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Randomized Control Trials

Probiotic *Lactobacillus plantarum* P8 alleviated stress and anxiety while enhancing memory and cognition in stressed adults: A randomised, double-blind, placebo-controlled study

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SUMMARY

Background & aims: To investigate the effects of probiotic in alleviation of stress in stressed adults, along our focus to identify and justify strain specificity on selected health benefits with a precisely targeted population.

Methods: This 12-weeks randomized, double-blind and placebo-controlled study investigated the effects of a probiotic (*Lactobacillus plantarum* P8; 10 log CFU daily) on psychological, memory and cognition parameters in one hundred and three (P8 n = 52, placebo n = 51) stressed adults with mean age of 31.7 ± 11.1 years old. All subjects fulfilled the criteria of moderate stress upon diagnosis using the PSS-10 questionnaire.

Results: At the end of study, subjects on P8 showed reduced scores of stress (mean difference 2.94; 95% CI 0.08 to 5.73; P = 0.048), anxiety (mean difference 2.82; 95% CI 0.35 to 5.30; P = 0.031) and total score (mean difference 8.04; 95% CI 0.73 to 15.30; P = 0.041) as compared to placebo after 4-weeks, as assessed by the DASS-42 questionnaire. Although plasma cortisol levels were only marginally different between placebo and P8 (mean difference 3.28 ug/dl; 95% CI -7.09 to 0.52; P = 0.090), pro-inflammatory cytokines such as IFN-γ (mean difference 8.07 pg/ml; 95% CI -11.2 to −4.93; P < 0.001) and TNF-α (mean difference 1.52 pg/ml; 95% CI -2.14 to −0.89; P < 0.001) showed higher reduction as compared to placebo over 12-weeks. These were accompanied by enhanced memory and cognitive traits such as social emotional cognition and verbal learning and memory upon administration of P8 as compared to the placebo, with different effects in women as compared to men.

Conclusions: The present data illustrated that *L. plantarum* P8 is a feasible and natural intervention for the alleviation of selected stress, anxiety, memory and cognitive symptoms in stressed adults.

Trial registration: Approved by the JEPeM-USM Review Panel on Clinical Studies (Approval number USM/JEPeM/16050195) and was registered at ClinicalTrials.gov (identifier number NCT03268447).

1. Introduction

Stress often arise from not only physiologically or emotionally challenging experiences, but also from short reactions in swift situations such as that of a traffic jam or merely meeting working deadlines. Although stress is triggered by an event while anxiety is

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a reaction to stress, sustained and untreated anxieties may lead to deeper mental illnesses such as depression. Globally, there are over 300 million people that are affected by depression, with nearly 800,000 suicidal deaths annually [1]. Prolonged rise in glucocorticoid levels as induced by stress and the activation of the hypothalamic-pituitary-adrenal axis, produces negative impacts on the immunological states and changes in hippocampal structure leading to alterations in neurogenesis, neuronal morphology and even cell deaths [2]. An impaired hippocampal structure and physiological function reportedly disrupt normal spatial learning, memory and cognition functions [3], indicating an association between an imbalanced psychological state with loss of mental abilities. Up to now, there is no ultimate treatment for mental disorders such as depression, with success rates below ten percent in many countries globally [1]. Much medical interventions revolve around the use of antidepressants such as selective serotonin reuptake inhibitors, that have detrimental side effects such as reduced sexual dysfunction, weight gain and sleep disturbance [4].

Probiotic are “live microorganisms that exert health effects to the host if consumed in sufficient amounts” [5]. *Lactobacillus* remain one of the most commonly administered probiotic genera with a long history of safe use, and comprehensively documented to exert gut health and protection properties. There has been much postulations on the roles of gut health towards brain health, where gut microbiota was hypothesized to be its own endocrine organ. The term “microbial endocrinology” was first coined by Lyte in 1993 as a “conceptual framework to understand interactions between the microbiota and the host” [6]. This has led to the new concept of “gut-brain-axis”, a bidirectional and dynamic communication system that maintains homeostasis. While bridging the gap for brain-body, this axis also comprises of neural pathways, cytokines, hormones and neuropeptides as signaling molecules that are regulated at the neural (both central and enteric nervous systems), hormonal and immunological levels [7]. A lack of conventional microbiota in germ-free mice as compared to specific pathogen-free mice, also led to higher levels of plasma corticosterone and anxiety behaviors [8], indicating the influence of gut microbiota against brain health and behaviors of the host. It is therefore of upmost interests that probiotics could be used as a natural agent to influence gut health, brain health and psychological wellbeing. In a randomized, double-blind, placebo-controlled pilot study, chronic fatigue syndrome patients treated with *Lactobacillus casei* Shirota exhibited significant decrease in anxiety symptoms and behavior [9], while healthy human subjects administered with a probiotic formulation consisting of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R017 exhibited reduced psychological distress with reduced scores of hospitalized anxiety and depression [10].

Lactobacillus plantarum P8 was isolated from traditionally fermented sour milk samples in Inner Mongolia, China. While over 500 isolates were obtained, *L. plantarum* P8 showed higher tolerance against conditions of the gastrointestinal tract such as gastric acids, intestinal fluid and bile. The stable characteristics of P8 in the human gut was reflected in a recent human study; upon consumption of a single dose, P8 remained detected in fecal samples after 4–5 weeks, while upon consumption of 4-weeks, P8 was detected up to 17 weeks after consumption ceased [11]. Although P8 remains stable in the gut, safety assessments performed had confirmed mutational safety aspects of P8. Comparative genomic analysis from human fecal samples detected 19 single nucleotide polymorphisms indicating neutral evolution in the core genome, while loss of one to three plasmids in nearly half of the samples (n = 39, 42%) indicated reductive evolution in the accessory genome under selection pressure within the gastrointestinal tract [11]. The administration of P8 at a concentration of 10 log CFU/day for 4-weeks increased fecal levels of secretory immunoglobulin-A and short chain fatty acids in adults [12], accompanied by increased population of beneficial gut microbiota such as *Lactobacillus and* decreased population of gut opportunistic pathogens such as *Shigella*, *Escherichia* and *Enterobacter* [13].

Considering that P8 exerted gut health properties, we aimed to investigate the effects of P8 in alleviation of stress in stressed adults, along our focus to identify and justify specific strains for selected health benefits with a precisely targeted population. In addition, a better understanding of the mechanisms of actions would yield better implementation of probiotics in the expanding fields of healthcare and alternative medicine. The primary outcome of this study was to evaluate the efficacy of P8 in alleviating stress, including subsequent reactions of stress such as anxiety, depression, memory and cognitive abilities.

2. Materials and methods

2.1. P8 and placebo products

L. plantarum P8 was isolated from traditionally fermented sour milk samples in Inner Mongolia, China. Intervention consisted of daily administration of 2 g probiotic *L. plantarum* P8 or placebo (no probiotic) at a fixed dosage of 2×10^{10} CFU/sachet/day and continued for 12 weeks. The probiotic P8 and placebo were manufactured by JinHua YinHe Biological Technology Co. Ltd., China. Each dose was supplied in an aluminum sachet and all sachets appeared as light yellow powder and were identical in taste and appearance. Sachets were stored away from direct sunlight and below 30 °C. P8 did not contain any porcine or bovine ingredients and was manufactured under ISO9001 and HALAL in China. HALAL certification was provided by ARA HALAL Development Services Center Inc. (ARA), which is recognized by JAKIM, Malaysia. The product contained *L. plantarum* P8 and maltodextrin as excipient while placebo contained only maltodextrin.

2.2. Selection of subjects

Subjects were recruited from Penang and Kubang Kerian, Malaysia, and screened based on inclusion and exclusion criteria. Inclusion criteria included men or women, aged 18–60 years old, body mass index within a healthy range, no severe illnesses, willing to commit throughout the experiment, and a score of moderate stress level on Cohen's Perceived Stress Scale (PSS-10) [14]. Exclusion criteria include type-I diabetes, long term medication due to certain severe illness, HIV/AIDS, and glucose-6-phosphate dehydrogenase deficient, and subjects who, in opinion of the investigator, were not likely to complete the trial for whatever reasons. Written informed consent was obtained from all subjects prior to the start of the study.

2.3. Study protocol

This was a double-blind, randomized and placebo-controlled design study. Randomization was performed upon checking of the inclusion and exclusion criteria. Eligible subjects were randomized 1:1 ratio to the two arms of the study according to a computer generated list [15], assigned to the probiotic group (P8) and placebo group with treatment codes. Randomization was performed by the study statistician, who had no contact with the participants. The allocation sequence was not available to any member of the research team until the completion of the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the JEPeM-USM Review Panel on Clinical

Studies (Approval number USM/JEPeM/16050195) and was registered at ClinicalTrials.gov (identifier number NCT03268447).

The sample size was calculated for a parallel group study design involving one prevention arm and one placebo arm and was based on power design analysis. As this study involved subjects with mental health conditions, the determination of a dropout rate was crucial, to ensure that the final number of subjects fulfilled the required statistical strength. Limited information is available on the actual number of dropout in human studies involving natural products and mental health. Thus, references were based on dropout of patients undergoing treatments and/or care for anxiety and/or depression. While a study on medical care for anxiety and depression in older adults reported a lower dropout of 34% [16], other studies have reported a higher dropout. Outpatient mental health care units of the general medical sector in the USA showed nearly 60% of patients dropped out with a median of only three visits [17], while a dropout rate of 60% was reported in the first six months of women undergoing psychotherapy [18]. A total of 110 subjects were needed for this study, comprising of 55 subjects in each group (P8 and placebo). With an inclusion of 60% dropout, a total of 176 subjects were recruited. This calculation was based on the need for a continuous response variable from independent control and experimental subjects, with a ratio of control to subject fixed at 1:1, probability (power) of 0.95 and Type-I error probability associated with this test of null hypothesis of 0.05. In addition, previous data have shown that for a similar intervention using natural supplement to promote brain health measured by the DASS-21 stress questionnaire, a standard deviation of 3.32 within group was observed, accompanied by a mean reduction of 2.33 between treatment and placebo groups [19].

2.4. Analyses

2.4.1. Questionnaires

Potential subjects who fulfilled all the inclusion and exclusion criteria were tested for levels of psychological distress using the Perceived Stress Scale (PSS) questionnaire. Subjects with moderate levels of stress were recruited to join the study. Different language versions of the PSS-10 questionnaire were used to assess stress perception; the original English language version, the Malay and Chinese languages translated and validated versions [20,21]. All subjects were given the choice to answer the questionnaires on their own, in the presence of our team of psychologists and/or medical educationists, or completely given by our team of psychologists and/or medical educationists. The roles of psychologists and/or medical educationists while administrating the questionnaires were limited to only explaining on the questions should the subjects had difficulties in understanding the questions and are competent in all three languages. Outside the boundaries of questionnaires, our team of psychologists also provided psychological and medical advice and services throughout the study when needed. PSS-10 consisted of ten-items on a 5-point Likert scale (0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often), where six items were negatively stated while four items that were positively stated (items 4, 5, 7, and 8) were reversely scored (0 = very often, 1 = fairy often, 2 = sometimes, 3 = almost never, 4 = never). The sum of the 10 items represented the total score, with scores of 0–13 indicating low stress, 14–26 indicating moderate stress and 27–40 indicating high perceived stress.

All subjects that were successfully recruited were also assessed for stress, anxiety and depression via the Depression, Anxiety and Stress Scale (DASS-42) questionnaire [22]. DASS-42 is a 42 item self-report validated inventory comprising of three scales designed to measure the negative emotional states of depression, anxiety and stress, where each of the three scales contained 14 items. The depression scale assessed dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia. The anxiety scale assessed autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect, while the stress scale assessed difficulty in relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatience. Subjects were assessed based on a 4-point Likert scale (0 = did not apply to me at all, 1 = applied to me to some degree or some of the time, 2 = applied to me to a considerable degree or a good part of time, 3 = applied to me very much or most of the time). Scores for each subscale were categorized into five severity ranges, namely normal, mild, moderate, severe and extremely severe. All questionnaires were used for assessment at baseline (week-0) and at intervals of 4-weeks (week-4, 8, 12).

2.4.2. Cortisol, cytokines and full blood count

All subjects were invited to provide blood samples voluntarily. Blood samples (6 ml) were drawn from an antecubital vein directly into a K^2EDTA tube, two times throughout the study (week-0, 12). Plasma samples were analyzed for the concentrations of stress hormone cortisol, interleukin-1β, 4, and 10, tumor necrosis factor (TNF)-α and interferon (IFN)-γ using enzyme-linked immunoabsorbent assay (ELISA) kits (Immunodiagnostik, Germany) following the manufacturer's instructions. Whole blood was analyzed for full blood count tests (Gribbles Pathology, Penang, Malaysia).

2.4.3. CogState Brief Battery (CBB)

At the end of study (week-12), all subjects were assessed for memory and cognitive functions using the computerized CogState Brief Battery (CBB) [23]. Administration of the CogState battery test was conducted in a computer laboratory and/or personal laptops, installed with the CogState ClinicalTrials software. All subjects had an initial practice prior to the actual test battery. The study coordinator was available to help the subjects understand the tasks during the practice session. During the test session, the coordinator provided minimal supervision or assistance. Composition of each battery and their respective outcomes are listed in Table 1. Card tasks involving correct responses were randomly chosen for each trial, while maze tasks comprised of 20 possible hidden pathways matched for number of tiles and turns. Each task of the test battery was randomly chosen from the large number of equivalent alternative forms at any one time, resulting in a different set of exemplars for each individual.

2.4.4. Statistical analyses

Data were analyzed using SPSS version 20.0 (SPSS Inc, Chicago, USA). The primary hypothesis of this study involved differential efficacy between the two treatment groups of P8 and placebo. Comparisons between treatment groups as a measure of time were assessed using between-group repeated measures analysis of variance (ANOVA; general linear model) with group (the two treatment groups) and time (0, 4, 8, 12 weeks) as main effects. The ANOVA model also included a group-by-time interaction term. An independent t-test was used to compare the difference of P8 and placebo at specific time points. The score differences between different time points were examined using one sample t-test, where mean of the differences had a hypothesized value of zero. Considering the skewed distribution and non-parametric nature of our data, the associations between variables in different groups were evaluated using Spearman's rank correlations with rho (r) as the correlation coefficient. All tests were two-sided with $P < 0.05$ as considered statistically significant.

Table 1
Descriptions and outcomes of tests as assessed via the CogState Brief Battery (CBB).

Test/Task	Description	Outcome
Detection	Immediate click upon turning of a card	Speed, accuracy, correct, and error based on responses
Identification	Immediate left or right click of mouse button to determine whether the card was red or black upon turning	Speed, accuracy, correct, and error based on responses
One card learning	Immediate click to determine whether the card was seen previously upon turning	Speed, accuracy, correct, and error based on responses
One card back	Immediate left or right click of mouse button to determine whether the card was same or different from the previous card upon turning	Speed, accuracy, correct, and error based on responses
Groton maze chase (GMC)	Prompt click on tiles of a grid to trace the path towards the final destination grid	Duration and error of moves
Groton maze final recall	Same as GMC, but performed after all tasks have been completed to determine if a subject remember the hidden path as previously learned	Duration and error of moves
Social emotional	Prompt click on one of the four facial expressions that are different compared to the others	Speed, accuracy, correct, error
Continuous paired	Remember and recognize random shapes hidden beneath different locations	Speed, accuracy, and error based on responses
International shopping list	Remember and recall a list of 16 shopping items read to the subject in 3 consecutive trials	Total items recalled over 3 trials

3. Results

3.1. Baseline

Insignificant differences were observed between the general characteristics and full blood count parameters of P8 and placebo subjects for both women, men and the overall populations ($P > 0.05$; Table 2). A total of 132 subjects were successfully recruited, where 2 subjects dropped-out during the 12-weeks period, while 27 subjects did not fully comply in answering monthly questionnaires, providing blood samples and/or completing the computerized CogState test, yielding 103 subjects that fully complied (P8 n = 52, placebo n = 51) (Fig. 1). The PSS questionnaire was used as a tool for the diagnostic of stress, where a subject was confirmed to have moderate stress levels based on total scores of 14–26. Subjects from both groups fulfilled the inclusion criteria of moderately stressed (Table 2). The study started on 5th August 2017 and completed on 31st January 2018.

3.2. Stress, anxiety, depression

The primary outcome measured stress levels as assessed via questionnaires to determine effects of probiotic treatment, while the secondary outcome intended to measure gut microbiota profiles. However, due to financial constraints, gut microbiota analyses were not performed. Stress was assessed via the 10-items questionnaire of PSS-10 and the 42-items questionnaire of DASS-42. Based on PSS-10 (Fig. 2A), both P8 and placebo showed reduction in total scores over 12-weeks, with a significant influence from the effects of time ($P < 0.001$). However, the effects of treatments were insignificant across 12-weeks, and remained insignificantly different from each other at the evaluated time points of weeks-0, 4, 8, 12. The 42 items in DASS-42 were randomly sequenced, with 14 items each designated to evaluate stress (items number 1, 6, 8, 11, 12, 14, 18, 22, 27, 29, 32, 33, 35, 39), anxiety (items number 2, 4, 7, 9, 15, 19, 20, 23, 25, 28, 30, 36, 40, 41) and depression (items number 3, 5, 10, 13, 16, 17, 21, 24, 26, 31, 34, 37, 38, 42). Time significantly affected stress ($P < 0.001$) where both placebo and P8 reduced stress levels from moderate at week-0 to normal at week-12 (Fig. 2B). Although both treatments significant reduced stress levels ($P = 0.030$), P8 significantly exhibited lower scores as compared to the placebo at week-4, 8 and 12 for stress ($P < 0.05$). The current determination of sample size was based on a previous study utilizing the intervention of a natural supplement that promoted brain health as measured by the DASS-21 stress questionnaire, where a mean reduction of 2.33 between treatment and placebo groups was observed [19]. In our present study, although less than 110 subjects completely complied, reductions of 2.94, 2.57 and 3.08 were observed between placebo and P8 over 4, 8 and 12-weeks, respectively.

Table 2
Baseline characteristics of one hundred and three (n = 103) adult subjects randomly assigned to 12-weeks of double blind treatment with either P8 or placebo.

Baseline characteristics	Women		P-value	Men		P-value	Total		P-value
	Placebo	P8		Placebo	P8		Placebo	P8	
Sample size (n)	39	40		12	12		51	52	
Age	32.4 ± 11.3	31.2 ± 10.9	0.644	31.3 ± 12.2	31.3 ± 10.8	0.986	32.1 ± 11.4	31.3 ± 10.8	0.692
PSS-10 score	21.9 ± 4.8	22.5 ± 5.0	0.571	23.2 ± 4.5	21.1 ± 3.8	0.208	22.2 ± 4.7	22.2 ± 4.7	0.964
Full blood count parameters:									
Hemoglobin (g/L)	130.38 ± 14.41	130.78 ± 13.94	0.903	147.50 ± 15.33	146.17 ± 18.25	0.848	134.41 ± 16.22	134.33 ± 16.23	0.979
Red blood count (x 10^{12}/L)	4.70 ± 0.42	4.72 ± 0.49	0.858	5.52 ± 0.59	5.45 ± 0.87	0.827	4.89 ± 0.58	4.89 ± 0.67	0.967
Packed cell volume (L/L)	0.40 ± 0.04	0.41 ± 0.04	0.659	0.44 ± 0.04	0.44 ± 0.05	0.740	0.41 ± 0.04	0.42 ± 0.04	0.614
Mean corpuscular volume (fL)	86.18 ± 4.97	86.88 ± 7.10	0.615	80.33 ± 10.32	82.92 ± 12.45	0.586	84.80 ± 6.96	85.96 ± 8.65	0.456
Mean corpuscular hemoglobin (pg)	27.72 ± 1.85	27.93 ± 2.56	0.681	27.00 ± 3.81	27.33 ± 4.23	0.841	27.55 ± 2.43	27.79 ± 2.99	0.656
Mean corpuscular hemoglobin concentration (g/L)	322.46 ± 14.83	320.15 ± 12.94	0.463	335.58 ± 12.67	328.50 ± 11.57	0.167	325.55 ± 15.30	322.08 ± 13.02	0.218
Red cell distribution width (%)	13.93 ± 1.15	13.80 ± 1.50	0.653	13.82 ± 1.96	15.05 ± 4.08	0.356	13.90 ± 1.36	14.08 ± 2.37	0.635
White cell blood count (x 10^9/L)	7.33 ± 2.66	7.38 ± 2.28	0.930	7.43 ± 2.00	8.03 ± 2.43	0.510	7.35 ± 2.50	7.53 ± 2.31	0.708
Neutrophils (x 10^9/L)	4.04 ± 2.14	4.27 ± 1.73	0.615	4.13 ± 1.54	4.31 ± 1.79	0.791	4.06 ± 2.00	4.28 ± 1.73	0.566
Lymphocytes (x 10^9/L)	2.52 ± 0.79	2.38 ± 0.79	0.425	2.43 ± 0.66	2.68 ± 0.98	0.486	2.50 ± 0.76	2.44 ± 0.84	0.733
Monocytes (x 10^9/L)	0.53 ± 0.18	0.52 ± 0.15	0.776	0.62 ± 0.17	0.68 ± 0.24	0.496	0.55 ± 0.18	0.56 ± 0.19	0.894
Basophils (x 10^9/L)	0.14 ± 0.16	0.09 ± 0.13	0.120	0.15 ± 0.12	0.20 ± 0.51	0.743	0.14 ± 0.15	0.11 ± 0.26	0.515
Platelets (x 10^9/L)	326.23 ± 62.82	338.23 ± 71.70	0.431	306.67 ± 59.50	310.42 ± 64.75	0.884	321.63 ± 62.04	331.81 ± 70.54	0.438

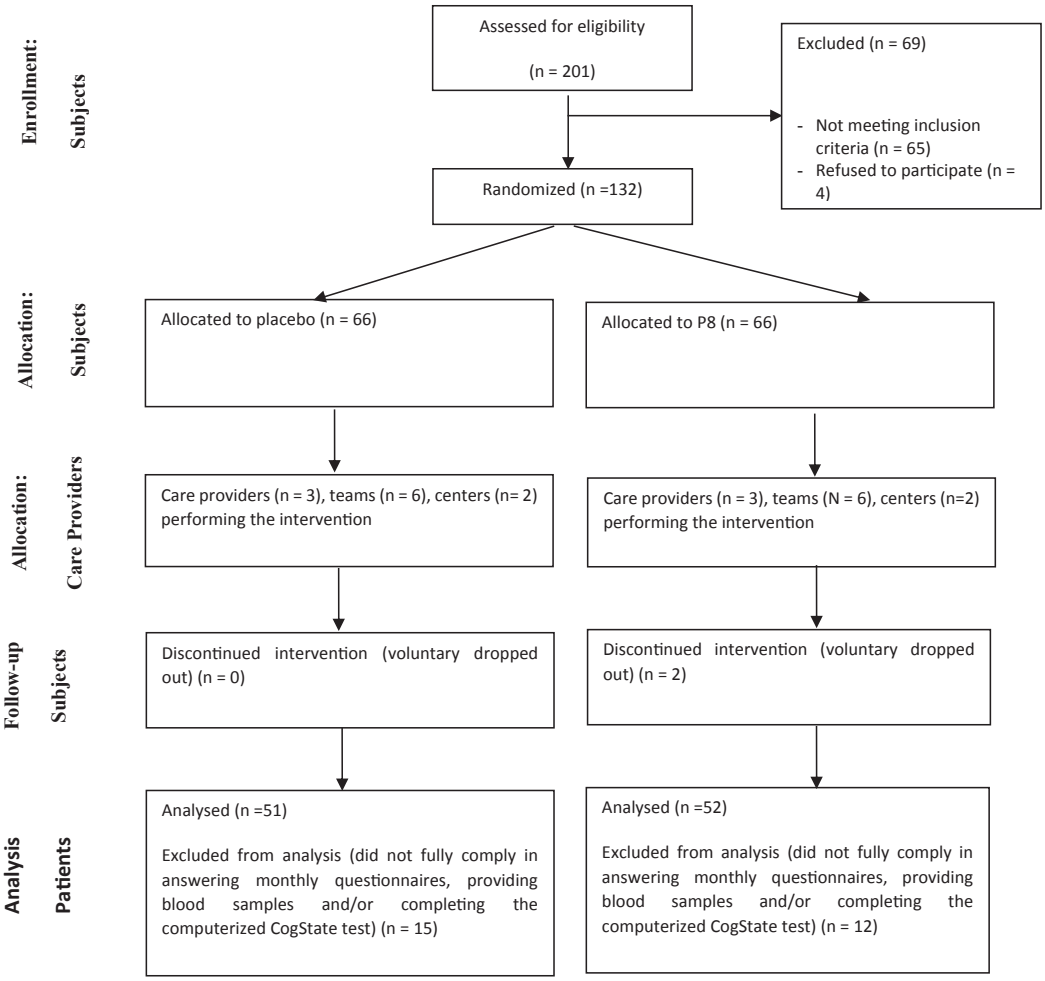

Fig. 1. Flow Chart detailing participants' recruitment, randomization and allocation.

The time factor significantly reduced scores for anxiety ($P < 0.001$; Fig. 2C), where P8 reduced anxiety levels from moderate at week-0 to normal at week-12 while the placebo only reduced from moderate at week-0 to mild at week-12. Although the effects of treatments against reduction of anxiety was marginal ($P < 0.10$), P8 significantly exhibited lower anxiety scores as compared to the placebo at week-4 and 12 ($P < 0.05$). The total scores for DASS-42 reduced over time for both groups ($P < 0.001$; Fig. 2D). Although the effects of treatments against reduction of total score was marginal ($P < 0.10$), P8 significantly exhibited lower total scores as compared to the placebo at week-4 and 12 ($P < 0.05$). The placebo-effect remained strong towards depression, where both placebo and P8 groups showed a reduction in scores over 12-weeks (Fig. 2E), reducing the symptoms of depression from mild at week-0 to normal at week-12. The effects of treatments against reduction of depression was insignificant, while P8 only marginally reduced depression as compared to the placebo at week-8 ($P < 0.10$).

To better understand individual component effects, we evaluated individual aspects of each item that were significantly improved. Although all 14 items designated for stress in the DASS-42 questionnaire were significantly reduced in both groups ($P < 0.05$; Fig. 3), P8 significantly exhibited a higher reduction effect in four items ($P < 0.05$), while marginally reduced one item ($P < 0.10$) as compared to the placebo. The efficacy of P8 in reducing stress as compared to the placebo was predominately attributed to reduction of touchiness (item no. 18, $P = 0.005$), reduced irritation (item no. 27, $P = 0.006$), increased calmness (item no. 29, $P = 0.015$) and increased tolerance against interruptions (item no. 32, $P = 0.010$). At a lesser degree, P8 also suggestively reduced upset (item no. 11, $P = 0.083$) as compared to the placebo. A total of 13 items designated for anxiety in the DASS-42 questionnaire were significantly reduced in the P8 group ($P < 0.05$; Fig. 4), while only 10 items were reduced in the placebo group over 12-weeks. The efficacy of P8 in reducing anxiety as compared to the placebo was predominately attributed to reduction in breathlessness (item no. 4, $P = 0.004$) and abnormal heart beats (item no. 25, $P = 0.028$) unrelated to physical activities, and decreased fear of the unfamiliar and unknown (item no. 30, $P = 0.029$). At a lesser degree, P8 also suggestively reduced physical shakiness (item no. 7, $P = 0.052$) as compared to the placebo.

3.3. Plasma levels of cortisol and cytokines

Plasma cortisol levels in total subjects were decreased upon administration of P8 while it increased in the placebo group over 12-weeks, attributed to a similar trend as observed in the women while men subject showed a decrease in both groups over 12-weeks (Table 3). However, this difference between P8 and placebo was merely marginal in total and women ($P < 0.10$). IFN-γ was reduced over 12-weeks in women, men and total subjects in the P8 group while those in the placebo group showed an increase over

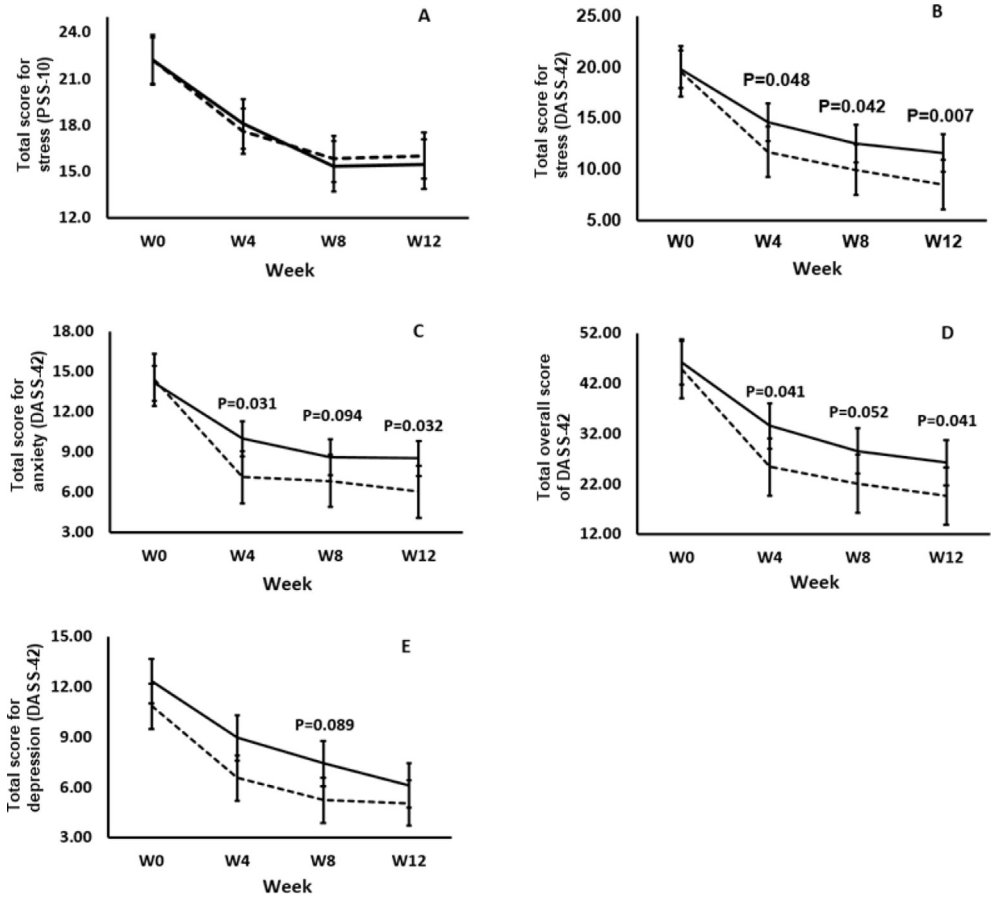

Fig. 2. Effects of a 12-week administration of probiotic *L. plantarum* P8, P8 (---) or placebo (—) on the total scores of (A) stress based on the PSS-10 questionnaire (W: $P < 0.001$, T: $P = 0.788$, TxW: $P = 0.820$), (B) stress (W: $P < 0.001$, T: $P = 0.030$, TxW: $P = 0.293$), (C) anxiety (W: $P < 0.001$, T: $P = 0.077$, TxW: $P = 0.170$), (D) total scores (W: $P < 0.001$, T: $P = 0.054$, TxW: $P = 0.427$), and (E) depression (W: $P < 0.001$, T: $P = 0.163$, TxW: $P = 0.787$) based on the DASS-42 questionnaire. P-values indicated difference between treatment groups at individual time points. Results are expressed as mean; error bars (SEM); n = 103. Repeated measures ANOVA provided statistical significance on W: effect of weeks; T: effect of treatment groups P8 and placebo; TxW: interaction between weeks and treatment.

12-weeks (mean difference 8.07 pg/ml; 95% CI −11.2 to −4.93; $P < 0.001$). Plasma levels of TNF-α was increased in both groups over 12-weeks but those administered with P8 showed a lower increase as compared to those on placebo (mean difference 1.52 pg/ml; 95% CI −2.14 to −0.89; $P < 0.001$), mainly attributed to the women which showed a similar trend (Mean difference 3.28; 95% CI −7.09 to 0.524; $P < 0.001$), while men did not exhibit any significant difference with the placebo. Both treatment groups did not yield any changes against plasma IL-10, IL-1β and IL-4 levels over 12-weeks. However, women administered with P8 showed a marginal decrease of IL-1β over 12-weeks as compared to the placebo which showed an increase (Mean difference 2.37; 95% CI -5.17 to 0.441; $P = 0.097$).

3.4. Cognition and memory

Cognition and memory parameters were less affected in the overall population of subjects involved in this study upon administration of P8, due to large variations among subjects. The intervention of P8 has increased the speed for social emotional cognition (Mean difference 0.079; 95% CI −0.128 to −0.03; $P = 0.002$), typically attributed to improvement in women while the men did not exhibit any differences as compared to the placebo (Table 4). The administration of P8 also increased total scores from the international shopping list memory test (Mean difference 2.32; 95% CI 0.0657 to 4.57; $P = 0.044$) as compared to the placebo, mainly attributed to the marginal improvement in men (Mean difference 4.14; 95% CI −0.519 to 8.8; $P = 0.079$), while insignificant differences were observed in women as compared to the placebo. Speed of the identification task was also marginally improved (Mean difference 50.1; 95% CI −109 to 8.62; $P = 0.093$) upon administration of P8 compared to the placebo in women. Meanwhile, men benefited marginally from the administration of P8 via correct input from the total overall scores (Mean difference 26.3; 95% CI −54.4 to 1.75; $P = 0.065$) as compared to the placebo.

3.5. Correlation analysis

Several parameters showed significant correlations ($P < 0.05$) with medium ($r > 0.30$) to high ($r > 0.50$) Spearman's rho values despite having low R^2 values (Fig. 5), indicating that significant trends were observed despite a high-variability among subjects. Plasma pro-inflammatory parameters of IFN-gamma and TNF-alpha were significantly ($P < 0.05$) and highly correlated ($r > 0.50$) with each other but not correlated with stress hormone cortisol. Cortisol also showed insignificant correlation with anxiety, although significantly associated with stress ($P < 0.05$). Both IFN-gamma and TNF-alpha showed higher correlation with stress than anxiety, although stress was highly correlated with anxiety ($r > 0.50$). Plasma levels of cortisol and cytokines did not affect social emotional cognition, and verbal learning and memory. However, these cognitive and memory traits were significantly

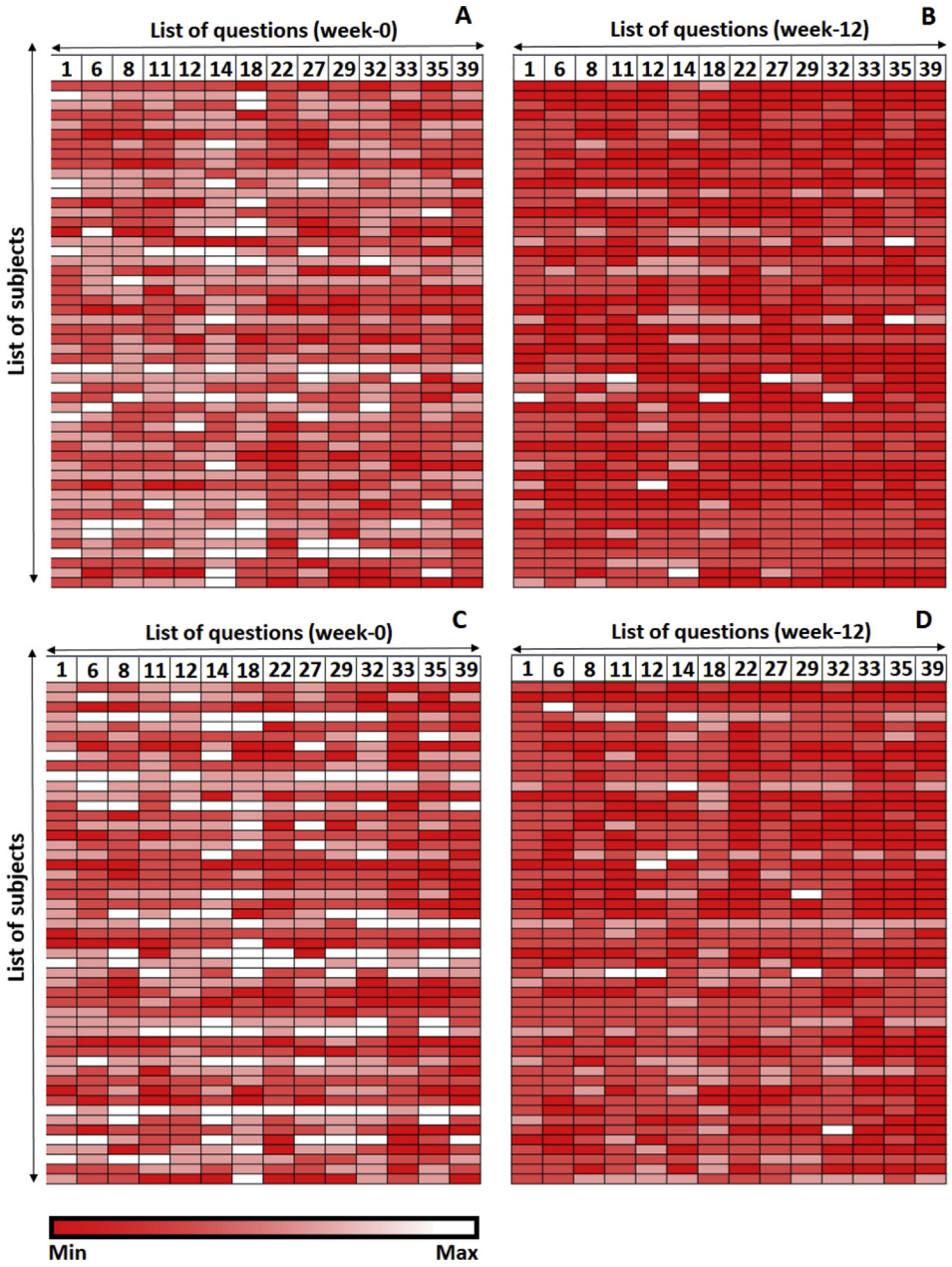

Fig. 3. Heat map illustrating the impacts of a 12-week administration of probiotic *L. plantarum* P8 (P8) or placebo on scores of individual subjects across individual items for the parameters of stress using the DASS-42 questionnaire. Data are presented as scores for P8 at week-0 (A) and week-12 (B), and for placebo at week-0 (C) and week-12 (D); n = 103. A darker color indicated lower stress score value. Both groups significantly ($P < 0.05$) reduced scores for all 14 items. However, P8 showed a significantly higher reduction for items no. 18 ($P = 0.005$), no. 27 ($P = 0.006$), 29 ($P = 0.015$) and no. 32 ($P = 0.010$), while marginally reduced for item no. 11 ($P = 0.083$) as compared to the placebo. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

correlated with stress and anxiety ($P < 0.05$). Social emotional cognition was also significantly correlated with verbal learning and memory ($P < 0.05$).

4. Discussion

Probiotics are conventionally discovered and developed for purposes of maintaining gut health and homeostasis. Recent evidences have reported the effects of probiotics on modulating behaviors and activities of animals that were stressed, implying the significance of the "gut-brain-axis" and subsequently motivating the development of probiotic-based products with emphases on brain health. Our present study utilized a probiotic strain, *L. plantarum* P8 with gut modulatory effects against possible alleviation of psychological disorders. Two stress questionnaires were used to assess the states of stress. PSS-10 is a validated and widely used psychological instrument for measuring the degree of appraised stress. Scores from PSS have been shown to correlate well with stress measures, self-reported health and health services measures, health behavior measures, smoking status, and help seeking behavior [14]. DASS-42 is another validated tool that was constructed to measure defined emotional states, in addition to further define, understand and measure the ubiquitous and clinically significant emotional states usually described as depression and anxiety [22]. All subjects were recruited based on moderate stress levels as determined via scores from PSS-10. Over 12-weeks,

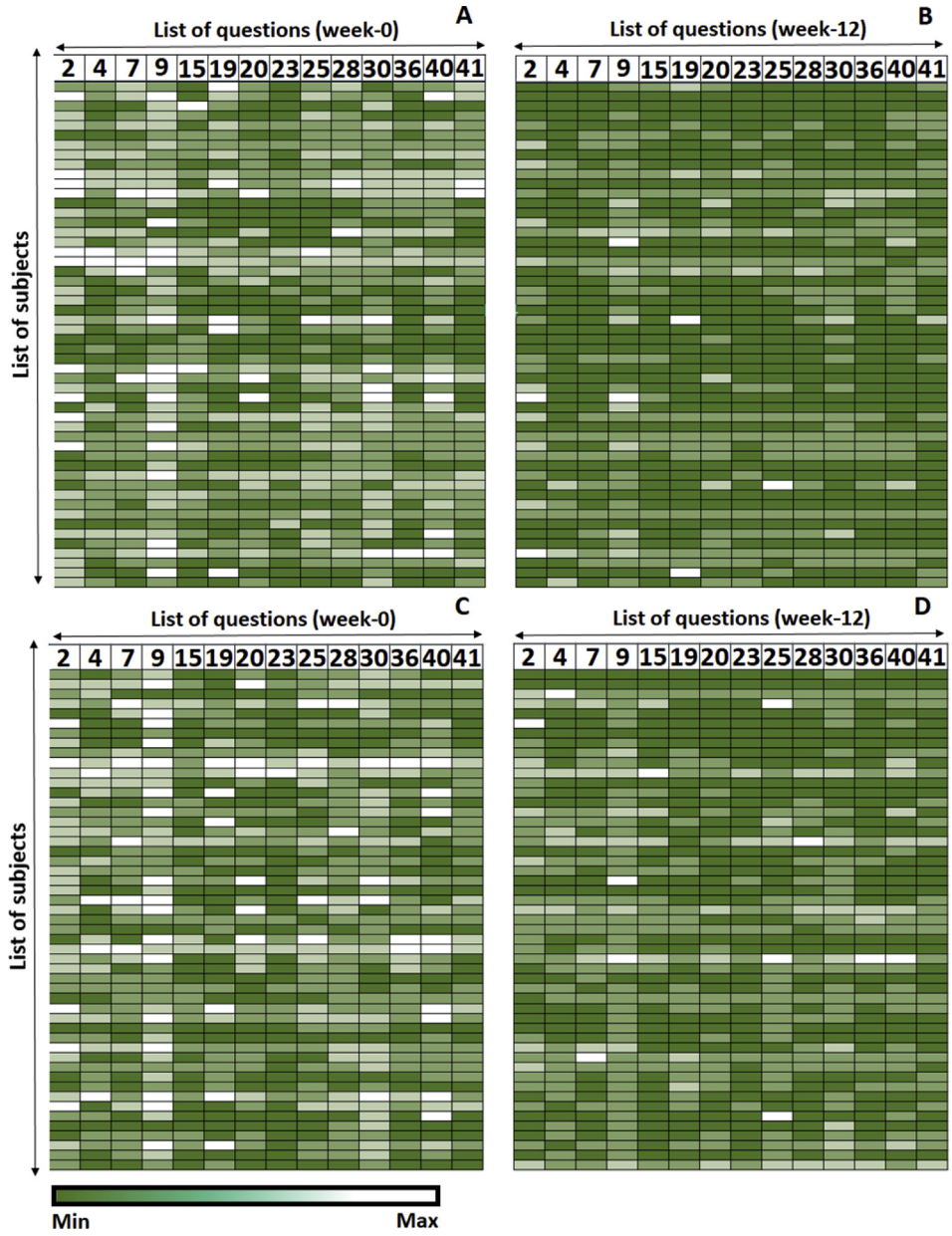

Fig. 4. Heat map illustrating the impacts of a 12-week administration of probiotic *L. plantarum* P8 (P8) or placebo on scores of individual subjects across individual items for the parameters of anxiety using the DASS-42 questionnaire. Data are presented as scores for P8 at week-0 (A) and week-12 (B), and for placebo at week-0 (C) and week-12 (D); n = 103. A darker color indicated lower stress score value. All 14 items except item no. 23 were significantly reduced in scores for the P8 group, while all items except items no., 15, 23, 25 and 41 were significantly reduced in scores for the placebo group after 12-weeks. P8 showed a significantly higher reduction for items no. 4 (P = 0.004), no. 25 (P = 0.028), and no. 30 (P = 0.029), while marginally reduced for item no. 7 (P = 0.052) as compared to the placebo. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Changes of plasma cortisol, interferon-gamma (IFN-γ), interleukin-10 (IL-10), interleukin-1-beta (IL-1β), interleukin-4 (IL-4), and tumor necrosis factor alpha (TNF-α) levels over 12-weeks in women, men and overall total subjects, upon administration of probiotic *L. plantarum* P8 or placebo.

Plasma parameter	Women			Men			All subjects		
	P8	Placebo	P-value	P8	Placebo	P-value	P8	Placebo	P-value
	Changes over 12-weeks								
Cortisol (ug/dl)	−3.01 ± 11.46	0.89 ± 8.29	0.097	−3.03 ± 4.35	−1.51 ± 9.19	0.612	−3.01 ± 10.19	0.27 ± 8.49	0.090
IFN-γ (pg/ml)	−0.47 ± 2.47	8.54 ± 12.20	<0.001*	−1.11 ± 4.35	4.40 ± 4.91	0.008*	−0.62 ± 2.98	7.46 ± 10.88	<0.001*
IL-10 (pg/ml)	0.85 ± 4.56	1.23 ± 7.29	0.784	2.81 ± 9.45	2.01 ± 5.60	0.802	1.31 ± 6.01	1.44 ± 6.84	0.924
IL-1β (pg/ml)	−1.52 ± 8.13	0.84 ± 1.12	0.097	−0.18 ± 0.95	−1.40 ± 8.03	0.610	−1.21 ± 7.13	0.26 ± 4.20	0.228
IL-4 (pg/ml)	5.22 ± 15.65	3.47 ± 10.59	0.583	9.51 ± 29.45	7.98 ± 19.86	0.883	6.23 ± 19.50	4.65 ± 13.51	0.646
TNF-α (pg/ml)	0.06 ± 1.02	1.75 ± 1.84	<0.001*	0.53 ± 1.94	1.53 ± 1.74	0.196	0.17 ± 1.29	1.69 ± 1.80	<0.001*

Results are expressed as mean ± SEM; n = 103. *P < 0.05.

Table 4
Cognition and memory parameters as assessed via the computerized CogState Brief Battery at week-12 for women and men upon administration of probiotic *L. plantarum* P8 or placebo.

Cognition and memory parameters		Women			Men			All subjects		
		P8	Placebo	P-value	P8	Placebo	P-value	P8	Placebo	P-value
Detection (Measures psycomotor)	Speed	385.45 (339.44–431.46)	410.54 (367.4–453.7)	0.423	389.77 (291.27–488.27)	364.23 (315.4–413)	0.617	386.55 (345.91–427.19)	398.5 (364.5–432.5)	0.652
	Accuracy	89.23 (80.79–97.66)	87.75 (79.83–95.67)	0.797	87.97 (71.86–104.08)	89.15 (77.67–100.6)	0.898	88.91 (81.69–96.12)	88.11 (81.73–94.49)	0.869
	Correct	34.82 (32.86–36.78)	33.92 (32.3–35.54)	0.478	30.38 (22.33–38.44)	35.85 (35.03–36.66)	0.155	33.69 (31.29–36.09)	34.42 (33.20–35.64)	0.588
	Error	5.84 (−1.76–13.45)	9.65 (1.55–17.74)	0.589	12.85 (−1.64–27.34)	8.08 (−3.13–19.28)	0.576	7.63 (1.07–14.19)	9.24 (2.77–15.71)	0.726
Identification (measures basic attention)	Speed	515.87 (471.14–560.59)	565.97 (526.6–605.4)	0.093	614.77 (560.32–669.22)	561.15 (501.5–602.8)	0.161	541.08 (503.83–578.33)	564.72 (532.7–596.7)	0.336
	Accuracy	86.47 (76.96–95.97)	88.48 (81.96–95.01)	0.725	85.38 (70.14–100.63)	88.42 (78.36–98.47)	0.721	86.19 (78.38–94.00)	88.47 (83.16–93.77)	0.631
	Correct	29.05 (27.53–30.58)	30.14 (28.05–32.22)	0.397	23.23 (15.46–31.01)	29.31 (27.80–30.82)	0.108	27.57 (25.35–29.79)	29.92 (28.35–31.49)	0.086
	Error	6.29 (−1.02–13.60)	7.05 (1.30–12.81)	0.869	14.69 (0.05–29.33)	5.15 (−0.91–11.22)	0.202	8.43 (2.02–14.85)	6.56 (2.12–11.00)	0.632
One card learning (measures visual learning & memory)	Speed	2.99 (2.95–3.03)	3.02 (2.97–3.07)	0.338	2.99 (2.93–3.04)	2.96 (2.89–3.03)	0.52	2.99 (2.95–3.02)	3.00 (2.97–3.04)	0.544
	Accuracy	0.96 (0.83–1.09)	0.91 (0.87–0.94)	0.434	0.83 (0.69–0.97)	0.92 (0.85–0.99)	0.199	0.93 (0.82–1.03)	0.91 (0.88–0.94)	0.760
	Correct	57.21 (53.18–61.24)	54.92 (51.89–57.95)	0.362	50.08 (40.12–60.04)	56.54 (50.95–62.12)	0.23	55.39 (51.56–59.23)	55.34 (52.77–57.91)	0.982
	Error	35.00 (29.22–40.78)	34.38 (30.99–37.77)	0.852	43.54 (28.99–58.09)	33.31 (27.16–39.45)	0.171	37.18 (31.68–42.67)	34.1 (31.24–36.96)	0.324
One card back (measures working memory)	Speed	843.87 (756.26–931.48)	894.86 (821.8–968.0)	0.369	913.92 (840.84–987.01)	845.08 (714.4–975.7)	0.326	861.73 (794.56–928.90)	881.9 (820.1–943.7)	0.658
	Accuracy	84.01 (78.51–89.52)	83.11 (76.93–89.3)	0.826	77.84 (60.71–94.97)	80.51 (70.66–90.36)	0.771	82.41 (76.69–88.12)	82.43 (77.36–87.51)	0.993
	Correct	30.13 (28.49–31.77)	29.84 (28.30–31.37)	0.792	25.69 (19.07–32.31)	29.85 (28.43–31.26)	0.194	29.00 (26.99–31.01)	29.84 (28.67–31.01)	0.472
	Error	6.97 (4.18–9.76)	7.38 (4.11–10.65)	0.849	14.08 (0.75–27.40)	8.38 (3.39–13.38)	0.392	8.78 (5.01–12.56)	7.64 (4.98–10.30)	0.621
Maze (measures executive function)	Duration	276,718 (210,414 −343,022)	252,819 (226,730 −278,907)	0.503	207,404 (170,007 −244,802)	229,768 (157,483 −302,053)	0.555	259,050 (208,768 −309,332)	246,826 (221,237 −272,414)	0.666
	Total scores	101.89 (99.45–104.34)	100.08 (98.12–102)	0.264	97.92 (86.97–108.87)	100.85 (96.38–105.30)	0.595	100.88 (97.77–104.00)	100.28 (98.51–102.10)	0.738
	Errors	56.42 (45.70–67.15)	58.19 (52.96–63.42)	0.264	55.54 (45.63–65.45)	56.23 (44.84–67.62)	0.598	56.20 (47.98–64.41)	57.68 (53.02–62.34)	0.754
Maze final recall (measures long term memory)	Duration	36,268 (30,524–42,012)	38,669 (32,524 −44,815)	0.564	28,888 (21,381–36,395)	34,047 (23,211 −44,883)	0.402	34,387 (29,735–39,039)	37,468 (32,285 −42,650)	0.376
	Error	8.68 (6.62–10.75)	8.19 (6.68–9.70)	0.697	8.23 (4.50–11.96)	8.69 (5.27–12.11)	0.844	8.57 (6.83–10.31)	8.32 (6.96–9.68)	0.822
Social emotional task (measures social emotional cognition)	Speed	3.48 (3.44–3.52)	3.56 (3.53–3.60)	0.004*	3.44 (3.37–3.50)	3.51 (3.42–3.61)	0.186	3.47 (3.44–3.50)	3.55 (3.51–3.59)	0.002*
	Accuracy	1.11 (1.06–1.16)	1.11 (1.08–1.15)	0.949	1.03 (0.88–1.17)	1.11 (1.05–1.17)	0.229	1.09 (1.04–1.14)	1.11 (1.09–1.14)	0.412
	Correct	38.53 (36.50–40.55)	39.30 (38.2–40.39)	0.502	34.69 (28.50–40.88)	38.85 (36.59–41.11)	0.182	37.55 (35.44–39.65)	39.18 (38.22–40.41)	0.163
	Error	10.00 (7.81–12.20)	9.32 (8.12–10.53)	0.589	14.00 (7.60–20.40)	9.77 (7.48–12.06)	0.188	11.02 (8.79–13.25)	9.44 (8.41–10.47)	0.203
Continuous Paired associate learning)	Speed	3.33 (3.28–3.39)	3.38 (3.33–3.44)	0.191	3.32 (3.21–3.43)	3.26 (2.98–3.54)	0.675	3.33 (3.28–3.38)	3.35 (3.27–3.43)	0.615
(measures	Accuracy	0.81 (0.73–0.89)	0.79 (0.71–0.87)	0.686	0.75 (0.61–0.88)	0.81 (0.66–0.97)	0.509	0.80 (0.73–0.86)	0.79 (0.73–0.86)	0.993
	Correct	56 (56–56)	56 (56–56)		56 (56–56)	56.00 (56–56)				
	Error	75.29 (54.52–96.06)	75.68 (58.87–92.48)	0.977	98.00 (51.02–144.98)	80.15 (70.66–90.36)	0.549	81.08 (62.20–99.56)	76.84 (60.94–72.94)	0.731
Shopping list (measures verbal learning & memory)	Total scores from 3x	27.41 (25.26–29.55)	25.76 (24.37–27.15)	0.207	26.31 (23.36–29.25)	22.17 (18.13–26.20)	0.079	27.12 (25.40–28.84)	24.83 (23.33–26.27)	0.044*
	Scored from recall	10.86 (10.13–11.59)	11.03 (10.65–11.41)	0.687	10.69 (10.07–11.32)	9.80 (7.37–12.23)	0.378	10.82 (10.27–11.37)	10.74 (10.14–11.33)	0.846
Total scores (Total Cognition)	Accuracy	6.79 (6.48–7.10)	6.63 (6.32–6.94)	0.449	5.93 (5.02–6.84)	6.62 (6.20–7.04)	0.147	6.57 (6.25–6.90)	6.63 (6.38–6.87)	0.794
	Correct	273.79 (265.45–282.13)	272.05 (266.4–277.7)	0.730	248.08 (219.24–276.91)	274.38 (267.6–281.2)	0.065	267.24 (257.69–276.78)	272.66 (268.2–277.1)	0.306
	Error	204.50 (162.59–246.42)	209.84 (181.1–238.6)	0.833	260.92 (187.51–334.33)	209.77 (152.3–267.30)	0.244	218.88 (183.09–254.67)	209.82 (184.9–234.7)	0.678

Results are expressed as mean (95% CI); n = 103. *P < 0.05.

Correlation		Plasma cortisol & cytokines			DASS-42 psychological traits		CogState Battery cognition & memory traits
		Cortisol	IFN-gamma	TNF-alpha	Anxiety	Stress	Social emotional cognition
Plasma cortisol & cytokines	IFN-gamma	P=0.701 *r*=0.04 R^2=0.00					
	TNF-alpha	P=0.523 *r*=0.07 R^2=0.00	P<0.001 *r*=0.78 R^2=0.21				
DASS-42 psychological traits	Anxiety	P=0.080 *r*=0.18 R^2=0.05	P=0.017 *r*=0.24 R^2=0.04	P=0.008 *r*=0.27 R^2=0.04			
	Stress	P=0.033 *r*=0.22 R^2=0.04	P=0.007 *r*=0.27 R^2=0.03	P<0.001 *r*=0.35 R^2=0.07	P<0.001 *r*=0.80 R^2=0.69		
CogState Battery & cognition memory traits	Social emotional cognition	P=0.617 *r*=0.05 R^2=0.02	P=0.751 *r*=0.03 R^2=0.00	P=0.242 *r*=0.12 R^2=0.02	P=0.004 *r*=0.29 R^2=0.08	P=0.014 *r*=0.24 R^2=0.05	
	Verbal learning & memory	P=0.799 *r*=0.03 R^2=0.00	P=0.277 *r*=0.12 R^2=0.00	P=0.139 *r*=0.16 R^2=0.01	P=0.019 *r*=0.25 R^2=0.03	P=0.014 *r*=0.26 R^2=0.05	P=0.028 *r*=0.23 R^2=0.01

Fig. 5. Correlation analysis between plasma cortisol and cytokines, DASS-42 psychological traits and CogState Battery cognition and memory traits in all subjects (n = 103) upon a 12-week administration of probiotic *L. plantarum* P8 (P8) or placebo. Data on cortisol, cytokines and DASS-42 traits were based on difference in scores between week-12 and week-0. Data on CogState Battery traits were based on scores at week-12. A darker shade of red indicated higher statistical significance, while a darker shade of blue indicated lower statistical significance at P < 0.05. *r*: Spearman's rho. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the intervention of P8 yielded reduced stress levels after week-4 as compared to the placebo as observed via DASS-42 but insignificant changes were observed as assessed via PSS-10. Such a difference may be attributed to the different assessment natures of both tools. Each item in the PSS-10 questionnaire was designed to specifically include circumstances that were deemed unpredictable, uncontrollable and overloaded of currently experienced stress levels, while the stress scale of DASS-42 had broader natured items which were sensitive to levels of chronic non-specific arousal, such as difficulty in relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient. Due to its broader scopes of assessments, DASS-42 has been widely used in both clinical and non-clinical samples, while PSS-10 is primarily used in research settings. Our further evaluations of individual stress items in DASS-42 also showed that the effects of P8 were more pronounced in four items that were primarily associated with agitation, irritation and nervous arousal.

While P8 exerted beneficial effects in reducing scores of anxiety after week-4 as compared to the placebo, insignificant effects were observed against depression. The anxiety scale of DASS-42 assessed autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect, while the depression scale assessed dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia. In general, the subjects that were recruited for this study experienced mild levels of depression and thus changes over 12-weeks remained insignificant. Our further evaluations of individual anxiety items in DASS-42 exhibited that the effects of P8 were more pronounced in three items that were primarily associated with subconscious arousal and anxiousness.

Conventionally, hypothalamic-pituitary-adrenal axis-mediated hormones namely glucocorticoids have been recognized as agents that trigger a myriad of stress effects on the hippocampus and as contributing factors to stress-associated psychopathologies. However, recent reports have shown that glucocorticoid-mediated alterations of the hippocampus remain inconclusive with large variations across studies, mainly attributed to the functions of glucocorticoid in regulating other broad cellular metabolic processes [24]. Our present study also exemplified such a trait, where both placebo and P8 groups yielded very marginal differences of plasma cortisol levels over 12-weeks, while other than stress levels, cortisol also did not show an association with other parameters studied.

At neuronal levels, stress reportedly altered neuronal morphology, suppressed neuronal proliferation, reduced synaptic plasticity and firing properties, which ultimately reduced hippocampal volume. All these eventually impaired various hippocampal-dependent memory tasks, leading to impaired memory, learning, and/or cognitive abilities [25]. Stress subjects on P8 showed lower plasma pro-inflammatory cytokines such as IFN-gamma and TNF-alpha than the placebo, accompanied by better cognitive and memory potentials. Correlation analyses showed that pro-inflammatory

cytokines were positively correlated with psychological traits, while psychological traits were correlated with cognition and memory. These indicated that inflammation may have caused stress and anxiety, leading to impaired mental potentials. Increased inflammation has been found in cases of metabolic disorders such as diabetes, cardiovascular and obesity [26], and correlated with reduced volume in brain regions such as post-central gyrus, frontal lobe, putamen, and middle frontal gyrus [27], linking the roles of inflammation in brain pathophysiology. Our current data illustrated that P8 exhibited a stress and anxiety reducing potential together with improved cognition and memory primarily via targeting anti-inflammatory properties. P8 has been previously reported to benefit the gut environment via suppressing gut pathogens while increasing population of beneficial gut microbiota, accompanied by increased concentrations of short chain fatty acids in adults [12,13]. Considering that increased abundance of gut pathogens often leads to increased accumulation of pro-inflammatory metabolites, while probiotics primarily benefit gut health, we strongly believe that inflammation plays a crucial role in brain pathophysiology along the gut-brain-axis.

Stress, either acute or chronic, often elicit different responses in men and women. Women have higher rates of internalizing disorders such as depression and anxiety, with a global annual prevalence of 1.7-fold greater incidences than men [28], while men often externalize symptoms such as aggressiveness, substance abuse, antisocial, attention deficit and defiance amid stress [29]. Our present data also illustrated the different effects of P8 in stressed men and women. In women, enhanced cognitive properties such as social emotional cognition (encoding, memory, and interpretation of social information) and basic attention (ability to sustain attention) were observed upon administration of P8, indicating better management of anxiety disorders, anxiousness and mindful attention [30]. The administration of P8 benefited men primarily via improved verbal learning and memory (coding of information in memory by sound) and overall correct input. These traits are crucial indicators of verbal memory functions and mental focus, and were reportedly deficit in chronic alcohol and cannabis users [31]. Although women tend to outperform men in verbal memory tasks [32], P8 exerted a stronger effect in men than women in this aspect, most probably attributed to the higher tendency of externalization symptoms in men.

5. Conclusions

Stress and anxiety involves a broad spectrum of behavioral symptoms that are individual dependent with low medical success rates amid various reports of side effects. Various efforts and natural therapies are initiated to tackle this health disorder. Our present data illustrated that *L. plantarum* P8 reduced some stress and anxiety symptoms via anti-inflammatory properties, followed by enhanced memory and cognitive abilities. P8 also exerted different memory and cognitive functions in men and women. Taken altogether, our data presented a feasible and natural approach using a specific probiotic P8 to manage stress and anxiety.

Author contributions

MTL and HZ conceived and designed the experiments. YYH, LCL, NAAY, MSBY, NSR, AA, JAMM and MTL performed the study and analyzed the data. MFILA, NZ and NW provided psychological and medical advice and services. YYH, LCL, SBC, MTL, LYK, ZS and HZ drafted the work, critically revised for intellectual content and wrote the manuscript.

Conflict of interest

The authors declare no competing financial or conflict of interest.

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