (STAI state) | 45.4 | 8.2 | 39.3 | 9.6 | 46.0 | 9.3 | 38.5 | 9.1 | <0.001 | 0.97 | 0.55 |
| Fatigue (MFI total score) | 58.5 | 11.9 | 47.5 | 14.6 | 60.1 | 12.6 | 50.0 | 13.5 | <0.001 | 0.50 | 0.66 |
| Reduced motivation | 10.5 | 3.2 | 8.0 | 3.0 | 10.7 | 3.1 | 8.5 | 3.5 | <0.001 | 0.64 | 0.64 |
| Reduced activities | 10.1 | 3.4 | 8.4 | 3.6 | 11.0 | 3.5 | 8.6 | 3.2 | <0.001 | 0.45 | 0.45 |
| Mental fatigue | 12.6 | 3.4 | 10.4 | 3.9 | 13.0 | 3.6 | 11.3 | 3.9 | <0.001 | 0.46 | 0.55 |
| Physical fatigue | 10.8 | 3.8 | 8.8 | 3.3 | 10.7 | 3.0 | 9.6 | 2.7 | <0.001 | 0.61 | 0.20 |
| General fatigue | 14.5 | 2.7 | 12.0 | 3.4 | 14.6 | 2.7 | 12.0 | 2.9 | <0.001 | 0.89 | 0.92 |
| VAS stress | 48.8 | 17.1 | 37.5 | 22.7 | 46.1 | 20.5 | 38.4 | 21.7 | 0.003 | 0.89 | 0.51 |
| Sleep disturbance (PSQI) | 8.0 | 2.7 | 6.2 | 2.4 | 8.0 | 2.6 | 7.0 | 3.2 | <0.001 | 0.61 | 0.25 |
| NRS total | 39.3 | 10.4 | 26.0 | 14.4 | 35.4 | 14.9 | 26.9 | 16.8 | <0.001 | 0.66 | 0.18 |
| Cognitive alterations | 6.2 | 2.8 | 4.0 | 2.9 | 4.5 | 3.5 | 4.5 | 3.5 | <0.001 | 0.94 | 0.09 |
| Altered sleep | 4.8 | 2.1 | 3.5 | 1.9 | 4.9 | 2.0 | 3.9 | 2.7 | 0.002 | 0.64 | 0.57 |
| Sickness symptoms | 6.2 | 2.4 | 5.9 | 3.0 | 4.3 | 3.1 | 5.1 | 3.1 | 0.006 | 0.74 | 0.21 |
| Anxiety | 17.1 | 4.2 | 11.0 | 6.0 | 14.6 | 7.0 | 10.7 | 6.6 | <0.001 | 0.33 | 0.16 |
| Quality of life (MOS SF-12) | 61.3 | 11.8 | 67.4 | 9.6 | 62.4 | 10.3 | 69.6 | 11.1 | <0.001 | 0.53 | 0.79 |
| Mental HEALTH component score | 50.0 | 12.6 | 57.3 | 10.4 | 50.8 | 10.9 | 61.4 | 13.0 | <0.001 | 0.44 | 0.40 |
| Physical health component score | 63.6 | 12.6 | 68.0 | 9.3 | 63.7 | 10.6 | 68.0 | 11.5 | 0.006 | 0.99 | 0.95 |
| Role-emotional | 61.5 | 21.5 | 74.0 | 19.8 | 69.4 | 19.7 | 79.3 | 23.4 | 0.002 | 0.21 | 0.62 |
| Social functioning | 69.8 | 20.8 | 80.2 | 19.5 | 69.4 | 24.4 | 83.7 | 21.1 | 0.002 | 0.79 | 0.65 |
| Energy/vitality | 22.5 | 18.0 | 27.5 | 17.5 | 21.5 | 15.6 | 32.3 | 20.5 | 0.002 | 0.67 | 0.23 |
| General health perception | 55.2 | 16.5 | 60.4 | 12.6 | 51.9 | 11.9 | 57.7 | 13.7 | 0.004 | 0.37 | 0.87 |
| Pain | 68.3 | 14.4 | 72.5 | 12.9 | 71.1 | 17.0 | 70.8 | 19.0 | 0.48 | 0.87 | 0.42 |
| Mental health | 40.8 | 9.7 | 44.6 | 11.4 | 41.8 | 13.6 | 53.8 | 12.7 | <0.001 | 0.07 | 0.04 |
| Role-physical | 80.2 | 23.3 | 82.8 | 16.8 | 83.3 | 15.9 | 87.0 | 15.6 | 0.35 | 0.33 | 0.86 |
| Physical functioning | 91.7 | 14.1 | 96.9 | 8.5 | 90.7 | 17.2 | 92.3 | 15.4 | 0.15 | 0.37 | 0.29 |

Values are means ± SD. One missing value in the saffron group at V2 ($n = 26$, lost of follow-up). *P* values were obtained using a linear mixed model with time and treatment as fixed effects and subject as random effect.
BDI, Beck Depression Inventory; MOS SF, Medical Outcome Study Short-Form; NRS, neurotoxicity rating scale; PSQI, Pittsburgh Sleep Quality Index; STAI, State-Trait Anxiety Inventory; VAS, visual analog scale.

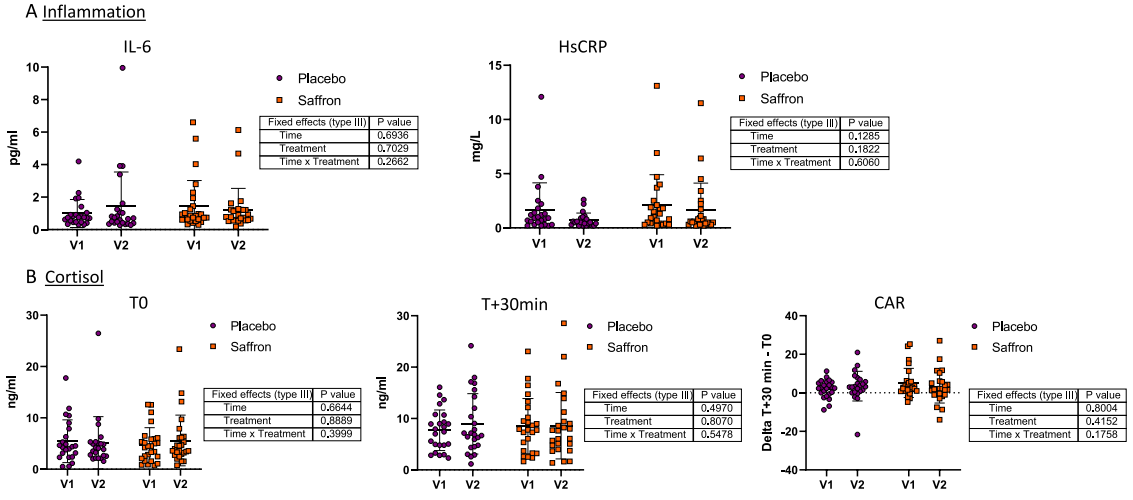


**FIGURE 3.** Effect of saffron extract supplementation on biological outcomes ($N = 51$). (A) Effect of saffron extract supplementation on inflammatory markers, IL-6 and high-sensitivity C-reactive protein (hsCRP). (B) Effect of saffron extract supplementation on cortisol awakening response (CAR). T0, immediately after awakening in the morning; T30, 30 min after awakening in the morning. *P* values were obtained using a linear mixed model with time and treatment as fixed effects and patient as random effect; $n = 23$ in the placebo group and $n = 26$ in the saffron group. One participant exhibited an abnormally high hsCRP concentration (>30 mg/L) at visit (V)1 in the saffron group and 1 participant in the placebo group reported flu-like symptoms at V2. These participant were also excluded from the inflammatory marker analysis.

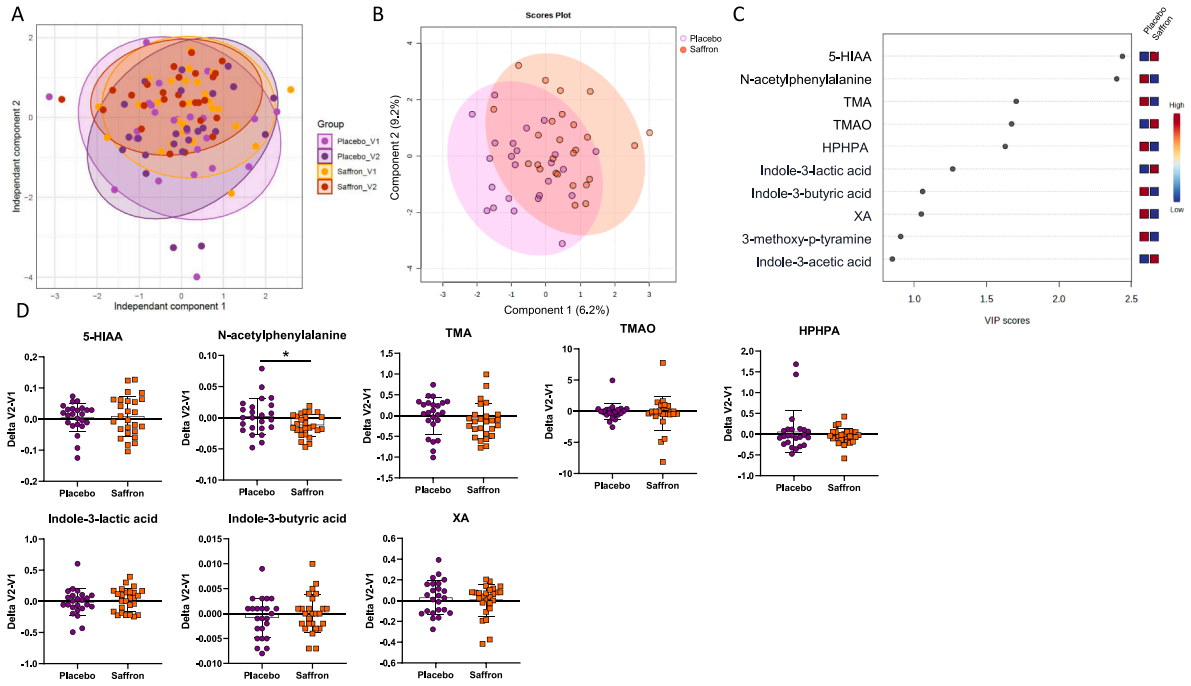


**FIGURE 4.** Effect of saffron extract supplementation on aromatic amino acid derivatives ($n = 50$). (A) Individual plot of the independent component analysis (ICA). (B) Individual plot of the partial least square discriminant analysis (PLS-DA). (C) Variable importance in projection (VIP) plots with the top discriminating plasma metabolites (VIP score $> 1$) identified through PLS-DA analyses in descending order of importance. (D) Comparison between placebo and saffron groups of the metabolites with a VIP score $> 1$. $P$ values were obtained using Mann–Whitney Wilcoxon test; $^*P < 0.05$. 5-HIAA, 5-hydroxyindole-3-acetic acid; HPHPA, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid; TMA, trimethylamine; TMAO, trimethylamine N-oxide; XA, xanthurenic acid.

indicates saffron's potential for enhancing emotional well-being, even at subclinical symptom levels. Jackson et al. [53] have shown that 8-wk supplementation with a standardized saffron extract induced decreased scores on the depression subscale of the Profile of Mood States (POMS) questionnaire in subjects with feelings of low mood and anxiety and/or stress. However, they did not report any effect on individual symptoms evaluated with the STAI state or the Hospital Anxiety and Depression Scale [53].

Saffron did not significantly influence concentrations of IL-6 or hsCRP. This finding diverges from clinical studies suggesting anti-inflammatory effects of saffron [54–58]. However, it is noteworthy that this effect was specifically detected in conditions of chronic inflammatory diseases, such as diabetes, rheumatoid arthritis, or multiple sclerosis. In contrast, participants of the current study displayed low levels of inflammation, at baseline, which made it challenging to detect a potential reduction in inflammatory marker concentrations. In addition, in vitro studies conducted on human cell cultures report an anti-inflammatory and neuroprotective impact of saffron, but only under conditions of immune stimulation [19,59]. Akin with these findings, a recent preclinical study revealed beneficial effects of saffron extract against neuroinflammation and associated behavioral alterations in mice infected with bacterial lipopolysaccharides, whereas no significant effect was detected in the absence of immune stimulation [28]. Importantly, saffron appeared to preferentially target the mechanisms involved in resolving inflammation, such as promoting anti-inflammatory cytokines, rather than directly affecting inflammatory cytokines themselves. This may also explain why no significant reduction in IL-6 and hsCRP concentrations was measured in the present study.

Consistent with its lack of detectable effect on inflammatory markers, saffron supplementation also did not alter HPA axis reactivity, as assessed by salivary cortisol concentrations. The effect of long-term saffron supplementation on the HPA axis has so far been little explored in clinical studies. One study found that 28 d of supplementation in adults with unsatisfactory sleep failed to affect evening cortisol [60]. To our knowledge, no study has assessed the effect of saffron on CAR. However, preclinical and clinical evidences suggest that saffron may preferentially interfere with the HPA axis under stressful rather than basal conditions, which could explain the lack of effect in our study [27,30,61,62]. Methodological differences, such as the study population and saffron extract formulation, as well as the subclinical nature of symptoms in our sample, could also contribute to these discrepancies. It is plausible that more pronounced effects on inflammatory and HPA axis pathways may be observed in subjects experiencing more severe depressive symptoms or following longer supplementation durations.

To our knowledge, this is the first study assessing, beyond inflammation and HPA axis reactivity, the effect of saffron extract on metabolome, by using a highly performing method of targeted quantification of dietary essential AA such as L-tryptophan, L-phenylalanine, and L-tyrosine, as well as BCAA, their derivatives, and the contribution of gut-microbial metabolism [51]. This investigation was needed as these metabolites are known to play a crucial role in human health, especially in mental health [38,51,63]. In contrast to our preclinical studies [27,28], we did not observe any significant effect of saffron on tryptophan metabolism along the kynurenine pathway, nor on tyrosine and phenylalanine metabolism. These metabolic pathways are well-known to contribute to the pathophysiological mechanisms of

inflammation-driven depression, particularly by disrupting monoamine synthesis and inducing neurotoxicity [38,64]. Although the reasons for this lack of effect of saffron remain to be determined, as well as the potential implications for improving the emotional profile of the supplemented participants, it is important to note that this finding is consistent with the absence of any significant effect on inflammation.

Based on the present data, it could be argued that the dose of saffron and/or the duration of supplementation chosen in this work could be not sufficient to alleviate the reported subclinical neuropsychiatric symptoms and related biological changes. However, it is worth mentioning that long-term saffron supplementation seems to improve mental health scores on the SF-12 questionnaire compared with placebo, whereas reducing N-acetylphenylalanine. N-acetylphenylalanine is an amphipathic derivative of phenylalanine, an essential AA that plays a role in the synthesis of neurotransmitters such as dopamine, norepinephrine, and serotonin. An accumulation of phenylalanine and its metabolites, including N-acetylphenylalanine, has been observed in patients with phenylketonuria and was associated with emotional and neurocognitive disturbances [65]. This metabolite has also been found to be altered in the blood of patients with bipolar disorders and in postmortem brain tissues of patients with Alzheimer disease patients compare with that in the blood of controls [66,67]. The observed reduction in N-acetylphenylalanine concentrations following saffron administration and the negative correlations observed between these metabolites and mood scores may provide insight into the mechanism by which saffron exerts its reported neuropsychiatric benefits. However, N-acetylphenylalanine itself is not widely studied in psychiatric disorders and more researches are needed to fully elucidate its role.

A notable strength of this study is its randomized, placebo-controlled design in a subclinical population, offering insights into saffron's potential as a preventive strategy for neuropsychiatric conditions. This study has the advantage of examining the effects of saffron supplementation on various symptom dimensions observed in depressive disorders, including mood symptoms and neurovegetative symptoms. This approach is novel compared with other studies in the field. Additionally, we tested the effect of saffron on several biological markers that have been identified as being involved in the pathophysiology of depression. However, several limitations should be acknowledged. The relatively small sample size and short supplementation period of 6 wk, although consistent with prior studies on saffron, may have limited the ability to detect significant effects on some outcomes measured, especially for subclinical conditions. Additionally, the dose of saffron extract used in this study may not have been optimal for eliciting more robust effects on neuropsychiatric symptoms and underlying mechanisms. Finally, this study reports a substantial number of secondary mental health outcomes and exploratory metabolomic outcomes. As these analyses were not adjusted for multiple comparisons, the reported *P* values should be interpreted with caution.

In conclusion, this RCT did not demonstrate a significant effect of saffron supplementation on emotional well-being assessed using a composite score. However, we showed that supplementation with saffron extract for 6 wk may improve overall mental health in individuals with subclinical neuropsychiatric symptoms, potentially through modulation of specific AA metabolic pathways. Further larger-scale and adequately powered studies are needed to confirm these observations in populations with subclinical symptoms of depression.

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## Author contributions

The authors' responsibilities were as follows – CA, NC, LC: conceived and designed the research that led to the submission and were involved in writing the manuscript; LC, NC, CA, SD: managed the project administration and ethical approvals; CA, QL, MF, NC, LC, AA, FM, JM, LP, DG, LC: critically contributed to the interpretation of the results; CA, MF, JM, MF, EB, AA: conducted the biological experiments; CA, QL, MF: performed data analysis; AA, FM: assisted with the target metabolomics; and all authors: critically revised the manuscript, agreed on all aspects of the work, and approved the final version.

## Conflict of interest

The authors declare no conflicts of interest.

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## Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending approval.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2025.09.050.

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