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Original Article

A randomized trial of the immediate effect of bee-humming breathing exercise on blood pressure and heart rate variability in patients with essential hypertension

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ABSTRACT

Objectives: Bee-Humming Breathing (BHB) exercise is a simple yogic practice recommended for its favorable effect on cardiac physiology, including blood pressure (BP) and autonomic nervous system. However, strong evidence supporting its effectiveness is lacking. The present study was designed to evaluate the immediate effect of BHB exercise on blood pressure parameters and heart rate variability (HRV) in patients with essential hypertension.

Study methods: We conducted a randomized control trial including 70 patients with essential hypertension, randomly allocated to perform either BHB exercise (n=35) or placebo slow breathing exercise (n = 35) for 5-minutes duration. Blood pressure and HRV were measured before, during, and after the practice.

Results: There was no significant decrease in systolic [effect size (95% CI): 2.22 (-13.20, 17.64); p 0.77], diastolic [4.54 (-17.40, 26.48); p 0.68] and mean blood pressures [1.37 (-8.78, 11.52); p 0.78] after BHB exercise in comparison to the control group in our study. The HRV analysis showed a significant increase in the HF power [6.8 (1.47, 12.12); p 0.01], and decrease in the LF power [-26.47 (-34.25, -18.68); p < 0.01] during the recovery phase of the 5-minute BHB exercise in comparison to the control group.

Conclusions: This is the first randomized controlled trial to show that though a single short session of BHB exercise in hypertensive patients does not significantly reduce BP, it significantly augments the parasympathetic tone as indicated by a significant improvement in HRV parameters.

Clinical trial registration number: CTRI/2018/08/015215

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Introduction

The cardiovascular system is regulated by a complex interplay of intrinsic myocardial contractility, neural factors as well as other factors like baroreceptor reflexes, chemoreceptors reflexes, and hormones. Of these, the main neural factor is the autonomic nervous system (ANS), which consists of the sympathetic and parasympathetic nervous systems. Both components of the ANS play a major role in maintaining and regulating systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR). The sympathetic nervous system releases norepinephrine (NE) and increases the heart rate, blood pressure, and myocardial contractility, mainly during excitement, exercise, emotional stress, and various pathological

conditions (like heart failure). On the other hand, the parasympathetic nervous system counteracts the sympathetic activity by the release of acetylcholine predominantly during relaxed and restful conditions.¹ Imbalances in any of the component of the autonomic nervous system can lead to a variety of cardiovascular disorders such as hypertension, hypotension, syncope, arrhythmia, myocardial ischemia, and infarction. Moreover, numerous studies suggest a strong association between autonomic dysfunction (e.g., decreased vagal activity and/or increased sympathetic activity) and sudden and non-sudden cardiac death.²

Lifestyle modification is an underrated but essential strategy in the treatment, prevention, and rehabilitation of cardiovascular disorders. The main aim is to achieve a balanced and peaceful mind-body state by adopting a disciplined and healthy behavior. Yoga is one of the most popular methods of lifestyle modifications. It is an ancient Vedic science thought to have originated in India in 5000 BC.³ It includes the practice of specific postures (asana), regulated breathing

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techniques (pranayama), and concentration techniques (meditation).⁴ Among them, controlled slow breathing exercise or 'pranayama' has been considered and practiced as one of the most effective ways to maintain healthy physiology of the human body. The beneficial effects of slow breathing are mostly due to the modulation of autonomic nervous activities. Both sympathetic and parasympathetic pathways of the autonomic nervous system are actively controlled by central respiratory centers where autonomic drives from the reflex mechanisms and lung stretch receptors converge. According to the respiratory gate theory, the autonomic outflow is inhibited during inspiration and disinhibited during expiration.⁵ As a result, controlled slow breathing (~6/minute) exercise synchronizes the cardiovascular and respiratory rhythmic fluctuations leading to augmentation of vagal tone, sinus arrhythmia, baroreflex sensitivity, blood oxygenation, and exercise tolerance apart from its profound effects on calmness and wellbeing.⁶

'Pranayama' is a Sanskrit word that literally means control of vital force ('Prana' = vital force; 'Yama' = control). Basically, it consists of three steps: inhalation (purak), retention (kumbhak), and exhalation (rechak). The steps of pranayama can be modified to perform different varieties of slow breathing exercises to improve its effectiveness.⁷ Bee-Humming Breathing Exercise (BHB) or 'Bhramari pranayama' is one such modification which involves inhalation through both nostrils and while exhalation producing the sound of humming bee as long as possible. It is a simple technique and can be performed by everyone irrespective of age and sex.⁷ BHB exercise has been reported to improve pulmonary function⁴, blood pressure,^{8–10} heart rate variability,⁸ tinnitus,¹¹ anxiety, depression, and drug-dependency¹² by its meditative relaxing effect as well as its action on the autonomic nervous system.

According to the recent hypertension guideline, stress reduction by behavioral therapy like guided breathing exercise is an easy and non-expensive method of blood pressure reduction.¹³ However, studies verifying the effectiveness of BHB or similar breathing techniques are very few. Most of these are observational studies with very small sample sizes leading to a significant risk of biased reporting. Though various studies on BHB have shown to produce a substantial reduction in blood pressure in healthy volunteers,^{8–10} to the best of our knowledge, there is no known observational or randomized controlled study reporting the effect of BHB in patients with essential hypertension. Since the benefits of BHB observed in healthy volunteers are expected to be present in pathological conditions as well, we hypothesized that the practice of BHB might be useful in reducing blood pressure in patients with essential hypertension. Moreover, there is an inconsistency in reporting the possible mechanism behind the reduction of blood pressure after BHB. For example, few studies have reported that the reduction in blood pressure was due to parasympathetic dominance on cardiovascular system^{9,10} while another study has demonstrated parasympathetic withdrawal during the practice of BHB. Thus, the reduction in blood pressure might not be explained by parasympathetic dominance alone and might include other mechanisms such as changes in cardiac output or peripheral resistance.⁸ Hence, there is a need to find out the mechanism behind the effect of BHB.

Materials and methods

Study design

The study was a single-center, open-label, randomized active-control trial to assess the immediate effects of BHB exercise on blood pressure, heart rate, heart rate variability, cardiac output, and peripheral resistance in patients with essential hypertension. The patient recruitment was started on 25th August 2018 and completed on 1st March 2019. The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of

Helsinki) for experiments involving humans. The study protocol was approved by the institutional ethical committee. The committee did not raise any objection on the study protocol and methodology. The BHB exercise was considered uniformly safe as no adverse events has been reported by any previous studies on this exercise. Written consent was obtained from all the participants.

Patient population

Both male and female hypertensive patients were eligible for the enrolment in the study if they were 30–70 years of age, having SBP between 140–180 mmHg, DBP between 90–110 mmHg, and not taking more than two anti-hypertensive medications. The other eligibility criteria were willingness to participate in the trial and provision of written informed consent. The exclusion criteria were poor drug compliance, presence of secondary hypertension, coronary artery disease, heart failure, arrhythmia, rheumatic heart disease, congenital heart disease, and other significant systemic (renal, hepatic, pulmonary, neurological and, psychiatric) diseases or complications. Those who were practicing yoga regularly for more than one month, and subjects who were unable to perform BHB exercise were also excluded from the trial.

Patient recruitment and randomization

Patients with essential hypertension attending cardiology outpatient clinics at a tertiary care referral hospital in New Delhi, India, were screened for inclusion in the study. Patients satisfying eligibility criteria were enrolled in the study after taking informed consent. All the participants were randomized in a 1:1 ratio to the BHB arm and the control arm by a computer-generated table of random numbers (Fig. 1).

Intervention

A detailed demographic and clinical assessment were done for all patients at the beginning of the trial by a yoga physician. The participants were made to sit on an easy chair comfortably with their spine supported and eyes closed throughout. After a five minutes rest, the yoga group participants were asked to perform BHB exercise, which involves deep inhalation through both nostrils followed by exhalation along with the production of a humming bee sound ('MMMM') for as long as possible. The approximate duration of one round for both breathing exercises (BHB exercise and control exercise) was 10 to 15 seconds—leading to a breath rate of 4–6/minutes. This was repeated for a duration of 5 minutes (**Supplementary material**).

The participants of the control group assumed the same posture and duration as the yoga group. But they were asked to inhale deeply through both the nostrils followed by exhalation along with the production of a hissing sound ('SSSS') for as long as possible. This controlled slow breathing exercise was designed to evaluate the exact benefit of bee-humming sound over and above the beneficial effect of slow deep breathing. Both BHB and control breathing exercises were conducted under the direct supervision of a yoga instructor. The blood pressure, heart rate, and HRV parameters were assessed before (resting phase), during (active phase), and after (recovery phase) the intervention in both groups.

Cardiovascular function assessment

The cardiovascular variables such as systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), arterial stiffness, and total peripheral resistance (TPR) were assessed in sitting position using non-invasive ambulatory blood pressure monitoring system (**Mobil-O-Graph®**, **PWA, IEM GmBH**).¹⁴ The cuff was placed on the upper arm of the left

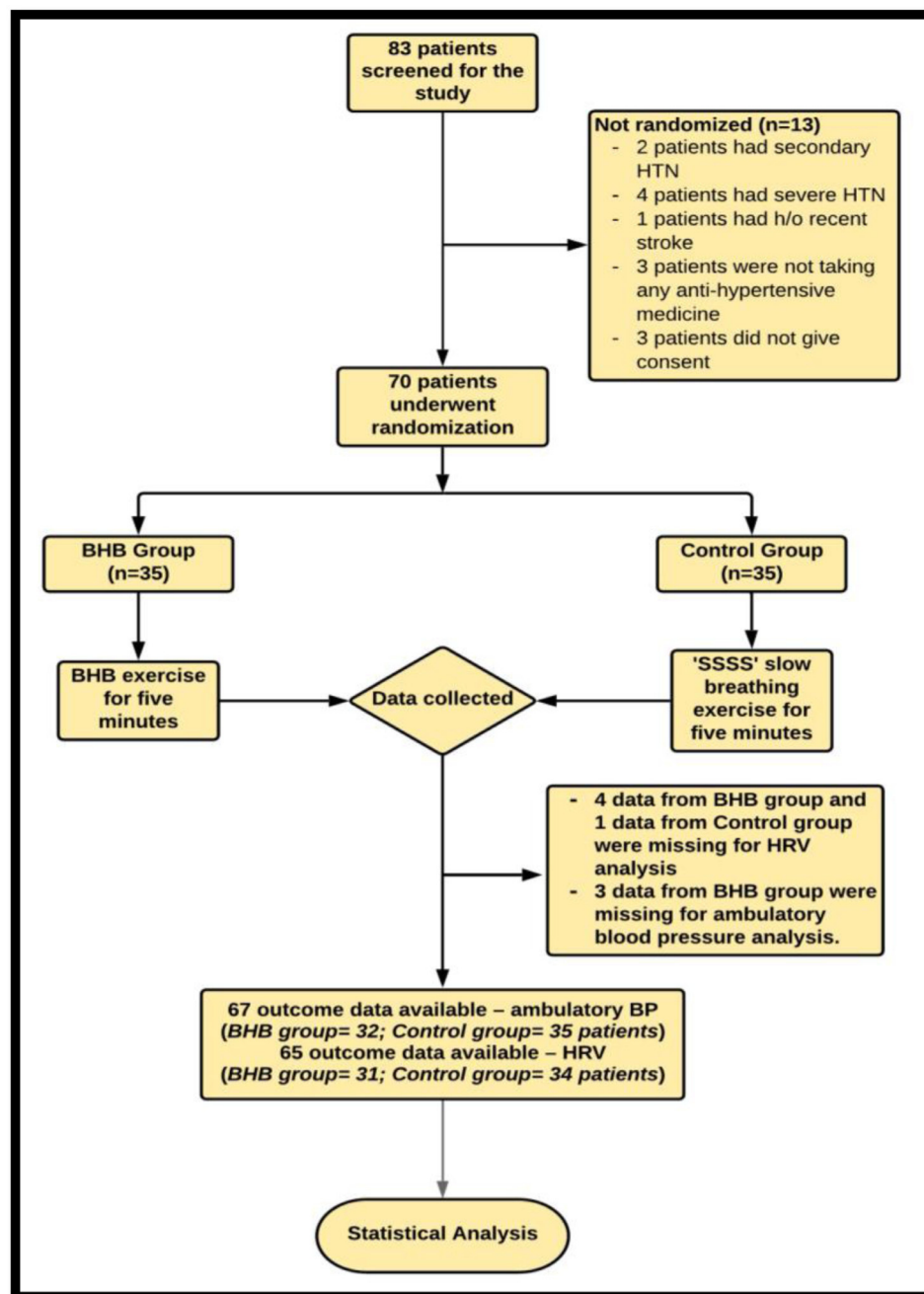


Fig. 1. Flow diagram showing screening, recruitment, and randomization of the study population. BHB = Bee humming breathing, HRV = heart rate variability, BP = blood pressure, HTN = hypertension.

hand at the level of the heart and the marker on the cuff directly above the brachial artery.

Heart rate variability assessment

Parameters of heart rate variability (HRV) of both yoga and control groups were measured before, during, and after the intervention using Digital Holter recorder (*Medilog® FD12plus, Schiller*) machine. The frequency domain and time domain components of the HRV were analyzed separately for each phase. Among the frequency domain parameters, low frequency (LF) band (0.04–0.15 Hz), high-frequency (HF) band (0.15–0.4 Hz), and LF/HF ratio were calculated. The time-domain HRV parameters that were analyzed included standard deviation of RR

Intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent normal-to-normal (NN) intervals (rMSSD), the proportion derived by dividing NN50 by the total number of NN intervals (pNN50). The data recorded were visually inspected off-line, and only noise-free data were included for the analysis.

Study endpoints

The primary endpoint was to assess the change in blood pressure parameters during and after the BHB exercise in patients with essential hypertension taking anti-hypertensive medications. The assessment of the heart rate variability parameter changes during and immediately after the BHB exercise was the secondary endpoint.

Table 1
Baseline characteristics of the study populations.

Characteristics	Study groups		p-value
	Control (n = 35)	BHB (n = 32)	
Age (Years)	48.25 ± 8.45	49.59 ± 7.88	0.50
Gender			
Male	19 (54.29)	17 (53.13)	0.73
Female	16 (45.71)	15 (46.87)	0.85
Weight (kg)	70.64 ± 12.21	73.6 ± 14.26	0.36
BMI (kg/m²)	27.47 ± 3.69	29.30 ± 5.23	0.10
Systolic blood pressure (mmHg)	127.8 ± 12.91	131.75 ± 9.67	0.16
Diastolic blood pressure (mmHg)	88.05 ± 9.81	91.37 ± 7.72	0.13
Heart rate (per minutes)	76.51 ± 10.43	74.28 ± 9.58	0.36
Number of anti-hypertensive medicines			
1	26 (74.29)	25 (78.12)	0.88
2	9 (25.71)	7 (21.88)	0.61
Medication			
Calcium channel blocker	10 (28.57)	14 (43.76)	0.41
ACEI	1 (2.86)	3 (9.37)	0.31
ARB	22 (62.86)	11 (34.37)	0.05
Beta blockers	2 (5.71)	4 (12.50)	0.48

Values are mean ± SD or number (%); P values in bold indicate $P \leq 0.05$.

ACEI = Angiotensin converting enzyme inhibitors, ARB = Angiotensin receptor blocker, SD = Standard deviation, BMI = Body mass index, BHB= Bee humming breathing.

Statistical analysis

The sample size calculation was based on the single-group observational study done by L. Nivethitha et al.⁸ in which a reduction in systolic blood pressure with an effect size of 0.61 (Cohen's d per standard deviation) was observed due to BHB exercise (Bhramari pranayama) in a small group of healthy volunteers. Consequently, considering the reduction in systolic blood pressure as the primary outcome measure with an alpha of 0.05 and power of 0.8, the calculated sample size was 70 patients (35 in each group).

The data was analyzed statistically using the **STATA ver. 14.2** software. Categorical variables were reported as frequency (percentages) and subsequently compared using Chi-Square test. Continuous variables were tested for approximate normality and reported as Mean ± SD (standard deviation) or Geometric Mean (GM) ± SD as appropriate. Independent sample t-test was used to compare mean values across two groups. Effect size, adjusted for the baseline outcome variable, was calculated using Analysis of Covariance (ANCOVA), and it was reported as effect size (95% Confidence Interval). In this study, two-sided P value ≤ 0.05 has been considered as statistically significant.

Results

A total of 83 hypertensive patients were screened for the study, and among them, 70 patients fulfilled the eligibility criteria. They were randomly allocated in a 1:1 ratio to perform the BHB exercise ($n = 35$) or the control breathing exercise ($n = 35$) for five minutes duration (**Fig. 1**). As four HRV data from the BHB group and one data from the control group were missing, they were excluded during the final HRV analysis. Similarly, ambulatory blood pressure data could not be retrieved for three patients in the BHB group. Therefore, they were also excluded during the final assessment of the ambulatory blood pressure trend.

Baseline characteristics

Baseline characteristics of the BHB and the control populations are shown in **Table 1**. A significantly higher number of patients in the control arm were taking angiotensin receptor blocker than the BHB arm. The rest of the baseline parameters were not significantly different between the two study arms of the trial.

Ambulatory blood pressure monitoring parameters

The ambulatory blood pressure monitoring of the BHB exercise group did not show any significant decrease of systolic, diastolic, and mean blood pressure (**Table 2**, **Fig. 2**) during the resting, active, and recovery phase of BHB exercise in comparison to the control group. The heart rate of the BHB arm decreased during the recovery phase in contrast to the resting phase, but it was not statistically significant when compared with the control population [Effect size (95% CI): $-2.37 (-23.29, 18.55)$; $P = 0.82$]. There was no significant difference in the rest of the values of the stroke volume and total vascular resistance in the two study arms.

Heart rate variability parameters

Regarding the time domain heart rate variability measures, the adjusted SDNN [Effect size (95% CI): $-5.71 (-9.70, -1.71)$; $P < 0.01$] and rMSSD [Effect size (95% CI): $-4.53 (-8.52, -0.53)$; $P = 0.02$] values decreased significantly during the active phase of the BHB exercise in comparison to the control group. However, there was no significant difference in the same parameters during the recovery phase. Though not statistically significant, there was an increasing trend of the rMSSD and pNN50 values during the recovery phase of the BHB group in comparison to the control group (**Table 3**).

Table 2
Changes in the blood pressure parameters during and after the BHB exercise and the control exercise.

Blood pressure parameters	Groups	Mean ± SD			BHB effect during active phase		BHB effect during recovery phase	
		Pre (Resting)	During (Active)	Post (Recovery)	Effect size (95% CI)	p-value	Effect size (95% CI)	p-value
SBP (mmHg)	BHB (n = 32)	131.7 ± 9.6	131.7 ± 12.8	131.7 ± 10.9	2.16 (-23.12, 27.44)	0.86	2.22 (-13.20, 17.64)	0.77
	Control (n = 35)	127.8 ± 12.9	127.1 ± 16.3	125.8 ± 12.6				
DBP (mmHg)	BHB (n = 32)	91.4 ± 7.7	94.5 ± 8.81	92.3 ± 8.7	4.54 (-17.40, 26.48)	0.68	0.97 (-10.54, 12.48)	0.86
	Control (n = 35)	88.05 ± 9.8	87.4 ± 11.3	88.3 ± 9.2				
MAP (mmHg)	BHB (n = 32)	109.9 ± 7.7	112.3 ± 9.8	110.3 ± 8.5	2.94 (-16.57, 22.45)	0.76	1.37 (-8.78, 11.52)	0.78
	Control (n = 35)	106.1 ± 10.0	105.6 ± 12.2	105.4 ± 9.6				
HR (/min)	BHB (n = 32)	74.3 ± 9.6	76.7 ± 10.3	71.7 ± 13.1	0.96 (-17.81, 19.73)	0.91	$-2.37 (-23.29, 18.55)$	0.82
	Control (n = 35)	76.5 ± 10.4	77.6 ± 10.9	76.1 ± 10.3				
SV (ml)	BHB (n = 32)	66.9 ± 8.7	63.2 ± 7.8	67.0 ± 9.6	$-0.41 (-18.12, 17.30)$	0.96	$-1.09 (-22.50, 20.32)$	0.91
	Control (n = 35)	62.9 ± 10.2	61.2 ± 8.9	64.9 ± 11.5				
TVR (PRU)	BHB (n = 32)	1.4 ± 0.13	1.4 ± 0.17	1.4 ± 0.17	0.06 (-0.33, 0.45)	0.76	0.04 (-0.32, 0.40)	0.82
	Control (n = 35)	1.4 ± 0.15	1.36 ± 0.17	1.3 ± 0.17				

Effect size adjusted for resting values of outcome variables (using ANCOVA); P values in bold indicate $P \leq 0.05$.

BHB = Bee Humming Breathing, SBP = Systolic blood pressure, DBP = Diastolic blood pressure, MBP = Mean blood pressure, SV = Stroke volume, HR = Heart rate, TVR = Total vascular resistance, PRU = Peripheral resistance unit, CI = Confidence interval, SD = Standard deviation.

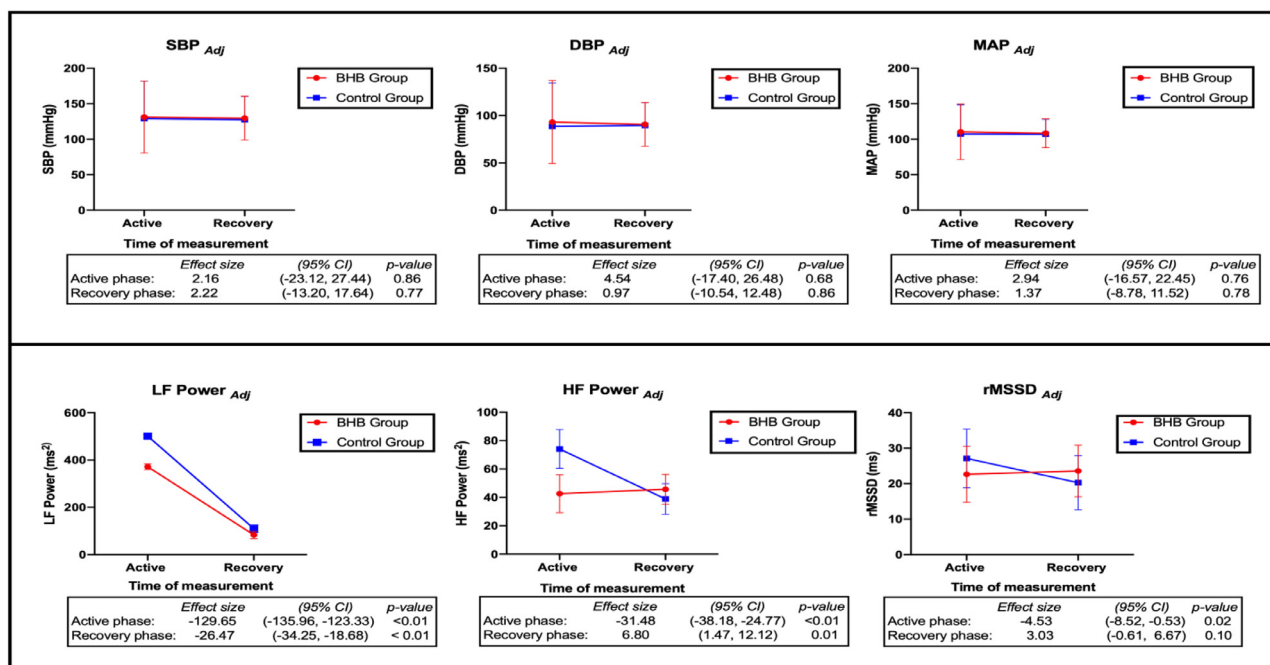


Fig. 2. Graph showing the trend of adjusted blood pressure and heart rate variability parameters during and after the BHB exercise and the control exercise. Effect size adjusted for resting values of outcome variables (using ANCOVA). SBP = Systolic blood pressure, DBP = Diastolic blood pressure, MAP = Mean blood pressure, HF = High frequency, LF = Low frequency, rMSSD = Square root of the mean of the sum of the squares of differences between adjacent normal-to-normal (NN) intervals, Adj. = Adjusted, BHB = Bee humming breathing.

Among the frequency domain measures, BHB resulted in significant decrease in the LF power both during the active [Effect size (95% CI): -129.65 (-135.96, -123.33); $P < 0.01$] and recovery phase [Effect size (95% CI): -26.47 (-34.25, -18.68); $P < 0.01$] of the exercise (Table 3, Fig. 2). In addition, the HF power of the BHB exercise arm significantly decreased during the active phase [Effect size (95% CI): -31.48 (-38.18, -24.77); $P < 0.01$]. However, the BHB exercise significantly increased the HF value during the recovery phase [Effect size (95% CI): 6.80 (1.47, 12.12); $P = 0.01$]. The Log LF/HF values did not show any statistically significant difference between the BHB arm and the control arm.

Discussion

In this study, a single BHB session in hypertensive patients who were not regular yoga practitioners did not lead to any significant

decrease in blood pressure during or immediately after performing the pranayama. However, it resulted in a significant increase in parasympathetic tone, as indicated by a significant increase in the HF power and a decrease in the LF power values immediately after the practice.

Though BHB exercise is one of the popular pranayama techniques being practiced since ancient times, evidence on its health benefits and explanations behind its action are very scarce in the literature. There are few hypothesis on the mechanism behind the beneficial effects of BHB exercise (Fig. 3). Firstly, being a pranayama technique with short inhalation and prolonged exhalation phase, BHB exercise leads to stretching of the lung tissue and a decrease in the dead space ventilation. This leads not only to an improvement in the lung function but also produces inhibitory signals by the action of slowly adapting stretch receptors and hyperpolarization current by the action of fibroblast resulting in parasympathetic system activation. It

Table 3

Changes in the heart rate variability (HRV) parameters during and after the BHB exercise and the control exercise.

HRV parameters	Groups	Geometric Mean $\pm$ SD			BHB effect during active phase		BHB effect during recovery phase	
		Pre (Resting)	During (Active)	Post (Recovery)	Effect size (95% CI)	p-value	Effect size (95% CI)	p-value
LF Power (ms^2)	BHB ($n=31$)	87.09 $\pm$ 2.88	316.22 $\pm$ 2.81	74.13 $\pm$ 3.09	-129.65 (-135.96, -123.33)	<0.01	-26.47 (-34.25, -18.68)	<0.01
	Control ($n=34$)	134.89 $\pm$ 2.57	575.43 $\pm$ 2.81	125.89 $\pm$ 3.16				
HF Power (ms^2)	BHB ($n=31$)	36.30 $\pm$ 3.01	38.01 $\pm$ 2.95	38.90 $\pm$ 3.38	-31.48 (-38.18, -24.77)	<0.01	6.80 (1.47, 12.12)	0.01
	Control ($n=34$)	52.48 $\pm$ 2.81	81.28 $\pm$ 3.16	44.66 $\pm$ 2.81				
log LF/HF	BHB ($n=31$)	0.20 $\pm$ 4.57	0.85 $\pm$ 1.54	0.26 $\pm$ 2.39	0.21 (-4.07, 4.49)	0.92	-0.13 (-5.66, 5.40)	0.96
	Control ($n=34$)	0.17 $\pm$ 2.63	0.74 $\pm$ 1.54	0.40 $\pm$ 2.08				
SDNN (ms)	BHB ($n=31$)	31.62 $\pm$ 1.51	44.66 $\pm$ 1.51	31.62 $\pm$ 1.58	-5.71 (-9.70, -1.71)	<0.01	-0.79 (-4.60, 3.02)	0.68
	Control ($n=34$)	35.48 $\pm$ 1.58	54.95 $\pm$ 1.54	36.30 $\pm$ 1.58				
rMSSD (ms)	BHB ($n=31$)	20.89 $\pm$ 1.73	21.37 $\pm$ 1.62	22.38 $\pm$ 1.73	-4.53 (-8.52, -0.53)	0.02	3.03 (-0.61, 6.67)	0.10
	Control ($n=34$)	23.44 $\pm$ 1.73	28.18 $\pm$ 1.73	21.37 $\pm$ 1.58				
pNN50 (%)	BHB ($n=25$)	3.46 $\pm$ 5.37	5.01 $\pm$ 3.54	4.16 $\pm$ 6.02	0.62 (-6.39, 7.63)	0.86	1.05 (-6.29, 8.39)	0.77
	Control ($n=30$)	5.12 $\pm$ 3.54	5.88 $\pm$ 4.16	4.36 $\pm$ 3.23				

Effect size adjusted for resting values of outcome variables (using ANCOVA); P values in bold indicate $P \leq 0.05$.

BHB = Bee Humming Breathing, LF = Low frequency, HF = High frequency, SDNN = Standard deviation of RR Intervals, rMSSD = Square root of the mean of the sum of the squares of differences between adjacent normal-to-normal (NN) intervals, pNN50 = Proportion derived by dividing NN50 by the total number of NN intervals, SD = Standard deviation, CI = Confidence interval.

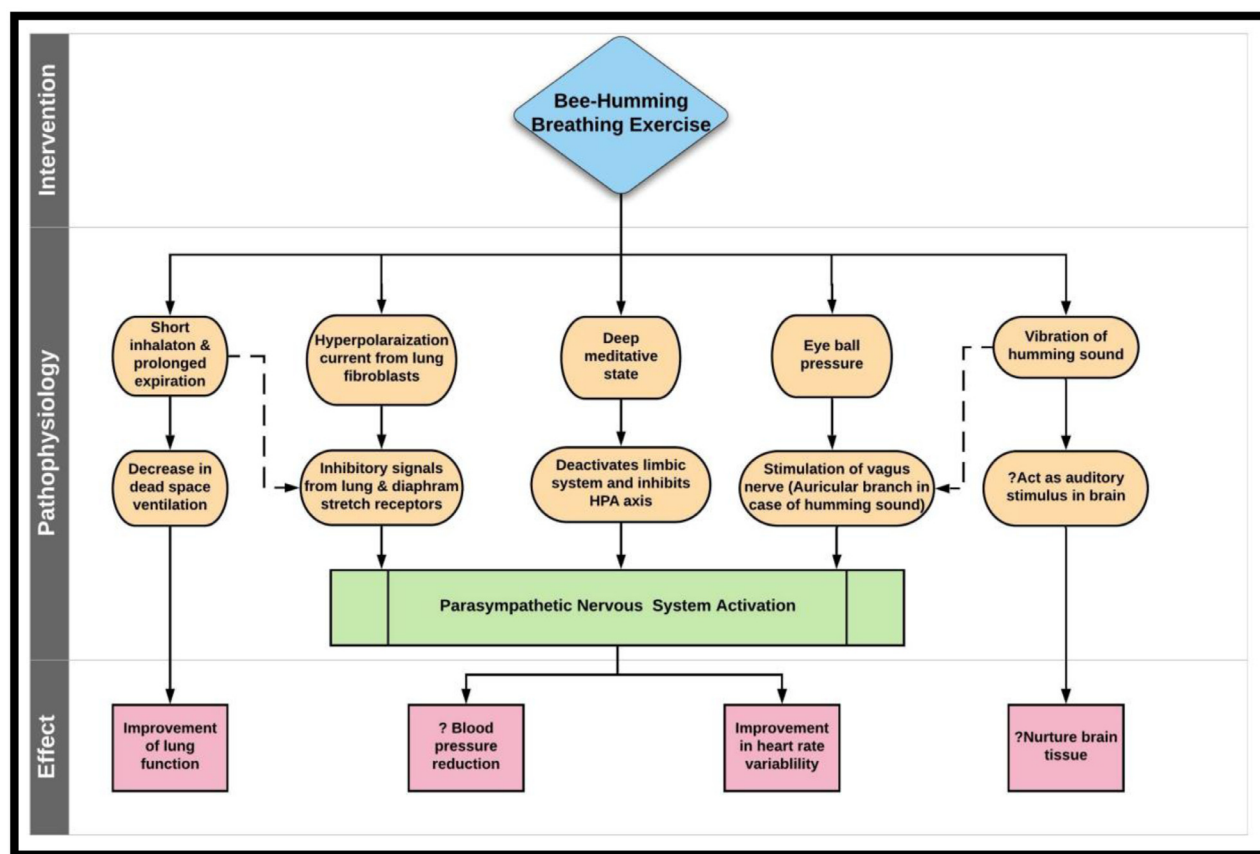


Fig. 3. Flowchart showing the pathophysiological effects of Bee-Humming Breathing (BHB) exercise.

has also been suggested that stretch-induced inhibitory signals from the abdominal muscles (mainly diaphragm) and the nose may also induce the same effect.¹⁵ Secondly, while performing the BHB exercise, subjects shut down their eyes, close the ears with the thumb of both hands, and hold attention at the philosophical third eye point. So, BHB exercise is not only a breathing technique but also a form of meditation technique resulting in a state of deep relaxation and mental refreshment.^{7,16} Third, the practitioner presses the eyeballs with their fingers while doing the BHB exercise. This may lead to stimulation of the vagus nerve tipping the balance toward the parasympathetic nervous system.¹¹ Fourth and the most distinctive factor is the humming sound produced during the practice of BHB exercise. The vibration due to the humming sound acts as an auditory stimulus and leads to changes in the scaling exponents of the raw Electroencephalogram (EEG) data in the temporal and frontal regions of the brain.¹⁶ The slow pranayamic breathing has been seen to increase the amplitude of the theta and delta waves of EEG, indicating parasympathetic activity.¹⁵ In addition, the humming sound may stimulate the auricular branch of the vagus nerve contributing to the parasympathetic nervous system activation.¹⁷ Another theory is that the humming sound of the BHB exercise may act like a metronome to keep the frequency of the respiration close to 10 seconds each cycle (~6/minutes) without concentrating on the breathing rate while doing pranayama. This results in efficient synchronization of the cardiovascular and respiratory rhythmic fluctuation causing significant improvement in the sympathovagal balance.⁶ Bernardi L et al.⁶ have also shown similar mechanism behind the beneficial effects of the 'Ave Maria' prayer and 'Om-mani-padme-om' mantra on autonomic nervous system. However, the exact pathophysiology behind the autonomic regulation of the BHB exercise and its effect on human physiology is still a matter of investigation.

Few studies have tried to evaluate the effect of BHB exercise on blood pressure parameters. Most of them were observational studies and recruited a small number of healthy volunteers. Two studies used cold pressure tests to evaluate the autonomic modulation of the BHB exercise, and one of them recruited pregnant women.^{18,19} Both of the studies showed a reduction in the hyper-reactivity reaction as well as a significant decrease in blood pressure parameters. Pramanik et al.¹⁰ tested the immediate effect of BHB exercise in fifty healthy subjects, and they showed a significant reduction in diastolic and mean pressure. The only randomized control trial done by Kuppusamy et al.⁹ showed a substantial reduction in SBP, DBP, pulse pressure, mean pressure, rate pressure product, and the double product immediately after performing BHB exercise. However, our study did not show any significant change in SBP, DBP, and MBP during or immediately after the BHB exercise in comparison to the control subjects. There are a few factors that might be responsible for this effect. Firstly, the subjects were already taking adequate anti-hypertensive drugs with controlled blood pressure at the baseline. This may have reduced the influence of BHB on blood pressure parameters. Secondly, this was the first study which used ambulatory blood pressure monitoring system in comparison to the previous trials which used periodic manual sphygmomanometric measurement. Thirdly, the control subjects of this study performed slow breathing exercise without the bumble-bee humming sound. This may also have contributed to the insignificant difference between the study groups as pranayama (slow breathing exercise) itself has been shown to modulate the autonomic nervous system and cardiovascular parameters. Fourthly, the study subjects performed a single short session of BHB exercise without any previous training. Whether a regular and long-term practice of BHB exercise is capable of reducing blood pressure parameters over and above the effect of anti-hypertensive medicines needs to be evaluated in future trials.

Heart rate variability (HRV) is another important non-invasive indicator of autonomic nervous system function. Among cardiac patients, increased heart rate variability, as measured by SDNN > 100 ms, has been seen to be associated with low risk of mortality.²⁰ Among the other time-domain HRV components, the increased value of rMSSD and pNN50 indicates parasympathetic dominance even in a short-term measurement of heart rate variability. Frequency domain components like very low frequency (VLF), high frequency (HF), low-frequency power (LF), and log of LF/HF has been used to evaluate the balance between sympathetic and parasympathetic activity. Among them, VLF is not generally reported for short term studies, but the higher value of HF indicates increased parasympathetic nervous system activity. The autonomic influence of low-frequency power (LF) is complex. Although it is classically considered as a measure of sympathetic activity, significant influence of parasympathetic and baroreceptor activity has also been described.^{21,22} Slow breathing techniques like nadisuddhi pranayama have been shown to increase parasympathetic tone in multiple trials.^{23–25} Whereas, the fast breathing type pranayama increases sympathetic activity and decreases the vagal tone.²⁵ Very few studies have analyzed the influence of BHB exercise on heart rate variability. One observational study by MU Sujana et al.²⁵ showed a significant increase in the LF/HF ratio during the BHB exercise. But there was a significant fall in the LF/HF value during the recovery phase suggesting an increased parasympathetic activity. In another small observational trial, there was a significant increase in the heart rate, LF power, and significant decrease in the HF power during the BHB exercise, suggesting parasympathetic withdrawal, which reverted to the normal value during the recovery phase.⁸ In our study, the BHB exercise of five minutes duration resulted in increased parasympathetic activity and decreased sympathetic activity as indicated by a significant increase in the HF power, and decrease in the LF power during the recovery phase in comparison to the control group. Another parameter, rMSSD, also had an insignificant increasing trend supporting parasympathetic dominance during the recovery phase of BHB exercise. Although the influence of the BHB exercise on the other time-domain measures of heart rate variability, i.e., HR, SDNN, pNN50, did not support the above findings in our study. Probably a short duration of analysis resulted in such a mismatch as physiological interpretation is better with frequency-domain parameters than the time-domain parameters during short term HRV analysis.²⁶ Overall, our study strengthens the fact that BHB leads to a significant increase in parasympathetic nervous system activity resulting in a relaxed and mindful state. A significantly higher number of patients in the control arm were taking angiotensin receptor blocker than the BHB arm. Angiotensin receptor blockers (ARB) decrease sympathetic nervous system activity by inhibition of the Renin-Angiotensin-Aldosterone System. Despite that 'advantage' there was an overall decreased sympathetic tone in the BHB group. This further strengthens the beneficial effect of the BHB exercise.

This study is the first randomized control trial to evaluate the immediate effect of BHB exercise on blood pressure in hypertensive patients taking adequate anti-hypertensive medications. Objective assessment and validated devices have been used in this trial to evaluate the ambulatory blood pressure and heart rate variability reducing the chance of error in the data collection. However, there are few limitations to this study. Firstly, it is a single-center study involving a relatively small number of patients. Though the sample size is more than most of the previous trials on this subject, the predictive power of the result would have been better with a larger sample size. Secondly, the participants were naïve to BHB exercise before the study resulting in a high chance of underperformance. Thirdly, analysis of long term BHB exercise instead of a single five-minutes session might have resulted in more clarification on the effectiveness of Bhramari pranayama.

Conclusions

For the first time in an RCT, this study demonstrates that a short single session of bee-humming breathing exercise can lead to a significant increase in the parasympathetic nervous system activity in non-yoga practitioners with essential hypertension taking anti-hypertensive medication. However, the one-time performance of BHB exercise did not result in any significant change in systolic, diastolic, and mean blood pressure.

Author contribution

GS did conceptualization, project administration, funding acquisition, and supervision of the study; GS, NG, AKK, DS participated in project design, investigation, methodology, implementation, data curation, and data analysis; BK, SM, NL participated in project implementation and data curation; NG, GS participated in original draft writing, review, and editing; AA, RMP, NG helped in validation, visualization of resources, and formal analysis; NG, AKK, GS, DS helped with manuscript revision and language editing. All authors read and approved the final manuscript.

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Declaration of Competing Interest

None.

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Supplementary materials

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