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Ujjayi pranayama in systemic lupus women: randomized-controlled effect on cortisol, stress, depression, anxiety, and fatigue

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Abstract

Introduction. The aim of this study was to investigate the effects of ujjayi pranayama on non-lethal problems and cardiorespiratory functions in women with systemic lupus erythematosus (SLE).

Methods. This randomized controlled trial involved a home-based training of ujjayi pranayama for 6 weeks. Forty SLE women were randomly assigned to either the ujjayi-pranayama group (n = 20), who performed 30 min of ujjayi pranayama daily (15 min in the morning and evening), or the waitlist control group (n = 20). Various measures, including State-Trait Anxiety Inventory (STAI), cortisol, Stress Vulnerability Scale (SVS), Beck Depression Inventory II (BDI-II), Fatigue Severity Scale (FSS), Pittsburgh Sleep Quality Index (PSQI), pulse rate (Pul-r), respiratory rate (Res-r), systolic blood pressure (SBPr), and diastolic blood pressure (DBPr) were assessed.

Results. Significant reductions were observed via paired tests in Pul-r (75.95 ± 3.63 vs 72.95 ± 3.17; $p < 0.001$), Res-r (16.70 ± 3.62 vs 14.80 ± 2.94; $p < 0.001$), SBPr (126.60 ± 4.75 vs 121.30 ± 4.30; $p < 0.001$), DBPr (82.15 ± 3.57 vs 78.05 ± 3.44; $p < 0.001$), PSQI (7.45 ± 1.27 vs 5.10 ± 1.20; $p < 0.001$), FSS (4.80 ± 1 vs 3.75 ± 1.11; $p < 0.001$), BDI-II (13.70 ± 2.77 vs 6.80 ± 1.50; $p < 0.001$), STAI (66.70 ± 4.72 vs 47.55 ± 6.15; $p < 0.001$), cortisol (8.51 ± 1.36 vs 6.34 ± 1.36; $p < 0.001$), and SVS (12.65 ± 1.78 vs 6.45 ± 1.57; $p < 0.001$) in the ujjayi pranayama group only.

Conclusions. Ujjayi pranayama significantly improved non-lethal problems and cardiorespiratory functions in women with lupus.

Key words: pranayama, stress, fatigue, anxiety, lupus

Introduction

Although a combination of genetic, hormonal, immunologic, and environmental variables is necessary for the development of autoimmune disorders [1], stress plays a role in the onset of systemic lupus erythematosus (SLE), one of the most prevalent autoimmune diseases in women. SLE is associated with joint pain, high body temperature, skin rashes, and organ damage [2].

The sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis (stress axis), both engage in intricate/reciprocal communication with immune-system cytokines, hormones, and neurotransmitters. These pathways have been demonstrated to interact directly and/or indirectly with immune cell activity through chronic stress stimulation or repetitive stress exposure, potentially contributing to the development of autoimmunity, immunological dysregulation, and recurrent flare-ups of autoimmune diseases, including SLE [3].

In addition to the traditional diagnostic criteria of SLE, which include joint inflammation/pain, skin lesions, photosensitivity, and various organ-specific manifestations (renal, hepatic, cardiac, etc.), patients with SLE are also more likely to experience symptoms such as exhaustion, vulnerability to stress and fatigue, generalized body pain, sadness, anxiety, and sleep disturbances. Unfortunately, these symptoms typically do not improve with immunosuppression or corticosteroid therapy, even with increased doses [4].

Patients with SLE have become increasingly interested in utilizing the profound effects of non-pharmacologic complementary treatments over the past 20 years. Yoga is one such treatment that is considered safe and helpful for people with SLE [5]. Yoga has been shown to assist SLE patients with stress, sadness/depression, sleep patterns/disturbances, muscle strength, and exhaustion or vulnerability to fatigue [6].

In addition to asanas (physical yogic postures) and dhyana (meditation techniques), pranayama (regulation/control of vital energy) is a key technique used in yogic training [7].

According to yogic teachings, ujjayi pranayama, which involves the regulation of the incoming and outgoing flow of breath with retention, has a profoundly calming and relaxing impact on the psychological level [8]. Ujjayi pranayama is a deep breathing technique where the epiglottis is gently contracted, creating a soft hissing sound during inhalation and exhalation. The term "ujjayi" is derived from the Sanskrit word "ujj," meaning to conquer or acquire by conquering [9].

Ujjayi pranayama, as commonly understood, involves more than just a relaxing breathing procedure. Due to the connection between consciousness and breathing, ujjayi pranayama stabilizes the body's energy and consciousness. Accord-

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ing to traditional yoga teachings, this stabilization promotes the internalization of the five senses and offers mental control while aiding in their conquest [8].

Studies have reported various positive effects of practicing ujjayi pranayama. It calms the mind, reduces anxiety, improves attention [9], and alleviates mental fatigue [10] by relaxing the parasympathetic, respiratory, and cardiovascular systems [9, 11]. Additionally, as part of a yogic breathing regimen that includes various pranayama techniques, ujjayi pranayama improves respiratory function [12], depression [11], fatigue [13], and sleep disturbances [14].

Despite the reported benefits of ujjayi pranayama, its effects on stress, anxiety, depression, fatigue, sleep disturbances, and cardiorespiratory functions in SLE patients have not been studied in the lupus literature. Therefore, this complementary intervention study aims to investigate these effects.

Subjects and methods

Design

This two-arm randomized controlled study was evaluated as a pre-post comparison in lupus women (body mass index < 30 kg/m^2). The detailed methodology (inclusion criteria and interventions) of this single-blinded trial was registered on www.clinicaltrials.gov (NCT05748899).

Settings of recruitment

Cairo University hospitals.

Inclusion criteria for women

Forty SLE females were randomly allocated to one of two clinical trial groups: 20 women in the experimental ujjayi-pranayama group and 20 in the waitlist control group. According to the standards of the American College of Rheumatology, women with SLE were admitted [15].

Exclusion criteria

The following were the exclusion criteria for SLE women: (1) illiteracy; (2) inability to attend online pranayama sessions or adhere to authors' recommendations; (3) administration of antipsychotics or antidepressants (to investigate the effect of ujjayi pranayama alone without any effect from supporting antipsychotics or antidepressants); (4) SLE comorbidities (cardiovascular, systemic, renal, neurological, or respiratory comorbidities); (5) receiving complementary treatments in the 3 months before starting our intervention (ujjayi pranayama); and (6) drug or alcohol abuse.

Randomization

The study's design and methods were not disclosed to the person (physiotherapy assistant) who carried out the randomization (computer-generated randomization list) (Figure 1). The authors ensured that this person did not have a role in the other steps of this ujjayi-pranayama trial.

The ujjayi-pranayama group was supervised daily during the 6-week application of ujjayi pranayama. Meanwhile, the waitlist control lupus group was instructed not to alter their regular daily routine or use traditional prescriptions or new mobile applications for rest, relaxation, meditation, or sleep during the same period.

Intervention (ujjayi pranayama)

The study was conducted from December 15, 2022, to March 15, 2023. The steps of the ujjayi pranayama procedure were as follows:

– In a sitting position with an erect spine and closed eyes, lupus women were asked to perform slow, deep breathing by maintaining an equal ratio of inspiration and expiration between both nostrils [16].

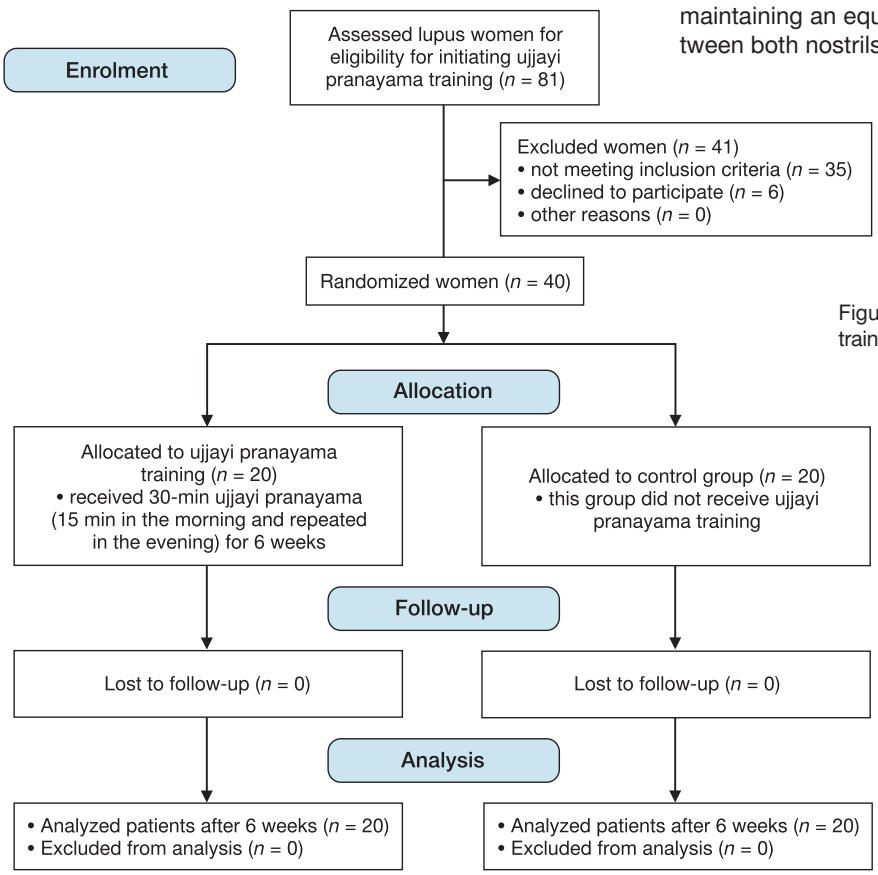

Figure 1. Consort flow chart of ujjayi pranayama training in lupus women

– Lupus women were instructed to make a specific ocean-wave-like sound (a faint hissing snoring sound) in their throat with a half-closed glottis during the inspiration phase of ujjayi pranayama [16].

– Slight tightening of the woman's throat during inspiration allowed air to pass through the glottis, creating the ocean wave-like sound [16].

– Exhalation was performed through both nostrils without straining the neck area, producing the sound "HHHH-HAAAA" [17].

– The glottis muscle and epiglottis were slightly under tension throughout the ujjayi pranayama procedure [16].

– Every 3–5 breathing cycles (one set of ujjayi pranayama), women were given a 1-min rest before starting the next set.

– For 30 min daily (15 min in the morning and 15 min in the evening), over 6 weeks, lupus women were instructed to perform the ujjayi-pranayama intervention at home. This was supervised online by the authors using Zoom video conferencing to ensure adherence. The first two days of the intervention were conducted face-to-face to teach the correct steps, ensuring error-free performance during subsequent online sessions.

Outcomes

Primary outcome

Cortisol

For the waitlist control or ujjayi-pranayama groups, ELISA kits were used to assess cortisol levels in the morning of lupus women. This morning cortisol level was the primary outcome of this lupus study on ujjayi pranayama.

Secondary outcomes

The State-Trait Anxiety Inventory (STAI)

STAI is a psychological self-reported inventory used to assess or differentiate between two forms of anxiety: general/long-standing quality of anxiety (i.e., trait anxiety) and temporary quality of anxiety (i.e., state anxiety). Both forms were evaluated with a 40-item inventory (20 separate items for each form on a 4-point Likert scale). In this lupus study, because "trait anxiety" represents general/long-standing anxiety, the authors evaluated the response of "trait anxiety" to ujjayi pranayama with a score ranging from 20–80 [18].

Stress Vulnerability Scale (SVS)

Identifying vulnerabilities to stress was the main aim of this 20-item scale. With a 5-point Likert scale for each item and a total score ranging from 20–100, lupus women were asked to assess the "vulnerability quotient" across three main areas of the scale (habitual, lifestyle, and basic needs). A score over 50 denotes a serious vulnerability or susceptibility to stress [19].

Beck Depression Inventory II (BDI-II)

BDI-II is not a diagnostic instrument for depression but is used to assess the depth of depression in any diagnosed illness with a score ranging from 0–63. Instead of focusing on behavioral and physical features, the main aim of BDI-II is to evaluate cognitive features of depression with different categories (no, slight, moderate, or severe depression) [20].

Fatigue Severity Scale (FSS)

Examining the degree/severity of fatigue in various circumstances from the week preceding our evaluation was the aim of this 9-item self-reported questionnaire. Each item was evaluated with a score ranging from 1 to 7, where 1 denotes disagreement with the question (i.e., no fatigue) and 7 denotes full agreement (i.e., severe fatigue). At the end of assessing FSS, the assessor can obtain a total score representing the mean value of the total asked for nine self-reported items [21].

Pittsburgh Sleep Quality Index (PSQI)

Assessing sleep quality in many diseases, including SLE, was the aim of this 19-item questionnaire, PSQI. Seven dimensions/features of sleep quality were covered during our evaluation: subjective sleep quality, latency of women's sleep, sleep duration, habitual sleep efficiency of women, disturbances in lupus women's sleep, consumption of sleeping medication, and lupus women's daytime dysfunction. The possible total range of this evaluation in lupus women was 0–21 points [22].

Cardiorespiratory autonomic functions

Cardiorespiratory autonomic functions were considered during the evaluation of outcomes in women with SLE before and after the 6-week duration of this study. These functions included pulse rate (Pul-r), respiratory rate (Res-r), and blood pressure (systolic blood pressure, SBPr, and diastolic blood pressure, DBPr).

Blinding precautions

The authors took all possible blinding precautions during the pre- and post-evaluations of outcomes. The study's purpose or methods were not disclosed to the assessors who carried out the assessments of outcomes. Lupus women were instructed not to provide information about the study to the assessors during the pre- and post-evaluations of outcomes.

Sample size

Under the supervision of a PhD-holding statistician, 34 lupus participants were the required sample size for this 6-week ujjayi-pranayama trial (settings of the G*power analysis were 80% power, 1.02 cortisol effect size, and 16 pilot-test lupus women). To account for potential participant dropout (20%), the authors added six women to the initially determined sample size of 34 women from the G*power analysis.

Details of statistical analysis

The measured demographic parameters (age, body mass index, waist circumference, and educational level) and medical data (dose of corticosteroid therapies, duration of lupus disease, complement system, and anti-nDNA levels) of women were statistically analyzed between lupus groups using unpaired *t*-tests before starting the 6-week ujjayi pranayama. Using SPSS 18 with $p < 0.05$, the significance of outcomes (Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS) was tested within and between groups using paired or unpaired *t*-tests, respectively. The PhD-holding statistician chose these tests due to the normal distribution of demographic parameters, medical data, and tested outcomes, as determined by Smirnov's test.

Table 1. Basic data of ujjayi-pranayama group and control group

Basic data	Basic data of ujjayi pranayama mean ± *SD*	Basic data of control group mean ± *SD*	*p*-value
Age, year	42.55 ± 8.29	45.05 ± 8.41	0.349
Duration of disease (year)	8.85 ± 1.95	9.35 ± 1.34	0.350
Education levels (year)	10.20 ± 1.54	9.65 ± 1.18	0.212
Women's body mass index (kg/m^2)	24.26 ± 2.97	25.81 ± 3.23	0.122
Women's waist circumference (cm)	94.75 ±17.83	95.70 ± 17.13	0.864
Lupus women's anti-nDNA (IU/ml)	31.20 ± 3.30	30.25 ± 3.35	0.372
Cumulated dose of corticosteroid therapies (g)	38.65 ± 4.43	39.90 ± 3.61	0.334
Lupus women's C3 (mg/dl)	98.95 ± 20.23	96.10 ± 19.64	0.653
Lupus women's C4 (mg/dl)	21.20 ± 2.66	20.20 ± 2.54	0.231

All *p*-values of this table were non-significant before applying ujjayi pranayama.

Table 2. Outcomes of both SLE groups after ujjayi pranayama

Parameters of women	Group A (ujjayi-pranayama group) mean ± *SD*	Group B (control women group) mean ± *SD*	*p*-value (between groups)
SVS			
base-line measurement	12.65 ± 1.78	13.15 ± 1.53	0.346
post-measurement	6.45 ± 1.57	13.30 ± 2.45	0.0001*
p-value (within women group)	< 0.001*	0.767	
STAI (trait anxiety)			
base-line measurement	66.70 ± 4.72	65.45 ± 7.18	0.519
post-measurement	47.55 ± 6.15	65.75 ± 6.01	0.0001*
p-value (within women group)	< 0.001*	0.759	
BDI-II			
base-line measurement	13.70 ± 2.77	13.85 ± 1.98	0.844
post-measurement	6.80 ± 1.50	14.15 ± 3.01	0.0001*
p-value (within women group)	< 0.001*	0.659	
Cortisol (mg/dl)			
base-line measurement	8.51 ± 1.36	8.46 ± 1.23	0.903
post-measurement	6.34 ± 1.36	8.64 ± 1.46	0.0001*
p-value (within women group)	< 0.001*	0.427	
Fatigue severity scale			
base-line measurement	4.80 ± 1	4.75 ± 1.06	0.878
post-measurement	3.75 ± 1.11	5.10 ± 1.16	0.0006*
p-value (within women group)	< 0.001*	0.260	
Pittsburgh Sleep Quality Index			
base-line measurement	7.45 ± 1.27	7.15 ± 1.34	0.471
post-measurement	5.10 ± 1.20	7.50 ± 1.50	0.0001*
p-value (within women group)	< 0.001*	0.635	
SBPr (mm Hg)			
base-line measurement	126.60 ± 4.75	127.80 ± 5.35	0.457
post-measurement	121.30 ± 4.30	128.15 ± 5.27	0.0001*
p-value (within women group)	< 0.001*	0.504	
DBPr (mm Hg)			
base-line measurement	82.15 ± 3.57	83 ± 3.97	0.480
post-measurement	78.05 ± 3.44	83.20 ± 3.03	0.0001*
p-value (within women group)	< 0.001*	0.761	
Pul-r (beats/min)			
base-line measurement	75.95 ± 3.63	78.10 ± 3.87	0.077
post-measurement	72.95 ± 3.17	78.50 ± 4.35	0.0001*
p-value (within women group)	< 0.001*	0.494	
Res-r (beats/min)			
base-line measurement	16.70 ± 3.62	17.50 ± 2.92	0.446
post-measurement	14.80 ± 2.94	17.65 ± 2.53	0.002*
p-value (within women group)	< 0.001*	0.711	

SVS – Stress Vulnerability Scale, STAI – State-Trait Anxiety Inventory (trait anxiety is the measured variable), BDI – Beck Depression Inventory II; * *p*-value is significant

Results

No statistically significant difference was affirmed between groups regarding the measured demographic parameters (age, body mass index, waist circumference, and educational level), medical data (dose of corticosteroid therapies, duration of lupus disease, complement system, and anti-nDNA levels) (Table 1), and assessed outcomes (Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS) (Table 2) before starting this 6-week ujjayi-pranayama clinical study.

The PhD-holding statistician found a significant difference/improvement between pre- and post-values of Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS within the ujjayi-pranayama group only, whereas the other group did not show such improvement. Additionally, in favor of the ujjayi-pranayama training, the PhD-holding statistician found a highly significant difference/improvement between post-values of both groups for Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS (Table 2).

Discussion

It has been proposed that SLE-related neuropsychiatric (depression and anxiety) and physical (fatigue) complaints are consequences of autoimmune system dysregulation/dysfunction, repeated daily exposures to stress, which has been proposed to affect SLE symptoms and disease activity, and dysregulation/dysfunction of the HPA axis. Dysregulation of the HPA axis may act as a biological setting event in SLE because it is an autoimmune condition, making individuals with SLE more vulnerable to neuropsychiatric and physical complaints [23].

The main component of yoga, pranayama, has not been specifically evaluated in SLE women [24]. Our study was the first to report that performing 6 weeks of ujjayi pranayama (as a main yogic breathing technique) can improve SLE women's Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS. However, the exact mechanism of these improvements after ujjayi pranayama is difficult to explain.

Physical (fatigue) and neuropsychiatric (disrupted psychosocial functioning) complaints are frequent untreated demands [25, 26] that are shared by many chronic illness patients [27, 28], including SLE women. Yoga has demonstrated efficacy in reducing physical and neuropsychiatric symptoms of several chronic illnesses [23]. A systematic review conducted in 2006 on 82 clinical trials aimed to assess the efficacy of different meditation techniques in the treatment of medical conditions supported this point of view because it concluded that yogic meditation may be an effective complementary procedure in treating nonpsychotic mood disorders and anxiety disorders [29].

The reported significant reduction of state-trait anxiety among participants performing ujjayi pranayama [9] may be attributed to increased cardiac output, decreased Pul-r, reduced stress [30, 31], induced relaxation of the mind, and regulated connectivity and activities/functions of brain areas (e.g., amygdala, anterior cingulate and insula, and prefrontal and ventrolateral cortex involved in negative affect and/or emotional processes), which are consequences of inhibiting the sympathetic system and activating the parasympathetic system.

The adherence of patients with rheumatic diseases, including SLE, to breathing exercises connected with progressive muscle relaxation training may be the cause of the improved health-related quality of life, reduced articular pain and tenderness, and improved muscle functions and strength of the lower extremities; hence, the highly reported fatigue in those patients improves [32].

Supporting the results of this SLE study, a new systematic review conducted by de Orleans Casagrande et al. [33] reported that yoga is an effective alternative tool in improving depression, anxiety, and sleep problems in rheumatic disease patients. Also, the 9-month follow-up to the stress-reduction program (biofeedback-assisted cognitive behavioral training) in SLE patients showed that this program was significantly effective in improving fatigue, depressive symptoms, self-efficacy, and perceived stress of those sufferers [34].

Supporting our results, stress management (relaxation training and breathing exercises) as part of the 8-Week Fatigue and Activity Management Education Program significantly improved fatigue and activities of daily living in lupus patients [35]. In agreement with our results, mental quality of life and psychological symptoms (depression and anxiety) significantly improved in response to an eight-session mindfulness training in lupus patients [36]. Again, illness perception, depression, and SLE-related pain improved following a mindfulness-based stress reduction protocol in lupus patients [4]. Also, a self-management program aimed at better health (exercise and coping strategies for pain, fatigue, and frustration) in lupus patients improved their pain, depression, and fatigue, but cortisol did not improve [37]. In allogeneic hematopoietic stem cell transplantation patients, 6 weeks of relaxation breathing exercises significantly reduced the patients' perceived fatigue [38]. Cancer-related fatigue is significantly improved after pranayama training for 6 weeks in breast cancer women [39]. Cortisol and reported stress, anxiety, depression, and sleep difficulties were improved after 8 weeks of relaxation breathing training in women who complained of functional dyspepsia [40]. Cortisol and reported stress, anxiety, and depression were improved after one session of relaxation breathing training in men [41].

Ujjayi pranayama – practiced along with other pranayama forms – is very helpful for improving sleep disturbance, anxiety, and mental quality of life among patients undergoing chemotherapy [14]. Niranjan and Balaram [9] supported our results, especially after their reported significant immediate effect of ujjayi pranayama on reducing stress and anxiety (assessed by STAI) among university students. Similarly, a significant improvement was recorded during the pre-to-post comparison of the comprehensive anxiety test (SCAT) after a 45-day training of ujjayi pranayama (performed 15 min in the evening) in healthy individuals [42]. Another study also reported that slow breathing practice improves comfort and pleasantness, alertness and confusion, arousal and vigor, anxiety and depression, and anger [43]. Similarly, the practice of diaphragmatic breathing exercises was also found to improve healthy adults' sustained attention, negative affect, and cortisol levels [44]. Following 1 week of detoxification management in alcohol-dependent individuals, 2 weeks of ujjayi pranayama in combination with other pranayama forms showed a significant reduction in individuals' BDI and cortisol [45].

Supporting our results, the practice of ujjayi pranayama without combining other pranayama forms can reduce weightlifters' mental fatigue [10]. Also, ujjayi pranayama – practiced along with other pranayama forms – reduces depression in alcohol-dependent individuals [11] and physical fatigue in women in their third trimester of pregnancy [13].

Regarding improved lupus-related cardiovascular and respiratory autonomic functions, the suggested mechanism of improvement is related to the ability of regular ujjayi training to inhibit sympathetic activity, decrease resting Pul-r, increase heart muscle strength, increase efficacious transportation of oxygenated blood throughout the body, and decrease respiratory demand [46].

In accordance with our lupus women's results, healthy individuals' Pul-r, Res-r, SBPr, and DBPr showed significant improvements after 6 months of ujjayi-pranayama training [11]. Also, ujjayi pranayama, along with yogic postures, decreased SBPr, DBPr, pulse pressure, mean arterial pressure, and rate pressure product [47]. Ujjayi pranayama – as part of a different-type pranayama program – in university-level kho-kho players showed a significant decrease in SBPr, DBPr, Res-r, and Pul-r [48].

Contrary to our results, the effect of 6 weeks of ujjayi pranayama on strengthening immunity did not support our viewpoint due to the resultant non-significant difference between pre- and post-levels of white blood cells [49]. Also, DBPr and Pul-r did not significantly differ after involving healthy individuals in a 6-week ujjayi pranayama program [50]. Despite the insignificant decrease in blood pressure and Res-r, the significant decrease in Pul-r of participants (physical education females) who received an 8-week ujjayi pranayama supported our results [31]. Also, Pul-r and Res-r showed significant improvement after ujjayi-pranayama training for 6 weeks (45 min of training daily), whereas SBPr and DBPr, despite the improvements, did not show the same significance [46]. Unlike our reported significant difference in between-group post-values of fatigue, Bal et al. [51] found a non-significant difference in post-values of muscle strength and endurance between girls who underwent long-term ujjayi pranayama and those who did not.

Limitations

The main limitation of this pranayama study was that it did not analyze the remission period/effect of the results (i.e., long-term follow-up to ujjayi pranayama results including Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS. The four authors recommend addressing this gap in future ujjayi pranayama studies in lupus women. Analyzing the response of the assessed outcomes to ujjayi pranayama versus standard respiratory/breathing exercises is recommended.

Conclusions

Lupus women's Pul-r, Res-r, SBPr, DBPr, PSQI, FSS, BDI-II, STAI, cortisol, and SVS significantly improved after adherence to 6-week ujjayi-pranayama training.

Ethical approval

The research related to human use has complied with all the relevant national regulations and institutional policies, has followed the tenets of the Declaration of Helsinki, and has been approved by the Local Institutional Review Board, Cairo University, Faculty of Physical Therapy (approval No.: IRB P.T.REC/012/004288). A detailed methodology (criteria of inclusion and interventions) of this single-blinded trial was registered on www.Ccinicaltrials.gov (NCT05748899).

Informed consent

Informed consent was obtained from all individuals included in this study.

Disclosure statement

No author has any financial interest or received any financial benefit from this research.

Conflict of interest

The authors state no conflict of interest.

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