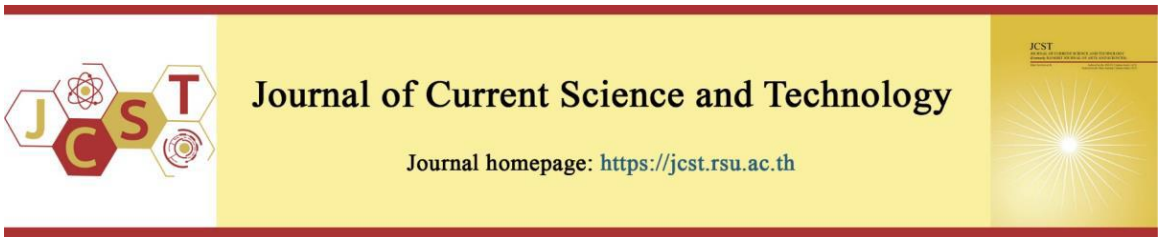
Journal of Current Science and Technology, May - August 2024 Vol. 14 No. 2, Article 25
ISSN 2630-0656 (Online)

Cite this article: Noiprasert, S., Buttagat, V., Sittiprapaporn, P., Sivaphongthongchai, A., & Hongsing, P. (2024). Effects of acupuncture on autonomic nervous system parameters and salivary cortisol level among mental stress university students: A pilot randomized controlled trial. *Journal of Current Science and Technology*, *14*(2), Article 25. https://doi.org/10.59796/jcst.V14N2.2024.25



Effects of Acupuncture on Autonomic Nervous System Parameters and Salivary Cortisol Level Among Mental Stress University Students: A Pilot Randomized Controlled Trial

Sulukkana Noiprasert[1,2], Vitsarut Buttagat[1,2], Phakkharawat Sittiprapaporn[3], Akarat Sivaphongthongchai[4] and Parichart Hongsing[1,2*]

[1]School of Integrative Medicine, Mae Fah Luang University, Chiang Rai 57100, Thailand
[2]Mae Fah Luang University Hospital, Mae Fah Luang University, Chiang Rai 57100, Thailand
[3]Neuropsychological Research Laboratory, Neuroscience Research Center, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok 57100, Thailand
[4]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand

*Corresponding author; E-mail: parichart.hon@mfu.ac.th

Received 26 October, 2023; Revised 14 November, 2023; Accepted 27 November, 2023
Published online 2 May, 2024

Abstract

Stress is a common and often unavoidable aspect of daily life that can negatively impact the autonomic nervous system, which controls the functions of internal organs and glands. Chronic stress has been associated with an imbalance in autonomic nervous system function, leading to a range of health conditions. Acupuncture is a medical treatment modality that has been applied to health care in different dimensions including physical, mental, and spiritual. This study aimed to investigate the effect of acupuncture on blood pressure, heart rate, and salivary cortisol level in mild stress university students, a population particularly vulnerable to stress. Twenty-eight university students were recruited and divided into two groups, a real acupuncture group and a sham acupuncture group. Heart rate, blood pressure, and salivary cortisol level were evaluated before and after a 30-minute acupuncture session on the ST36 acupoint. The results showed that the real acupuncture group had a significant decrease in blood pressure and heart rate, whereas the sham acupuncture group showed no change. Both groups had a slight decrease in salivary cortisol levels with no statistical significance. There was a statistically significant difference in the decrease of systolic blood pressure and heart rate after acupuncture between the two groups, but there was no significant difference between the groups in salivary cortisol level after acupuncture. The findings suggest that acupuncture can be a valuable treatment for restoring balance to the symptoms of the autonomic nervous system in university students experiencing stress. A larger sample size, non-intervention control, as well as different sources and times of cortisol collection, could contribute to the validation of the findings in this study.

Keywords: *Acupuncture; acupoint; autonomic nervous system; stress level; alternative medicine; traditional Chinese medicine; cortisol level*

1. Introduction

Stress is a common mental health disorder that can lead to illness and disability among adolescents. University or college students are among the age group of adolescents which are at greater risk of mental health conditions (Mohan et al., 2022). Stress frequently affects university students, leading to considerable consequences on their mental and physical well-being, along with their academic achievements (Asif et al., 2020). Prior research has shown strongly linked stress-inducing factors among medical students included personal relationships, physical well-being, mental health, challenges in social interactions,

contentment with academic performance, and a sense of monotony in education (Phomprasith et al., 2022). The stress experienced by university students can be interpreted as the body's response, both in terms of neurological and physiological aspects, to acclimate to the unfamiliar situation (Dyrbye et al., 2005). Previous study affirmed that chronic stress and anxiety exert an adverse impact on mental health, while also establishing a connection to thoughts of suicide among medical students (Rosiek et al., 2016). Numerous research studies have documented a significant prevalence of stress-related conditions in university students. A cross-sectional study of university students in Thailand revealed that the prevalence of high stress levels among the students was found to be 44.3%, with the main sources of stress being academic demands, financial problems, and personal relationships (Srichan et al., 2020). Similarly, the prevalence of moderate to high stress levels among medical students in Thailand was found to be 44.9% and 38.6%, respectively (Norphun et al., 2020) Moreover, those students with high stress levels were significantly associated with their engagement in ineffective coping techniques (Norphun et al., 2020). Taken together, the literature reviewed reiterated the importance of considering the need for interventions to address stress among university students in Thailand and to provide support for their mental health needs.

The autonomic nervous system (ANS) has a direct role in physical response to stress. The ANS is in the peripheral division of nervous system which controls the functions of internal organs and glands (Goldstein et al., 2002). There are the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) in ANS that work in opposition to each other to maintain the homeostasis of the body (Jänig, 2006). Stress is a prevalent and often unavoidable aspect of daily life that can negatively impact the ANS and leads to various health issues (McEwen, 2005). Chronic stress has been linked to an imbalance in ANS function, characterized by increased sympathetic activity and decreased parasympathetic activity, which has been associated with a range of health problems, including hypertension, cardiovascular disease, and gastrointestinal disorders (Esler et al., 2003; Konturek et al., 2011). Therefore, assessment of ANS parameters can be used to evaluate and indicate individual's stress (Bian et al., 2022). Moreover, employing biological indicators is an objective approach to the diagnosis of stress and mental illnesses. Cortisol, a hormone synthesized by the adrenal cortex, is frequently employed as a stress biomarker. Examination of saliva cortisol is highly preferred because of its quickness, cost-effectiveness, and non-invasiveness. However, caution should be exercised when utilizing saliva to identify markers, given that its composition can fluctuate based on variables such as circadian rhythms, age, gender, dietary habits, tobacco consumption, and medication usage (Chojnowska et al., 2021).

Acupuncture is a traditional Chinese medicine technique that involves the insertion of thin needles into specific points on the body to stimulate the flow of energy and restore the balance of the body. Acupuncture has been used to treat a wide range of mental health conditions, including chronic pain, anxiety, and depression (Sniezek, & Siddiqui, 2013; You et al., 2021). It's important to note that acupuncture is a personalized treatment that involves a comprehensive diagnosis and individualized treatment plan. Therefore, numerous specific acupoints were selected based on principles from traditional Chinese medicine. The aim was to target the diagnostic patterns associated with Shaoyin and Jueyin syndromes, which commonly observed in individuals experiencing heightened levels of stress (Fleckenstein et al., 2018; Huang et al., 2011; Oh et al., 2012). This study selected Zu-San-Li (ST36) acupoint according to the acupuncture theory as this point is believed to have calming and balancing effects on the mind and body, and may help to reduce symptoms of stress, anxiety, and insomnia (Eshkevari et al., 2012).

Acupuncture has been proposed as a potential therapy for autonomic nervous system (ANS) imbalances, which can manifest as a variety of conditions such as hypertension, anxiety, and digestive disorders. Some studies have suggested that acupuncture may help to regulate ANS activity and restore balance. Acupuncture has been applied in addressing diverse ailments, including cardiovascular disorders like hypertension. Many investigations have indicated that acupuncture can reduce blood pressure (BP) in individuals with hypertension (Liu et al., 2015; Wang et al., 2013). In cases of chronic stress, levels of the sympathetic neuropeptide, neuropeptide Y (NPY), have been observed to rise, leading to the suggestion that it plays a role in the body's physiological reaction to stress. There are literatures explaining that the

Hypothalamic-Pituitary-Adrenal (HPA) axis is a key component of the stress response system related to NPY. When the brain perceives stress, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH then triggers the release of cortisol from the adrenal glands. NPY has been shown to inhibit the release of CRH in the hypothalamus, reducing the activation of the HPA axis. This modulation helps regulate the release of stress hormones such as cortisol, thereby influencing the body's overall stress response (Smith & Vale, 2006). Cortisol takes on the role of the primary adrenal stress hormone in humans whereas corticosterone (CORT) serves as the primary corticosteroid hormone in rodents (Joëls et al., 2018). The aforementioned mechanism was investigated in a rat model of ethanol withdrawal. Acupuncture was employed to investigate its impact on plasma levels of CORT, ACTH, and the protein levels of CRH in the paraventricular nucleus of the hypothalamus. The study revealed that acupuncture at the ST36 point prevented the rise in plasma CORT and ACTH levels during ethanol withdrawal and counteracted the excessive expression of CRH protein in the hypothalamus (Zhao et al., 2014). NPY is produced and secreted in the central and peripheral nervous systems. In the peripheral nervous system, it acts as a neurotransmitter influencing the release of norepinephrine. This can lead to vasoconstriction, increased heart rate, and other physiological responses associated with the stress response (Ruohonen et al., 2012). In the central nervous system, NPY interacts with Y1 receptor in the brain which causes vasoconstriction leading to high blood pressure. Thus, regulation of NPY is associated with heart rate and blood pressure (Vella, 2016). Previous mouse model study reported that the treatment of chronic stress using electroacupuncture (EA) on ST36 acupoint, both administering EA to the ST36 acupoint before and simultaneously with the cold stress effectively inhibited the elevation of both peripheral and central neuropeptide Y (NPY) levels after a 14-day period ($P < 0.05$) (Eshkevari et al., 2012). In summary, diminishing heart rate and blood pressure through NPY and cortisol levels which are related to stress were mostly studied in animal models. In a systemic review of six randomized control trials reports only two studies exhibited the reduction on cortisol level after receiving acupuncture; however, no studies use single ST36 acupoint for the intervention (Lim et al., 2010). Thus, the evaluation of using single acupoint made it an interesting topic to study as this will be beneficial for stress management.

2. Objectives

This study aimed to evaluate the potential of ST36 acupoint with manual acupuncture to reduce the salivary cortisol level and ANS parameters (blood pressure and heart rate) in university students with mild stress level.

3. Materials and methods

3.1 Clinical trial

This study was a randomized controlled trial that employed a single-blind methodology with the participants being blinded. Ethical approval was obtained from the Mae Fah Luang Ethics Committee on Human Research under Certificate No. 110/202. The study was conducted at Mae Fah Luang University Hospital. All participants were offered one therapy session for their participation and were required to sign an informed consent form.

3.2 Intervention and procedure

The participants were students at Mae Fah Luang University—both genders, aged 18–23 years. On the first arrival for the screening meeting, participants were given more detailed information about the procedures of the study. The recruitment was carried out by using Thai stress test to obtain mild stress students, with no psychiatric disorders (schizophrenia and other psychotic disorders, bipolar disorder, obsessive-compulsive disorder substance-related disorders, and addictive disorders) or physical ailments that the researcher considers significant and inappropriate or risky for involvement in the study. Pregnant woman and participants who had received acupuncture or electroacupuncture treatment in the past 3 months were excluded in this study. Preliminary investigations, commonly known as pilot studies, typically mark the initial phase of experimental research. Conducted on a smaller scale, this study provided insights into intervention development, assess study feasibility, and offer a preview of how the larger main study would unfold if undertaken.

Twenty-eight participants were evaluated stress level by Thai stress test before the intervention day and underwent the concealment of group allocation using a computer-generated number. The mild stress participants were provided

with instructions and appropriate material for salivary cortisol collection. The collection was done at home prior to the experimental site, between 7.00 – 7.30 a.m., then keep in suitable container and bring to the facility. The protocol for saliva collection was done following the previous study (Amorim et al., 2022). After saliva collection, ANS physiological parameters (heart rate and blood pressure) were collected by the researchers and then the participants were blinded to the type of intervention (real acupuncture and sham acupuncture), and the treatment session lasted for 30 minutes. After treatment session, salivary cortisol, blood pressure, and heart rate were collected immediately before 9.00 a.m. in the same day.

3.3 Thai stress test

The self-report stress test used to evaluate stress level of the participants was the Thai stress test (Ngamthipwathana & Sukhatungkha, 2000). The assessment comprises a set of 24 questions depicting psychological responses (both positive and negative) linked to everyday occurrences in the lives of Thai individuals. All 24 questions within the evaluation were utilized to gauge an individual's emotions and thoughts (both positive and negative) throughout the previous month. The score was calculated following the matrix table of the Thai stress test interpreting 4 levels of stress as excellent mental health, normal mental health, mild stress and stressful. People with mild stress were selected for this study.

3.4 Salivary cortisol collection and analysis

Participants were given guidance, demonstration and appropriate materials to collect saliva samples before the collection date at the experimental site by researchers. The first collection process took place at home, and the sample was kept in a suitable container upon reaching the facility. Participants were instructed as follows: (1) Refrain from physical activity for 3 hours before collecting the sample; (2) Avoid eating, drinking, chewing gum, or brushing teeth in the 30 minutes prior to sample collection; (3) Inform researchers of any medication being taken; (4) If experiencing oral diseases, inflammation, injuries, or recent tooth extraction, refrain from sample collection to prevent contamination with blood; (5) Do not use substances that could increase saliva production during sample collection; (6) Rinse mouth, drink half a glass of water, and wait 5 minutes before collecting the sample; (7) Wash hands with soap and water, and dry with a clean towel before starting collection; (8) Initiate the first saliva collection 30 minutes after waking, using the provided container; (9) Gather small amounts of saliva in the mouth and transfer to the collection container, ensuring at least 2.5 mL of saliva is collected; (10) Store samples in the refrigerator (2°C–8°C) until delivering to researchers; (11) Note the collection time on the label of the container; (12) Inform researchers if any deviations from these instructions occur. The second collection of saliva samples after treatment session took place at the experimental site by the researchers. The concentration of cortisol in saliva was analyzed using ELISA (range 0.005–3 μg/dl, IBL International Cortisol Saliva ELISA, RE52611, Hamburg, Germany) according to the manufacturer's procedure. Saliva samples were tested in duplicates and the mean coefficient of variation was <10%. The samples acquired from an individual were examined together in a single batch to prevent any discrepancies between different analysis runs.

3.5 Acupuncture session

Both groups of participants were blinded to the type of intervention (real acupuncture and sham acupuncture), and the treatment session lasted for 30 minutes. The participants received the intervention in the seated knee flexion position. They were kept unaware of the intervention, with a hospital trolley table arranged over their knees to obstruct their view of the intervention. In the real acupuncture group, a disposable stainless-steel needle (diameter 0.25 mm, length: 25 mm, BOENMED brand, China) was used. After disinfecting the acupoint area with alcohol, the needle was inserted bilaterally into the ST36 acupoint over the tibialis anterior muscle to a depth of approximately 1 cm. The needle was then left in place for 30 minutes without additional manipulation and was then removed. In the sham acupuncture group, a non-penetrating needle was used to simulate acupuncture without actually puncturing the skin. The non-penetrating needle was placed on the skin surface of the ST36 acupoint without insertion, and the same concealment technique and time duration as the real acupuncture group was performed.

3.6 Evaluation of ANS physiological parameters

At the arrival of the experimental site, participants were asked to rest for 5 minutes before blood pressure and heart rate measurement. The recording of ANS physiological parameters (blood pressure and heart rate) were measured by the Biolight M7000 Multiparameter Patient Monitor (BIOM7000) before and after an intervention in the same participants.

3.7 Data analysis

Changes in each variable were examined for significance using a t-Test. SPSS version 22 was used for data analysis. The comparison between and within the real and sham acupuncture groups was performed by a paired t-test (parametric). A p-value less than 0.05 was considered statistically significant. Values were presented as mean and standard deviation.

4. Results

A total of 28 university students who were evaluated as having mild stress level, aged 18-23 years, participated in the study. Majority of students were female in both groups (Table 1). All recruited participants completed the trial and there was no report of adverse effects associated with the data collection process.

Table 1 Baseline characteristics of mild stress level participants.

Characteristics	Real acupuncture (n=14)	Sham acupuncture (n=14)	p-value
Age (years), mean ± S.D.	20.00 ± 0.63	20.28 ± 0.75	0.65
Gender, Male/Female	2/12	2/12	1.00
BMI, mean ± S.D.	20.73 ± 2.11	20.67 ± 2.04	0.94
Systolic BP, mean ± S.D.	117.93 ± 16.00	102.93 ± 13.18	1.08
Diastolic BP, mean ± S.D.	77.21 ± 11.34	68.57 ± 12.10	0.06
Heart rate, mean ± S.D.	88.14 ± 11.34	77.07 ± 8.55	0.81
Cortisol, mean ± S.D.	0.69 ± 0.18	0.67 ± 0.18	0.82

Measurement of body mass index in kg/m^2, blood pressure in mmHg (BP); Heart rate in beat per minute; salivary cortisol concentration in µg/dL

Table 2 Pre-Post intervention outcome of real and sham acupuncture groups.

Parameters	Pre-test		Post-test		t	p-value
	$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Real acupuncture						
- Systolic BP	117.93	16.00	107.71	13.03	3.20	0.01*
- Diastolic BP	77.21	11.34	72.21	11.38	2.65	0.02*
- Heart rate	88.14	11.34	79.14	12.66	2.99	0.01*
- Cortisol	0.69	0.18	0.68	0.19	2.16	0.25
Sham acupuncture						
- Systolic BP	102.93	13.18	100.64	15.30	1.44	0.18
- Diastolic BP	68.57	12.10	66.71	11.71	0.89	0.40
- Heart rate	77.07	8.55	76.79	8.20	0.27	0.80
- Cortisol	0.67	0.18	0.66	0.20	2.16	0.82

Measurement of blood pressure in mmHg (BP); Heart rate in beat per minute; salivary cortisol concentration in µg/dL evaluated before (Pre) and after (Post) a 30-minute acupuncture session on the ST36 acupoint.

Table 3 Comparison the decrease of each parameter between the real and sham acupunctures

Parameters	Real acupuncture		Sham acupuncture		t	p-value
	$\bar{x}$	S.D.	$\bar{x}$	S.D.		
Systolic BP	10.21	11.94	2.29	5.95	2.22	0.04*
Diastolic BP	5.00	7.06	1.86	7.8	1.11	0.28
Heart rate	9.00	11.27	0.29	3.93	46.00	0.02*
Cortisol	0.01	0.02	0.01	0.16	0.43	0.67

Comparison of the reduction in blood pressure in mmHg (BP); Heart rate in beat per minute; salivary cortisol concentration in μg/dL compared in real acupuncture and sham acupuncture on the ST36 acupoint.

4. 1 Results of ANS parameters and salivary cortisol analysis

The ANS parameters in the real acupuncture group revealed statistically significant decreases in systolic blood pressure (SBP), diastolic blood pressures (DBP) and heart rate (HR) (p-value < 0. 05). However, there was a slight decrease in salivary cortisol levels without statistical significance (p- value = 0. 25). In the sham acupuncture group, there were no significant differences in ANS parameters and salivary cortisol level, as shown in table 2. The comparison of the differences in all parameters between the real and sham acupuncture groups was demonstrated in table 3. It was found that the systolic BP (p-value = 0.04) and heart rate (p- value = 0. 02 in the real acupuncture group were significantly lower than those in the sham acupuncture group. Unfortunately, there was no significant difference observed in the minor decrease of salivary cortisol levels between the real acupuncture group and the sham acupuncture group.

5. Discussion

This study aimed to investigate the effectiveness of acupuncture on ST36 acupoint to treat university students with mild stress. When the body senses stress, the hypothalamic-pituitary-adrenal axis produces a steroidal hormone cortisol and excreted into saliva during periods of stress (Aguilar Cordero et al., 2014). The high level of salivary cortisol can cause an increase in heart rate and blood pressure. Concerning the slight decrease in salivary cortisol levels observed during this study, no statistically significant findings were present when comparing values before and after the tests in both sets of participants. There are numerous factors cause the variation of cortisol level including collecting time, duration of the therapy, and acupoint (Lim et al., 2010). Previous study of acupuncture treatment applied ST36 acupoint along with 7 different acupoints in patients with irritable bowel syndrome revealed the reduction of cortisol level (Schneider et al., 2007). Thus, the use of various acupoints along with ST36 acupoint may have more potential in cortisol level reduction than that of single acupoint in this study. Moreover, longer duration of the treatment may assist in the reduction of cortisol level.

Current research and available evidence show that acupuncture has the potential to decrease blood pressure through various pathways, including acupoints, afferent nerves, the central nervous system, efferent nerves, and neurotransmitters (Fan et al., 2020). Systematic analyses revealed a broad spectrum of outcomes regarding the impact of acupuncture on lowering BP. The degree of BP reduction varied from 3 to 40 mm Hg. Nevertheless, the overall credibility of these findings is compromised due to the generally inadequate quality of the trials encompassed in these systematic reviews (Kim, & Zhu, 2010; Li et al., 2014; Wang et al., 2013; Zhao et al., 2015). Regarding the results of BP and HR in this present study, systolic BP, diastolic BP, and HR were significantly lower after 30-min acupuncture session on the ST36 acupoint in mild stress university students. The finding is similar to the previous study of sixteen healthy medical students who received 15-min period of two different frequencies EA (2 Hz and 100 Hz) on the ST36 acupoint while resting. It was found that the application of both frequencies to the ST36 acupoints led to a reduction in HR, which possibly causes by triggering increased parasympathetic nerve activity in heartbeats (Hsieh et al., 1999). Similar effect on lowering BP was found in previous study of ST36 acupuncture on normal adults. The mechanism of ST36 acupoint to diminish BP, HR, and muscle sympathetic nerve activity (MSNA) were studied in eight healthy adult

males. Microneurography was used to evaluate the reduction of MSNA. MSNA causes vasoconstriction, thus lowering MSNA induces a reduction of BP (Katayama & Saito, 2019). After 15-min period of acupuncture, there was a significant reduction in BP comparing to the sham acupuncture group ($p < 0.05$). However, no significant reduction of HR and MSNA was found in this study. Hence, the lowering BP in this study was not associated with MSNA reduction (Kimura et al., 2017). Another EA study using the combination of many acupoints (P5, P6, ST37) with ST36 acupoint was studied with hypertensive patients with no medication. It was found that the reduction of systolic BP and diastolic BP was long-lasting for 24 hours when compared to patients in control group (Li et al., 2015). Obviously, combination of many acupoints with ST36 acupoint or single use of ST36 acupoint can lower the BP. Previous studies in animal models might be the potential scenario to explain the mechanism of the fall in BP. The mechanism of lowering BP in laboratory animals receiving acupuncture at the ST36 acupoint might causes by regulation of NPY level (Eshkevari et al., 2012) and/or regulation of a major neuroendocrine system, HPA axis that controls reactions to stress (Zhao et al., 2014). Although the result of a fall in BP in this study were consistent with previous research; however, the effect of ST36 acupuncture on the HR remains controversial.

There are limitations in this pilot study that should be noted. Firstly, placebo controls are frequently employed to eliminate psychological variables. Research indicates the challenge of implementing appropriate controls in clinical acupuncture studies, leading to skepticism about acupuncture's efficacy. Selecting a point and demonstrating its lack of impact on the body is highly challenging, given that the human body is viewed as a network interwoven with meridians in traditional Chinese medicine theory. If sham acupuncture is not a truly inactive control and has the potential to influence outcomes, and the primary goal is to assess acupuncture's effectiveness, a nonintervention control or relaxation control might be more appropriate. Secondly, previous systemic review of cortisol level after acupuncture revealed that source of cortisol and cortisol sampling time exhibited different results (Lim et al., 2010). Therefore, different source and sampling time for cortisol collection should be investigated in the future.

6. Conclusion

Acupuncture using only ST36 acupoint in university students with mild stress level leads to a fast reduction of the ANS parameters including blood pressure and heart rate. Cortisol is commonly known as the stress hormone, and it results in an elevation of your heart rate and blood pressure; however, a minor reduction of salivary cortisol level was found in this study. Thus, future studies with larger sample size with nonintervention group as well as comparison of different source and sampling time for cortisol collection may help confirm the current study's results.

7. Acknowledgements

This research was supported by research grants from Mae Fah Luang University Fiscal Year 2021.

8. References

Aguilar Cordero, M. J., Sánchez López, A. M., Mur Villar, N., García García, I., Rodríguez López, M. A., Ortegón Piñero, A., & Cortés Castell, E. (2014). Salivary cortisol as an indicator of physiological stress in children and adults; a systematic review. *Nutricion Hospitalaria, 29*(5), 960-968. https://doi.org/10.3305/nh.2014.29.5.7273

Amorim, D., Brito, I., Caseiro, A., Figueiredo, J. P., Pinto, A., Macedo, I., & Machado, J. (2022). Electroacupuncture and acupuncture in the treatment of anxiety - A double blinded randomized parallel clinical trial. *Complementary Therapies in Clinical Practice, 46*, Article 101541. https://doi.org/10.1016/j.ctcp.2022.101541

Asif, S., Mudassar, A., Shahzad, T. Z., Raouf, M., & Pervaiz, T. (2020). Frequency of depression, anxiety and stress among university students. *Pakistan Journal of Medical Sciences, 36*(5), 971-976. https://doi.org/10.12669/pjms.36.5.1873

Bian, W., Zhang, X., & Dong, Y. (2022). Autonomic Nervous System Response Patterns of Test-Anxious Individuals to Evaluative Stress. *Frontiers in Psychology, 13*, Article 824406. https://doi.org/10.3389/fpsyg.2022.824406

Chojnowska, S., Ptaszyńska-Sarosiek, I., Kępka, A., Knaś, M., & Waszkiewicz, N. (2021). Salivary Biomarkers of Stress, Anxiety and Depression. *Journal of Clinical Medicine, 10*(3), Article 517. https://doi.org/10.3390/jcm10030517

Dyrbye, L. N., Thomas, M. R., & Shanafelt, T. D. (2005). Medical student distress: causes, consequences, and proposed solutions. *Mayo Clinic Proceedings, 80*(12), 1613-1622. https://doi.org/10.4065/80.12.1613

Eshkevari, L., Egan, R., Phillips, D., Tilan, J., Carney, E., Azzam, N., Amri, H., & Mulroney, S. E. (2012). Acupuncture at ST36 prevents chronic stress-induced increases in neuropeptide Y in rat. *Experimental Biology and Medicine, 237*(1), 18-23. https://doi.org/10.1258/ebm.2011.011224

Esler, M., Lambert, G., Brunner-La Rocca, H. P., Vaddadi, G., & Kaye, D. (2003). Sympathetic nerve activity and neurotransmitter release in humans: translation from pathophysiology into clinical practice. *Acta Physiologica Scandinavica, 177*(3), 275-284. https://doi.org/10.1046/j.1365-201X.2003.01089.x

Fan, H., Yang, J. W., Wang, L. Q., Huang, J., Lin, L. L., Wang, Y., Zhang, N., & Liu, C. Z. (2020). The Hypotensive Role of Acupuncture in Hypertension: Clinical Study and Mechanistic Study. *Frontiers in Aging Neuroscience, 12*, Article 138. https://doi.org/10.3389/fnagi.2020.00138

Fleckenstein, J., Krüger, P., & Ittner, K. P. (2018). Effects of single-point acupuncture (HT7) in the prevention of test anxiety: Results of a RCT. *PLoS One, 13*(8), Aricle e0202659. https://doi.org/10.1371/journal.pone.0202659

Goldstein, D. S., Robertson, D., Esler, M., Straus, S. E., & Eisenhofer, G. (2002). Dysautonomias: clinical disorders of the autonomic nervous system. *Annals of Internal Medicine, 137*(9), 753-763. https://doi.org/10.7326/0003-4819-137-9-200211050-00011

Hsieh, C. L., Lin, J. G., Li, T. C., & Chang, Q. Y. (1999). Changes of pulse rate and skin temperature evoked by electroacupuncture stimulation with different frequency on both Zusanli acupoints in humans. *The American Journal of Chinese Medicine, 27*(1), 11-18. https://doi.org/10.1142/s0192415x99000033

Huang, W., Howie, J., Taylor, A., & Robinson, N. (2011, Feb). An investigation into the effectiveness of traditional Chinese acupuncture (TCA) for chronic stress in adults: a randomised controlled pilot study. *Complementary Therapies in Clinical Practice, 17*(1), 16-21. https://doi.org/10.1016/j.ctcp.2010.05.013

Jänig, W. (2006). *The Integrative Action of the Autonomic Nervous System: Neurobiology of Homeostasis*. Cambridge University Press. https://books.google.co.th/books?id=iNPjzQEACAAJ

Joëls, M., Karst, H., & Sarabdjitsingh, R. A. (2018). The stressed brain of humans and rodents. *Acta Physiologica, 223*(2), Artile e13066. https://doi.org/10.1111/apha.13066

Katayama, K., & Saito, M. (2019). Muscle sympathetic nerve activity during exercise. *The Journal of Physiological Sciences, 69*(4), 589-598. https://doi.org/10.1007/s12576-019-00669-6

Kim, L. W., & Zhu, J. (2010). Acupuncture for essential hypertension. *Alternative Therapies in Health & Medicine, 16*(2), 18-29. PMID: 20232615

Kimura, K., Ishida, K., Takahashi, N., Toge, Y., & Tajima, F. (2017). Effects of acupuncture at the ST-36 point on muscle sympathetic nerve activity and blood pressure in normal adults. *Autonomic Neuroscience, 208*, 131-136. https://doi.org/10.1016/j.autneu.2017.08.009

Konturek, P. C., Brzozowski, T., & Konturek, S. J. (2011). Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. *Journal Physiol Pharmacol, 62*(6), 591-599. PMID: 22314561

Li, D. Z., Zhou, Y., Yang, Y. N., Ma, Y. T., Li, X. M., Yu, J., Zhao, Y., Zhai, H., & Lao, L. (2014). Acupuncture for essential hypertension: a meta-analysis of

randomized sham-controlled clinical trials. *Evidence-Based Complementary and Alternative Medicine, 2014*, Article 279478. https://doi.org/10.1155/2014/279478

Li, P., Tjen, A. L. S. C., Cheng, L., Liu, D., Painovich, J., Vinjamury, S., & Longhurst, J. C. (2015). Long-Lasting Reduction of Blood Pressure by Electroacupuncture in Patients with Hypertension: Randomized Controlled Trial. *Medical Acupuncture, 27*(4), 253-266. https://doi.org/10.1089/acu.2015.1106

Lim, J., Song, K. T., & Lee, S. (2010). Acupuncture and Cortisol Levels: a Systematic Review. *The Journal of Korean Oriental Medicine, 31*(6), 21-28

Liu, Y., Park, J. E., Shin, K. M., Lee, M., Jung, H. J., Kim, A. R., Jung, S. Y., Yoo, H. R., Sang, K. O., & Choi, S. M. (2015). Acupuncture lowers blood pressure in mild hypertension patients: a randomized, controlled, assessor-blinded pilot trial. *Complementary Therapies in Medicine, 23*(5), 658-665. https://doi.org/10.1016/j.ctim.2015.06.014

McEwen, B. S. (2005). Stressed or stressed out: what is the difference?. *Journal of Psychiatry and Neuroscience, 30*(5), 315-318. PMID: 16151535

Mohan, A., Kostelecky, S. M., Sivakumar, A., Khalil, M., & Clark, H. (2022). Improving adolescent wellbeing is an urgent global priority. *Bmj, 379*, Article o2551. https://doi.org/10.1136/bmj.o2551

Ngamthipwathana, T., & Sukhatungkha, K. (2000, 01/01). The Development of the Thai Stress Test. *45*. Retrieved from https://www.psychiatry.or.th/JOURNAL/download/45-3-05.pdf

Norphun, N., Pitanupong, J., & Jiraphan, A. (2020). Stress and Coping Strategies among Thai Medical Students in a Southern Medical School. *Siriraj Medical Journal, 72*(3), 238-244. https://doi.org/10.33192/Smj.2020.32

Oh, Y. I., Yang, E. J., Choi, S. M., & Kang, C. W. (2012). The effect of electroacupuncture on insulin-like growth factor-I and oxidative stress in an animal model of renal failure-induced hypertension. *Kidney Blood Press Res, 35*(6), 634-643. https://doi.org/10.1159/000339640

Phomprasith, S., Karawekpanyawong, N., Pinyopornpanish, K., Jiraporncharoen, W., Maneeton, B., Phinyo, P., & Lawanaskol, S. (2022). Prevalence and Associated Factors of Depression in Medical Students in a Northern Thailand University: A Cross-Sectional Study. *Healthcare (Basel), 10*(3), Article 488. https://doi.org/10.3390/healthcare10030488

Rosiek, A., Rosiek-Kryszewska, A., Leksowski, Ł., & Leksowski, K. (2016). Chronic Stress and Suicidal Thinking Among Medical Students. *International Journal of Environmental Research and Public Health, 13*(2), Article 212. https://doi.org/10.3390/ijerph13020212

Ruohonen, S. T., Pesonen, U., & Savontaus, E. (2012). Neuropeptide Y in the noradrenergic neurons induces the development of cardiometabolic diseases in a transgenic mouse model. *Indian Journal of Endocrinology and Metabolism, 16*(Suppl 3), S569-576. https://doi.org/10.4103/2230-8210.105574

Schneider, A., Weiland, C., Enck, P., Joos, S., Streitberger, K., Maser-Gluth, C., Zipfel, S., Bagheri, S., Herzog, W., & Friederich, H. C. (2007). Neuroendocrinological effects of acupuncture treatment in patients with irritable bowel syndrome. *Complementary Therapies in Medicine, 15*(4), 255-263. https://doi.org/10.1016/j.ctim.2006.12.002

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience, 8*(4), 383-395. https://doi.org/10.31887/DCNS.2006.8.4/ssmith

Sniezek, D. P., & Siddiqui, I. J. (2013). Acupuncture for Treating Anxiety and Depression in Women: A Clinical Systematic Review. *Medical Acupuncture, 25*(3), 164-172. https://doi.org/10.1089/acu.2012.0900

Srichan, P., Ruanjai, T., Khunthason, S., & Apidechkul, T. (2020). Factors Associated with High-to-Severe Stress among University Students in Northern Thailand.

Journal of the Medical Association Thailand, 103(6), 594-603. https://doi.org/doi.org/10.35755/jmedassocthai.2020.06.10877

Vella, A. (2016). Chapter 38 - Gastrointestinal Hormones and Gut Endocrine Tumors. In S. Melmed, K. S. Polonsky, P. R. Larsen, & H. M. Kronenberg (Eds.), *Williams Textbook of Endocrinology (Thirteenth Edition)* (pp. 1701-1722). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-29738-7.00038-1

Wang, J., Xiong, X., & Liu, W. (2013). Acupuncture for essential hypertension. *International Journal of Cardiology, 169*(5), 317-326. https://doi.org/10.1016/j.ijcard.2013.09.001

You, J., Li, H., Xie, D., Chen, R., & Chen, M. (2021). Acupuncture for Chronic Pain-Related Depression: A Systematic Review and Meta-Analysis. *Pain Research and Management, 2021*, Article 6617075. https://doi.org/10.1155/2021/6617075

Zhao, X. F., Hu, H. T., Li, J. S., Shang, H. C., Zheng, H. Z., Niu, J. F., Shi, X. M., & Wang, S. (2015). Is Acupuncture Effective for Hypertension? A Systematic Review and Meta-Analysis. *PLoS One, 10*(7), Article e0127019. https://doi.org/10.1371/journal.pone.0127019

Zhao, Z., Lee, B. H., Lin, F., Guo, Y., Wu, Y., In, S., Park, S. M., Chan Kim, S., Yang, C. H., & Zhao, R. (2014). Effects of acupuncture at Zu-San-Li (ST36) on the activity of the hypothalamic--pituitary--adrenal axis during ethanol withdrawal in rats. *Journal of Acupuncture and Meridian Studies, 7*(5), 225-230. https://doi.org/10.1016/j.jams.2014.03.002