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Hatha Yoga is Associated with Improved Sleep Quality and Reduced Anxiety in Female Medical Students: A Quasi-Experimental Study

Abstract

Study purpose. Poor sleep and psychological stress are common among university students and may be linked to disruptions in circadian and neuroendocrine regulation. Mind–body interventions such as yoga have gained increasing attention as complementary approaches to improve sleep and emotional well-being. However, evidence linking yoga practice with endogenous melatonin regulation remains limited.

Materials and methods. This study involved 30 female medical students and used a one-group pretest–posttest quasi-experimental design with baseline anxiety scores ≥14 on the Hamilton Anxiety Rating Scale (HARS). Participants performed 30-minute Hatha Yoga sessions three times per week for four weeks. Salivary melatonin was analyzed from 22:00 saliva samples using ELISA, and sleep quality was measured with the Pittsburgh Sleep Quality Index (PSQI).

Results. Changes from baseline to post-intervention were tested using paired t-tests for normally distributed variables and Wilcoxon signed-rank tests for variables that did not meet the assumptions of normality. Salivary melatonin levels increased significantly after the intervention (18.72 ± 2.24 pg/mL vs. 22.30 ± 3.95 pg/mL; $p = 0.0001$). Mean anxiety scores decreased from 33.47 ± 7.29 to 19.57 ± 11.33 ($p < 0.001$). Sleep quality also improved, with PSQI scores decreasing from 13.97 ± 2.30 to 5.23 ± 2.76 ($p < 0.001$). After the intervention, 73.3% of participants achieved good sleep quality (PSQI < 5), whereas all participants had poor sleep quality at baseline.

Conclusions. A 4-week Hatha Yoga program was associated with improved subjective sleep quality. It increased nocturnal melatonin levels among female medical students, suggesting potential benefits of mind–body practice for sleep regulation in young adults under academic stress.

Introduction

Sleep is a fundamental biological process required for cognitive performance, emotional regulation, metabolic balance, and physiological recovery. Sleep quality is closely related to learning, emotional regulation, and daily functioning (Fayad et al., 2021). Sleep problems are often reported by young adults and can affect how they learn, manage emotions, and function during the day. This concern is especially important in medical students, who commonly face long study periods, frequent examinations, and limited time to rest. Studies on sleep health show that poor sleep quality is common in adults, although the proportion varies between populations and study settings. (Simonelli et al., 2018).

For medical students, this problem may be more pronounced because academic routines often involve long study hours, examination pressure, clinical responsibilities, and limited recovery time.

These academic demands may increase psychological stress and contribute to anxiety symptoms, which often occur alongside poor sleep. This concern is supported by evidence showing that depressive symptoms are more common among medical students than among age-matched peers in other academic fields (Shafiee et al., 2024). Anxiety is also a major global mental health problem and is reported more frequently among women (Javaid et al., 2023). Therefore, female medical students are a relevant group for studying the overlap among anxiety, sleep quality, and biological markers of circadian regulation.

Anxiety and poor sleep often reinforce each other. Students with anxiety may have difficulty falling asleep or maintaining sleep, while repeated sleep disruption may worsen next-day emotional regulation and increase vulnerability to further anxiety symptoms.(Gould et al., 2015). Repeated sleep problems may affect concentration, memory, academic performance, and the ability to cope with stress (Guan et al., 2024). Although stress-related pathways such as autonomic activity and the HPA axis may be involved, the present study focused specifically on sleep quality, anxiety symptoms, and salivary melatonin rather than measuring these mechanisms directly (Tafet & Nemeroff, 2020).

Melatonin was included as a biological marker related to nocturnal circadian regulation. (Vasey et al., 2021) Its secretion normally increases in the evening and is sensitive to circadian timing and environmental light exposure. (Pévet, 2016). Melatonin is not limited to sleep–wake regulation; it has also been related to anxiety, oxidative stress, and immune responses, making it relevant to the interaction between sleep, stress, and emotional health (Muñoz-Jurado et al., 2022; Reiter et al., 2016; Repova et al., 2022). In students with anxiety and poor sleep, salivary melatonin measurement may provide additional information beyond subjective questionnaires. Previous studies have linked altered melatonin secretion with sleep disturbance and stress-related physiological changes, although a single nocturnal measurement cannot fully represent the complete circadian profile (Coles et al., 2015; Poza et al., 2022).

For students who struggle with both stress and poor sleep, a non-drug approach may be helpful because it is relatively easy to apply and does not add medication use. Yoga was chosen in this study because it brings together simple body movements, breathing exercises, and relaxation. Earlier studies have shown that yoga practice can be followed by better perceived sleep and lower stress or anxiety, although the reasons behind these changes are still not fully clear.(Szaszkó et al., 2023).

However, studies that assess anxiety, sleep quality, and salivary melatonin together in medical students are still limited. This is important because questionnaire scores alone may not fully reflect changes related to circadian regulation. For this initial study, we used a one-group pretest–posttest design rather than a randomized controlled design because the study was conducted during an active academic semester and was intended as an exploratory intervention. Therefore, the results should be viewed as preliminary findings that need confirmation in future controlled studies.

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Study participants

This quasi-experimental study used a one-group pretest–posttest design and included 30 female medical students aged 17–25 years from the Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia. Participants were recruited using consecutive sampling. The sample size was estimated a priori using G*Power 3.1 software. Based on an anticipated large effect size (Cohen's d = 0.8), a two-tailed alpha of 0.05, and a desired statistical power of 0.80 for a paired-samples design, the minimum required sample was 27 participants. Thirty participants were recruited to provide a buffer against potential attrition, yielding an achieved power exceeding 0.85.

Participants were eligible if they were female medical students aged 17–25 years, had a baseline HARS score of 14 or higher, had never practiced yoga before, and agreed to participate by providing written informed consent. Students were excluded if they had musculoskeletal or respiratory conditions that could limit yoga practice, were taking medications that affect the central nervous system, had a previous diagnosis of psychiatric disorder, or consumed alcohol during the study period.

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This study examined changes in sleep quality, anxiety, and salivary melatonin after a 4-week Hatha Yoga program. At baseline, participants completed the Hamilton Anxiety Rating Scale (HARS) and the Pittsburgh Sleep Quality Index (PSQI), followed by saliva collection at 22:00 for melatonin analysis. Salivary melatonin was measured using a commercially available enzyme-linked immunosorbent assay kit (IBL International, Hamburg, Germany; catalog no. RE79001). The reported assay sensitivity was 0.5 pg/mL, with intra-assay and inter-assay coefficients of variation of <10% and <15%, respectively. Saliva samples were collected by passive drool into pre-chilled polypropylene tubes, stored at −80°C, and analyzed in duplicate.

To ensure consistency in melatonin measurements, participants received standardized instructions before each sampling day. They were asked to avoid caffeinated drinks after 14:00, limit bright light and screen exposure for at least 60 minutes before the 22:00 collection, maintain a regular sleep–wake schedule, and avoid vigorous exercise and food intake for at least two hours before sampling. Compliance was monitored using self-report diaries, which were reviewed during follow-up.

The intervention consisted of 30-minute Hatha Yoga sessions, three times per week, for four weeks. Each session included five minutes of breathing practice, 20 minutes of guided postures, and five minutes of relaxation. The breathing component included Nadi Shodhana and slow diaphragmatic breathing. The posture sequence included Tadasana, Marjaryasana–Bitilasana, Balasana, Uttanasana, Viparita Karani, and Supta Baddha Konasana. Relaxation was performed using guided Yoga Nidra. The same sequence was used throughout the study. Sessions were delivered via live video instruction by a certified Hatha Yoga instructor, and participants were advised to practice in a quiet, dimly lit room.

After the 4-week intervention, HARS and PSQI assessments were repeated, and saliva was collected again at 22:00 using the same procedure.

Statistical analysis

Data distribution was assessed using the Shapiro–Wilk test. Continuous variables are shown as mean ± standard deviation. Baseline and post-intervention values were compared within the same participants. Normally distributed variables were analyzed with paired t-tests, while variables with non-normal distribution were analyzed with Wilcoxon signed-rank tests. Statistical significance was set at $p < 0.05$.

Effect sizes were included to help interpret the size of the observed changes. Cohen's d was used for paired t-test outcomes, and r was used for Wilcoxon outcomes. The effect sizes were interpreted as small, moderate, or large using conventional cut-off values. Ninety-five percent confidence intervals were calculated for mean differences when appropriate. No participant withdrew from the study, and no missing outcome data were found.

Ethical clearance was granted by the Research Ethics Committee of the Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia (No. 115/KEP/V/2023). All participants gave written informed consent before joining the study.

Result

A total of 30 female medical students met the inclusion criteria and completed the 4-week Hatha Yoga intervention. All participants underwent baseline and post-intervention assessments of salivary melatonin concentration, anxiety severity (Hamilton Anxiety Rating Scale [HARS]), and sleep quality (Pittsburgh Sleep Quality Index [PSQI]). No missing data were identified for any outcome variable. All 30 participants completed baseline and post-

intervention assessments and were included in the final analysis; therefore, no data imputation was performed.

Salivary Melatonin

Baseline salivary melatonin concentration showed a mean value of 18.72 ± 2.24 pg/mL (range 15.69–27.21). Following the 4-week Hatha Yoga intervention, the mean melatonin concentration increased to 22.30 ± 3.95 pg/mL (range 16.89–34.71), representing a 3.58 pg/mL increase.

The melatonin values did not meet the normality assumption based on the Shapiro–Wilk test ($p < 0.05$). Therefore, the change between baseline and post-intervention melatonin levels was tested using the Wilcoxon signed-rank test. The increase in salivary melatonin levels was statistically significant, with a large effect size ($p = 0.0001$; $r = 0.70$; 95% CI for mean difference: 2.69–4.47 pg/mL) in table 1.

Table 1. Changes in Melatonin Levels, Anxiety Scores, and Sleep Quality After a 4-Week Hatha Yoga Intervention (n = 30)

Variable	Before intervention Mean ± SD	After intervention Mean ± SD	Statistical test	p-value
Salivary melatonin (pg/mL)	18.72 ± 2.24	22.30 ± 3.95	Wilcoxon signed-rank	0.0001
Anxiety (HARS score)	33.47 ± 7.29	19.57 ± 11.33	Paired t-test	<0.001
Sleep quality (PSQI score)	13.97 ± 2.30	5.23 ± 2.76	Paired t-test	<0.001

HARS: Hamilton Anxiety Rating Scale; PSQI: Pittsburgh Sleep Quality Index.

Anxiety

At baseline, anxiety severity among participants was predominantly moderate to severe. Based on HARS classification, 16.7% (n = 5) of participants had moderate anxiety, 70.0% (n = 21) had severe anxiety, and 13.3% (n = 4) had very severe anxiety. No participants were classified as having mild or no anxiety because the inclusion criterion required HARS scores ≥14.

After the intervention, a marked shift toward lower anxiety severity was observed. Post-intervention assessment showed that 26.7% (n = 8) had no anxiety, 23.3% (n = 7) mild anxiety, 26.7% (n = 8) moderate anxiety, 20.0% (n = 6) severe anxiety, and 3.3% (n = 1) very severe anxiety Table 2. The mean HARS score decreased from 33.47 ± 7.29 at baseline to 19.57 ± 11.33 after the intervention, corresponding to a mean reduction of 13.90 points. Shapiro–Wilk testing indicated that HARS scores were normally distributed ($p > 0.05$). Paired t-test analysis showed a significant decrease in anxiety severity after the yoga program, with a large effect size ($p < 0.001$; Cohen's $d = 1.47$; 95% CI for mean difference: 10.72–17.08 points) in table 1.

Table 2. Distribution of Anxiety Severity Before and After the Hatha Yoga Intervention

Anxiety category	Before intervention n (%)	After intervention n (%)
No anxiety	0 (0)	8 (26.7)
Mild anxiety	0 (0)	7 (23.3)
Moderate anxiety	5 (16.7)	8 (26.7)
Severe anxiety	21 (70.0)	6 (20.0)

Anxiety category	Before intervention n (%)	After intervention n (%)
Very severe anxiety	4 (13.3)	1 (3.3)

HARS: Hamilton Anxiety Rating Scale.

Sleep Quality

At baseline, all participants (100%) were classified as having poor sleep quality (PSQI >5). The mean PSQI score before the intervention was 13.97 ± 2.30 (range 10–19), indicating markedly impaired sleep quality.

Following the 4-week intervention, the mean PSQI score decreased to 5.23 ± 2.76 (range 1–14), representing a mean reduction of 8.74 points. Post-intervention classification showed that 73.3% (n = 22) of participants achieved good sleep quality (PSQI < 5), while 26.7% (n = 8) remained in the poor sleep quality category Table 3.

Normality testing confirmed that PSQI scores were normally distributed ($p > 0.05$). Paired t-test analysis demonstrated a statistically significant improvement in sleep quality after the intervention, with a very large effect size ($p < 0.001$; Cohen's $d = 3.50$; 95% CI for mean difference: 7.72–9.76 points) Table 1. All 30 participants provided complete data at both time points; no missing data or attrition occurred.

Table 3. Changes in Sleep Quality Classification Before and After the Hatha Yoga Intervention

Sleep quality	Before intervention n (%)	After intervention n (%)
Good sleep quality (PSQI <5)	0 (0)	22 (73.3)
Poor sleep quality (PSQI ≥5)	30 (100)	8 (26.7)

PSQI: Pittsburgh Sleep Quality Index.

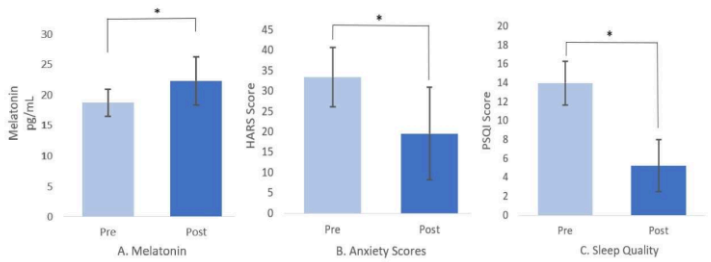

Figure 1. Changes in biological and psychological outcomes following the 4-week Hatha Yoga intervention.

(A) Increase in nocturnal salivary melatonin concentration after the intervention.
(B) Reduction in anxiety scores.
(C) Improvement in sleep quality as reflected by lower PSQI scores.

Data are presented as mean ± SD. *p < 0.05 compared with baseline.

Discussion

The present study examined changes in salivary melatonin concentration, anxiety severity, and sleep quality following a 4-week Hatha Yoga program among female medical students. The main findings were that salivary melatonin levels increased, anxiety scores decreased, and subjective sleep quality improved after the intervention. Given the quasi-experimental one-group pretest–posttest design, these findings should be interpreted as associations observed after the intervention rather than evidence of definitive causality.

The increase in salivary melatonin concentration is an important finding because melatonin is closely involved in circadian rhythm regulation and sleep–wake timing (Le et al., 2025; Waly & Hallworth, 2015) Melatonin is secreted by the pineal gland under circadian control and typically rises during the evening to facilitate sleep onset (Spinedi & Cardinali, 2019; Vasey et al., 2021) Previous studies have linked altered melatonin secretion with sleep disturbance, circadian misalignment, and stress-related physiological dysregulation (Poza et al., 2022; Repova et al., 2022) In the present study, the higher post-intervention melatonin level may indicate a favorable shift in nocturnal circadian regulation. However, because only salivary melatonin was measured, and markers such as cortisol, heart rate variability, salivary alpha-amylase, or objective circadian phase markers were not assessed, the underlying physiological pathway cannot be confirmed.

Anxiety severity also decreased after the 4-week Hatha Yoga program. This result is important because anxiety and poor sleep often occur together in students facing academic pressure. Anxious students may find it harder to relax at night, and repeated poor sleep can make mood regulation more difficult the next day (He et al., 2023; Wang & Chia, 2024). In this study, the decrease in HARS scores may have contributed to the improvement in PSQI scores. However, this interpretation should be made carefully. Since the study did not include a control group, the observed changes cannot be attributed only to Hatha Yoga. Expectation effects, natural symptom fluctuation, changes in academic workload, regression to the mean, or increased attention during the study may also have influenced the results.

Sleep quality improved substantially, as reflected by the reduction in PSQI scores and the shift of many participants from poor to good sleep quality. This result is consistent with previous reports suggesting that yoga and other mind–body practices may improve subjective sleep outcomes. However, the present study relied on PSQI as a self-reported measure and did not include objective sleep assessment such as actigraphy or polysomnography. Therefore, the findings should be interpreted as improvement in perceived sleep quality rather than confirmed changes in sleep architecture or sleep efficiency.

The present findings are generally consistent with previous studies reporting beneficial effects of yoga or meditation on melatonin regulation and sleep-related outcomes. A meta-analysis reported a moderate overall effect of yogic techniques on melatonin levels, and earlier experimental studies suggested that meditation or regular yoga practice may be associated with increased endogenous melatonin secretion (Govindasamy et al., 2025). However, the evidence is not uniformly positive. For example, Szaszkó et al. (2023) found that an 8-week Hatha Yoga intervention reduced self-reported stress but did not significantly improve state or trait anxiety (Szaszkó et al., 2023). These differences across studies suggest that yoga may not produce the same response in all groups. The results may depend on who participates, how long the intervention lasts, how regularly participants practice, the severity of symptoms at baseline, the outcomes measured, and the overall study design. This is why the present findings should be interpreted with caution and further tested in controlled studies.

One strength of this study is that it combined psychological outcomes with salivary melatonin measurement. Many yoga studies focus mainly on questionnaires, whereas this study

included both perceived changes in sleep and anxiety and a biological marker related to circadian regulation. Still, the biological explanation remains tentative. Hatha Yoga may support sleep and anxiety through relaxation, breathing practice, reduced arousal, or more regular bedtime routines, but these pathways were not measured directly. Therefore, mechanisms involving autonomic activity, the HPA axis, or pineal melatonin regulation should be viewed as possible explanations rather than confirmed findings from this study.

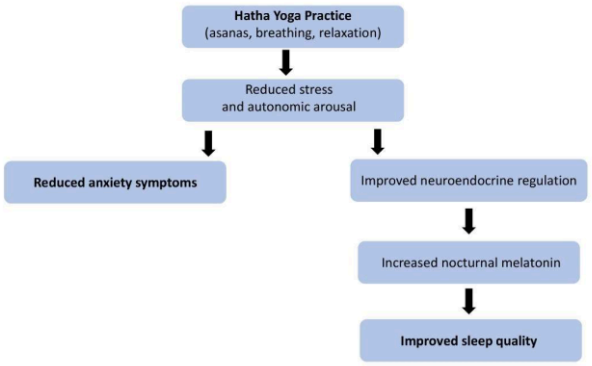

Figure 2. Proposed psychoneuroendocrine pathway linking Hatha Yoga with improved sleep quality.

These findings need to be read with some caution. The study did not use randomization or a control group, so the changes seen after the intervention cannot be linked to the yoga program with certainty. The sample was also small and limited to female medical students, which means the results may not apply to male students or to other student populations. Melatonin was measured only once at night, so the study could not describe the full daily pattern of melatonin secretion. Some factors that may affect sleep and melatonin, including evening light exposure, screen use, caffeine intake, sleep habits, menstrual cycle phase, and academic workload, were not objectively monitored. Adherence to the study instructions was also based on participants' self-reports, which may be affected by recall bias or the desire to give expected answers. In addition, because participants knew they were receiving a yoga intervention, expectation effects may have contributed to the reported improvements. Finally, sleep quality and anxiety were assessed using PSQI and HARS, without objective sleep measurements or physiological markers such as actigraphy, polysomnography, cortisol, or heart rate variability.

Within these limitations, the study shows that better sleep quality, lower anxiety severity, and higher salivary melatonin levels were observed after a 4-week Hatha Yoga program in female medical students. These results suggest that yoga may be a feasible non-pharmacological practice to support student well-being, but they should be confirmed through randomized controlled studies with larger samples, objective sleep assessment, repeated melatonin measurements, and direct markers of autonomic or HPA-axis activity.

Conclusion

In conclusion, salivary melatonin levels, anxiety severity, and sleep quality improved after a 4-week Hatha Yoga program among female medical students. However, the one-group pretest–posttest design without randomization or a control group limits causal interpretation. Therefore, these findings should be regarded as preliminary and require confirmation in controlled studies. Hatha Yoga may be a feasible non-pharmacological practice to support sleep and psychological well-being in academically stressed young adults. Future randomized controlled studies with larger and more diverse samples, objective sleep measurements, and repeated hormonal assessments are needed to confirm these findings and clarify the underlying mechanisms

Acknowledgment

The authors are grateful to all participants for their time and willingness to take part in this study. We also thank the Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia, for supporting the research process.

Conflict of interest

The authors declare that they have no conflicts of interest related to this study.

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This study examined changes in sleep quality, anxiety, and salivary melatonin after a 4-week Hatha Yoga program. At baseline, participants completed the Hamilton Anxiety Rating Scale (HARS) and the Pittsburgh Sleep Quality Index (PSQI), followed by saliva collection at 22:00 for melatonin analysis. Salivary melatonin was measured using a commercially available enzyme-linked immunosorbent assay kit (IBL International, Hamburg, Germany; catalog no. RE79001). The reported assay sensitivity was 0.5 pg/mL, with intra-assay and inter-assay coefficients of variation of <10% and <15%, respectively. Saliva samples were collected by passive drool into pre-chilled polypropylene tubes, stored at −80°C, and analyzed in duplicate.

To ensure consistency in melatonin measurements, participants received standardized instructions before each sampling day. They were asked to avoid caffeinated drinks after 14:00, limit bright light and screen exposure for at least 60 minutes before the 22:00 collection, maintain a regular sleep–wake schedule, and avoid vigorous exercise and food intake for at least two hours before sampling. Compliance was monitored using self-report diaries, which were reviewed during follow-up.

The intervention consisted of 30-minute Hatha Yoga sessions, three times per week, for four weeks. Each session included five minutes of breathing practice, 20 minutes of guided postures, and five minutes of relaxation. The breathing component included Nadi Shodhana and slow diaphragmatic breathing. The posture sequence included Tadasana, Marjaryasana–Bitilasana, Balasana, Uttanasana, Viparita Karani, and Supta Baddha Konasana. Relaxation was performed using guided Yoga Nidra. The same sequence was used throughout the study. Sessions were delivered via live video instruction by a certified Hatha Yoga instructor, and participants were advised to practice in a quiet, dimly lit room.

After the 4-week intervention, HARS and PSQI assessments were repeated, and saliva was collected again at 22:00 using the same procedure.

Statistical analysis

Data distribution was assessed using the Shapiro–Wilk test. Continuous variables are shown as mean ± standard deviation. Baseline and post-intervention values were compared within the same participants. Normally distributed variables were analyzed with paired t-tests, while variables with non-normal distribution were analyzed with Wilcoxon signed-rank tests. Statistical significance was set at $p < 0.05$.

Effect sizes were included to help interpret the size of the observed changes. Cohen's d was used for paired t-test outcomes, and r was used for Wilcoxon outcomes. The effect sizes were interpreted as small, moderate, or large using conventional cut-off values. Ninety-five percent confidence intervals were calculated for mean differences when appropriate. No participant withdrew from the study, and no missing outcome data were found.

Ethical clearance was granted by the Research Ethics Committee of the Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Indonesia (No. 115/KEP/V/2023). All participants gave written informed consent before joining the study.

Result

A total of 30 female medical students met the inclusion criteria and completed the 4-week Hatha Yoga intervention. All participants underwent baseline and post-intervention assessments of salivary melatonin concentration, anxiety severity (Hamilton Anxiety Rating Scale [HARS]), and sleep quality (Pittsburgh Sleep Quality Index [PSQI]). No missing data were identified for any outcome variable. All 30 participants completed baseline and post-

intervention assessments and were included in the final analysis; therefore, no data imputation was performed.

Salivary Melatonin

Baseline salivary melatonin concentration showed a mean value of 18.72 ± 2.24 pg/mL (range 15.69–27.21). Following the 4-week Hatha Yoga intervention, the mean melatonin concentration increased to 22.30 ± 3.95 pg/mL (range 16.89–34.71), representing a 3.58 pg/mL increase.

The melatonin values did not meet the normality assumption based on the Shapiro–Wilk test ($p < 0.05$). Therefore, the change between baseline and post-intervention melatonin levels was tested using the Wilcoxon signed-rank test. The increase in salivary melatonin levels was statistically significant, with a large effect size ($p = 0.0001$; $r = 0.70$; 95% CI for mean difference: 2.69–4.47 pg/mL) in table 1.

Table 1. Changes in Melatonin Levels, Anxiety Scores, and Sleep Quality After a 4-Week Hatha Yoga Intervention (n = 30)

Variable	Before intervention Mean ± SD	After intervention Mean ± SD	Statistical test	p-value
Salivary melatonin (pg/mL)	18.72 ± 2.24	22.30 ± 3.95	Wilcoxon signed-rank	0.0001
Anxiety (HARS score)	33.47 ± 7.29	19.57 ± 11.33	Paired t-test	<0.001
Sleep quality (PSQI score)	13.97 ± 2.30	5.23 ± 2.76	Paired t-test	<0.001

HARS: Hamilton Anxiety Rating Scale; PSQI: Pittsburgh Sleep Quality Index.

Anxiety

At baseline, anxiety severity among participants was predominantly moderate to severe. Based on HARS classification, 16.7% (n = 5) of participants had moderate anxiety, 70.0% (n = 21) had severe anxiety, and 13.3% (n = 4) had very severe anxiety. No participants were classified as having mild or no anxiety because the inclusion criterion required HARS scores ≥14.

After the intervention, a marked shift toward lower anxiety severity was observed. Post-intervention assessment showed that 26.7% (n = 8) had no anxiety, 23.3% (n = 7) mild anxiety, 26.7% (n = 8) moderate anxiety, 20.0% (n = 6) severe anxiety, and 3.3% (n = 1) very severe anxiety Table 2. The mean HARS score decreased from 33.47 ± 7.29 at baseline to 19.57 ± 11.33 after the intervention, corresponding to a mean reduction of 13.90 points. Shapiro–Wilk testing indicated that HARS scores were normally distributed ($p > 0.05$). Paired t-test analysis showed a significant decrease in anxiety severity after the yoga program, with a large effect size ($p < 0.001$; Cohen's $d = 1.47$; 95% CI for mean difference: 10.72–17.08 points) in table 1.

Table 2. Distribution of Anxiety Severity Before and After the Hatha Yoga Intervention

Anxiety category	Before intervention n (%)	After intervention n (%)
No anxiety	0 (0)	8 (26.7)
Mild anxiety	0 (0)	7 (23.3)
Moderate anxiety	5 (16.7)	8 (26.7)
Severe anxiety	21 (70.0)	6 (20.0)

Anxiety category	Before intervention n (%)	After intervention n (%)
Very severe anxiety	4 (13.3)	1 (3.3)

HARS: Hamilton Anxiety Rating Scale.

Sleep Quality

At baseline, all participants (100%) were classified as having poor sleep quality (PSQI >5). The mean PSQI score before the intervention was 13.97 ± 2.30 (range 10–19), indicating markedly impaired sleep quality.

Following the 4-week intervention, the mean PSQI score decreased to 5.23 ± 2.76 (range 1–14), representing a mean reduction of 8.74 points. Post-intervention classification showed that 73.3% (n = 22) of participants achieved good sleep quality (PSQI < 5), while 26.7% (n = 8) remained in the poor sleep quality category Table 3.

Normality testing confirmed that PSQI scores were normally distributed ($p > 0.05$). Paired t-test analysis demonstrated a statistically significant improvement in sleep quality after the intervention, with a very large effect size ($p < 0.001$; Cohen's $d = 3.50$; 95% CI for mean difference: 7.72–9.76 points) Table 1. All 30 participants provided complete data at both time points; no missing data or attrition occurred.

Table 3. Changes in Sleep Quality Classification Before and After the Hatha Yoga Intervention

Sleep quality	Before intervention n (%)	After intervention n (%)
Good sleep quality (PSQI <5)	0 (0)	22 (73.3)
Poor sleep quality (PSQI ≥5)	30 (100)	8 (26.7)

PSQI: Pittsburgh Sleep Quality Index.

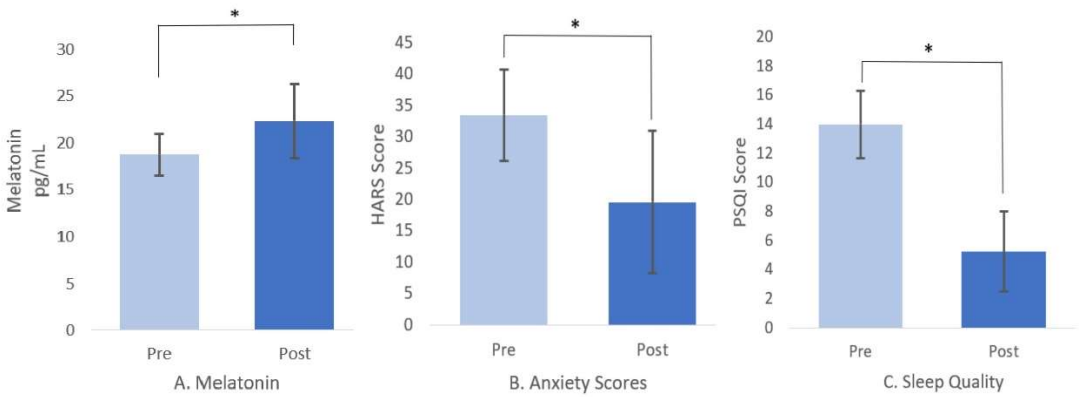

Figure 1. Changes in biological and psychological outcomes following the 4-week Hatha Yoga intervention.

(A) Increase in nocturnal salivary melatonin concentration after the intervention.
(B) Reduction in anxiety scores.
(C) Improvement in sleep quality as reflected by lower PSQI scores.

Data are presented as mean ± SD. *p < 0.05 compared with baseline.

Discussion

The present study examined changes in salivary melatonin concentration, anxiety severity, and sleep quality following a 4-week Hatha Yoga program among female medical students. The main findings were that salivary melatonin levels increased, anxiety scores decreased, and subjective sleep quality improved after the intervention. Given the quasi-experimental one-group pretest–posttest design, these findings should be interpreted as associations observed after the intervention rather than evidence of definitive causality.

The increase in salivary melatonin concentration is an important finding because melatonin is closely involved in circadian rhythm regulation and sleep–wake timing (Le et al., 2025; Waly & Hallworth, 2015) Melatonin is secreted by the pineal gland under circadian control and typically rises during the evening to facilitate sleep onset (Spinedi & Cardinali, 2019; Vasey et al., 2021) Previous studies have linked altered melatonin secretion with sleep disturbance, circadian misalignment, and stress-related physiological dysregulation (Poza et al., 2022; Repova et al., 2022) In the present study, the higher post-intervention melatonin level may indicate a favorable shift in nocturnal circadian regulation. However, because only salivary melatonin was measured, and markers such as cortisol, heart rate variability, salivary alpha-amylase, or objective circadian phase markers were not assessed, the underlying physiological pathway cannot be confirmed.

Anxiety severity also decreased after the 4-week Hatha Yoga program. This result is important because anxiety and poor sleep often occur together in students facing academic pressure. Anxious students may find it harder to relax at night, and repeated poor sleep can make mood regulation more difficult the next day (He et al., 2023; Wang & Chia, 2024). In this study, the decrease in HARS scores may have contributed to the improvement in PSQI scores. However, this interpretation should be made carefully. Since the study did not include a control group, the observed changes cannot be attributed only to Hatha Yoga. Expectation effects, natural symptom fluctuation, changes in academic workload, regression to the mean, or increased attention during the study may also have influenced the results.

Sleep quality improved substantially, as reflected by the reduction in PSQI scores and the shift of many participants from poor to good sleep quality. This result is consistent with previous reports suggesting that yoga and other mind–body practices may improve subjective sleep outcomes. However, the present study relied on PSQI as a self-reported measure and did not include objective sleep assessment such as actigraphy or polysomnography. Therefore, the findings should be interpreted as improvement in perceived sleep quality rather than confirmed changes in sleep architecture or sleep efficiency.

The present findings are generally consistent with previous studies reporting beneficial effects of yoga or meditation on melatonin regulation and sleep-related outcomes. A meta-analysis reported a moderate overall effect of yogic techniques on melatonin levels, and earlier experimental studies suggested that meditation or regular yoga practice may be associated with increased endogenous melatonin secretion (Govindasamy et al., 2025). However, the evidence is not uniformly positive. For example, Szaszkó et al. (2023) found that an 8-week Hatha Yoga intervention reduced self-reported stress but did not significantly improve state or trait anxiety (Szaszkó et al., 2023). These differences across studies suggest that yoga may not produce the same response in all groups. The results may depend on who participates, how long the intervention lasts, how regularly participants practice, the severity of symptoms at baseline, the outcomes measured, and the overall study design. This is why the present findings should be interpreted with caution and further tested in controlled studies.

One strength of this study is that it combined psychological outcomes with salivary melatonin measurement. Many yoga studies focus mainly on questionnaires, whereas this study

included both perceived changes in sleep and anxiety and a biological marker related to circadian regulation. Still, the biological explanation remains tentative. Hatha Yoga may support sleep and anxiety through relaxation, breathing practice, reduced arousal, or more regular bedtime routines, but these pathways were not measured directly. Therefore, mechanisms involving autonomic activity, the HPA axis, or pineal melatonin regulation should be viewed as possible explanations rather than confirmed findings from this study.

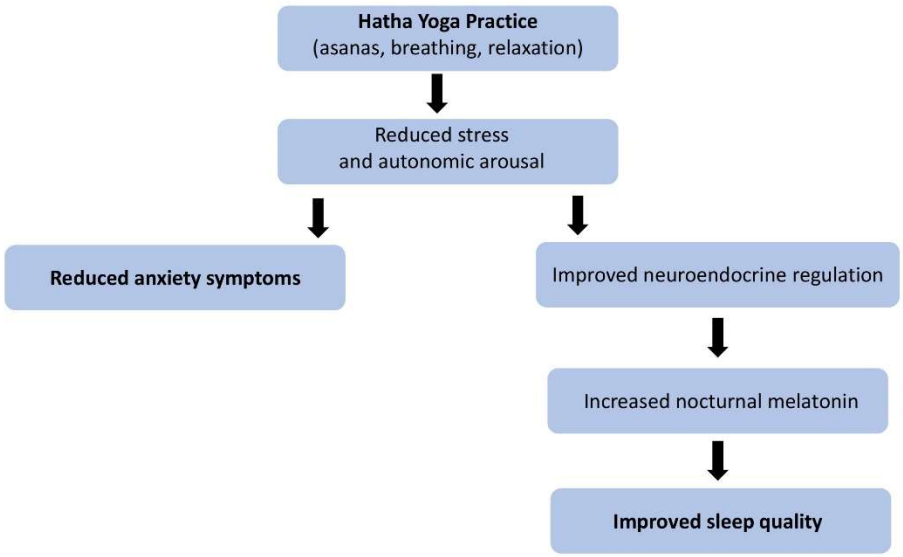

Figure 2. Proposed psychoneuroendocrine pathway linking Hatha Yoga with improved sleep quality.

These findings need to be read with some caution. The study did not use randomization or a control group, so the changes seen after the intervention cannot be linked to the yoga program with certainty. The sample was also small and limited to female medical students, which means the results may not apply to male students or to other student populations. Melatonin was measured only once at night, so the study could not describe the full daily pattern of melatonin secretion. Some factors that may affect sleep and melatonin, including evening light exposure, screen use, caffeine intake, sleep habits, menstrual cycle phase, and academic workload, were not objectively monitored. Adherence to the study instructions was also based on participants' self-reports, which may be affected by recall bias or the desire to give expected answers. In addition, because participants knew they were receiving a yoga intervention, expectation effects may have contributed to the reported improvements. Finally, sleep quality and anxiety were assessed using PSQI and HARS, without objective sleep measurements or physiological markers such as actigraphy, polysomnography, cortisol, or heart rate variability.

Within these limitations, the study shows that better sleep quality, lower anxiety severity, and higher salivary melatonin levels were observed after a 4-week Hatha Yoga program in female medical students. These results suggest that yoga may be a feasible non-pharmacological practice to support student well-being, but they should be confirmed through randomized controlled studies with larger samples, objective sleep assessment, repeated melatonin measurements, and direct markers of autonomic or HPA-axis activity.

Conclusion

In conclusion, salivary melatonin levels, anxiety severity, and sleep quality improved after a 4-week Hatha Yoga program among female medical students. However, the one-group pretest–posttest design without randomization or a control group limits causal interpretation. Therefore, these findings should be regarded as preliminary and require confirmation in controlled studies. Hatha Yoga may be a feasible non-pharmacological practice to support sleep and psychological well-being in academically stressed young adults. Future randomized controlled studies with larger and more diverse samples, objective sleep measurements, and repeated hormonal assessments are needed to confirm these findings and clarify the underlying mechanisms

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Conflict of interest

The authors declare that they have no conflicts of interest related to this study.