Brain, Behavior, & Immunity - Health 18 (2021) 100372

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Mindfulness meditation training effects on quality of life, immune function and glutathione metabolism in service healthy female teachers: A randomized pilot clinical trial

Daniela Rodrigues de Oliveira [a,b,*], David Wilson [a], Fanny Palace-Berl [b], Bárbara de Mello Ponteciano [b,c], Luciana Fungaro Rissatti [b], Flávia Sardela de Miranda [d], Valéria Piassa Pollizi [a], José Carlos Fuscella [e], Alex Mourão Terzi [f], Ana Paula Lepique [d], Vânia D'Almeida [g], Marcelo Demarzo [a]

[a] Mente Aberta - Brazilian Center for Mindfulness and Health Promotion, Departamento de Medicina Preventiva, Universidade Federal de São Paulo, São Paulo, SP, Brazil
[b] Departamento de Patologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil
[c] Laboratório de Investigação Médica (LIM-26), Departamento de Cirurgia, Universidade de São Paulo, SP, Brazil
[d] Laboratório de Imunomodulação, Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brazil
[e] Centro Educacional Objetivo, São Paulo, SP, Brazil
[f] Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais, Campus, São João del-Rei, MG, Brazil
[g] Departamento de Psicobiologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Mindfulness meditation
Teachers
Glutathione metabolism
Inflammatory cytokines
Stress
Quality of life

ABSTRACT

Background: Despite the crucial role of educators in encourage students' academic learning, addressing educator stress inside the classroom remains a significant challenge in the educational context. Mindfulness Meditation training (MM) has been recommended as an environmental enrichment strategy in schools to help teachers cope with stress and cultivating a state of awareness in daily life. Although studies have shown that MM can improve immune system dynamics the biological mechanism underlying glutathione metabolism in a healthy human is unclear

Objective: The purpose of this study was to determine whether MM training benefits psychological and behavioral response, immunological functions and glutathione metabolism in service healthy female teachers from public schools

Methods: We randomly assigned 76 teachers to an 8-week Mindfulness-Based Health Program for Educators (MBHPEduca) or Neuroscience for Education program (Neuro-Educa; active control group). Using the quality of life as our primary outcome, perceived stress, negative affectivity, and resilience as our secondary outcome, and pro-inflammatory cytokines and glutathione levels as our third outcome at baseline and post-intervention that occurred in public schools. Blood samples were collected for the measurement of three proinflammatory markers, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8) and three GSH metabolism, including Cysteine (Cys), Homocysteine (HCys) and GSH were conducted at pre-and post-intervention, with selfreported assessments over time. Treatment effects were analyzed using generalized estimating equations (GEE) with to intention to treat

Results: We observed statistically significant improvements to the MBHP-Educa group compared to active control in perceived stress, resilience, positive and negative affect, and quality of life after 8-weeks MM ($p < 0.0001$). Further, the MBHP-Educa group exhibited lower circulating IL-6 production accompanied by high circulating GSH, and Cys ($p < 0.0001$). Additional analyses indicated that enhancing quality of life through mindfulness meditation training was mediated by reducing perceived stress and serum levels of IL- 6 and increasing resilience and teachers ‘plasma GSH levels

* Corresponding author. Departamento de Patologia, Universidade Federal de São Paulo, Departamento de Medicina Preventiva, Universidade Federal de São Paulo, Laboratório de Investigação Médica (LIM-26), Departamento de Cirurgia, Universidade de São Paulo, São Paulo, SP, Brazil.

E-mail address: danielaoliveira.phd@gmail.com (D. Rodrigues de Oliveira).

https://doi.org/10.1016/j.bbih.2021.100372
Received 17 March 2021; Received in revised form 6 October 2021; Accepted 13 October 2021
Available online 21 October 2021

Conclusions: The present study is a pilot trial with low-power and provides preliminary evidence that mindfulness meditation training help teachers to cope with stress in the school environment with an impact on the quality of life, immune function, and glutathione metabolism.

1. Introduction

Stress in teachers is known to education authorities worldwide, and to a higher degree in emerging countries. Several different kinds of adversities surround the work of a teacher, among them, underpayment labor overtaxing, leave alone excessively long work shifts. Besides stressful demands and duties, other effects such as massification and serial production (such as in Industry), shared with other professions, bring on effects of a repetitive, monotonous and year-after-year working style which can, in their own turn, predispose teachers to boredom, which has been for long recognized as a potent stress-inducing kind of experience (Thackray, 1981). Thus, providing resources that, through stress reduction and emotional self-regulation, may enhance teachers' personal sense of self and ability to adapt successfully to stressful life events to which teachers are exposed (personal and family drama, violence within and outside school boundaries, defiance from students, among others) is essential. Therefore, stress reduction can help dilute the deleterious effects of sustained stress occurring in this population. Strategies designed to aid educators' ability to cope with demands inside and outside of class and strengthen their perception and insight in life are a must, since no efforts must be spared in keeping a classroom environment as healthy as possible.

We already have some mindfulness-based programs, most of which are designed for students (Bierman et al., 2015; Karcher, 2008), but to our knowledge, endeavors involving mindfulness meditation for teachers are far more scarce (despite their obvious potential for boosting both students' and teachers' quality of life). Besides, these fewer mindfulness intervention-based teacher-targeted interventions mainly have specific scopes and are designed to attend specific socio-cultural contexts (Hirshberg et al., 2020; Jennings, 2015; López-Gonzalez et al., 2016). However, a program specifically designed for health promotion in schools is yet to be offered, which, could in fact show itself feasible as part of the professional training and capacitation for education professionals. Mindfulness-Based Health Program for Educators (MBHP-Educa) was designed by Brazilian researchers along with educators and has been proposed as an environmental enrichment strategy for schools in order to strengthen and develop inherent awareness capacities, in order to cope with stress and increase resilience in the educational Brazilian context (Demarzo et al., 2020).

Mindfulness meditation (MM) encourages practitioners to cultivate several capacities of awareness, such as present-centered awareness, meta-awareness, nonreactivity, and dereification (Wielgosz et al., 2019). These capacities act as stress responses helping to better respond to environmental emergencies and changes. To do so, psychological responses (e.g., cognitive and affective) are first fired, and a multisystemic physiological response (e.g., neuroendocrine axis, immune systems and oxidative stress response pathways) is consequently triggered (Epel et al., 2018; McEwen, 2007). Taken altogether, these mechanisms strive to improve the ability to adapt in the face of adversity, through the maintenance of psychological and physiological equilibrium and well-being (Southwick et al., 2014) and, therefore, improving one's ability to perceive his or her quality of life in these moments. In this context, the practice of MM may stand as a preventive strategy to help the organism to better cope with the stress response.

Extensive evidence points out that MM modulates immune-inflammatory pathways in both clinical and non-clinical conditions (Black and Slavich, 2016; Buric et al., 2017). These modulations have been related to decreased levels of proinflammatory interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 1β (IL-1β) and Tumor Necrosis Factor-alpha (TNF-α) and increased levels of anti-inflammatory interleukin 10 (IL-10) (Bower et al., 2015; Chaix et al., 2020; Jedel et al., 2014; Rosenkranz et al., 2013). It is known that an imbalance of pro-and anti-inflammatory cytokines triggers an oxidative stress response (Dröge and Breitkreutz, 2000; Schmidt et al., 2005). In this regard, enhanced proinflammatory cytokines signaling promotes the generation of reactive oxygen species (ROS), which in turn leads to oxidative damage resulting in depletion of known antioxidant defenses such as glutathione (GSH) (De la Fuente, 2018; Dröge and Breitkreutz, 2000). GSH plays an important role in maintaining immune response and redox homeostasis in cells, and it is important in epigenetic regulation (García-Giménez et al., 2017; Markovic et al., 2010). Whether mindfulness meditation training could increase the GSH levels in the blood is a thrilling question.

With this background, the primary objective of the present randomized controlled trial (RCT) was to evaluate the efficacy of MBHP-Educa (a Mindfulness-Based Intervention) on enhancing the quality of life (primary outcome) in in-service active healthy female teachers in Brazilian Public Schools. We were secondarily interested in whether the intervention would reduce perceived stress and negative affectivity and increase resilience and positive affectivity (secondary outcomes). Furthermore, we were interested in whether the intervention reduced or not pro-inflammatory cytokine (IL-6, IL-8 and IL-12p70) levels and increased or not GSH levels (third outcomes). We hypothesize that 8 weeks of mindfulness meditation training (MBHP-Educa) could foster improvements in the scores of quality of life in healthy teachers, and that such an improvement of quality of life could be kept during the next 12 months following the training. Unfortunately, the follow-up procedures were forcefully interrupted due to the COVID-19 pandemic. We believe that COVID-19 pandemic poses as an extreme stressor (catastrophic, life-threatening and of severe social consequences), and thus would compromise the ability to perceive quality of life within the period. Based on the literature for mindfulness meditation interventions in teachers as well as those presenting chronic diseases, we also hypothesized that the training could reduce perceived stress, as well as negative affectivity and pro-inflammatory cytokines levels, while increasing resilience, positive affectivity, and GSH levels.

2. Method and materials

2.1. Study design

This study is a randomized controlled clinical trial (RCT) consisting of two arms: (1) an MBHP-Educa program conducted by a certified instructor with more than ten years of experience in meditation practices; and (2) an active control group (Neuro-Educa program) conducted by a neuroscientist. Active control group and MBHP-Educa intervention lasted eight weeks, and the outcome measures and biomarkers were collected at similar time points baseline (pre-intervention) and post-intervention assessments, and originally it would be collected at 12-month follow up assessment as well (see Fig. 1). Unfortunately, the follow-up procedures were forcefully interrupted due to the COVID-19 pandemic. Furthermore, we believe that COVID-19 pandemic poses as an extreme stressor (catastrophic, life-threatening and of severe social consequences), and thus would compromise the results of the follow-up anyway. Despite the Neuro-Educa program (active control group) having been designed to control for mindfulness content (awareness, a specific ingredient inherent to mindfulness meditation training), it in fact, does also include cognitive and sensorial stimulation as well as didactic material and lectures (the content of which unrelated to mindfulness) and group discussion.

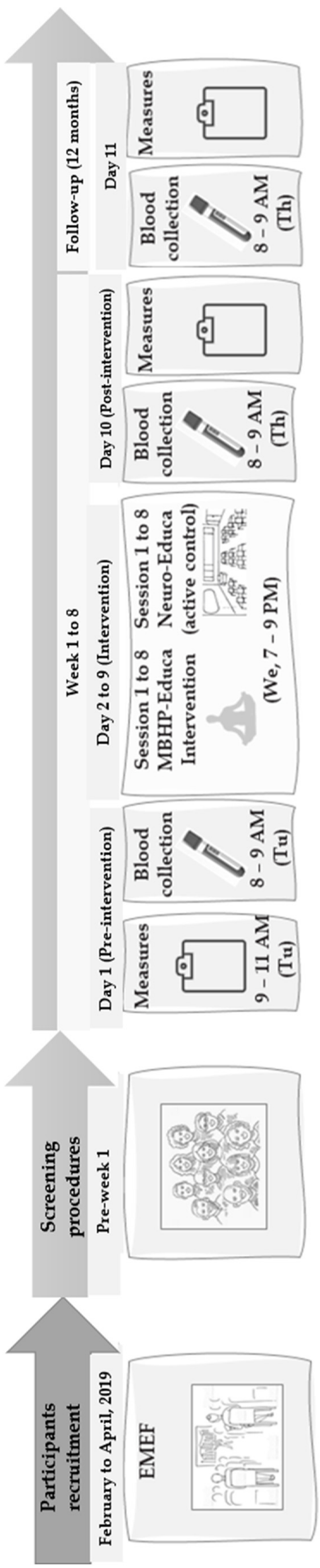

Fig. 1. Study design. Note: Note. Tu = Tuesday; We = Wednesday; Th= Thursday; EMEF = EMEF Carlos de Andrade Rizzini.

2.2. Procedure

All study procedures were approved by the Research Ethics Committee of the Universidade Federal de São Paulo – UNIFESP, Brazil (Number: 2.825.371 - CAAE: 92876418.2.0000.5505). The study design, measures, and analysis approach were preregistered (https://www.clinicaltrials.gov/ct2/show/NCT03839030) on February 12, 2019. Participants were recruited from public schools linked the "Diretoria Regional de Educação de Santo Amaro – DRESA" – a southern section of the São Paulo City Hall public education secretaryship between February to April 2019. Inclusion criteria were: (1) Brazilian female aged between 23 and 65 years and (2) currently working in the classroom. Exclusion criteria were: (1) the presence of medical conditions or medications use that might affect inflammatory pathways; (2) report of an infectious condition in the last 15 days before collecting a blood sample; (3) active psychiatric and/or clinical comorbidities potentially life-threatening such as psychosis or suicidal ideation; and (4) had previously practiced or currently practicing meditation, yoga or Tai-Chi. Those subjects who met the inclusion/exclusion criteria and gave their written informed consent before baseline procedures were subsequently included and randomized to both groups.

2.3. Randomisation procedure

A researcher who had not been involved in the study generated a simple random allocation sequence by using IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA) to determine group assignment. Participants were not informed of group allocation until after completion of baseline (pre-intervention) assessments and, the outcome assessor remained blind as to participant allocation.

2.4. Sample size and power analyses

Power analyses were performed using the G*Power 3.1 software (Faul et al., 2009) indicated that planned two-tailed test (post-test *minus* pre-test and follow-up test *minus* pre-test difference score models, i.e., GROUP x time point interaction) with α set to 0.05, power of 0.80, N = 70 would be required to detect moderate (d = 0.55) magnitude effects.

Based on effect sizes from prior mindfulness study on quality of life with students (Demarzo et al., 2014), the present study was designed for an anticipated sample of N = 70, sufficiently powered to detect moderate and large but not small magnitude effects.

2.5. Participants

One hundred forty-six teachers eligible for screening, seventy were excluded (see flow-chart, Fig. 2). The reasons for exclusion were (a) current depressive disorder (n = 20), (b) current anxiety disorder (n = 3), (c) reported use of nonsteroidal anti-inflammatory drugs (NSAIDs) in the last seven days (n = 17) and (d) current meditation, yoga or Tai-Chi practices (n = 11). Nineteen declined to take part in the study for personal reasons. Only 76 participants were recruited and randomly allocated to the intervention group, 38 were assigned to active control and 38 to MBHP-Educa. Thirty-five participants dropped out of the study because they did not complete more than three sessions. The most common reasons were having competing familiar time demands and time constraints. Therefore, a total of 20 participants in the active control (Neuro-Educa) group and 21 in the MBHP-Educa intervention group completed the study and were included in the analysis. The number of sessions attended was equal between groups (eight sessions completed). Finally, we had a total of 41 subjects (active control; n = 20 and MBHP-Educa; n = 21) who had completed the intervention after 8-weeks (see Table 1 for more details).

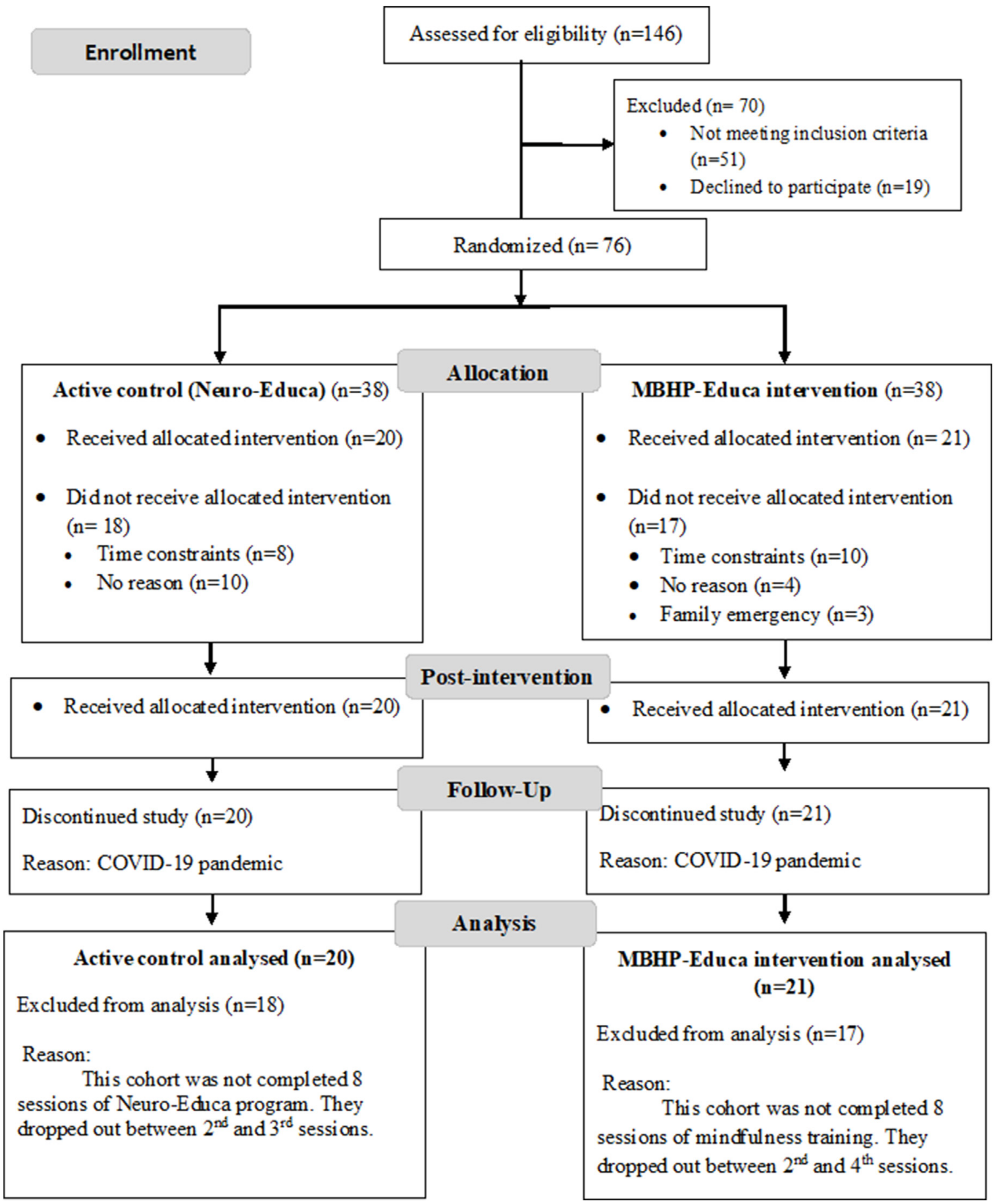

Fig. 2. CONSORT diagram.

2.6. Interventions

Both MBHP-Educa and Neuro-Educa were structurally equivalent, having a group format consisting of 8 weekly, 2-h meetings (16.0-h total). Participants from MBHP-Educa group were asked to meditate for 10–30 min/day via audio recording. We also asked to participants from Neuro-Educa (active control) group to read a book or listening to music for 10–30 min/day. Mindfulness training was conducted by two instructors with more than 30 years' experience in meditation practices (Zen and Tibetan Buddhist approach to mind-training) and extensive mindfulness teaching and practices (>5 years each) experiences. Neuro-Educa program was conducted by two neuroscientists (2–10 years teaching experience). Neuro-Educa program (active control) and MBHP-Educa intervention (mindfulness training, an environmental enrichment intervention) happened between April 2019 and June 2019.

2.6.1. Mindfulness-based health promotion program for educators (MBHP-Educa)

MBHP-Educa training program (Demarzo et al., 2020) is a mindfulness-based intervention (MBI) specifically designed for school context, socioculturally adapted to Brazil, and can be suitably understood as an environment-enriching experience as shown below. As a whole, it encompasses different practices which intend to exercise awareness by using mindfulness moment by moment. Thus, practices such as mindful

Table 1
Demographic, clinical and lifestyle and physical characteristics of participants at baseline.

Characteristics	Control	MBHP-Educa	Group Comparisons
	(n = 38)	(n = 38)	
Socio-demographic			
Age, mean (SD)	43.05 (8.50)	46.37 (8.08)	t(74) = 1.74, p = 0.086
Ethnicity, n (%)			
White	29 (76.3)	25 (65.8)	χ2 = 7.989, p = 0.157
Black	3 (7.9)	–	
Indian Brazilian	–	2 (5.3)	
Asian Brazilian	1 (2.6)	1 (2.6)	
Pardo Brazilian	5 (13.2)	8 (21.1)	
Not informed	–	2 (5.3)	
Relationship status, n (%)			
Single	13 (34.2)	11 (28.9)	χ2 = 3.424, p = 0.331
In a relationship	6 (15.8)	2 (5.3)	
Married	16 (42.1)	19 (50.0)	
Divorced	3 (7.9)	6 (15.8)	
Individual Income (R$ 954.00), n (%)			
1908.00–4770.00	17 (44.7)	22 (57.9)	χ2 = 2.033, p = 0.362
4770.00–9540.00	20 (52.6)	14 (36.8)	
>9540.00	1 (2.6)	2 (5.3)	
Working hours			
40	20 (52.6)	16 (42.1)	χ2 = 0.884, p = 0.643
41–50	12 (31.6)	14 (36.8)	
>50	6 (15.8)	8 (21.1)	
Clinical Characteristics			
Psychological health, n (%)			
BDI score, mean (SD)	11.68 (4.84)	12.24 (5.55)	t(74) = 0.462, p = 0.645
BAI score, mean (SD)	11.42 (6.51)	12.42 (6.90)	t(74) = 0.649, p = 0.518
Pathology, n (%)			
not diagnostic	22 (57.9)	22 (57.9)	χ2 = 0.000, p = 1.0
metabolic syndrome	16 (42.1)	16 (42.1)	
Lifestyle and physical Characteristics			
BMI, mean (SD)	26.12 (4.98)	26.29 (3.53)	t(74) = 1.69, p = 0.866
Alcohol consumption (gr/day), n (%)			
None	23 (60.5)	26 (68.4)	χ2 = 0.575, p = 0.750
Less than 50	13 (34.2)	10 (26.3)	
Over 50	2 (5.3)	2 (5.3)	
Physical activity (times per week), n (%)			
None	4 (10.5)	2 (5.3)	χ2 = 2.100, p = 0.350
Less than 3	17 (44.7)	13 (34.2)	
Over 3	17 (44.7)	23 (60.5)	
Smoking habits, n (%)			
Non-smoker	27 (71.1)	33 (86.8)	χ2 = 3.667, p = 0.160
Former smoker	5 (13.2)	1 (2.6)	
Smoker	6 (15.8)	4 (10.5)	

Note: Means with standard deviations (SD), min: minimum, max: maximum and number of case with percentages (%).

breathing, compassionate communication, loving-kindness, self--compassion, mindful listening, dealing with difficulties, the 3 step meditation, walking meditation, body scan with progressive relaxation, thoughts, emotions, gratitude, sounds and breathing all comprehend a global aim of developing awareness (see Supplementary Note 1 for example lessons).

2.6.2. Neuroscience for educators program (Neuro-Educa)

Neuro-Educa is an environmental enrichment program which does not specifically involve awareness, nor does it include any mindfulness training. It provides cognitive and sensorial stimuli by means of lectures about applied neuroscience for education. Lesson topics include: molecular and cellular biology of the neuron, development of the nervous system, neuroplasticity, neurobiology of memory, neurobiology of emotions, proprioception and motor systems, sensory systems, chronobiology and sleep, learning and attention issues and, social and emotional learning competencies (see Supplementary Table 1).

2.7. Measures

Measures were selected to assess: i) quality of life theoretically important to access the impact of stressful fife events on individual perception-related quality of life; ii) perceived stress, commonly elevated among active teachers in classroom; iii) resilience, which is the ability to adapt to adversity and stressful life events and, for this reason, is an important asset in the teaching profession; and iv) negative affect, which is commonly elevated among active teachers in the classroom and is related with lower quality of life.

2.7.1. Quality of life questionnaire (WHOQOL-Bref)

The quality of life questionnaire – WHOQOL-Bref (Fleck et al., 2000) is a generic instrument to evaluate the perception of the quality of life developed by the World Health Organization (WHO). The study used an abbreviated version, with 26 items grouped into 4 domains: physical health, psychological health, social relationship, environment health and general quality of life. It presents Likert scale answer (1–5 points). Cronbach's alpha coefficient for the Brazilian validation of WHOQOL-BREF domains were physical health domain (Cronbach's alpha = 0.84), psychological health domain (Cronbach's alpha = 0.79), social relationship domain (Cronbach's alpha = 0.69), environment health domain (Cronbach's alpha = 0.71) and general quality of life (Cronbach's alpha = 0.77) (Fleck et al., 2000).

2.7.2. Perceived stress scale (PSS-10)

The Perceived Stress (PSS-10) (Luft et al., 2007) is an instrument designed to evaluate the level of subjectively noticed stress, composed of 10 items, 6 of which scoring positively, and 4 scoring negatively, using a Likert-kind frequency scale. The answers range from “Never” (0) to “Always” (4), and the scoring shows a crescendo proportional to the experimented stress (0–7 indicating very low stress; 8–11, low stress; 12–15 require attention, 16–20, high stress; above 21, very high stress). Translated into Portuguese and adapted for Brazilian use, it was tested on a sample of 517 teachers, which revealed adequate adjustment, showing internal consistency (Cronbach's alpha = 0.82) *(Luft* et al., *2007)*.

2.7.3. Connor-davidson resilience rating scale (CD-RISC)

The CD-RISC is a 25-item measure of resilience. Each item ranges from 0 (“not true at all”) up to 4 (“true nearly all the time”). The final scores are obtained by summing the response to each of the items, with higher values indicating higher levels of resilience. Translated into Portuguese and adapted for Brazilian use, it was tested on a sample of 463 subjects, which revealed adequate adjustment, showing internal consistency (Cronbach's alpha = 0.82) (Lopes and Martins, 2011).

2.7.4. Positive affect and negative affect scale (PANAS)

The PANAS evaluates the predominance of positive and negative affects within one's emotional landscape. Measures are collected from a 0 (“not a bit”) up to 5 (“extremely”) range of answers to 20 questions, 10 of which versing about positive affects and dispositions, and 10 about negative emotions and dispositions. Translated into Portuguese and adapted for Brazilian use, it was tested on 115 male and 239 female subjects, showing excellent psychometric properties, consistent dimensions and reliable item functioning (Cronbach's alpha = 0.88) (Pires et al., 2013).

2.8. Biological measurements

Biological measures were selected to assess: i) pro-and-anti-inflammatory cytokines interleukin-8 (IL-8), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumornecrosis factor (TNF) e interleukin-12p70 (IL-12p70) serum levels, which are theoretically an important informative to access the immune response under psychosocial stress and ii) glutathione (GSH) plasma level, which is a potentially informative inflammatory biomarker and play an important role in immune response, for example.

2.8.1. Determination of cytokine levels in serum samples

Serum samples were obtained through the collection of peripheral blood of the volunteers in tubes without anti-coagulants (BD Vacutainer®), followed by centrifugation, after blood clotting. The serum samples were stored at −80 °C until analysis, for no longer than six months. The level of the inflammatory cytokines interleukin-8 (IL-8), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor (TNF) e interleukin-12p70 (IL-12p70) was determined using the Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit (BD Biosciences, San Jose, CA) and flow cytometry using the FACSCanto II (BD Biosciences, San Jose, CA) cytometer. The analysis of the acquisition was performed in the FCAP ArrayTM *Software* (Soft Flow Hungary Ltd., Pécs, HU).

The inter-assay coefficient of variability (%CV) was calculated to three replicate measures of each target analyte per assay tubes, tested according to the following equation %CV = [(standard deviations (SD) of tube means/mean of plate means) x100].

The inter-assay CV% were as follows: IL-8 = 4.75%, IL-12p70 = 6.81%, IL-6 = 10.83%, IL-1β = 12.56%, IL-10 = 29,71% and TNF = 25.57%.

Whereas intra-assay %CV was calculated to each replicate sample measure of each target analyte per assay tubes tested according to the following equation %CV = [(SD replicate sample mean/mean of sample) x100]. The intra-assay CV% were as follows: IL-8 = 2.77%, IL-12p70 = 4.97%, IL-6 = 5.83%, IL-1β = 13.09%, IL-10 = 24,77% and TNF = 16.87%.

The theoretical limit of detection of the kit for each protein, and the quantity of reads that were below it, are shown in Supplementary Table 2. Most of the reads for the cytokines TNF, IL-10 and IL-1β (6, 2, 5, respectively) yielded results bellow the theoretical limit of detection accepted for CBA Human Inflammatory Cytokines kit and, therefore, were excluded from the statistical analysis.

2.8.2. Determination of Hcys, Cys and GSH levels in plasma samples

Blood was collected in BD vacutainer® EDTA tubes and stored on ice up to 90 min, and centrifuged at 3000 rpm for 10 min at 4 °C. Plasma aliquots were stored at −80 °C for Hcy, Cys, and GSH measurements. Plasma Hcy, Cys and total GSH were quantified by high-performance liquid chromatography (HPLC) through fluorescence detection and isocratic elution. The method was developed by Pfeiffer and collaborators (Pfeiffer et al., 1999) with slight modifications, as follows: column C18 Luna (5 mm, 150 mm 64.6 mm), mobile phase (0.06 M sodium acetate, 0.5% acetic acid, pH 4.7 (adjusted with acetic acid), 2% methanol) and flow rate of 1.1 mL/min. The retention time was 3.6 min for Cys; 5.2 for Hcy and 9.0 for GSH (Galdieri et al., 2007). For reduced GSH quantification, the reducing agent was not added, and the concentrations were determined using GLICO-TECK®/GLICOHEMOGLOBINA (RealLab, Sao Paulo, Brazil).

2.9. Missingness and statistical analyses

Missing data is common in RCTs and can reduce the power and efficiency of study and it ends up impacting inferential statistics and can also lead to biased results (Bell et al., 2014; Graham, 2009; White et al., 2011). Multiple imputation (Graham, 2009; Rubin, 1987), mixed models (Fitzmaurice et al., 2011) and generalized estimating equations (GEE) (Bell et al., 2018; Dahmen et al., 2004; Schober and Vetter, 2018) can be valid and unbiased methods for missing at random (MAR) data. In the present study, we did not have missing data post-intervention (0%, n = 0; see Fig. 1). However, at the 12-month follow-up, control and intervention groups were forcefully interrupted due to the COVID-19 pandemic.

The socio-demographic, clinical, physical, and lifestyle variables were described using means and standard deviations (SD), frequencies, or percentages (%), according to the nature of the variables. Group comparisons in these variables were carried out using Student's independent *t*-test for continuous variables and Chi-Square (X^2) test (Fisher's test was used when adequate) for categorical data.

In the present study, we conducted Generalized Estimation Equation (GEE) model which is a robust approach to population-averaged for longitudinal data which are not normally distributed, and the variance of the outcome variables are not constant, and also includes extensions for missing data and covariates (Dahmen et al., 2004; Schober and Vetter, 2018). GEE was used to assess the efficacy of the MBHP-Educa on quality life (primary outcome), secondary outcomes such as perceived stress, resilience and positive and negative affectivity, and biological measurements (third outcomes) in service healthy female teachers. The models were adjusted for intra-individual variability (covariates/confounders factors: age, BDI, BAI and BMI). QIC (quasilikelihood under the independence model criterion) was used to select the best model in analyses (Pan, 2001) (see Supplementary Table 3). Based on the lower QIC value, we chose the "best-fit" model to carry on the statistical analyses that include the parameters below: missing values included, within-subject variables effect, maximum likelihood estimate (100 interactions), and unstructured working correlation matrix (see Supplementary Note 2). *P*-values were reported based on the Wald chi-square test and were adjusted by Bonferroni. Statistical analyses were conducted in IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and adjusted *P*-values were calculated by Bonferroni method in R (Team, 2013).

3. Results

3.1. Socio-demographic, clinical, lifestyle, and physical characteristics

Data revealed no significant differences between groups (p > 0.05) on any socio-demographic, clinical, lifestyle, and physical variables (Table 1).

3.2. Enhancing quality of life through mindfulness meditation

To assess the effects of mindfulness meditation on all aspects of quality of life (primary outcome) (Fig. 3A–F and Table 2), we carried on GEE analysis. The intervention group showed a significantly greater increase from pre-to post-test on the perception of physical healthy subscale of WHOQOL (main effect of Time*Group: β = −0.216, p = 0.000 or 4.8E-05, adjusted p = 0.001, 95% Wald CI [−0.320, −0.112]). A similar magnitude increase on the intervention group was evident on the psychological healthy subscale from pre-to post-test (main effect of Time*Group: β = −0.218, p = 0.000 or 1.4E-08, adjusted p = 2.2E-07, 95% Wald CI [−0.294, −0.143]), social-relationship subscale (main effect of Time*Group: β = −0.214, p = 0.000 or 6.0E-05, adjusted p = 0.001, 95% Wald CI [−0.319, −0.110]) and also to environmental health subscale (main effect of Time*Group: β = −0.247, p = 0.000 or 2.7E-08, adjusted p = 4.4E-07, 95% Wald CI [−0.334, −0.160]). We also observed a significantly greater increase from pre to post-test on the perception of self-evaluation subscale of WHOQOL (main effect of Time*Group: β = −0.215, p = 0.001, adjusted p = 0.009, 95% Wald CI [−0.337, −0.093]). In relation to general quality of life, we observed a significantly greater increase from pre to post-test (main effect of Time*Group: β = −0.215, p = 0.000 or 3.1E-08, adjusted p = 4.9E-07, 95% Wald CI [−0.291, −0.139]).

3.3. Mindfulness meditation enhance resilience to stress and reduce negative affectivity

The intervention group showed a significant reduction in perceived stress from pre to post-test on perceived stress scale (PSP) (main effect of Time*Group: β = 0.537, p = 0.000 or 3.1E-08, adjusted p = 4.9-E07, 95% Wald CI [0.438, −0.635], Fig. 3G and Table 2). Regarding to person's ability to successfully adapt under adversity and stress, intervention group showed a substantial increase from pre to post-test on resilience

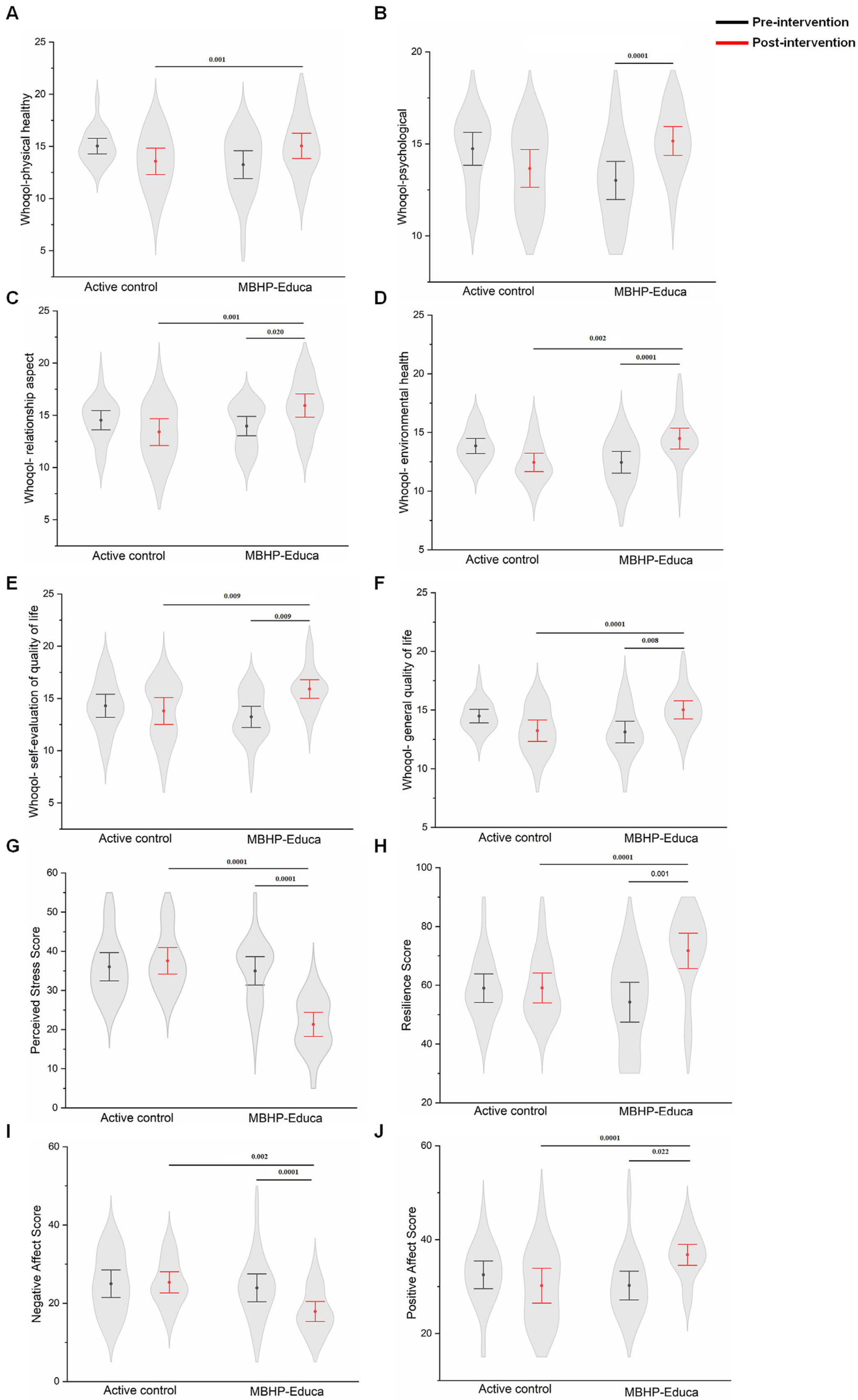

Fig. 3. Effect of MBHP-Educa on perceived stress, resilience, negative affect and positive affects and quality of life in healthy teachers, Note: Graphics are "violins" the relative kernel distribution across the entire range of values. Diamonds and box represent mean ± CI of each time point by day. The scales are different in this section to make them legible. Raw data were used in all measures. Active control = Neuro-Educa program.

Table 2
Summary statistic from generalized estimating equations model (GEE) for all variables.

	Neuro-Educa (Pre- n = 38 and Post- n = 20)		MBHP-Educa (Pre- n = 38 and Post- n = 21)		GEE models							
	Mean (SD)	Min - Max	Mean (SD)	Min - Max	Variables	β	SE	95% CI		Wald χ2	P-value	adjusted P-value
								Min	**Max**			
Primary outcome												
Whoqol-physical healthy												
Pre	14.60 (2.31)	5.71–19.42	13.23 (2.88)	5.71–17.14	*Time*	0.088	0.041	0.007	0.169	4.549	0.033	0.527
Post	13.57 (2.18)	8.00–17.71	15.04 (2.65)	10.28–20.00	*Group*	0.118	0.055	0.009	0.226	4.534	0.033	0.532
					*Time*Group*	−0.216	0.053	−0.320	−0.112	16.524	<0.0001a	**0.001**
Whoqol-psychological healthy												
Pre	14.47 (2.13)	10.00–18.00	13.07 (2.29)	9.33–17.33	*Time*	0.068	0.031	0.007	0.128	4.787	0.029	0.459
Post	13.66 (2.18)	10.00–16.66	15.15 (1.72)	11.33–18.00	*Group*	0.116	0.040	0.038	0.194	8.516	0.004	0.056
					*Time*Group*	−0.218	0.038	−0.294	−0.143	32.182	<0.0001 b	**<0.0001c**
Whoqol-social relationship												
Pre	14.56 (1.99)	10.66–18.66	13.96 (2.02)	10.66–16.00	*Time*	0.082	0.042	0.000	0.165	3.812	0.051	0.814
Post	13.40 (2.75)	8.00–18.66	15.93 (2.43)	12.00–20.00	*Group*	0.172	0.053	0.068	0.277	10.420	0.001	**0.020**
					*Time*Group*	−0.214	0.053	−0.319	−0.110	16.089	<0.0001 d	**0.001**
Whoqol-environmental health												
Pre	13.65 (1.77)	8.00–16.50	12.47 (1.96)	8.00–16.00	*Time*	0.097	0.033	0.033	0.161	8.839	0.003	**0.047**
Post	12.45 (1.67)	9.50–16.00	14.47 (1.95)	10.00–19.00	*Group*	0.156	0.040	0.077	0.235	15.002	<0.0001e	**0.002**
					*Time*Group*	−0.247	0.044	−0.334	−0.160	30.891	<0.0001f	**<0.0001g**
Whoqol-perception of self-evaluation												
Pre	14.26 (2.51)	8.00–18.00	13.26 (2.25)	8.00–16.00	*Time*	0.033	0.055	−0.074	0.140	0.369	0.543	1.000
Post	13.80 (2.74)	8.00–18.00	15.90 (1.94)	12.00–20.00	*Group*	0.142	0.051	0.043	0.241	7.918	0.005	0.078
					*Time*Group*	−0.215	0.062	−0.337	−0.093	11.948	0.000	**0.009**
Whoqol-general quality of life												
Pre	14.25 (1.66)	8.76–17.38	13.15 (2.25)	8.00–16.00	*Time*	0.081	0.031	0.021	0.141	6.925	0.009	0.136
Post	13.24 (1.95)	9.23–16.46	15.02 (1.69)	12.00–19.07	*Group*	0.134	0.039	0.059	0.210	12.081	0.000	**0.008**
					*Time*Group*	−0.215	0.039	−0.291	−0.139	30.672	<0.0001 h	**<0.0001i**
Secondary outcome												
PSP												
Pre	35.03 (6.41)	18.00–49.00	34.68 (6.53)	18.00–42.00	*Time*	−0.042	0.018	−0.078	−0.006	5.114	0.024	0.380
Post	37.20 (6.59)	28.00–49.00	21.33 (6.74)	6.00–34.00	*Group*	−0.547	0.065	−0.674	−0.420	71.262	<0.0001j	**<0.0001k**
					*Time*Group*	0.537	0.050	0.438	0.635	114.376	<0.0001 l	**<0.0001m**
RISC												
Pre	59.05 (9.69)	44.00–86.00	54.24 (14.53)	31.00–79.00	*Time*	−0.002	0.014	−0.029	0.026	0.011	0.915	1.000
Post	59.10 (10.88)	43.00–85.00	71.71 (13.22)	41.00–88.00	*Group*	0.193	0.047	0.100	0.285	16.627	<0.0001n	**0.001**
					*Time*Group*	−0.278	0.041	−0.358	−0.198	46.150	<0.0001°	<0.0001p
PANAS-N												
Pre	24.97 (8.00)	12.00–37.00	24.34 (7.35)	10.00–45.00	*Time*	−0.014	0.033	−0.079	0.050	0.190	0.663	1.000
Post	25.35 (5.77)	16.00–36.00	17.90 (5.61)	10.00–30.00	*Group*	−0.339	0.078	−0.491	−0.187	19.089	<0.0001q	**<0.0001r**
					*Time*Group*	0.313	0.081	0.155	0.472	15.030	<0.0001s	**0.002**
PANAS-P												
Pre	32.66 (6.31)	16.00–45.00	29.95 (5.82)	20.00–50.00	*Time*	0.075	0.041	−0.005	0.156	3.355	0.067	1.000
Post	30.20 (7.93)	18.00–47.00	36.76 (4.89)	27.00–45.00	*Group*	0.188	0.059	0.073	0.303	10.255	0.001	**0.022**
					*Time*Group*	−0.275	0.059	−0.389	−0.160	21.983	<0.0001t	**<0.0001u**
Third outcome												
IL-6 (pg/mL)												
Pre	3.25 (0.56)	2.02–4.09	3.87 (0.93)	2.49–6.31	*Time*	−0.030	0.043	−0.114	0.053	0.504	0.478	1.000
Post	3.33 (0.37)	2.39–3.70	2.74 (0.58)	2.15–4.43	*Group*	−0.199	0.050	−0.297	−0.101	15.810	<0.0001v	**0.001**
					*Time*Group*	0.375	0.060	0.258	0.492	39.237	<0.0001w	**<0.0001x**
IL-8 (pg/mL)												

(*continued on next page*)

Table 2 (*continued*)

	Neuro-Educa (Pre- n = 38 and Post- n = 20)		MBHP-Educa (Pre- n = 38 and Post- n = 21)		GEE models							
	Mean (SD)	Min - Max	Mean (SD)	Min - Max	Variables	β	SE	95% CI Min	95% CI Max	Wald χ2	P-value	adjusted P-value
Pre	7.77 (2.93)	2.28–17.57	8.17 (3.84)	2.54–18.99	*Time*	−0.120	0.095	−0.307	0.067	1.588	0.208	1.000
Post	8.91 (3.98)	2.48–19.96	6.08 (2.50)	2.63–10.76	*Group*	−0.369	0.118	−0.600	−0.137	9.762	0.002	**0.028**
					*Time*Group*	0.419	0.111	0.201	0.637	14.173	<0.0001 y	**0.003**
IL-12p70 (pg/mL)												
Pre	3.30 (1.00)	2.07–5.98	3.56 (1.49)	2.03–8.76	*Time*	0.025	0.092	−0.156	0.205	0.071	0.790	1.000
Post	3.22 (0.94)	2.18–5.39	3.50 (1.05)	2.23–6.34	*Group*	0.082	0.090	−0.095	0.259	0.826	0.364	1.000
					*Time*Group*	−0.007	0.121	−0.243	0.230	0.003	0.956	1.000
GSH (μmol/gHb)												
Pre	19.05 (5.65)	5.67–29.90	16.57 (5.22)	6.23–29.77	*Time*	0.076	0.031	0.016	0.137	6.180	0.013	0.207
Post	17.85 (4.84)	5.24–26.38	27.44 (6.66)	13.16–36.32	*Group*	0.444	0.071	0.305	0.582	39.359	<0.0001z	**<0.0001aa**
					*Time*Group*	−0.583	0.064	−0.709	−0.457	82.143	<0.0001 ab	**<0.0001ac**
Cys (μmol/L)												
Pre	314.92 (125.48)	170.33–564.72	421.72 (47.20)	347.12–498.62	*Time*	0.005	0.013	−0.020	0.030	0.129	0.720	1.000
Post	306.04 (132.82)	173.04–575.47	500.14 (74.97)	419.99–699.79	*Group*	0.480	0.075	0.333	0.626	41.206	<0.0001ad	**<0.0001ae**
					*Time*Group*	−0.188	0.027	−0.241	−0.134	47.350	<0.0001af	**<0.0001 ag**
Hcys (μmol/L)												
Pre	8.11 (0.68)	7.01–9.56	8.21 (0.85)	7.02–9.82	*Time*	0.020	0.009	0.002	0.039	4.509	0.034	0.540
Post	7.99 (0.75)	7.01–9.54	7.96 (0.57)	6.94–9.09	*Group*	−0.002	0.022	−0.046	0.042	0.011	0.916	1.000
					*Time*Group*	0.014	0.019	−0.022	0.051	0.585	0.444	1.000

a: 4.8E-05; b: 1.4E-08; c: 2.2E-07; d:6.0E-05; e: 1.1E-04; f: 2.7E-08; g:4.4E-07; h: 3.1E-08; i: 4.9E-07; j: 1.2E-05; k: 2.0E-04; l: 1.1E-11; m:4.9E-07; n:4.5E-05; o:1.1E-11; p: 1.8E-10; q: 1.2E-05; r: 2.0E-04; s: 1.1E-04; t: 2.8E-06; u: 4.4E-05; v: 7.0E-05; x: 3.8E-10; w:6.0E-09; y: 1.7E-04; z: 3.5E-10; aa:5.6E-09; ab: 3.8E-10; ac:6.0E-09; ad: 1.4E-10; ae: 2.2E-09; af:5.9E-12; ag: 9.6E-11.
Adjusted P-value by Bonferroni method.
Note: Means with standard deviations (SD), Min: minimum, Max: maximum; Δ is difference between post and pre; B: estimate; SE: standardized error; 95% CI: Wald Confidence interval.

scale (RISC) (main effect of Time*Group: β = −0.278, p = 0.000 or 1.1E-11, adjusted p = 1.8E-10, 95% Wald CI [−0.358, −0.198], Fig. 3H and Table 2). A similar magnitude increase on the intervention group was evident on positive affect subscale from pre-to-post-test (PANAS-P) (main effect of Time*Group: β = −0.275, p = 0.000 or 2.8E-06, adjusted p = 4.4E-05, 95% Wald CI [−0.389, −0.160], Fig. 3J and Table 2). We

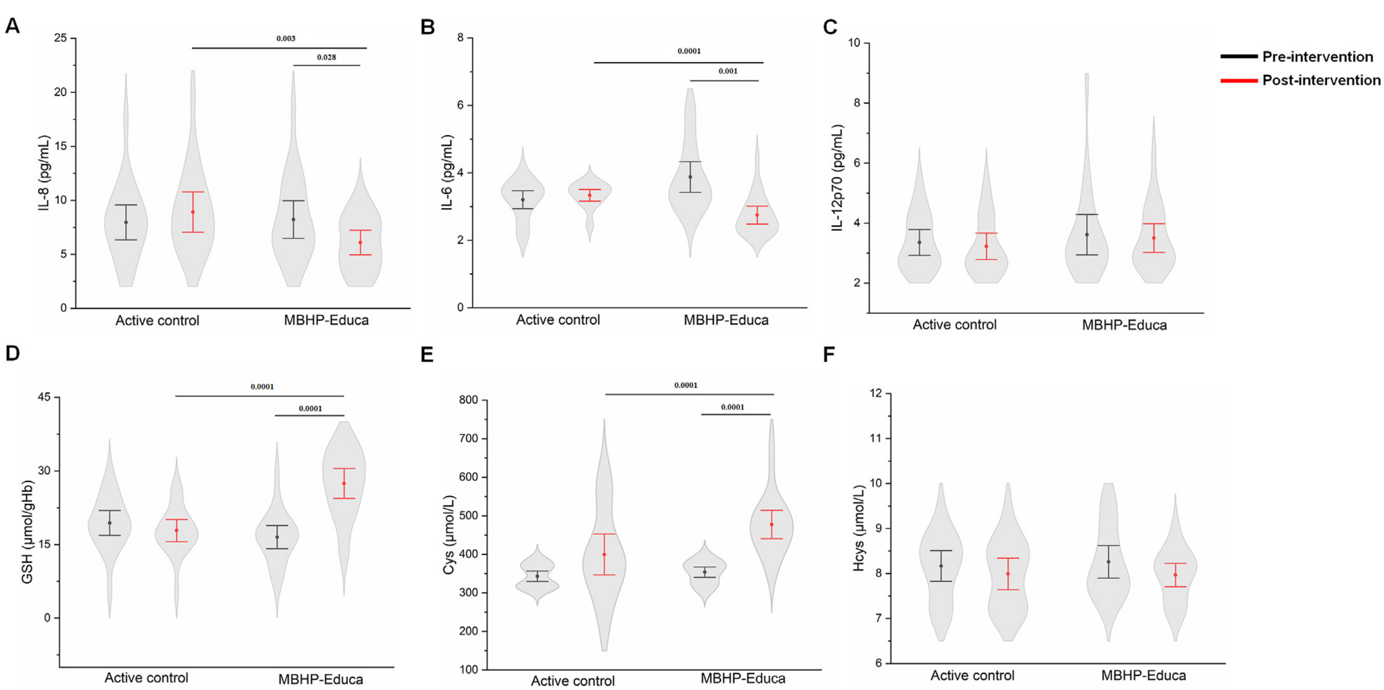

Fig. 4. Effect of MBHP-Educa on proinflammatory cytokines and GSH metabolism. Note: Graphics are "violins" the relative kernel distribution across the entire range of values. Diamonds and box represent mean ± CI of each time point by day. The scales are different in this section to make them legible. Raw data were used in all measures. Active control = Neuro-Educa program.

also observed in intervention group a decrease on negative affect subscale from pre-to-post-test (PANAS-N) (main effect of Time*Group: $\beta = 0.313$, $p = 0.0001$, adjusted $p = 0.002$, 95% Wald CI [0.15, 0.47], Fig. 3I and Table 2).

3.4. Mindfulness meditation alters inflammatory biomarkers and glutathione metabolism

Knowing that mindfulness meditation practices promote changes in the levels of circulating cytokines, we investigated the effects of mindfulness meditation from pre to post-test on inflammatory cytokines (see Fig. 4A–C). The intervention group showed lower serum IL-8 and IL-6 levels from pre to-post-test (effect of Time*Group: B = 0.419; 95% Wald CI = 0.201 to 0.637; p = 0.0002 or 1.7E-04, adjusted p = 0.003, see Fig. 4A and Table 2) and (effect of Time*Group: B = 0.375; 95% Wald CI = 0.258 to 0.492; p = 0.000 or 3.8E-10, adjusted p = 6.0E-09, see Fig. 4C and Table 2), respectively. No differences were found to serum levels of IL-12p70 (effect of Time*Group: B = −0.007; 95% Wald CI = −0.243 to 0.230; p = 0.956, adjusted p = 1.000, see Fig. 4B and Table 2). Overall, both TNF, IL-10 and IL-1β generated results bellow the theoretical limit of detection of the assay kit; since all participants were healthy and did not declared any chronic diseases, we already expected to detect low levels of cytokines, especially these mentioned (Kleiner et al., 2013). Moreover, the recoveries of IL-1β and TNF are lower than for the other cytokines measured by the CBA kit used.

To test whether mindfulness meditation practices may modulate glutathione metabolism (Fig. 4D–F), we analyzed glutathione (GSH), cysteine (CYS) and homocysteine (HCYS) on plasma levels. The intervention group showed a significant increase of GSH and CYS levels from pre to post-test (effect of Time*Group: B = −0.583; 95% Wald CI = −0.709 to −0.457; p = 0.0000 or 3.8E-10, adjusted p = 6.0E-09, see Fig. 4D and Table 2) and (effect of Time*Group: B = −0.188; 95% Wald CI = −0.241 to −0.134; p = 0.000 or 5.9E-12, adjusted p = 9.6E-11, see Fig. 4E and Table 2), respectively. No differences were found to plasma levels of HCys (effect Time*Group: B = 0.014; 95% Wald CI = −0.022 to 0.051; p = 0.444, adjusted p = 1.000, see Fig. 4F and Table 2).

4. Discussion

To our knowledge, this is the first study to evaluate the effects of the MBHP-Educa program on health promotion in a Brazilian education context and its potential for improving the teacher's quality of life via the cultivation of awareness. Three main findings emerge from this study. First, MBHP-Educa participants reported lower levels of perceived stress, higher levels of resilience, along with changes in negative affect to positive affect, and improvement on the perception in all aspects of quality of life compared to a Neuro-Educa group at post-intervention. Second, MBHP-Educa participants showed a significant enhancement in glutathione metabolism biomarkers levels (GSH and CYS) compared to the Neuro-Educa group at post-intervention. Moreover, the levels of proinflammatory cytokines such as IL-6 and IL-8 decreased.

4.1. Inner experience and stress reduction

Considering stress response, we observed that MM improves the ability of teachers to self-recognize their perceived stress, with levels falling over time. We also provide evidence that perceived stress has a significant mediating factor on quality of life. These results are consistent with previous studies showing that perceived stress is an important mediator for both quality of life (Valikhani et al., 2020) and reduced perceived stress at post-mindfulness intervention in healthy teachers (Jennings et al., 2011, 2013; Roeser et al., 2013). We believe that an environmental enrichment strategy such as a mindfulness-based intervention specifically designed to attend the teachers' specific necessities may promote the development of the core capacities gained through awareness. Together, these capacities influence one's resilience to stress with a strong impact on the quality of life, which could explain our findings in this study.

Through cultivating present-centered awareness (maintaining present-focused attention), mindfulness meditation improves the ability to recognize internal bodily states (interoception) (Farb et al., 2015; Gibson, 2019) and dynamic body positioning (proprioception) (Chatutain et al., 2019). For example, in the mindful breathing practices, phrases such as "bring your awareness inside you, to body sensations" and "bring your attention gently to your breathing, feeling your breathing" (Demarzo et al., 2020; Kabat-Zinn and Hanh, 2013), acted stimulating sensory perceptions. The same happens in walking meditation practices as "be aware of all the different sensations in your feet" (Demarzo et al., 2020; Kabat-Zinn and Hanh, 2013), in this case, stimulating proprioception. Taken together, multisensory stimulation enhances emotion awareness and reduces stress reactivity, thus promoting emotional regulation via an environmental enrichment intervention (MBHP-Educa).

Further extending this idea, mindfulness might promote changes on the coupling of perception and the appraisal of environmental stimuli through the cultivation of nonreactivity and dereification (Lutz et al., 2015), both being important aspects of the decentering process. In other words, mindfulness might act changing people's evaluative judgment concerning environmental situations or events previously appraised as stressful, leading to a more adaptive coping style and fostering insight about more subtle aspects of inner experience. The repeated experience of allowing unpleasant or undesired emotional and affective states, withdrawing avoidance behaviors usually fueled by elaborative thoughts (internal stimuli, i.e., rumination) may constitute a means for exposure to them, which may lead to habituation or extinction of conditioned emotional response (McSweeney and Swindell, 2002; Phelps et al., 2004; Watson and Rayner, 1920). In mindfulness meditation, this event can be facilitated by adopting a non-aversive stance toward present moment experience, encouraging nonjudgement, acceptance of one's present experience (Kabat-Zinn and Hanh, 2013), thus allowing emotional regulation. These events can explain the high levels of positive affect and lower level of negative affect observed in the MBHP-Educa group compared to the active control group at post-mindfulness intervention. Our results are in line with previous studies, which have shown that negative affect was reduced (Jennings et al., 2011, 2013), and positive affect was increased in teachers (Harris et al., 2016; Jennings et al., 2011, 2013). In addition, it was not observed that changes in negative affect and positive affect mediate the impact of MBHP-Educa on quality of life. Maybe affective response is a dynamic process facilitated directly by MBHP-Educa intervention.

We observed that MBHP-Educa intervention promoted a significant increase in the resilience levels within the healthy MBHP-Educa group compared to the active control group at post-intervention. One way this may occur is through cognitive reappraisal (Garland et al., 2011; Lutz et al., 2015), an emotion regulation mechanism involving reinterpreting the meaning of a situation, which can also be facilitated through the cultivation of nonreactivity and present-centered awareness (Garland et al., 2014). It is known that resilient individuals use cognitive reappraisal in a more frequent way (Goldin et al., 2008; Ochsner et al., 2004). Here, it is important to highlight that positive affect also supports resilience by promoting broader associative thinking and adaptive coping (Jennings et al., 2011, 2013), corroborating our findings.

The present study provides new evidence regarding resilience as a mediator of the MBHP-Educa program's effects on the general quality of life. These findings are in line with a previous study that supported resilience as a mediator of quality of life effect (Guccione, 2014; Zhang et al., 2017). Resilience is an important ability in the educational context by enhancing a person's sense of emotional control, altogether improving the ability to adapt in the face to adversity, maintaining psychological well-being (Southwick et al., 2014), and, therefore, improving the ability for the individual's perception of the quality of life.

4.2. Biological mechanisms

Hence, although the effect of mindfulness meditation on psychological response is well-characterized, several questions regarding the physiological response, as possible correlations between immune/oxidative stress responses, remain unanswered. So far, it can be stated that this unbalanced proinflammatory/anti-inflammatory ratio we found in this study can promote the generation of reactive oxygen species (ROS) and lead to oxidative damage, since oxidative and inflammatory stresses are closely related (De la Fuente, 2018). The present study demonstrated that MBHP-Educa intervention causes a significant reduction on the levels of IL-6 and IL-8 in the health MBHP-Educa group when compared to the active control group from pre-to post-analysis. These results are in agreement with other studies, which have found lower serum levels of IL-6 (Bower et al., 2015; Ng et al., 2020). Regarding IL-8, Rosenkranz et al. noticed in their study a trend towards IL-8 decrease which did not present statistical significance; here, the decrease of IL-8 with MBPH-Educa intervention showed to be significant. Both the studies of Bower et al., Ng et al. (2020) and Rosenkranz et al. (2013) obtained their results with volunteers with chronic diseases, but this study provided new evidence in non-clinical conditions.

Another major finding of our study is that MBHP-Educa intervention induced a significant enhancement in the levels of Cys and GSH in the healthy MBHP-Educa group. These results suggest that redox status can be shifted toward a reduced state by practicing mindfulness, which is an important adaptative strategy for minimizing oxidative stress and its effects in mind and body. The increase of Cys-induced by meditation training is probably due to increased turnover of GSH, as GSH synthesis requires increased Cys utilization. Similar effects in GSH levels were seen in long-term practitioners in yoga (Sinha et al., 2007), Tai Chi (Goon et al., 2009), and Zen meditation (Kim et al., 2005), but this study provided new evidence in naïve-meditator. To our knowledge, this is the first study to explore the influence of mindfulness in antioxidant defenses. Thus, the results obtained in the present study might serve as a heuristic force to inspire future studies, moving forward on a promising area of research. These findings are in line with the idea that enriched environment strategies can play an important role in the maintenance of homeostasis in the immune and redox systems, which are both important to enhance the perceived quality of life. A central question emerges from these data: what molecular mechanisms are modulated by MBHP-Educa intervention that underly the effects observed in this study? Understanding this question is essential, and new approaches are needed.

4.3. Limitations and future research

Future research with higher-powered designs should investigate the specific sequence of events related to changes in mindfulness, psychological flexibility and neuropsychological test (in particular interesting, episodic memory and attention). Furthermore, the results of this healthy sample do not necessarily reflect those that may eventually be found in clinical populations, such as those with high scores on anxiety, depression or metabolic syndrome. The behavior of the above biomarkers may be different in such populations. Perhaps in these specific, clinically compromised groups, a more detailed assessment of depressive or anxious symptoms should be proceeded via psychometric approaches other than self-report. Finally, future research 6 months and 12 months follow-up will be necessary to assess the effects over time.

Another limitation our study poses is the fact that it did not assess lifelong psychopathology, such as could have been done by applying instruments such as SCID (DSM 5), since such a resource showed itself unfeasible and beyond our budget, albeit certainly a great enrichment of our study of the sample. Nevertheless, the authors understand that the absence of such an evaluation does not invalidate the results and conclusions as a whole. Further studies, with better funding, certainly will shed greater light on such an endeavor.

Additional issue that deserves addressing is the fact that the COVID-19 pandemic confinement, which broke the chain of our follow-up, brought about a large, life-threatening, socially challenging stressful world-wide life event, which in turn disturbs the findings that could be investigated in order to better understand the longer-term effects of a Mindfulness-based Program for Educators. Consequently, new studies involving stress-reduction and emotion-regulation will have to be conceived and executed from square one, in the hope that no other catastrophic events interrupt the long-term evaluation of complex psychological studies.

4.4. Conclusions

Our findings point out that MBHP-Educa program promotes the development of abilities such as present-centered awareness, non-reactivity to experience, meta-awareness, and dereification, which together may encourage teachers to keep a close friendly engagement to adversity and stressful life events. Together, these capacities promote changes in psychological and physiological responses. Concerning psychological responses, they promote increases in resilience, increases in positive affect, and decreases in perceived stress responses. In physiological responses, they activate antioxidant systems (increase GSH levels) and promote regulation on immune systems by decrease of proinflammatory cytokine IL-6 and IL-8. All these benefic effects are achieved through the cultivation of awareness.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

DRO appreciates the support provided by grant 2017/16155-4 and 2019/07274-5, Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), and by 424329/2018-2 Conselho Nacional de Desencolvimento Científico e Tecnológico (CNPq). We appreciate the support by Diretoria Regional de Educação Santo Amaro (DRE), Núcleo de Apoio e Acompanhamento para Aprendizagem (NAAPA), and “Mente Aberta” – The Brazilian Center for Mindfulness and Health Promotion. We would also like to express our gratitude to Ana Claudia de Paula Correa, Kátia Maria Vergne, and Debora Lieber de Paula for the support and partnership of this study and all the teachers and staff in the Public Schools from São Paulo city. We thank all volunteers who participated in this study. We express our gratitude to Joyce Yamamoto, and Vânia D'Almeida. We also express our gratitude to our Bioinformatic, Fanny Palace-Berl, to do the statistical analysis presented in this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2021.100372.

Author contributions

DRO and MD contributed to the study design. DRO contributed the acquisition of data, analysis, and interpretation of data, and drafting/revising of the manuscript for content. DRO contributed to designing the Neuro-Educa program. FPB is involved in the statistical analysis of all data. DW and VPP, contributed to conducting the MBHP-Educa program for teachers. DRO, MD, VPP, JCF and AMT are authors of the MBHP-Educa program. BPM collected blood samples from research subjects. BPM, DW and LFR were involved in the application of all scales and questionnaires. APL, BPM, FSM, and DRO analyzed and interpreted data regarding the inflammatory biomarkers. VD'A, BPM and DRO analyzed and interpreted data regarding the GSH metabolism. MD and VD'A contributed to supervising the study and revising the manuscript content. All authors critically reviewed the manuscript. The authors read and approved the final manuscript.

References

Bell, M.L., Fiero, M., Horton, N.J., Hsu, C.-H., 2014. Handling missing data in RCTs; a review of the top medical journals. BMC Med. Res. Methodol. 14, 1–8.

Bell, M.L., Horton, N.J., Dhillon, H.M., Bray, V.J., Vardy, J.J.P.o., 2018. Using Generalized Estimating Equations and Extensions in Randomized Trials with Missing Longitudinal Patient Reported Outcome Data 27, pp. 2125–2131.

Bierman, K.L., Welsh, J.A., Heinrichs, B.S., Nix, R.L., Mathis, E.T.J.C.d., 2015. Helping Head Start Parents Promote Their Children's Kindergarten Adjustment: the Research-based Developmentally Informed Parent Program, vol. 86, pp. 1877–1891.

Black, D.S., Slavich, G.M., 2016. Mindfulness meditation and the immune system: a systematic review of randomized controlled trials. Ann. N. Y. Acad. Sci. 1373, 13.

Bower, J.E., Crosswell, A.D., Stanton, A.L., Crespi, C.M., Winston, D., Arevalo, J., Ma, J., Cole, S.W., Ganz, P.A., 2015. Mindfulness meditation for younger breast cancer survivors: a randomized controlled trial. Cancer 121, 1231–1240.

Buric, I., Farias, M., Jong, J., Mee, C., Brazil, I.A., 2017. What is the molecular signature of mind–body interventions? A systematic review of gene expression changes induced by meditation and related practices. Front. Immunol. 8.

Chatutain, A., Pattana, J., Parinsarum, T., Lapanantasin, S., 2019. Walking meditation promotes ankle proprioception and balance performance among elderly women. J. Bodyw. Mov. Ther. 23, 652–657.

Dahmen, G., Rochon, J., Konig, I.R., Ziegler, A., 2004. Sample size calculations for controlled clinical trials using generalized estimating equations (GEE). Methods Inf. Med. 43, 451–456.

De la Fuente, M., 2018. Oxidation and Inflammation in the Immune and Nervous Systems, a Link between Aging and Anxiety. Handbook of Immunosenescence. Springer.

Demarzo, M., Oliveira, D.R., Terzi, A.M., Campayo, J.G., 2020. Mindfulness para profissionais de educação: práticas para o bem-estar no trabalho e na vida profissional. Editora Senac São Paulo.

Demarzo, M.M., Andreoni, S., Sanches, N., Perez, S., Fortes, S., Garcia-Campayo, J., 2014. Mindfulness-based stress reduction (MBSR) in perceived stress and quality of life: an open, uncontrolled study in a Brazilian healthy sample. Explore 10, 118–120.

Dröge, W., Breitkreutz, R., 2000. Glutathione and immune function. Proc. Nutr. Soc. 59, 595–600.

Epel, E.S., Crosswell, A.D., Mayer, S.E., Prather, A.A., Slavich, G.M., Puterman, E., Mendes, W.B., 2018. More than a feeling: a unified view of stress measurement for population science. Front. Neuroendocrinol. 49, 146–169.

Farb, N., Daubenmier, J., Price, C.J., Gard, T., Kerr, C., Dunn, B.D., Klein, A.C., Paulus, M.P., Mehling, W.E., 2015. Interoception, contemplative practice, and health. Front. Psychol. 6, 763.

Faul, F., Erdfelder, E., Buchner, A., Lang, A.-G., 2009. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. Behav. Res. Methods 41, 1149–1160.

Fitzmaurice, G.M., Laird, N.M., Ware, J., 2011. Wiley Series in Probability and Statistics. Applied Longitudinal Analysis. John Wiley, Hoboken. NJ.

Fleck, M., Louzada, S., Xavier, M., Chachamovich, E., Vieira, G., Santos, L., Pinzon, V., 2000. Aplicação da versão em português do instrumento abreviado de avaliação da qualidade de vida" WHOQOL-bref. Rev. Saude Publica 34, 178–183.

Galdieri, L.C., Arrieta, S.R., Silva, C.M., Pedra, C.A., D'Almeida, V., 2007. Homocysteine concentrations and molecular analysis in patients with congenital heart defects. Arch. Med. Res. 38, 212–218.

García-Giménez, J.L., Romá-Mateo, C., Pérez-Machado, G., Peiró-Chova, L., Pallardó, F.V., 2017. Role of glutathione in the regulation of epigenetic mechanisms in disease. Free Radic. Biol. Med. 112, 36–48.

Garland, E., Froeliger, B., Howard, M., 2014. Mindfulness training targets neurocognitive mechanisms of addiction at the attention-appraisal-emotion interface. Front. Psychiatr. 4, 173.

Garland, E.L., Gaylord, S.A., Fredrickson, B.L., 2011. Positive reappraisal mediates the stress-reductive effects of mindfulness: an upward spiral process. Mindfulness 2, 59–67.

Gibson, J., 2019. Mindfulness, interoception, and the body: a contemporary perspective. Front. Psychol. 10.

Goldin, P.R., McRae, K., Ramel, W., Gross, J.J., 2008. The neural bases of emotion regulation: reappraisal and suppression of negative emotion. Biol. Psychiatr. 63, 577–586.

Goon, J.A., Aini, A.H., Musalmah, M., Anum, M.Y., Nazaimoon, W.M., Ngah, W.Z., 2009. Effect of Tai Chi exercise on DNA damage, antioxidant enzymes, and oxidative stress in middle-age adults. J. Phys. Activ. Health 6, 43–54.

Graham, J.W., 2009. Missing Data Analysis: Making it Work in the Real World. Annu. Rev. Psychol. vol. 60, 549–576.

Guccione, A.A., 2014. Resilience and self-efficacy as mediators of quality of life in geriatric rehabilitation. Top. Geriatr. Rehabil. 30, 164–169.

Harris, A.R., Jennings, P.A., Katz, D.A., Abenavoli, R.M., Greenberg, M.T., 2016. Promoting stress management and wellbeing in educators: feasibility and efficacy of a school-based yoga and mindfulness intervention. Mindfulness 7, 143–154.

Hirshberg, M.J., Flook, L., Enright, R.D., Davidson, R.J.J.L., Instruction, 2020. Integrating Mindfulness and Connection Practices into Preservice Teacher Education Improves Classroom Practices, vol. 66, p. 101298.

Jennings, P.A., 2015. Mindfulness for Teachers: Simple Skills for Peace and Productivity in the Classroom (The Norton Series on the Social Neuroscience of Education). WW Norton & Company.

Jennings, P.A., Frank, J.L., Snowberg, K.E., Coccia, M.A., Greenberg, M.T., 2013. Improving classroom learning environments by Cultivating Awareness and Resilience in Education (CARE): results of a randomized controlled trial. Sch. Psychol. Q. 28, 374.

Jennings, P.A., Snowberg, K.E., Coccia, M.A., Greenberg, M.T., 2011. Improving classroom learning environments by cultivating awareness and resilience in education (CARE): results of two pilot studies. J. Classr. Interact. 37–48.

Kabat-Zinn, J., Hanh, T.N., 2013. Full Catastrophe Living (Revised Edition): Using the Wisdom of Your Body and Mind to Face Stress, Pain, and Illness. Random House Publishing Group.

Karcher, M.J.J.P.S., 2008. The Study of Mentoring in the Learning Environment (SMILE): A Randomized Evaluation of the Effectiveness of School-Based Mentoring, vol. 9, p. 99.

Kim, D.H., Moon, Y.S., Kim, H.S., Jung, J.S., Park, H.M., Suh, H.W., Kim, Y.H., Song, D.K., 2005. Effect of Zen Meditation on serum nitric oxide activity and lipid peroxidation. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 29, 327–331.

Kleiner, G., Marcuzzi, A., Zanin, V., Monasta, L., Zauli, G., 2013. Cytokine levels in the serum of healthy subjects. Mediat. Inflamm. 2013.

Lopes, V.R., Martins, M.d.C.F., 2011. Validação fatorial da escala de resiliência de Connor-Davidson (CD-RISC-10) para brasileiros. Rev. Psicol. Organ. Trab. 11, 36–50.

López-Gonzalez, L., Gonzalez, M., Bisquerra, R., 2016. Mindfulness and action-research: the TREVA programa gestation. Revista Interuniversitaria del Formación del Profesorado 87, 75–91.

Luft, C.D.B., Sanches, S.d.O., Mazo, G.Z., Andrade, A., 2007. Versão brasileira da Escala de Estresse Percebido: tradução e validação para idosos. Rev. Saude Publica 41, 606–615.

Lutz, A., Jha, A.P., Dunne, J.D., Saron, C.D., 2015. Investigating the phenomenological matrix of mindfulness-related practices from a neurocognitive perspective. Am. Psychol. 70, 632.

Markovic, J., García-Giménez, J.L., Gimeno, A., Viña, J., Pallardó, F.V., 2010. Role of glutathione in cell nucleus. Free Radic. Res. 44, 721–733.

McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol. Rev. 87, 873–904.

McSweeney, F.K., Swindell, S., 2002. Common processes may contribute to extinction and habituation. J. Gen. Psychol. 129, 364–400.

Ng, T.K.S., Fam, J., Feng, L., Cheah, I.K.-M., Tan, C.T.-Y., Nur, F., Wee, S.T., Goh, L.G., Chow, W.L., Ho, R.C.-M., 2020. Mindfulness improves inflammatory biomarker levels in older adults with mild cognitive impairment: a randomized controlled trial. Transl. Psychiatry 10, 1–14.

Ochsner, K.N., Ray, R.D., Cooper, J.C., Robertson, E.R., Chopra, S., Gabrieli, J.D., Gross, J.J., 2004. For better or for worse: neural systems supporting the cognitive down-and up-regulation of negative emotion. Neuroimage 23, 483–499.

Pan, W., 2001. Akaike's information criterion in generalized estimating equations. Biometrics 57, 120–125.

Pfeiffer, C.M., Huff, D.L., Gunter, E.W., 1999. Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. Clin. Chem. 45, 290–292.

Phelps, E.A., Delgado, M.R., Nearing, K.I., LeDoux, J.E., 2004. Extinction learning in humans: role of the amygdala and vmPFC. Neuron 43, 897–905.

Pires, P., Filgueiras, A., Ribas, R., Santana, C., 2013. Positive and negative affect schedule: psychometric properties for the Brazilian Portuguese version. Spanish J. Psychol. 16.

Roeser, R.W., Schonert-Reichl, K.A., Jha, A., Cullen, M., Wallace, L., Wilensky, R., Oberle, E., Thomson, K., Taylor, C., Harrison, J., 2013. Mindfulness training and reductions in teacher stress and burnout: results from two randomized, waitlist-control field trials. J. Educ. Psychol. 105, 787.

Rubin, D.B., 1987. Multiple Imputation for Survey Nonresponse. Wiley, New York.

Schmidt, A.J., Krieg, J.-C., Vedder, H., 2005. Interleukin-6 induces glutathione in hippocampal cells. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 29, 321–326.

Schober, P., Vetter, T.R., 2018. Repeated measures designs and analysis of longitudinal data: if at first you do not succeed-try, try again. Anesth. Analg. 127, 569–575.

Sinha, S., Singh, S.N., Monga, Y.P., Ray, U.S., 2007. Improvement of glutathione and total antioxidant status with yoga. J. Alternative Compl. Med. 13, 1085–1090.

Southwick, S.M., Bonanno, G.A., Masten, A.S., Panter-Brick, C., Yehuda, R., 2014. Resilience definitions, theory, and challenges: interdisciplinary perspectives. Eur. J. Psychotraumatol. 5, 25338.

Team, R.C., 2013. R: A Language and Environment for Statistical Computing.

Thackray, R.I.J.P.m., 1981. The Stress of Boredom and Monotony: A Consideration of the Evidence, vol. 43, pp. 165–176.

Valikhani, A., Kashani, V.O., Rahmanian, M., Sattarian, R., Rahmati Kankat, L., Mills, P.J., 2020. Examining the mediating role of perceived stress in the relationship between mindfulness and quality of life and mental health: testing the mindfulness stress buffering model. Hist. Philos. Logic 1–15.

Watson, J.B., Rayner, R., 1920. Conditioned emotional reactions. J. Exp. Psychol. 3, 1.

White, I.R., Horton, N.J., Carpenter, J., Pocock, S.J.J.B., 2011. Strategy for Intention to Treat Analysis in Randomised Trials with Missing Outcome Data, 342.

Wielgosz, J., Goldberg, S.B., Kral, T.R., Dunne, J.D., Davidson, R.J., 2019. Mindfulness meditation and psychopathology. Annu. Rev. Clin. Psychol. 15, 285–316.

Zhang, H., Zhao, Q., Cao, P., Ren, G., 2017. Resilience and quality of life: exploring the mediator role of social support in patients with breast cancer. Med. Sci. Mon. Int. Med. J. Exp. Clin. Res.: international medical journal of experimental and clinical research 23, 5969.