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Effect of Pranayama on Anxiety, Depression and Stress Levels in Post-Graduate Students: Correlation with Serum Cortisol and Hemoglobin Levels

Lakshmikant Nagar[1], Chintaharan Betal[2]*, Ishan Chauhan[1] and Prerna Tyagi[1]

[1] *Research Scholar, Department of Naturopathy & Yoga, H.N.B. Garhwal A Central University, Srinagar Garhwal, Uttarakhand, India.*

[2] *Assistence Professor, Department of Naturopathy & Yoga, H.N.B. Garhwal A Central University, Srinagar Garhwal, Uttarakhand, India.*

*E-Mail: ***drcbetal@rediffmail.com***

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Background: Anxiety, depression and stress (ADS) are prevalent mental health disorders among university students due to the demanding nature of their academic pursuits. Pranayama yogic breathing (PYB), a controlled breathing technique has been suggested as a potential intervention to alleviate these psychological maladies. Aim: This study aimed to investigate the effect of PYB practices on ADS levels of post-graduate students (PGSs) while exploring the potential relationships with serum cortisol (SC) and hemoglobin (Hb) levels. Methods: 60 university students aged between 21 to 24 years were randomly assigned into two groups: an experimental group participant (n=30) who practiced PYB for 45 minutes daily for three months and a control group participant (n=30) who did not receive any intervention. ADS levels were assessed using a standardized questionnaire ADSS, while SC levels and Hb were measured using laboratory tests. Data were collected at three states pre (before intervention), mid (after 45 days of intervention) and post (after 90 days of intervention). Data were analyzed using SPSS version 2.5.11 (110) Results: The results demonstrated that PGSs who underwent the PYB intervention exhibited significantly reduced anxiety, depression and stress compared to the control group. Furthermore, the experimental group showed a significant decrease in SC levels and a significant increase in Hb levels after the intervention. Conclusion: PYB practice has been shown to significantly reduced ADS and positively influenced SC and Hb levels in PGSs. Further research is warranted to explore the long-term effects and broader applicability of PYB on diverse populations.

Key words: Pranayama, Anxiety, Depression, Stress, Serum Cortisol, Hemoglobin

University students face numerous challenges and pressures in their academic pursuits due to the demanding nature of academic, social and financial goals, which often contribute to higher levels of Anxiety, Depression and Stress (ADS) (Andrews & Wilding, 2004; Mofatteh, 2020). ADS and other mental illnesses of these students are critical factors that can influence their academic performance and overall quality of life (Andrews & Wilding, 2004; Zada *et al.*, 2021). Anxiety disorders entail ongoing uneasiness and worry, often lacking a clear trigger. In contrast, depression, a mood disorder, leads to emotional responses tied to health problems, affecting daily life with symptoms like sadness, guilt and loss of interest. Stress, which arises from non-compliance with environmental conditions, triggers psychological and biological changes, elevating the risk of illness (Mirzaei *et al.*, 2019).

ADS can significantly impact students' academic performance, leading to concentration problems, memory issues, decreased motivation and poor decision-making. Social withdrawal and substance abuse can worsen these conditions (Mofatteh, 2020; SAMHSA, 2021). Additionally, chronic ADS harm physical health, including headaches, digestive problems, weakened immunity and increased cardiovascular risk (SAMHSA, 2021). University students facing ADS have a higher risk of suicidal thoughts and self-harm (Asif *et al.*, 2020; Martínez-Líbano *et al.*, 2023). A survey was conducted among 500 university students which showed that a significant proportion experienced ADS, with percentages of 88.4%, 75%, and 84.4%, respectively (Asif *et al.*, 2020). In another study involving 1,062 higher education students, a notable prevalence of ADS was observed, with rates of 69.2%, 63.1% and 57%, respectively (Martínez-Líbano *et al.*, 2023).

Pranayama yogic breathing (PYB), a controlled breathing technique rooted in ancient Indian traditions, has emerged as a potential intervention to reduce ADS and achieving overall mental well-being (Zope & Zope, 2013). It is the fourth limb of ‘Ashtanga Yoga’, as outlined in the Yoga Sutras of Patanjali (Bryant, 2015). The term of pranayama originates from Sanskrit words, where "Prana" means breath or life force and "Ayama" means expansion and extension. PYB is the expansion of the life force through control of the breath. It is about extending and expanding the life force (prana) within the practitioner through controlled breathing, leading to heightened energy and awareness (Satyānanda, 2009).

Studies showed that PYB offers various psycho-physiological benefits which is closely tied to nervous system. This practice also contributes to mood enhancement, promoting better sleep and increasing parasympathetic tone (Jayawardena *et al.*, 2020). Therefore, PYB may reduce stress via serum cortisol (SC) regulation, enhancing relaxation through autonomic system balance and potentially improving hemoglobin (Hb) indirectly by boosting oxygen uptake. These interconnected benefits highlight the holistic benefits of PYB (Sengupta, 2012).

MATERIALS AND METHODS

Participants

The study is mainly focused on the Post Graduate Students (PGSs) who have the high ADS score. The participants were recruited from various departments of H.N.B. Garhwal A Central University. A total of 200 subjects were included in this study using the ADSS (Anxiety, Depression and Stress Scale) assessment and 60 participants who met the inclusion criteria were randomly assigned to the experimental group (n=30) and the control group (n=30). The experimental group engaged in PYB practice for three months, with daily practice duration of 45 minutes, excluding Sundays and gazette holidays. The sessions were conducted under the supervision of the researchers. The control group participants did not have any specific tasks and they continued their normal daily routine activities. Data were collected at three time points: Pre (before the intervention), Mid (after 45 days) and Post (after 90 days). Informed written consent was obtained from all participants prior to the study, ensuring their voluntary participation and understanding of the study's purpose and procedures. Figure 1 presents summarized screening, allocation, data collection and analysis of the study.

Inclusion/ Exclusion Criteria

Post Graduate Students (PGSs) with high ADS (Anxiety, Depression and Stress) score and age ranging from 21 to 24 (mean age 22.95±1.15) years with general health status but no prior exposure to PYB practice were included in the study. PGSs with major psychological problems, low ADS and unwillingness to participate in PYB practice were excluded from the study. But the PGSs of both groups were forbidden strictly to take any kind of medicine, counseling, psychotherapy and physical exercise or physiotherapy, so that the result of the treatment variable may not be contaminated and thus the experiment was conducted in a completely controlled condition.

Assignment of Treatment

Three days introductory training was given by the investigators for the experimental group subjects before starting the PYB practices. After completing the pre-test and providing the introductory training, the participants in the experimental group received PYB therapy as presented in Table 1 (Saraswati & Hiti, 1996).

Measurements

The data on ADS of the PGSs were collected by administering the Anxiety Depression Stress Scale (ADSS), developed by Pallavi Bhatnagar of the National Psychological Corporation, Agra (U.P.), Similarly, data on SC level in ug/dl were collected by using Electro Chemi Luminescent Immuno Assay (ECLIA) technique in pathology lab. Blood samples were collected from both the experimental and control groups between 8.00 to 10.00 AM and promptly sent to the laboratory for further estimation. The data on Hb level in gm/dl were collected by using same blood sample.

Data Analysis

Table 4 displayed the Mean ± SD values for ADS, SC and Hb levels of both the experimental and control groups. A repeated-measures analysis of variance (RM-ANOVA) was adopted using SPSS version 2.5.11 (110), considering two factors: groups with two levels (experimental and control) and states (pre, mid and post). Post-hoc analysis with Bonferroni correction were performed to account for multiple comparisons and the Huynh-Feldt epsilon was applied to adjust the degrees of freedom.

RESULTS

Table 2 presents the baseline characteristics of the selected participants, comprising 27 males and 33 females, with age ranging from 21 to 24 years (mean age: 22.95±1.15). At the beginning of the study, there were no statistically significant differences between the groups for all variables. All the 60 subjects successfully completed the study and no adverse events were reported during the intervention period.

The outcomes of the repeated-measures analysis of variance (RM-ANOVA) are as follows:

The results of the repeated-measures analysis of variance (RM-ANOVA) revealed significant differences between the groups for anxiety ($p<0.001$), depression ($p<0.001$), stress ($p<0.001$), serum cortisol (SC) ($p<0.001$) and hemoglobin (Hb) ($p<0.001$). Additionally, significant differences were observed between the states for anxiety ($p<0.001$), depression ($p<0.001$), stress ($p<0.001$), SC ($p<0.001$) and Hb ($p<0.001$). Notably, there were significant interactions between the groups and states for anxiety ($p<0.001$) depression ($p<0.001$), stress ($p<0.001$), SC ($p<0.001$) and Hb ($p<0.001$), indicating interdependence among these variables. Specific values for F, df, Huynh-Feldt epsilon and p-values can be found in Table 3.

Post hoc analysis within-groups yielded the following findings:

(i) In the experimental group, there were significantly lower anxiety scores from the pre to mid-state ($p<0.001$, 95% CI [0.83, 1.90]) and from the pre- to post-state ($p<0.001$, 95% CI [2.16, 3.63]). However, no significant difference was observed in the control group.

(ii) The experimental group displayed significantly lower depressions scores from the pre to mid-state ($p=0.001$, 95% CI [0.38, 1.61]) and from the pre- to post-state ($p<0.001$, 95% CI [2.49, 3.70]). Conversely, no significant difference was found in the control group.

(iii) The stress scores were significantly lower in the experimental group from the pre to mid-state ($p=0.001$, 95% CI [0.43, 1.90]) and from the pre- to post-state ($p<0.001$, 95% CI [2.41, 4.05]), while no significant difference was observed in the control group.

(iv) The experimental group displayed significantly lower SC levels from the pre to mid-state (P<0.001, 95% CI [1.51, 2.49]) and from the pre- to post-state (P<0.001, 95% CI [4.17, 5.35]). Conversely, no significant difference was found in the control group.

(v) Similarly, the Hb levels were significantly higher in the experimental group from the pre to mid-state (P<0.001, 95% CI [-1.07, -0.57]) and from the pre- to post-state (P<0.001, 95% CI [-2.72, -2.24]), while no significant difference was observed in the control group.

Post hoc analysis between groups revealed the following findings:

(i) The experimental group displayed significantly lower anxiety scores as compared to the control group at mid-state (P<0.001, 95% CI [-2.23, -0.76]) and post-state (P<0.001, 95% CI [-3.78, -2.47]). However, there was no significant difference between the groups at pre-state.

(ii) The experimental group exhibited significantly lower depression scores as compared to the control group at the mid-state (P=0.001, 95% CI [-1.83, -0.49]) and post-state (P<0.001, 95% CI [-4.03, -2.56]). Conversely, there were no significant differences between the groups at pre-state.

(iii) The stress scores were significantly lower in the experimental group compared to the control group at the mid-state (P=0.01, 95% CI [-1.97, -0.36]) and post-state (P<0.001, 95% CI [-4.08, -2.72]). However, there were no significant differences between the groups at pre-state.

(iv) The experimental group exhibited significantly lower SC levels as compared to the control group at the mid-state (P<0.001, 95% CI [-2.72, -1.17]) and post-state (P<0.001, 95% CI [-5.19, -3.75]). Conversely, there were no significant differences between the groups at pre-state.

(v) Similarly, the Hb levels were significantly higher in the experimental group compared to the control group at the mid-state (P<0.001, 95% CI [0.47, 1.59]) and post-state (P<0.001, 95% CI [2.18, 3.34]). However, there were no significant differences between the groups at pre-state.

The post hoc comparisons within groups and between groups are presented in Table 4 and graphical presentations can be found in Figure 2.

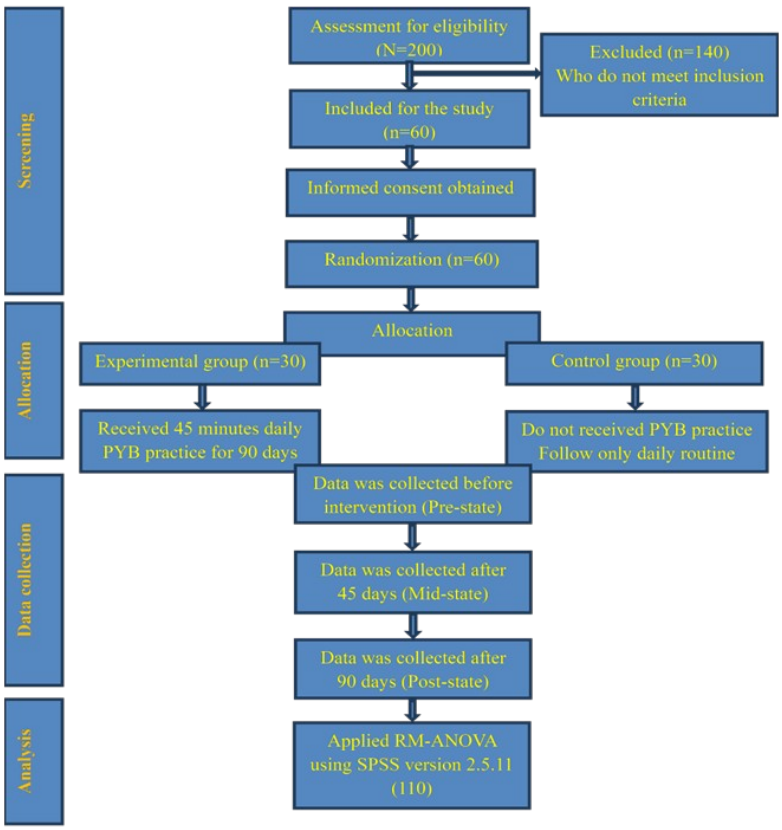

Figure 1. Flow chart summarized screening, allocation, data collection and analysis.

Table 1: Assignment of pranayama yogic breathing (PYB) in experimental group.

S.no	Name of PYB practice	Duration (Minutes)	Method	Benefits
1	Sahaja PYB	05	Observe natural breath without controlling it, shifting awareness through different areas	Increases mindfulness, concentration, creativity, energy levels, inner peace, thought control, emotional stability
2	Anuloma-viloma PYB	10	Alternate nostril breathing with equal inhalation and exhalation	Balances breath and brain hemispheres, calms anxiety, heals cardiovascular and nervous disorders
3	Bhramari PYB	10	Humming sound while breathing, focus on ajna chakra	Relieves stress, anger, anxiety, insomnia, enhances healing capacity, strengthens voice,
4	Ujjayi PYB	10	Breathing through the throat with a soft snoring-like sound, slow and deep breaths	Tranquilizing practice, soothes nervous system, calms the mind, cure insomnia, benefits in high blood pressure
5	Bhastrika PYB	10	Forceful inhalation and exhalation with diaphragm and abdomen movements	Burns toxins, aids women during labor, increases oxygen exchange and metabolism, strengthens the nervous system

Table 2: Baseline characteristics of post-graduate students (PGSs) (n=60).

		Experimental	**Control**	**Total**
Age Group (mean±SD)		22.93 (1.04)	22.96 (1.27)	22.95 (1.15)
Gender (%)	Male	16 (53.33)	11 (36.66)	27 (45)
	Female	14 (46.66)	19 (63.33)	33 (55)
Anxiety		**Pre (mean±SD)**	**Pre (mean±SD)**	
Depression		11.90(1.21)	12.27(1.23)	
Stress		11.13(1.30)	11.17(1.20)	
SC (µg/dl)		10.50(1.33)	10.60(1.35)	
Hb (gm/dl)		23.17(1.80)	22.85(1.59)	
		11.57(1.03)	11.15(1.05)	

Note; SC-Serum Cortisol; Hb -Hemoglobin

Table 3: Summary of Analysis of Variance (RM ANOVA) of Psycho-Physiological Variables.

Variables	**Factors**	**F(df)**	**Huynh-Feldt (ε)**	**P**	**Partial Eta Squared (ηp2)**
ADSS					
Anxiety	Group	40.67(1, 58)	0.935	<0.001	0.41
	States	33.32(1.87, 108.47)	0.935	<0.001	0.36
	Group X States	27.99(1,58 X 1.87,108.47)	0.935	<0.001	0.32
Depression	Group	30.63(1, 58)	1.000	<0.001	0.34
	States	34.26(2.00, 116.00)	1.000	<0.001	0.37
	Group X States	41.50(1,58 X 2.00,116.00)	1.000	<0.001	0.41
Stress	Group	38.34(1, 58)	1.000	<0.001	0.39
	States	24.46(2.00, 116.00)	1.000	<0.001	0.29
	Group X States	27.30(1,58 X 2.00,116.00)	1.000	<0.001	0.32
SC	Group	32.35(1, 58)	0.983	<0.001	0.35
	States	126.37(1.96, 113.99)	0.983	<0.001	0.68
	Group X States	126.89(1,58 X 1.96,113.99)	0.983	<0.001	0.68
Hb	Group	28.07(1, 58)	1.000	<0.001	0.32
	States	161.49(2.00, 116.00)	1.000	<0.001	0.73
	Group X States	136.71(1,58 X 2.00,116.00)	1.000	<0.001	0.70

Note; ADSS- Anxiety, depression, Stress Scale, SC-Serum Cortisol; Hb -Hemoglobin

Table 4: Results of Outcome Measures at Pre, Mid and Post of Experimental and Control Groups. Values are Given in Mean and Standard Deviation.

Variables	Experimental Group (n=30)			Control Group (n=30)		
	Pre	Mid	Post	Pre	Mid	Post
ADSS						
Anxiety	11.90(1.21)	10.53(1.54) ***$$$	9.00(1.23) ***$$$	12.27(1.23)	12.03(1.29)	12.13(1.30)
Depression	11.13(1.30)	10.13(1.30) ***$$$	8.03(1.49) ***$$$	11.17(1.20)	11.30(1.29)	11.33(1.34)
Stress	10.50(1.33)	9.33(1.66) ***$$	7.27(1.31) ***$$$	10.60(1.35)	10.50(1.43)	10.67(1.32)
SC (ug/dl)	23.17(1.80)	21.16(1.33) ***$$$	18.40(1.28) ***$$$	22.85(1.59)	23.11(1.66)	22.88(1.49)
Hb (gm/dl)	11.57(1.03)	12.39(1.05) ***$$$	14.05(1.07) ***$$$	11.15(1.05)	11.36(1.10)	11.29(1.15)

Note; ADSS- Anxiety, depression, Stress Scale, SC-Serum Cortisol; Hb -Hemoglobin

*Note; (***=P<0.001, **=P<0.01, *=P<0.05) Based on Bonferroni adjusted Post hoc analysis when Mid and Post States were compared with respective Pre-value. ($$$=P<0.001, $$=P<0.01, $=P<0.05) Based on Bonferroni adjusted Post hoc analysis when Pre, Mid and Post States of the Experimental Group were compared with the respective States of the Control Group.*

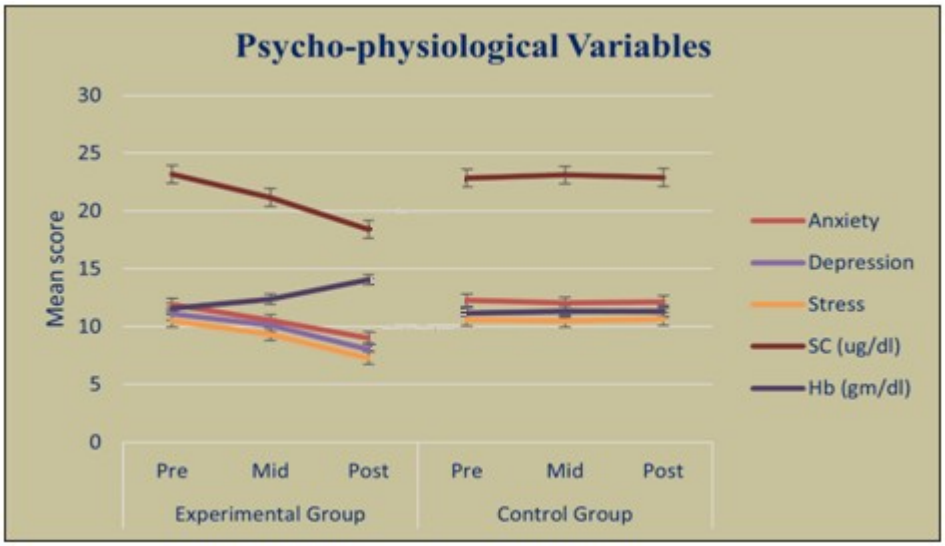

Figure 2. Graphical presentation of psycho-physiological variables.

DISCUSSION

The findings of this study indicate that the experimental group, which received the pranayama yogic breathing (PYB), exhibited significant improvements in psycho-physiological variables compared to the control group. Specifically, the experimental group showed lower anxiety, depression and stress (ADS) scores as well as lower serum cortisol (SC) levels and higher hemoglobin (Hb) levels. These results suggest that the intervention had a positive impact on the psycho-physiological well-being of the Post Graduate Students (PGSs).

PYB practice reduced the sympathetic activity, which is associated with the "fight or flight" response which helped in reducing ADS (Sengupta, 2012). Voluntary slow pranayamic breathing involves stretching tissues

during inhalation and retention of breath, generating inhibitory signals and hyperpolarizing currents. These signals synchronize neural elements, shifting the autonomic balance towards parasympathetic dominance and slowing down the physiological processes (Jerath *et al.*, 2006; Zaccaro *et al.*, 2018). Fast PYB like Bhastrika probably impacts brain regions tied to emotions (amygdala, cingulate, insula, prefrontal cortex) and reduces connectivity in the anterior insula and prefrontal cortex with changes in this connectivity linked to anxiety levels (Novaes *et al.*, 2020). Regular practice of PYB and yoga has been shown to down-regulate the HPA (Hypothalamus-Pituitary-Adrenal) axis and the sympathetic nervous system (SNS) response to stress (Sengupta, 2012). This can have a positive impact on overall health. PYB practice helped in decreasing the levels of salivary cortisol, a hormone produced by the adrenal glands that is involved in the stress response which is similar to the results of another studies (Sengupta, 2012; Sharma *et al.*, 2016; Maheshkumar *et al.*, 2022). Bhramari PYB, in particular has been found to improve adaptive capability to stress, as indicated by the initial rise in salivary cortisol response (Maheshkumar *et al.*, 2022). By regulating the functions of HPA axis and cortisol levels, PYB practice contributed to stress management and improve overall well-being of the practitioners. Furthermore, the significant increase in Hb levels in the experimental group suggests improved blood circulation, O_2 and improved cardiovascular functions because a specific part of Hb plays a crucial role in increasing blood flow when tissues don't get enough O2 (Premont & Stamler, 2020). Overall, the findings of this study provide evidence for the effectiveness of the intervention in improving both psychological as well as physiological well-being in PGSs. The reductions in ADS along with the lower SC levels and higher Hb levels indicate positive outcomes associated with PYB practice.

The reduction in ADS scores in the experimental group of present study aligns with previous researches. Jagadeesan *et al.* (2022) found that Bhramari PYB reduced ADS scores significantly ($p<0.05$) in COVID-19 patients in home isolation. Similarly, Shah & Kothari (2019) demonstrated that Nadi-shodhana PYB, along with conventional physiotherapy, improved lung functions and lowered ADS levels significantly ($p<0.01$) in post CABG patients. Other studies also support the positive effects of PYB on lowering ADS (Brown & Gerbarg, 2005; Chandrababu *et al.*, 2019). Maheshkumar *et al.* (2022) found that yoga participants had higher salivary cortisol responses initially but showed reduced levels ($p=0.03$) over time due to practice of PYB. Similarly, other studies back up our findings in positive effect of PYB on cortisol (Abedi Amiri *et al.*, 2018; Balaji & Varne, 2017; Kamei *et al.*, 2000; Ma *et al.*, 2017). Sahu and Kishore (2015) found significant impacts ($p<0.001$) of Bhramari PYB and Jyoti Dhyan on Hb levels and alpha EEG in college students, suggesting improved blood circulation and oxygen delivery.

LIMITATION OF THE STUDY

The research was carried out with a limited sample size within a single university campus located in H.N.B. Garhwal A Central University. ADS patterns could potentially vary based on geographical location and institutional management and social structure also. The second limitation is that the study is confined only to post graduate students' population. Therefore, the limited sample size restricts the generalizing outcomes to broader section of populations. Circadian cortisol variations further limit generalizability. Thirdly, research is confined to ADS, SC and Hb like psycho-physiological parameters among postgraduates, excluding age, gender and diverse exercises' effects. Fourth limitation is that the inadequate physiological assessment hinders direct correlation between ADS and serum cortisol and hemoglobin levels. Therefore, more substantial studies are needed for conclusive insights into pranayama's impact, including larger samples and encompassing yoga asanas, meditation and other yogic practices.

CONCLUSION

In fine, it can be stated that this study confirmed the positive impact of pranayama yogic breathing (PYB) on postgraduate students' well-being. PYB led to lower anxiety, depression and stress (ADS) scores, reduced cortisol levels and increased hemoglobin (Hb) levels. These results align with previous research and highlighted PYB's potential in enhancing mental and physical health. Therefore, Pranayama Yogic Breathing

(PYB) can be suggested as a most promising and potential means of alleviating ADS in Post Graduate students. However, further research is needed to explore its long-term effects and applicability in different populations, underscoring its value as a tool for well-being promotion.

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CONFLICTS OF INTEREST

The authors declares that they have no potential conflicts of interest.

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