EFFECT OF ALPHA-S1-CASEIN TRYPTIC HYDROLYSATE AND L-THEANINE ON SLEEP QUALITY IN ACADEMIC STAFF AT UTAR, KAMPAR

By

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ABSTRACT

EFFECT OF ALPHA-S1-CASEIN TRYPTIC HYDROLYSATE AND L-THEANINE ON SLEEP QUALITY IN ACADEMIC STAFF AT UTAR, KAMPAR

Kokila Thiagarajah

Poor sleep quality is frequently related to poor mental health and is a common medical disorder. This study was conducted to measure the prevalence of poor sleep quality among academic staff at Universiti Tunku Abdul Rahman (UTAR) Kampar Campus and investigate the effects of a supplement containing 150 mg of alpha-s1-casein tryptic hydrolysate and 50 mg of L-theanine in those affected with poor sleep quality. In total, 344 randomly selected academic staff were approached to answer the Pittsburgh Sleep Quality Index (PSQI) questionnaire. The supplement or placebo was randomly and double blindly assigned to 39 subjects with poor sleep quality (global PSQI score >5) in a crossover design for four weeks with a 1-week washout period. The changes in the subjective sleep assessment via PSQI questionnaire, heart rate, blood pressure, salivary cortisol via high-performance liquid chromatography method and alpha power of awake electroencephalogram (EEG) were studied. Unexpectedly, 42.7% of the academic staff were affected by poor sleep quality in the sleep quality screening. The average actual sleep duration was recorded at 6.68 ± 1.24 hours. Age and global PSQI scores were not significantly correlated. Female staff had poorer subjective sleep quality (P = 0.027). The elder age (41 years old and

above) group (P = 0.012) and associate professors and professors (P = 0.006) consumed more sleep medications. Non-Doctor of Philosophy (Ph.D.) holders had poorer subjective sleep quality (P = 0.008) and sleep latency (P = 0.032) as well as global PSQI score (P = 0.045) compared to Ph.D. holders. The data obtained via the intervention of the supplement was analysed in two ways, crossover and crossover summed up. The latter depicted that the supplement improved global PSQI score, sleep latency, sleep duration, sleep habitual efficiency, daytime dysfunction, and increased total and frontal alpha power significantly ($P < 0.05$). The supplement prolonged the total sleeping time by 45 min in the supplement-receiving group compared to the placebo group ($P < 0.001$). However, only sleep duration and sleep habitual efficiency showed profound effects in both analyses ($P < 0.05$). This study highlights a notably high prevalence of poor sleep quality among academic staff at UTAR Kampar Campus, emphasising the potential impact on their overall health and work performance. The findings demonstrate that a supplement containing alpha-s1-casein tryptic hydrolysate and L-theanine may offer significant improvements in certain aspects of sleep quality, particularly sleep duration and sleep efficiency. These results suggest that targeted nutritional interventions could be a promising strategy to alleviate sleep disturbances in populations with poor sleep quality. Further research with extended washout periods, various dosages and longer follow-up periods is warranted to confirm the long-term efficacy and safety of such interventions.

Keywords: alpha-s1-casein tryptic hydrolysate, L-theanine, sleep quality, academic staff, alpha power, salivary cortisol.

Subject Area: RA643-645 Disease (Communicable and noninfectious) and public health.

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LIST OF ABBREVIATIONS

CBT	Cognitive behavioural therapy
CBT-I	Cognitive behavioural therapy for insomnia
CDB	Conditioned defensive burying
CTH	Alpha s1-casein tryptic hydrolysate
CZP	Alpha-casozepine
EEG	Electroencephalogram
EPM	Elevated plus maze
HPLC	High-performance liquid chromatography
i.p.	Intraperitoneally administered
IS	Internal Standard
ISI	Insomnia severity index
LDB	Light/dark box
min	Minute
mL	Millilitre
mL/min	Millilitre per minute
nm	Nanometre
p	Probability
p.o.	Orally administered
PSQI	Pittsburgh Sleep Quality Index
SE	Sleep efficiency
SOL	Sleep onset latency
SPE	Solid-phase extraction

SPSS	Statistical Package for the Social Sciences
TSH	Total sleep hours
TST	Total sleeping time
UV	Ultraviolet
WASO	Wake after sleep onset
μL	Microlitre
$\chi 2$	Chi-Square

CHAPTER 1

INTRODUCTION

1.1 Research Background

Healthcare professionals have emphasised the importance of assessing sleep quality in addition to sleep quantity. Sleep quality is better related to sleepiness, health, and well-being than sleep quantity in a non-clinical population (Pilcher, Ginter and Sadowsky, 1997). Meanwhile, poor sleep quality is a defining criterion of insomnia (Edinger, et al., 2004; Sohn, et al., 2012), although more requirements are needed to diagnose insomnia. Symptoms of poor sleep quality such as tiredness upon waking and throughout the day, feeling rested and restored upon waking, and the number of awakenings they experienced in the night, were similar among people with insomnia and poor sleep quality (Harvey, et al., 2008), yet affected people are often not aware of their problem and may not be getting appropriate treatments.

A sleeping duration of 7 hours or more is crucial to maintain optimal health among adults aged 18 to 60 years. Besides, sleeping less than 7 hours per night was associated with impaired physical and psychological health (Roth, 2007). Young adults or those who are ill may need more than 9 hours of sleep. Besides age and medical factors, sleep problems can be influenced by gender, genetics, ethnicity, socioeconomic status, environment, or geographical factors (Morin, et al., 2015). Few studies have been carried out to explore the

epidemiological nature of sleep problems among Malaysian populations. Nevertheless, a study conducted among 2049 adult primary care patients in Malaysia revealed that 60% had insomnia symptoms. It was stated that for those 50 years old and above, anxiety and depression were risk factors for chronic insomnia with daytime dysfunction (Zailinawati, Mazza and Teng, 2012). Insomnia may lead to anxiety and stress, and vice versa, which can be a risk factor for a psychotic disorder. People with persistent sleep disturbances are more prone to accidents, have higher rates of work absenteeism, diminished job performance, decreased quality of life, and increased health care utilisation (Roth, 2007).

A list of pharmacological treatment options such as benzodiazepines (e.g., estazolam, temazepam, and triazolam), non-benzodiazepine hypnotic agents (e.g., eszopiclone, zaleplon, and zolpidem) and orexin receptor antagonists (e.g., suvorexant) are readily available for the treatment of insomnia. Unfortunately, they do have numerous side effects, such as dizziness, drowsiness, memory damage, cognitive impairment, dependence, tolerance, and withdrawal symptoms such as anxiety. Besides, patients might abuse the drugs, which could lead to injuries and accidents due to the sedation effect of the prescribed sleep medication (Srinivasan, et al., 2012; Bond and Lader, 2012; Procyshyn, Bezchlibnyk-Butler and Jeffries, 2023). These medications may also be inappropriate for those suffering from mild to moderate sleep disorders or acute insomnia. In addition, most of the affected people do not prefer a pharmacological approach to insomnia treatment (Vincent and Lionberg, 2001) and often switch to alternative treatments such as nutritional supplements.

Alpha-s1-casein tryptic hydrolysate (CTH) is a unique bioactive decapeptide within a milk protein that has been claimed to have calming properties. Messaoudi, et al. (2005) showed that CTH can improve mental conflict and physical stress in study subjects. In the experimental stress test, consuming CTH could significantly reduce systolic blood pressure, diastolic blood pressure, and cortisol levels. The anxiolytic-like effect of CTH could be due to the presence of casozepine (CZP), specifically the s1-CN(f91–100) in it. This decapeptide serves as a positive modulator for the gamma-aminobutyric acid type A ($GABA_A$) receptor. Similar to benzodiazepines, it increases the inhibitory responses of gamma-aminobutyric acid (GABA) which can produce anxiolytic but not sedative effects. GABAergic transmission is being targeted for induction and maintaining sleep as a treatment for insomnia (Luppi, Peyron and Fort, 2017; Masiulis, et al., 2019). In a preclinical study, rats treated with 300 mg/kg/day of CTH showed an increase in $GABA_A$ receptor activation as well as increased total sleeping hours and decreased time awake (Yayeh, et al., 2018). Meanwhile, a 4-week course of 300 mg/day of CTH produced no adverse events in people (Giuseppe, et al., 2017; Kim, et al., 2019).

On the other hand, L-theanine is a type of amino acid found mainly in green tea. It is claimed to have anti-stress or relaxing properties, helps maintain normal sleep, improves memory function, neutralises the effects of neurotoxin, and ameliorates cardiovascular disease (Juneja, et al., 1999; Yoto, et al., 2012; Zukhurova, et al., 2013; Adhikary and Mandal, 2017). The ability of this amino acid to initiate an alpha wave pattern in the brain implies a relaxed physical and mental state without drowsiness or impaired motor skills. Besides, it is involved

in forming the inhibitory neurotransmitter GABA (Mason, 2001) and acts as a competitive glutamate antagonist (Xu, et al., 2024). In healthy subjects, the intake of L-theanine has been shown to reduce their heart rate and serum immunoglobulin A level which attenuates sympathetic nervous activation by blocking the binding of glutamic acid to the N-Methyl-D-Aspartate (NMDA) receptors in the brain. Since NMDA receptors are involved in excitatory neurotransmission, preventing glutamic acid from activating them may have a calming and stress-relieving effect (Kimura, et al., 2007). In preclinical studies, L-theanine is neither mutagenic nor carcinogenic, and has low toxicity, as its median lethal dose (LD_{50}) value is 5000 mg/kg. At the same time, the no-observed-adverse-effect-level (NOAEL) for L-theanine is 4000 mg/kg/day (Borzelleca, Peters and Hall, 2006; Adhikary and Mandal, 2017).

There are other nutritional supplements available in the market for sleep issues, such as melatonin and magnesium. Melatonin is often effective for short-term sleep issues such as jet lag and shift workers, as it can help improve both stress and sleep (Yeom and Cho, 2024). However, sleep medications containing melatonin can potentially induce ventricular arrhythmias in structurally normal hearts (de Vries, Géczy and Szili-Torok, 2017). Observational studies showed associations between magnesium intake and sleep quality, such as sleep duration. Nevertheless, randomised clinical trials showed an uncertain association (Arab, et al., 2023). Moreover, magnesium supplementation seemed to be effective in treating mild anxiety and insomnia in those with low magnesium (Rawji, et al., 2024)

1.2 Significance of Study

Most sleep studies are conducted in developed countries and sleep has been studied largely in older populations or clinical populations associated with comorbidities. Instead of focusing on a particular working population, majority of these studies looked at the relationship between the adult population and sleep in general. This results in a paucity of understanding of the prevalence and effects of sleep insufficiency among workers whose sleep may be especially sensitive (Lee, et al., 2019).

To date, limited studies pertaining to sleep quality have been conducted among working adults, such as academic staff in Malaysia. Many sleep-related studies have been focused on shift workers such as police officers, nurses, and doctors (Slaven, et al., 2011; Chen, Kuo and Chueh, 2010). Sleep is very much essential for academic staff as they require high cognitive skills to carry out both teaching and research (Ismail and Noor, 2016). Moreover, they have multiple roles as a lecturer, clinician, researcher, supervisor, academic advisor, consultant, and even administrators, and play a central role in producing high-quality graduates. A study of 278 academic staff members in Malaysia revealed that more than one-fourth of them suffered from depression and anxiety. Inadequate workplace amenities, low-tier job category, working on an urban campus, and low income are all predictors of depression (Razali, et al., 2019).

The American Academy of Sleep Medicine and the Sleep Research Society recommend that both healthcare personnel and the general public be educated on the importance of obtaining sufficient sleep for optimal health. They

encourage research related to the role of sleep and health (Watson, et al., 2015).

Therefore, this cross-sectional study investigated sleep quality and the association between sleep quality and basic demographic profile among academic staff of Universiti Tunku Abdul Rahman (UTAR), a private university in Malaysia. The academic staff were randomly chosen in this study and screened for sleep quality by using the Pittsburgh Sleep Quality Index (PSQI), as it is a reliable screening tool with high specificity and sensitivity in differentiating between good and poor sleep quality. In addition, all the components of PSQI can distinguish poor sleep quality or primary insomnia from a healthy population (Sohn, et al., 2012; Backhaus, et al., 2002).

Knowing that sleeping problems can lead to stress or anxiety and vice versa, it can be postulated that the combination of CTH and L-theanine can be apt to relieve the symptoms. The two active ingredients were formulated into a single capsule by LiveLife Bioscience AG, Zurich, Switzerland. Those with poor sleep quality were randomly assigned in this double-blind, placebo-controlled, and crossover clinical trial to explore the effect of the supplement on sleep quality by assessing PSQI, blood pressure, heart rate, salivary cortisol, and alpha power. Thus, four weeks of treatment with either the supplement or placebo were used in this study. Figure 1.1 shows the conceptual framework of how CTH and L-theanine may improve poor sleep.

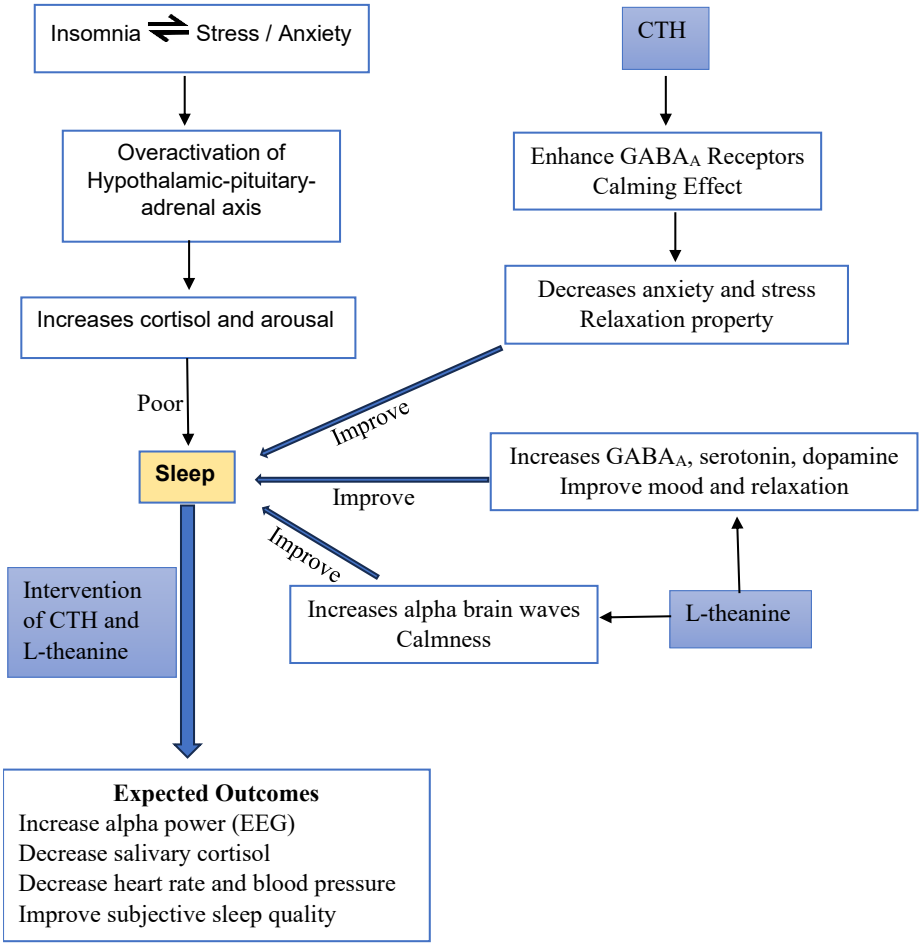


Figure 1.1: The conceptual framework of how CTH and L-theanine may improve poor sleep

1.3 Objectives

i. To conduct a systematic literature review on ‘preclinical and clinical anxiolytic studies of casein tryptic hydrolysate and casozepine’.

ii. To determine the prevalence of poor sleep quality among Universiti Tunku Abdul Rahman (UTAR) academic staff using the Pittsburgh Sleep Quality Index (PSQI).

iii. To study the effect of a supplement containing alpha-s1-casein tryptic hydrolysate and L-theanine on the subjective sleep quality of subjects with poor sleep quality.

iv. To determine the effect of a supplement containing alpha-s1-casein tryptic hydrolysate and L-theanine on the subjects’ salivary cortisol concentration, blood pressure, heart rate and alpha power.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Poor Sleep Quality and Insomnia

2.1.1 Definition and Sleep Recommendation

In the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, the term "insomnia" refers to the struggle to fall asleep, stay asleep or wake up too early, which happens at least three times per week for a minimum of one month along with functional impairment, including social and occupational distress (American Psychiatric Association, 2013). Insomnia may interfere with mental, normal physical and emotional functioning, yet affected people are not aware of it. Poor sleep quality is a key feature of insomnia, although more requirements are needed to diagnose it. The symptoms of poor sleep quality, such as tiredness on waking and throughout the day, feeling rested and restored on waking, and the number of awakenings they experienced at night were similar among people with insomnia and poor sleep quality (Harvey, et al., 2008).

A clinical interview comprising a sleep history, the use of sleep diaries and sleep questionnaires, and inquiries regarding physical and mental health should be part of the diagnostic process for insomnia and its co-morbidities. A physical examination and additional measures such as blood tests, electrocardiogram (ECG), and electroencephalogram (EEG) are strongly

recommended and act as moderate- to high-quality evidence. Despite the fact that polysomnography is considered the gold standard for diagnosing sleep disorders, it is not always the case for insomnia. Polysomnography is more helpful in evaluating other sleep disorders, such as respiratory disorders connected to sleep and periodic limb movement disorders, if suspected (Riemann, et al., 2017).

On average, one-third of our life has been spent sleeping. The National Sleep Foundation recommends different sleep duration ranges across the lifespan, as shown in Table 2.1. According to the foundation, 7 hours of sleep is essential for adults above 18 years old (Hirshkowitz, et al., 2015). American Academy of Sleep Medicine and the Sleep Research Society also recommend 7 or more hours of sleep per night for adults to support optimal health (Watson, et al., 2015). Working individuals are classified as having short sleep if they sleep for fewer than 7 hours, regular sleep if they sleep for 7-8 hours, and long sleep if they sleep for 9 hours or longer (Weston, et al., 2024).

Table 2.1: Sleep durations recommended by the National Sleep Foundation

Age group	Recommended sleep duration (hours)
New-borns 0-3 months	14 to 17
Infants 4 to 11 months	12 to 15
Toddlers 1-2 years old	11 to14
Pre-schoolers 3-5 years old	10 to 13
School-aged children 6 -13 years old	9 to 11
Teenagers 14-17 years old	8 to 10
Young adults 18-25 years old	7 to 9
Adults 26-64 years old	7 to 9
Older adults >64 years old	7 to 8

(Modified from Hirshkowitz , et al., 2015)

2.1.2 Epidemiology

Out of 1611 subjects in four states in Malaysia, 33.8% had insomnia associated with impaired daily function among adults within the range of 30-70 years old. Subjects with insomnia had significantly higher prevalences of feeling depressed (12.7%), loss of concentration (19.1%), exhaustion (17.2%), poor memory (9.2%), decreased work productivity (6.4%), and perceived poor health status (40.9%) (Zailinawati, et al., 2008). A study conducted among 2049 adult primary care patients in Malaysia revealed that 60% had insomnia symptoms (Zailinawati, Mazza and Teng, 2012).

The prevalence of insomnia among the working population is increasing across the world. The prevalence of chronic insomnia-related symptoms among Finns was 9.0% in 2007, and it increased to 9.6% in 2012 (Kronholm, et al., 2016). The age-adjusted prevalence of adult insomnia in the United States rose from 17.4% in 2002 to 18% in 2007 to 18.8% in 2012 (Ford, et al., 2015).

In 2018, 54.7% of the working adults from 117 organisations had insufficient sleep, less than 7 hours in a cross-sectional study conducted among 11356 Malaysians. The average sleep duration for the past 30 days was 6.49 hours (Chan, et al., 2021) while the National Sleep Foundation, the United States of America (USA) recommends 7 hours for adults (Hirshkowitz, et al., 2015).

In Lebanon, it was reported that 39% had a sleeping duration of less than 6 hours when a cross-sectional study was conducted among 501 adults (Chami, et al., 2020). People with higher body mass index and considered overweight or obese, smokers, those who are taking more than two sugary drinks a day, people performing less than 120 min of physical activity per week and medium to high risk of mental health issues are the lifestyle factors that have been linked to shorter sleep duration (Hafner, et al., 2017). Meanwhile, inadequate sleep was shown to occur due to smoking and the presence of children among Malaysian working adults (Chan, et al., 2021).

2.1.2.1 Age

Older age, starting from 24 years old, was significantly associated with shorter sleeping hours in Malaysian working adults. As the population's age

increases, the odds risk (OR) of getting insufficient sleep also increases from 1.2 to 2.2 (Chan, et al., 2021). Out of 123 Malaysians aged 60 and above, 47.2% had poor sleep quality (PSQI >5) with an average age of 69.5 ± 4.55. Gender, ethnicity and living arrangements were not associated with their sleep quality (Razali et al., 2016).

In a study conducted by Chami and colleagues (2020), it was reported that short sleep duration (<6 h) was associated with older age (OR = 1.02 per year). However, the short sleep duration was insignificant in older individuals during weekdays.

2.1.2.2 Gender

In developed countries such as the United Kingdom, the USA, Japan, Germany, and Canada, men had 9 min less sleep than women (Hafner, et al., 2017). However, females were significantly (P = 0.01) associated with insufficient sleep in Malaysian working adults (Chan, et al., 2021). In Nepal, men tend to go to bed earlier than women (Bhatta, Kattel, and Bhatta, 2018). This could be due to cultural norms and gender roles. In many societies, including Nepal, traditional gender roles may influence daily routines and sleeping time. Women may have responsibilities such as household chores or childcare, which could lead to differences in sleep patterns.

Women aged 18 and above in the USA seemed to have a stronger association between negative mood and sleep deprivation than men (Sullivan and Ordiah, 2018). The prevalence of insufficient sleep duration (<6 h) among

Lebanese women (43.2%) was significantly greater compared with men (31.8%), regardless of weekdays or weekends. In addition, 53% of women suffered from daytime fatigue compared to men (34%). Similarly, women had a significantly poorer feeling of not getting enough sleep than men (39% vs 31%) (Chami, et al., 2020).

2.1.2.3 Ethnicity

Researchers have employed ethnicity or race as a research variable in the social and biological sciences for millennia. The term "ethnic" has its origins in Greece, where it was used to refer to a race, people, band, tribe, or swarm (Baumann, 2004). The term refers to groupings of humans who share genetic characteristics that may contribute to the differences in behaviours. Many studies attempt to show that ethnicity or race is an important aspect of sleep studies (Grandner, et al., 2016).

It has been found that the sleep of Blacks/African Americans was less deep and restful compared to non-Hispanic whites. Among non-Hispanic Whites, very short sleep was associated with an increased risk of hypertension, hyperlipidemia, and diabetes, but only hypertension and obesity among self-identified Blacks/African Americans. Meanwhile, short sleep was associated with only hypertension among Mexican Americans and hyperlipidemia among Asians and others. Negative outcomes due to inadequate sleep may vary depending on ethnicity. Thus, cultural and behavioural factors have the capacity to either mitigate or magnify the negative consequences of insufficient sleep. These findings emphasise how crucial it is to comprehend the various routes

connecting sleep (Grandner , et al., 2016).

2.2 Health Risk of Poor Sleep Quality

Sleep is essential for physical, emotional, and psychological health, as well as cognitive functions such as memory, coordination, and concentration.

2.2.1 Physical Health Risk

Insufficient sleep has been closely linked to mortality risk during the past few decades, with 7 to 9 hours of sleep at night associated with a lower mortality risk (Chattu, et al., 2018). Heart diseases were significantly associated (χ^2 = 4.40, P = 0.04, OR = 2.5) with poor sleep quality among elderly primary care patients in Malaysia. Other medical conditions, such as Type 2 diabetes, arthritis, urinary problems, stroke, and hypertension were not associated with poor sleep quality (Razali, et al., 2016).

Experimental sleep deprivation could increase heart rate and blood pressure. Chronic sleep deprivation could result in increased average 24-hour blood pressure, as well as an increased risk of hypertension due to cardiovascular system adaptation to function at a heightened blood pressure equilibrium. Both cross-sectional and longitudinal epidemiological research have shown that shorter sleep duration was associated with increased blood pressure and hypertension. Nonetheless, the association seemed more pronounced in women and middle-aged people (Gangwisch, 2014). Gangwisch (2014) hypothesised that interventions to lengthen sleep duration and improve sleep quality could act as effective preventive measures for hypertension. A recent meta-analysis demonstrated that when compared to both normal sleepers and insomnia patients

with objectively normal sleep duration, insomnia patients with short sleep duration had a greater significant association with hypertension. Objectively measuring a short sleep duration could therefore be a valid and clinically useful measure of insomnia's impact on hypertension (Dai, et al., 2024). This is akin to a cohort study where short-duration sleep (less than 6 hours), longer sleep-onset, and sleep medication usage were associated with an increased risk of coronary heart disease (Lao, et al., 2018).

Although diabetes mellitus is a multifactorial metabolic syndrome, including genetic factors but inadequate sleep may play a role. There is a strong correlation between a heightened risk of diabetes and inadequate sleep duration and quality. Obesity and type 2 diabetes mellitus are linked to insufficient sleep (Antza, et al., 2021). In a prospective study, Japanese workers without a family history of diabetes who slept for 5 hours or less had a 5.37-fold increased risk of developing diabetes compared to those who slept for more than 7 hours (Kita, et al., 2012). Sleep restriction is associated with changes in energy homeostasis, insulin resistance, and β-cell function (Antza, et al., 2021).

Both cross-sectionally and longitudinally, short sleep duration as well as other aspects of poor sleep have been linked to obesity. These findings point to a possible association between higher rates of weight gain and inadequate sleep, which may be related to physical activity or food intake (Ogilvie and Patel, 2017). In addition to sleep duration, obesity has also been linked to other associated types of sleep dysfunction, such as night awakenings and excessive daytime sleepiness, which are markers of poor sleep quality (Norton, et al.,

2018). Sun, et al. (2016) found a significant negative association between sleep quality and overweight/obesity in men. The study suggested that a one-point rise in sleep quality would increase the risk of obesity/overweight in men by 7%. Poor sleep contributes to obesity through sympathetic activation, cortisol and ghrelin secretions, which contribute to fat accumulation and increased appetite (Ding, et al., 2018).

Insufficient sleep was associated with daytime fatigue in Lebanese adults. It was observed that 46.6% of participants were fatigued throughout the daytime and 36.1% felt they usually did not get enough sleep (Chami, et al., 2020).

2.2.2 Psychological Health

Poor sleep quality may act as a precursor to psychological issues. In a stratified random sampling undertaken by the Centres for Disease Control and Prevention in the United States, 20,851 participants responded out of 400,000 individuals approached annually with questions about sleep, anxiety, and depression. Covariates that were being controlled were age, gender, race, education status, marital status, body mass index, employment and income. Less than 6 hours was deemed mild insufficient sleep. Controlling for other covariates, each additional hour of sleep was associated with a lower odds of nervousness (0.80), hopelessness (0.79), depression (0.77), and feeling restless (0.75). One hour less than the recommended amount of sleep was associated with an increased risk of nervousness, hopelessness, depression, and feeling restless. Besides, sleep duration had an inverse relationship with the number of

days with poor mental health controlling other covariates. Mental health symptoms were 3 to 4 times worse when individuals' sleep duration was shorter than 5 hours (Sullivan and Ordiah, 2018).

There appear to be two pathways for the relationship between anxiety, depression, and insomnia. Firstly, there is a bidirectional relationship between insomnia and anxiety. Secondly, insomnia, particularly daytime dysfunction, was a predictor of the onset of depression. This could be due to decreased positive and increased negative emotionality (Baglioni, et al., 2010).

In Malaysia, psychological distress was associated with inadequate sleep duration (less than 7 hours) among adults. Although only 9.87% out of 11356 working adults exhibited psychological stress, they had 1.32 times higher odds of inadequate sleep (Chan, et al., 2021). Besides, 23.6% of elderly primary care patients experienced significant psychosocial distress, and it was positively correlated with sleep quality (Razali, et al., 2016).

In a Malaysian government hospital, 32 doctors on a regular on-call work schedule with a working period of more than 12 hours during the overnight calls were studied. The doctors experienced significant mental fatigue after the on-call duty, where the doctors had an average of 2.2 hours of sleep. Following an overnight on-call shift, the theta rhythm in an EEG analysis significantly increased in total, suggesting drowsiness to sleep. Moreover, there was a noticeable temporal region decrease in the alpha and beta rhythms, indicating a fall in overall power indicative of mental fatigue (Su, Xavier and Kuan, 2023).

2.3 Impacts of Poor Sleep Quality

2.3.1 Occupational Functioning

The majority of adults dedicate two-thirds of their everyday lives to work and sleep. Work can influence health behaviours, such as sleep patterns, which are significant for organisations due to the connection between sleep patterns and employee performance, safety, workplace relationships/attitudes, and health in the future (Lee, et al., 2019). A meta-analysis concluded that sleep quality and quantity were negatively associated with workload and health, attitudinal and affective outcomes (Litwiller, et al., 2017).

Certain working environments or job tasks may affect sleep quality. For instance, academic staff of a university in Japan were found to be in poor mental health due to occupational stress and ineffective coping style (Kataoka, et al., 2014). Lower job satisfaction and job control among academic staff in the university led to anxiety and insomnia (Kataoka, et al., 2014). In a previous study conducted in Malaysia, work-related stress was also found among public universities' academic staff due to performance pressure, role ambiguity, homework interface, and workload pressure (Ahsan, et al., 2009). Meanwhile, poor sleep quality was strongly associated with perceived stress and job dissatisfaction (Doi, Minowa and Tango, 2003).

Insufficient sleep significantly reduced psychological capital and job satisfaction among South Korean employees. Psychological capital refers to positive psychological resources such as efficacy, hope, optimism and resiliency. Additionally, burnout and psychological capital served as serial mediators in the

relationship between inadequate sleep and job satisfaction. The link between sleep insufficiency and serial mediators is shown in Figure 2.1 (Opoku, Kang and Choi, 2023).

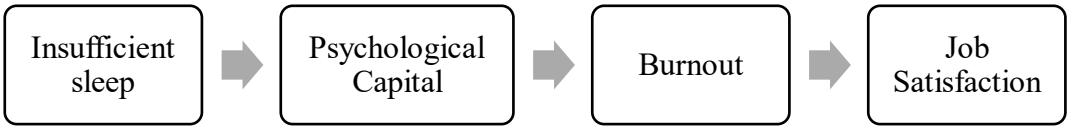


Figure 2.1: The link between sleep insufficiency and serial mediators (Opoku, Kang and Choi, 2023).

In white-collar workers, long work hours correlate with reduced sleep quality in a duration-dependent manner and daytime dysfunction. The prevalence of the global Pittsburgh Sleep Quality Index (PSQI) score was the highest in the workers with overtime hours of ≥50 hours a week (Nakashima, et al., 2011). A longitudinal study conducted among 25605 employees in the UK showed that working 55 hours a week or longer was related to inadequate sleep (less than 7 hours) and sleep disturbances compared to standard 35 to 40 hours a week of working hours. Moreover, working most or all the weekends was associated with short sleep, long sleep, and sleep disturbances. Furthermore, the authors recommended that policymakers should consider fair remuneration for individuals working irregular work schedules (Weston, et al., 2024).

A study was conducted among 340 mothers who worked in multiple levels of educational institutes in Pakistan. The study revealed that sleep insufficiency (less than 6 or 7 hours) was significantly positively correlated with workplace deviance (r = 0.386) and negatively correlated with worker

performance (r = 0.354). Thus, the study indicates that sleep deprivation makes it more difficult for working mothers to finish the assigned tasks in the recommended way. These working mothers may be forced to be involved in activities such as deceitful behaviour, antagonism, and frequent absence that are not deemed desirable in the organisations due to their sleep deprivation, which could negatively impact their performance at work (Deng, et al., 2022).

Presenteeism is the term used to describe the reduced productivity that happens when employees are unable to perform their duties at work due to physical or mental dysfunction. This is a significant indicator contributing between 50% and 75% of the overall cost of health-related expenses to corporate management. Japanese workers with short sleep duration between 5 and 6 hours ($P = 0.004$) and less than 5 hours ($P < 0.001$) saw a much larger drop in output due to presenteeism than those with sufficient sleep duration (7-8 hours). Presenteeism was found to be strongly impacted by subjective sleep quality, sleep latency, sleep disruption, daytime dysfunction and usage of sleep medication among 2897 workers from various sectors (Ishibashi and Shimura, 2020).

According to the results of a meta-analysis that included 27 study research papers, workers who experienced sleep issues in terms of quality and quantity were 1.62 times more likely to encounter an injury than those who did not. Sleep issues account for 13% of work-related injuries excluding medical origin and commuting accidents. It is interesting to note that sleepiness during the day had the lowest association with work injuries (relative risk = 1.33),

problems during the night had a moderate association (relative risk = 1.69) with work injuries, and problems during the day and night had the highest association (relative risk = 2.10) with work injuries. The possibility that sleep issues will result in a job injury was greater in the working population as a whole than in the groups representing particular occupations (Uehli, et al., 2014).

In a study involving 55 young adults without established sleep disorders, it was discovered that there was a negative correlation (r = -0.28, P = 0.039) between motor skill acquisition and waking after sleep onset (48.95 min), as determined by actigraphy. Even though the subjects had normal sleep duration (7.31 hours/night) and a global PSQI score of 3.95 (good sleep), sleep continuity appeared to be essential for retaining motor ability and might be vital for later motor memory encoding (Appleman, et al., 2016).

2.3.2 Social Functioning

In a survey of 501 Lebanese adults, 29.5% reported sleeping more hours on weekends than on weekdays. They sleep more than 8 hours on weekends compared to weekdays. Short sleep is more prevalent in the urban population of a low- to medium-income country (Chami, et al., 2020). This may affect the work-life balance.

Insomnia patients were not be able to recognise facial expression categories such as anger, fear, happiness, and sadness when compared to healthy controls. However, they tended to assess faces as less emotionally intense when they saw expressions of fear and sadness (Kyle, et al., 2014). Neuroimaging

studies show most clearly that sleep deprivation appears to increase emotional sensitivity to emotional stimuli. Sleep deprivation also appears to diminish emotional expressiveness, which has obvious effects on social functioning (Beattie, et al., 2015). Reduced empathy and emotional sensitivity make it more difficult to emotionally connect with others, which may result in miscommunication and relationship strain be at home or in an organisation.

A longitudinal study looking at 4988 teachers in the Finnish public sector found that 53% of them had been subjected to both verbal and nonverbal forms of violence. Sleep disruptions were slightly more common after the violent event than they were before, and this difference lasted for up to two years following the event. It is evident that workplace violence and sleep disturbances were associated. Teachers who were not exposed to violence at work did not experience a significant rise in sleep disruptions. Moreover, verbal acts of violence were associated with an increase in sleep disturbances among teachers working in low-justice conditions compared to those in high-justice conditions. This evidence suggests that working in unfair conditions increases the risk of poor sleep quality. In addition to preventing violence, organisations are recommended to treat employees with justice to maintain good sleep quality (Gluschkoff, et al., 2017).

Given the relationship between emotional valence and sleep, low positive and high negative emotions appeared to be correlated with poor sleep quality. Meanwhile, good sleep quality seemed to be associated with high positive emotions, irrespective of the strength of negative emotions. Emotional

disturbances are caused by a malfunction in the brain circuits that regulate sleep and wakefulness. Getting less sleep seems to be linked to having trouble handling stressful situations (Baglioni, et al., 2010). Adequate sleep is crucial for the optimal functioning of emotional intelligence. Inadequate sleep negatively affects the ability to perceive, understand, and manage emotions, which are essential components of emotional intelligence (Nowack, 2017).

Good sleep has been linked to enhanced interpersonal effectiveness as well. After adjusting for gender and perceived work/life stress, workers who reported having poor sleep quality and quantity scored significantly lower on interpersonal effectiveness by their peers. This is due to the fact that sleep has an impact on mood control, cognitive functions, and emotional stability, all of which have an impact on how people behave in social situations and maintain their relationships (Nowack, 2017).

2.3.3 Economic Burden

Sleep deprivation has a detrimental impact on individuals' health and well-being, and it is also costly for companies due to lost working time from their staff, which is connected with huge economic losses. Hafner, et al. (2017) estimated the economic loss due to sleep insufficiency in five countries: the USA, the UK, Canada, Japan and Germany. Out of these, in 2015, the USA was largely affected by the economic loss of $411 billion and may increase up to $467.7 billion in 2030, and the trend was followed by Japan ($138.6 to $156.2 billion), Germany ($60 to $69.1 billion), the UK ($50.2 to $58.7 billion), and Canada ($21.4 to $23.4). Nevertheless, in terms of overall economic magnitude,

Japan's predicted loss is bigger than that of the United States.

In Australia, where there are 24.8 million people, the estimated cost of insufficient sleep from 2016 to 2017 was $45.21 billion. Out of the total amount, $17.88 billion was due to financial costs comprised of productivity losses (29.19%) including reduced employment, presenteeism, and absenteeism; nonmedical accident costs (13.87%); deadweight loss (8.72%); direct health costs for sleep disorders and associated conditions (6.94%); premature death (3.41%) and informal care costs (2.29%). Reduced well-being came at a non-financial cost of $27.33 billion (Hillman, et al., 2018).

Chattu, et al. (2018) highlighted the significance of sleep for public health as a component of the non-communicable disease agenda on a national, international, and global scale, considering that insufficient sleep syndrome contributes significantly to the economic loss that affects the countries' gross domestic products.

2.4. Conventional Pharmacological Treatments and Limitations for Insomnia

A list of pharmacological treatment options is readily available to manage insomnia. Table 2.2 shows the major drug classes commonly prescribed for insomnia. For insomnia, benzodiazepines and benzodiazepine receptor agonists work well in the short term (less than four weeks). They have shorter half-lives and may have fewer side effects concerning sedation in the morning. They are not recommended for long-term treatment due to the lack of evidence and possible side effects/risks. Meanwhile, antidepressants do have

contraindications that should be carefully evaluated, and they are only useful for short-term treatment. Antipsychotics are not advised for the treatment of insomnia due to a lack of evidence and associated side effects (Riemann, et al., 2017). Antihistamines are only mildly to moderately effective in treating insomnia, and tolerance to these drugs occurs rapidly (Griend and Anderson, 2012). Numerous sedative antidepressants most likely use the histaminergic pathway to produce their hypnotic effects. Due to its poor efficacy, melatonin is not typically advised for treating insomnia (Riemann, et al., 2017).

In summary, these drugs have numerous side effects such as dizziness, drowsiness, memory damage, cognitive impairment and dependence, and withdrawal symptoms such as anxiety and tolerance development. Besides, patients might abuse the usage, which could lead to injuries and accidents due to the sedation effect of the prescribed sleep medication (Bond and Lader, 2012; Srinivasan, et al., 2012).

Table 2.2: Major drug classes commonly prescribed for insomnia

Drug Classes	Drugs
Benzodiazepines	Diazepam, flunitrazepam, flurazepam, lormetazepam, nitrazepam, oxazepam, temazepam, triazolam
Benzodiazepine receptor agonists	Zaleplone, zolpidem, zopiclone
Antidepressants	Agomelatine, amitriptyline, doxepin, mianserin, mirtazapine, trazodone, trimipramine
Antipsychotics	Chlorprothixene, levomepromazine, melperone, olanzapine, pipamperone, prothipendyl, quetiapine
Antihistamines	Diphenhydramine, doxylamine, hydroxyzine, promethazine
Melatonin receptor agonists	Melatonin, ramelteon

(modified from Riemann, et al., 2017)

Most of these medications might also be inappropriate for those suffering from mild to moderate sleep disorders or acute insomnia. In addition, most of the affected people do not prefer a pharmacological approach for insomnia treatment (Vincent and Lionberg, 2001) and switch to complementary and alternative treatments or non-pharmacological approaches (Pearson, Johnson and Nahin, 2006; Omvik, et al., 2010).

2.5 Alternative Treatments or Preventive Measures for Poor Sleep

2.5.1 Sleep Hygiene

Sleep hygiene is an education given to those affected with sleep disorders to promote sleep and discourage habits that lead to poor sleep. It can be a standalone therapy but often integrates with cognitive behavioural therapy (CBT) as a stimulus control element. Less evidence has proven that it can work as a single therapy, as it has not been studied in the general population. However, empirical evidence revealed an association between individual sleep hygiene recommendations and nocturnal sleep (Stepanski and Wyatt, 2003; Irish, et al., 2015).

According to the assessment for sleep hygiene, the sleep hygiene index (SHI) or score can be measured by taking into account the behaviours that are unfavourable for good sleep such as taking caffeine, tobacco or alcohol four hours before sleep, staying too long in bed, using bed not only for sleeping and having sex but for doing other daily tasks such as browsing internet, paying bills, eating, studying, playing games and so on. Going to bed at a different time or in a bad mood, exercising that causes sweating within 1 hour before sleep or taking more than 2 hours of naps are considered as unfavourable behaviours as well. Creating a conducive bedroom environment will promote better sleep, such as a comfortable bed, absolute darkness, less noise, and not too cold, hot or stuffy (Bootzin, 1972; Mastin, Bryson and Corwyn, 2006).

Numerous research showed that sleep hygiene such as self-help books was less successful than other strategies and was typically used in combination

with behavioural therapy (Kaku, et al., 2012). Avoiding caffeine or alcohol may only improve sleep for insomniacs with high sensitivity to those substances, although mostly the effects of sleep of those substances are dose dependent. Besides, not much significant relationship was found between nocturnal sleep and napping. Managing stress is also individual-based (Irish, et al., 2015). Different populations might need different or customised sleep hygiene. Children suffering from neurodevelopmental disabilities were also affected by poor sleep, but sleep hygiene was less effective (Jan, et al., 2008). Thus, further research should be conducted to develop more concise sleep hygiene.

2.5.2 Cognitive Behavioural Therapy and Mindful Meditation

Cognitive behavioural therapy (CBT) alters one's thinking and behaviours to treat associated health problems. In insomnia therapy, the cognitive approach is to determine and change beliefs or negative thoughts that affect the ability to sleep whereas the behavioural approach is to develop good sleep habits and eliminate bad habits that lead to insomnia. There are many intervention categories under CBT for insomnia (CBT-I) such as stimulus control therapy (re-associate the bedroom environment and reduce anxiety associated with sleep), sleep restriction therapy (merging fragmented sleep and increasing sleep deprivation to regulate the sleep cycle), and relaxation therapies or multi-component approaches that assimilate behavioural treatments with cognitive therapy (Hood, Rogojanski and Moss, 2014).

For a person to be treated using this approach, he or she must have severe trouble initiating or maintaining sleep, be affected with symptoms that are not

exclusively due to a chronobiologic sleep disorder, and establish an unstable primary medical or psychiatric condition that would not contraindicate treatment, and maladaptive coping mechanisms or presleep hyperarousal (Smith and Perlis, 2006).

Manber and co-researchers (2008) studied the effect of CBT-I in a combination of antidepressant, and escitalopram on depression among 30 subjects affected by both insomnia and major depressive disorder. The experimental group was treated with 12 weeks of escitalopram of 5 mg to 20 mg along with seven sessions of CBT-I while the control group only received antidepressants. A higher rate of remission of insomnia was noticed in CBT-I (50%) than in the control group (8%). In addition, CBT-I also reduced symptoms of depression by 62% versus the control group (33%). However, studies with a larger sample size involved must be conducted to posit that pharmacological treatment alone is not sufficient for insomnia or depression.

Mindfulness is a psychological process that creates awareness of the present moment and accept it without being judgmental. This would aid a person with stress to be less reactive to the unpleasant experience he or she is suffering from and the sense of well-being will be improved (Germer, 2004). A combined effect of CBT and mindful meditation might be able to sustain its benefit for up to 12 months (Ong, Shapiro and Manber, 2009). When co-morbidities are present, clinical judgment should be used to determine whether to treat the co-morbid disease or insomnia first or both at the same time. For individuals of any age, CBT-I is advised as the initial line of treatment for chronic insomnia

(Riemann, et al., 2017).

2.5.3 Acupuncture

Auricular therapy or auricular acupuncture induces stimulation at specific acupoints by piercing with fine needles for therapeutic purposes on the outer ear. It can be administered within a minute and has been studied to reduce anxiety with fewer side effects reported (Wang, Peloquin and Kain, 2001). Common adverse events that could occur in acupuncture are tenderness or pain at insertion, local discomfort, minor bleeding, dizziness, and nausea, and they are considered transient and tolerable (Tan, et al., 2014).

A meta-analysis involving six studies and 673 insomnia patients in China and Hongkong, showed that acupuncture significantly increased the quantity and quality of their sleep. Six commonly used auricular acupoints were Shen men, Heart, Occiput, Subcortex, Brain, and Kidney with different modalities such as needles, pellets and/or magnetic pearls. They were able to sleep for up to six hours, which was even better than the groups that received diazepam in two of the trials, and this study did not mention any adverse effects (Chen, et al., 2007). Subsequent meta-analyses demonstrated that acupuncture enhanced sleep parameters in comparison to placebo, such as PSQI, insomnia severity index (ISI), total sleep time (TST), wake after sleep onset (WASO), sleep-onset latency (SOL) and sleep efficiency (SE) (Zhang, et al., 2020).

Hot flashes and sleep disruptions among post-menopausal women have been associated, although less supporting evidence. In a systematic review and

meta-analyses involving 2433 subjects, acupuncture has been shown to alleviate menopause-related sleep disturbances among perimenopausal and postmenopausal women (Chiu, Hsieh and Tsai, 2016). In an independent study, menopausal women suffering from hot flashes following treatment for breast cancer received acupuncture treatment. The intervention study showed that acupuncture resulted in a concomitant reduction in sleep disturbances (Falsetti, et al., 2011).

Studies that evaluated the effectiveness of acupuncture on insomnia have many biases and limitations, such as not reporting adverse events, not being randomised and placebo-controlled trials to prove as an adjunct therapy to other treatments, small sample size and so forth. Excessive pressure exertion also might lead to infection, ischemia and tissue necrosis. Therefore, only trained therapists can conduct the procedure (Cheuk, et al., 2012).

2.5.4 Exercise

A systematic review revealed that aerobic exercise improved sleep or insomnia, and it was as effective as hypnotic medication for sleep, although more prospective studies should be carried out to support this finding. Most of the studies showed that exercise can reduce sleep latency onset. Exercise could improve insomnia by providing antidepressant-like effects such as increasing serotonin release, reducing anxiety, lowering body temperature in the late evening, followed by sleep onset triggering, and improving immunity (Passos, et al., 2012).

In another long-term study was conducted among 21 volunteers were subjected to 50 min of moderate aerobic exercise three times a week for four months. The study revealed that exercise significantly improved total sleeping time (TST), sleep onset latency (SOL), sleep efficiency (SE), and total wake time of polysomnographic measurements. PSQI subjective analysis shows improvements in total score, TST and SOL but not in SE. Fasting blood analysis showed that cortisol concentration, CD4, and CD8 counts decreased significantly. While CD4 and CD8 levels demonstrated a decrease in inflammation, a fall in cortisol suggested a decrease in stress (Passos, et al., 2014).

However, a bidirectional study done by Baron, Reid and Zee (2013) showed that good sleep influenced exercise participation rather than exercise inducing better sleep quality, although a larger sample size should be studied to prove this, as the study only involved 11 women (mean age- 61.27). They were required to engage in aerobic exercise for 30 min, three times a week.

A reciprocal relationship was also found between exercise and sleep quality when a study was conducted among 79 older adults with a mean age of 63.58 years. The 18-week study revealed that chronic exercise was shown to reduce WASO in these subjects. The same study proved that location (indoor versus outdoor) did not have a significant difference in sleep parameters and this indicated that sunlight exposure was not associated with exercise and insomnia. This study also supported the hypothesis that temperature regulation and mood improvement occurred after exercise promoted better sleep quality

(Dzierzewski, et al., 2014).

2.5.5 Yoga

Although yoga is considered mild exercise according to Godin leisure-time exercise questionnaire (Godin and Shephard, 1997), it can be categorised as a multicomponent practice including meditation and relaxation. It is a comprehensive system that promotes physical, psychological, and spiritual health and integrates postural exercise, meditation, and breathing practices (Goyeche, 1979). The body positions in yoga are called asanas, the respiratory exercises are called pranayama, and the meditation is called dhyana.

There are yoga postures able to increase blood supply to the brain, particularly to the sleep centre such as the pineal gland and hypothalamus that aids in achieving a better sleep cycle. Practicing yoga was able to improve self-confidence and alleviate stress among insomnia subjects (Sobana, et al., 2013). Brown and Gerbarg (2005) postulated that yoga was able to alleviate menopausal symptoms by increasing autonomic tone and the stress response system, reducing chemoreflex sensitivity, increasing baroreflex response sensitivity and increasing prolactin and oxytocin. Generally, insomnia reduces cognitive functions such as focus, but yoga improves brain concentrations by releasing GABA (Streeter, et al., 2007).

Yoga is beneficial not only as an adjunct therapy for cancer survivors but also for those in palliative care to improve their sleep quality and pain management (Deshpande, 2018). Table 2.3 shows the other studies of yoga and

the respective sleep outcomes. Many trials were conducted to study the effect of yoga but mostly integrated with other approaches. Nevertheless, without a standardised protocol and the presence of different types of yoga practices, it is very difficult to adapt them as a standard treatment for insomnia.

Table 2.3: Characteristics of studies examining yoga and sleep outcomes

Source	Intervention and duration	Population Subject	Age (years)	Outcome Related to Sleep
Khalsa, 2004	Kundalini yoga for 30 and 45 min a day for 8 weeks	20 (2 men and 18 women) 2 groups – 30 min and 45 min Method- sleep diaries	Mean = 48.1	Significantly improved TWT, TST, SE, SOL and WASO
Afonso, et al., 2012	Yoga HT for menopause, one hour twice a week for 4 months	44 postmenopausal women's Randomised control study Yoga- 15 Passive stretching- 14 Control- 15 Method- Insomnia severity Index, polysomnography	50-65	ISI significantly reduced in yoga and passive stretching group. Kupperman Menopausal Index significantly reduced only in yoga group
Mustian, et al., 2013	Yoga for Cancer Survivors (YOCAS), 75 min twice a week for 4 weeks	410 cancer survivors Yoga-168 Control-153 Method- PSQI and actigraphy	Mean= 54	Global sleep quality, daytime dysfunction, subjective sleep quality, and sleep medication use were improved In actigraphy, WASO and SE were improved

TWT= total wake time, TST= total sleeping time, SE= sleep efficiency, SOL= sleep onset latency, WASO= wake after sleep onset, ISI=insomnia severity index.

2.5.6 Tai Chi Chih

Tai Chi Chih (TCC) is a moving meditation comprising 19 movements and a single pose performed to obtain physical and mental health benefits. TCC is not only able to improve sleep quality in healthy individuals especially older adults but also those with chronic diseases such as cerebrovascular disorders and fibromyalgia (Raman, et al., 2013). Besides, TCC can improve sleep quality and reduce the associated risk factors for metabolic, cardiovascular, and inflammatory diseases by improving sleep quality after 16 months of practice although CBT achieved it right after the 4th month. TCC seems to have more benefits than regular exercise because of its use of mind-body integration yet the mechanisms of action remain unknown (Raman, et al., 2013; Carroll, et al., 2015a).

It has been postulated that TCC is capable of reducing sympathetic activity and at the same time stimulating parasympathetic activity in a similar way to how yoga functions. This will release endogenous neurohormones that promote deep states of relaxation and thus alleviate insomnia or sleep disorder-related symptoms (Jahnke, et al., 2010). A later study by Irwin and researchers (2017) involved breast cancer survivors, showed that both CBT-I and TCC improved insomnia, but TCC was statistically non-inferior to CBT-I.

However, a randomised clinical trial, comparing the effect of CBT versus TCC relative to a sleep seminar providing sleep hygiene education as a control group. It was a 4-month study that involved 2 hours of an intervention session per week. The study revealed that CBT had a twofold better remission rate

compared to the other two interventions. Yet, TCC was significantly associated with better sleep quality, depression, and fatigue. Meanwhile, the researchers recommended that TCC could be utilised before the severe clinical onset of chronic insomnia appears in older adults (Irwin, et al., 2014).

2.5.7 Supplements for Poor Sleep Problems

Given the potential risks and limitations associated with pharmacological treatments, herbal or synthetic supplements have led to their increasing appeal as alternative treatments for poor sleep problems. The supplements that are frequently used to improve sleep quality include valerian, hops, kava, German chamomile, tart cherry, tryptophan, theanine, melatonin, magnesium, and zinc. Most of these supplements including valerian, hops, kava and tryptophan modulate GABA receptors, increase melatonin and/or serotonin secretion which contribute to relaxation and better sleep quality. Meanwhile, tryptophan showed mixed results and improved certain sleep parameters only (Yeom and Cho, 2024). Tart cherries have been related to their high melatonin content, which could help in regulating the sleep-wake cycle. Melatonin supplements are generally considered safe for short-term use and are generally prescribed for jet lag and shift workers (Yeom and Cho, 2024). Due to its poor effectiveness, melatonin is not typically advised for the treatment of insomnia (Riemann, et al., 2017). On the other hand, magnesium improves sleep and regulates stress via GABA, melatonin and hypothalamic-pituitary-adrenal (HPA) activity (Yeom and Cho, 2024).

However, some of them may cause adverse effects for example, valerian

may cause headaches, dizziness, and gastrointestinal disturbances (Taibi, et al., 2007). Meanwhile, hops could cause gastrointestinal complaints, dizziness, and allergic reactions and interact with other sedative medications and alcohol, leading to drowsiness and functional impairments (Benkherouf, et al., 2020). Although kava has been proven to improve anxiety as well as insomnia with promising results, it could lead to hepatotoxicity and several countries have withdrawn kava products from the market (Sarris, LaPorte and Schweitzer, 2011). Leach and Page's (2015) systematic review and meta-analysis revealed that there was no statistically significant difference between herbal and placebo treatments or between herbal medicine and active control for all thirteen clinical efficacy indicators for insomnia cases. The common herbal mono preparations that were taken orally in those studies were wuling, kava, chamomile, and valerian.

These supplements are considered safe in general but they might be associated with serious adverse effects, including the possibility of interactions with prescription drugs or body metabolism. Due to a lack of stringent regulation, there is also a considerable variation in the quality and purity of the supplements. For clinical decision-making, larger, well-designed clinical trials are required to confirm the safety and efficacy of these supplements (Leach and Page, 2015; Yeom and Cho, 2024).

2.5.7.1 Alpha-S1-Casein Tryptic Hydrolysate

Milk hydrolysate has been incorporated into many food supplements. The calming effect is observed in babies following ingestion of milk where the milk has been digested distinctly. Trypsin is the enzyme that is predominantly involved in babies unlike in adults where pepsin is more active (Hamosh, 1997). Enzymatic hydrolysis of primary cow's milk protein, casein produces CTH, decapeptide known as alpha-casozepine (CZP), YLGYLEQLLR, f91-f100 and other peptides.

Since CTH is derived from milk, the relaxing decapeptide is classified as food and obtained the status of generally recognised as safe (GRAS) by the Food and Drug Administration in a self-declaration process (FDA-21 CFR §184.1553; NDI #rpt242). The commercial products that incorporate the bioactive peptide as an active ingredient are registered with different trademark names such as Lactium™, Zylkene, rlx2™, Calm Canine and Royal Canin's Calm Canine.

Anxiety or psychological stress is a serious health issue not only in humans but also in pets or farm animals. Such behavioural problems in pets are one of the reasons owners relinquish their pets. Pet keepers or owners would prefer natural bioactive compounds over psychotropic drugs to treat anxiety disorders. Besides, veterinarians have to manage anxiety in animals, as anxiety may cause adaptability. Nutraceutical agents such as CTH may aid them in anxiety management but regrettably, not many veterinarians opt for this agent. The same reluctance was found in pharmacological options to deal with stress

and anxiety in humans. Anxiolytic agents such as benzodiazepines are prescribed due to their quick action, but prolonged use of this agent may cause cognitive impairment, addiction and tolerance. Scientists are constantly in search of newer anxiolytic agents with fewer known risks (Bandelow, 2020).

The effect of the CTH and its bioactive peptides were studied in healthy and anxious subjects. In many animal studies, stress was induced and the behavioural response was recorded to test for the effectiveness of the biopeptides. Among the stress inducers are elevated plus maze (EPM), light/dark box (LDB), conditioned defensive burying (CDB) tests following an electric shock, paying a visit to a veterinary clinic, or introducing a new environment or human to the animal.

EPM was used to study anxiety in rats and mice. The test was designed based on the natural desire of the animals to explore and their fear of open and high spaces. The rodent with anxiety prefers a dark/closed space. The exploration in the maze for 5 minutes will be recorded. This includes the number of entries into open/closed arms, the time spent in each arm, and the latency time before entering into a closed arm (Miclo, et al., 2001; Violle, et al., 2006; Cakir-Kiefer, et al., 2011; Mizushige, et al., 2013). Figure 2.2 shows the elevated plus maze used for testing rats.

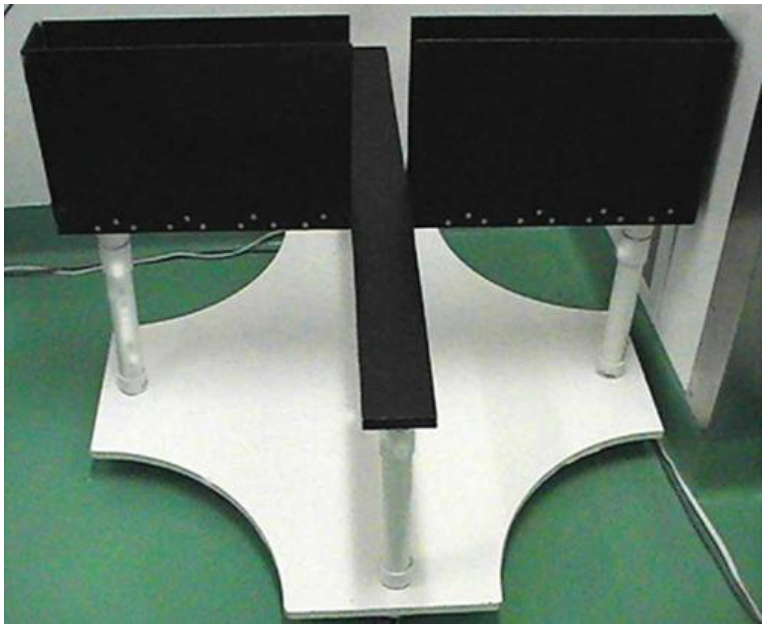


Figure 2.2: The elevated plus maze used for testing rats (Walf and Frye, 2007)

The LDB test uses two compartments: a familiar dark compartment illuminated with low-intensity white light and an aversive unfamiliar compartment illuminated with high-intensity white light. The rodents' exploration of the box for 10 minutes will be recorded. Parameters such as the number of entries and the time spent in each compartment will be measured (Benoit, et al., 2020; Cakir-Kiefer, et al., 2011).

CDB experiment usually uses electric shock around 1 mA to induce anxiety, and the anxiety behaviour following shock was evaluated by measuring the time taken to bury the aversive stimuli. The action of burying is linked with an anxiety response. Effective anxiolytic treatment may reduce or prevent the burying behaviour of rodents (Miclo, et al., 2001; Violle, et al., 2006; Messaoudi, et al., 2009; Cakir-Kiefer, et al., 2011).

In animal models, the effect of the milk hydrolysates was examined through sleep assessment (Yayeh, et al., 2018), sleep cycles (Yayeh, et al., 2018), neuronal and receptor activation in the brain (Benoit, et al., 2017, Yayeh, et al., 2018), cortisol level (Kato, et al., 2012; Miyaji, et al., 2015) and other behavioural and physiological changes (Kanso, et al., 2016; Landsberg, et al., 2017).

In humans, there are no specific biomarkers for anxiety disorders (Bandelow, 2020). Particular diseases or health conditions associated with stress, such as acne (Kerure, Udare, and Vispute, 2022) and poor sleep quality (Campbell and Neill, 2016; Scholey, et al., 2017; Kim, et al., 2019; Phing and Chee, 2019) were used to explore the anxiolytic activity. In a study by Messaoudi, et al. (2005), stress was induced in healthy human subjects through the Stroop test and cold pressure test. The effect of the milk hydrolysate was observed in terms of cortisol levels (Messaoudi, et al., 2005; Kerure, Udare, and Vispute, 2022) with various anxiety or sleep quality assessments (Campbell and Neill, 2016; Scholey, et al., 2017; Kim. et al., 2019; Kerure, Udare, and Vispute, 2022), and cardiovascular measures (Messaoudi, et al., 2005). Table 2.4 shows the summary of CTH and CZP research findings based on different models.

Table 2.4: The summary of CTH and CZP research findings based on different models

Author	Model	Treatment	Findings	Safety
ANIMAL				
d'Angelo, et al., 2022	7-year-old dog with tail chasing behaviour, case report.	450 mg of CZP, 25 mg of fluoxetine and associated with a behavioural recovery program (3 months)	No symptoms of frustration and anxiety, as well as tail-chasing behaviour. The compulsive disorder was resolved completely.	Not reported
Makawey, Iben and Palme, 2020	60 adult cats, visits to a veterinary clinic were used as the stressor.	15 mg/kg/day of CZP for 6 days, 75 mg/kg/day CZP for 3 days or placebo (fructose) for 3 days	The absence of sweaty paws was found in the higher dose of the CZP group in the waiting and examination room.	Not reported
Benoit, et al., 2020	48, 9-week-old, male Swiss mice, LDB served here as an anxiety-inducing situation.	Vehicle, α -CZP (1 mg/kg), YLGYL (0.5 mg/kg), or diazepam (1 mg/kg), i.p. α -CZP (1 mg/kg) and YLGYL (0.5 mg/kg) and diazepam (1 mg/kg), i.p.	**LDB** YLGYL contributed to anxiolytic-like properties in the LDB similar to CZP and diazepam during stressful conditions. Expression of c-Fos in brain regions was different among the peptides compared to diazepam.	Not reported
Naarden and Corbee, 2020	31 cats (domestic	A therapeutic diet to reduce crystal formation	The episode of recurrence of idiopathic cystitis in the treated group (5/17) was significantly lower compared	Not reported

	and shorthair) over 1 year age with acute non-obstructive idiopathic cystitis	enriched with α-CZP and L-tryptophan vs a non-therapeutic diet for 5 weeks.	to the control (11/14). CZP and L-tryptophan were given for anxiolytic properties.	
Ijichi, et al., 2019	10 horses, transport as a stressor.	1000 mg (<500 kg) and 2000 mg (>500 kg) of α -CZP, single dose.	Loading time was significantly shorter than control. No other behavioural or physiological changes.	Not reported
Yayeh, et al., 2018	C57BL/6 mice	A single dose of 30-240 mg/kg, p.o. of CTH, 1 mg/kg, i.p. of diazepam or saline	**Sleep Assessment** CTH increased sleep duration dose-dependently. Although 120 and 240 mg/kg were significantly different from the control, diazepam recorded the longest significant sleep duration.	-
	Sprague-Dawley rats	150 mg/kg of CTH, 300 mg/kg of CTH or saline, p.o. once per day for 3 days	**EEG recording** The shortest significant sleep-wake cycle number was achieved with the shortest wake time and the longest sleeping time at 300 mg/kg.	
	Sprague-Dawley rats	150 mg/kg of CTH, 300 mg/kg of CTH or saline, p.o. once per day for 3 days	**Western blotting of hypothalamic tissue** 150 mg/kg or 300 mg/kg of CTH increased the $GABA_A$ $ß_1$ receptor expression of, no significant changes in α_1 or γ_3.	

Benoit, et al., 2017	48, 9-week-old, Swiss mice LDB as a stressor	CZP (1 mg/kg), diazepam (1 mg/kg) and vehicle, i.p.	In LDB, CZP, and diazepam-received rats stayed longer in the lit area but only CZP increased the number of rears in the lit box compared to vehicle. In the non-stressful condition, CZP showed no difference but diazepam showed difference in neuronal activation compared to vehicles. When compared to diazepam, CZP imposed similar c-Fos expression in the hippocampus, hypothalamus and nucleus accumbens but the expressions were different in the amygdala and prefrontal cortex.	-
Landsberg, et al., 2017	24 short-haired cats with mildly fearful and fearful conditions	Diet supplemented with CZP and L-tryptophan (Royal Canin Feline Calm diet) and control diet	**Inactivity duration** A significant increase was found in the control but not in the test group. **Inactivity frequency** Significant increase between baseline and after 2 weeks in the test diet group and no significant changes in the control group. No differences between groups in the homeroom and human interaction test.	-
Meyer and Becvárová, 2016	10 cats with feline idiopathic cystitis	Cat food enriched with 15.3-15.7 mg/kg of CTH and 12 – 14 mg/kg of L-tryptophan and other nutrients	Lower urinary tract disorder signs and emotional and quality of life improved significantly	2 cats had gastrointestinal signs.
Kanso, et al.,	36-week-old	800 mg/kg/day for 15	**Vasorelaxant effect on WKY and SHR**	Not Reported

2016	Wistar Kyoto (WKY) normotensive rats and spontaneous hypertensive rats (SHR)	days of camel (CCTH) and bovine casein tryptic hydrolysate (BCTH), p.o	The two treatments induced relaxation in both groups of endothelium-intact aortic rings, but better in WKY. The relaxation was endothelium-dependent via nitric oxide (NO) pathway activation. Relaxation was impaired in the SHR thoracic aorta. **Antihypertensive effect in awake SHR** CCTH decreased blood pressure and heart rate only during the first week of treatment. BCTH marked no changes.
dela Peña, et al., 2016	Male ICR mice and Sprague-Dawley rats (n = unknown)	CTH (75, 150, 300 or 500 mg/kg), saline, p.o. and diazepam (1 mg/kg), i.p. The control group received distilled water.	**Open-field test (OFT)** Diazepam demonstrated a sedative effect as it decreased in distance moved and movement duration. No significant changes in CTH. **Rota-road test** Diazepam decreased endurance and increased falling frequency. No significant changes in CTH. **Pentobarbital-induced sleeping test** Diazepam decreased sleep onset and increased total sleep duration. CTH increased sleep duration at 150 mg/kg. In EEG analysis, diazepam increased the delta waves and decreased the alpha waves. CTH increased delta waves without significant changes in alpha waves during sleep. **Intracellular chloride ion measurement assays** CTH increased chloride ion influx in a dose-dependent

		Neuroblastoma cells (*in vitro*)	manner and bicuculline, a competitive GABAA receptor antagonist inhibited chloride ion influx induced by CTH	
Miyaji, et al., 2015	21 healthy indoor cats, hospital visits and blood sampling were the stressors.	CZP (15 mg/kg) and tryptophan (3.6 g/kg) fortified CALM Feline diet and control diet containing 3.4 g/kg of tryptophan but without CZP for 8 weeks for each group.	The study diet increased the ratio of plasma tryptophan to large neutral amino acids and decreased urinary cortisol concentration significantly compared to the control. No changes were observed in plasma cortisol and behavioural assessment in both groups.	No adverse effects and indicated safe
McDonnell, Miller and Vaala, 2014	3 to 13-year-old 10 (450-600 kg) light horse mares, aversive to routine healthcare procedures	2000 mg/day of CZP, and control supplement for 5 days, p.o.	Compliance and/or comfort scores improved significantly for 7/10 aversions in the treated group compared with 1/10 for the matched control during routine procedures	Not reported
Mizushige, et al., 2013	5-week-old male ddY mice (n=6-13)	1 mg/kg, 3 mg/kg, i.p. of YLG or saline	**Elevated plus-maze (EPM) Test** Dose-dependent anxiolytic activity was recorded, significantly different from saline	-
		2.4-10 μmol/kg, i.p. of YL, YLG or CZP and	Dose-dependent anxiolytic activity was shown in all three YL analogues with YLG exhibiting the highest.	

		saline		
		3.5 μmo/kg p.o. of YL, YLG, CZP and saline	All three peptides showed better anxiolytic activity than saline with CZP demonstrated the highest.	
		0.1 mg/kg, i.p. of YLG	**Open field test** 0.1 mg/kg, i.p. of YLG showed anxiolytic activity without affecting the locomotor activity	
		3 mg/kg of YLG i.p. followed by WAY100135, and bicuculline, i.p.	YLG-induced anxiolytic activity was inhibited.	
		Pepsin-pancreatin digest of α_{S1}-casein (0.1–1 g/kg) and saline, i.p.	**EPM** Dose-dependent anxiolytic activity was exhibited in all the concentrations tested and significantly higher than saline.	
McDonnell, Miller and Vaala, 2013	6 small semi-feral Shetland-type (160-205 kg) ponies	Zylkene, 1000 mg daily 5 days, p.o. before acclimation and training.	The treated ponies appeared alert and scored significantly higher than the control counterparts which tested for calmness, compliance, and acclimation/skill progress.	Not reported

Kato, et al., 2012	44 anxious dogs, visit for toenail clipping was used as the stressor	Control diet containing tryptophan to large neutral amino acid (LNAA) at a ratio of 0.043 without CZP followed by diet with CZP, 1.3 g/kg dry matter and tryptophan to LNAA at a ratio of 0.048 for 7 weeks each treatment with 1-week washout period.	The CZP-enriched diet lowered the stressor-induced increase of the urine cortisol to creatinine ratio significantly compared to the control diet.	No adverse effect was observed
Cakir-Kiefer, et al., 2011	6-week-old male Wistar rat, (n = 320)	0.7 mg/kg of CZP, 0.5 mg/kg of f91 – 97, 0.5 mg/kg of diazepam and saline by i.p. 1.0 mg/kg of CZP, 0.7 mg/kg of f91 – 97, 1.0 mg/kg of diazepam and saline i.p.	**CDB** Diazepam and f91 – 97 were significantly better than saline-treated rats but not CZP. **Open Field** Locomotor activity was not affected irrespective of the treatments. **EPM** Percentages of time spent in the open arm in the CZP or f91-97 group were similar to diazepam. Only diazepam group recorded a longer latency period to enter the closed arm.	-

			LDB The percentage of entries, the time spent and the number of rears in the lit area were significantly greater in the groups diazepam, CZP and f91-f97 compared to the control.	
Palestrini, et al., 2010	32 female Beagle dogs with or without anxiety.	Anxious and non-anxious dogs were fed with either CTH or a placebo diet for 4 weeks.	Anxiety score was higher in anxious than non-anxious dogs at baseline but no significant difference was shown at the end of the study between anxious CTH and non-anxious CTH or placebo diet. In CTH-fed anxious dogs, cortisol levels decreased and the behaviour of anxious dogs were improved. The diet did not affect heart rate, lysozyme concentration and white blood cell count.	Not reported
Messaoudi, et al., 2009	48 male Wistar HsdBrlHan rats	<u>Acute</u> 5 and 15 mg/kg of CTH, 3 mg/kg of diazepam, and vehicle (control), p.o., 60 min before the test.	**CDB** 15 mg/kg of CTH and diazepam showed significantly lower probe burying time than the vehicle.	
	36 male Wistar HsdBrlHan rats	150 mg/kg of CTH, 9 mg/kg of diazepam, and vehicle, p.o., 60 min before the test	**Tolerance** On day 8, a significantly lower burying time was recorded for 15 mg/kg of CTH compared to diazepam and control while there was no difference between control and diazepam.	Lack of tolerance for CTH not diazepam when given repeatedly
	36 male Wistar	<u>Subchronic</u> 15 mg/kg of CTH, 3	**Passive avoidance (Safety)** CTH and control rats displayed significantly longer	Diazepam caused antegrade

	HsdBrlHan rats	mg/kg of diazepam, and vehicle (control), p.o., twice daily for 7 days.	latency periods to enter the dark area than diazepam.	amnesia
	36 male Wistar HsdBrlHan rats	Phase 1- acclimation for 3 days Phase 2- 30 mg/kg of CTH, 6 mg/kg of diazepam or vehicle, and vehicle on alternate days, p.o., up to day 13. Phase 3- no treatment on day 14	**Conditioned place preference (Dependence)** The time spent in the non-preferred compartment of the CTH group was reduced between the first and final phases but diazepam increased the time spent in the earlier non-preferred compartment. No preference for control group.	Diazepam was addictive but not CTH.
Beata, et al., 2007a	34 cats with anxiety	15 mg/kg of CZP or placebo, p.o. for 56 days	The number of cats responded to treatment successfully (10/17) was higher than the placebo (4/17).	Not reported
Beata, et al., 2007b	38 dogs, with anxiety	15mg/kg of CTH or 0.5 mg/kg of selegiline hydrochloride (positive control) p.o. daily for 56 days	**Evaluation of a dog's emotional disorder** Both treatments were significantly effective over time. **Owner's assessment** The treatments were equally efficacious and no significant difference	No adverse effect
Violle, et al., 2006	40 male Wistar rats	5, 15, 30 and 50 mg/kg of CTH and	**CDB** 15, 30 and 50 mg/kg displayed lower probe burying	

		vehicle (control), p.o.	durations and latency of the first probe approach after the shock than control.	
	30 male Wistar rats	15 mg/kg of CTH, 3 mg/kg of diazepam, and vehicle, p.o.	**CDB** CTH and DZP showed lower probe burying durations and latency of the first probe approach than control. DZP induced a significant ten-fold decrease in this delay compared to control, whereas the CTH did not.	
	30 male Wistar rats	15 mg/kg of CTH, 3 mg/kg of diazepam, and vehicle, p.o.	**EPM** CTH and DZP significantly increased the percentage of entries and time spent in the open arm and decreased the percentage of the closed arm entries compared to the control.	Diazepam induced disinhibition and increased locomotor activity but not in CTH.
Guesdon, et al., 2006	42 male Wistar rats, sonorous and light-dark disturbances with tilting to the cage as stressors	15 mg/kg of CTH and bovine milk protein (control) for the stressed and 15 mg/kg of CTH for the unstressed group, p.o. for 8 days	**Short Term (2 days)** The reduction in total sleep duration in the control stressed group was greater than in the CTH group. CTH treatment did not affect sleep in unstressed rats. No significant differences in corticosterone or glucose levels. **Long term (8 days)** Significant decrease in total sleep duration in the control group while sleep was preserved in the CTH group.	Non-sedative

Miclo, et al., 2001	Wistar rats (n is not reported)	1 and 3 mg/kg of CTH, i.p. 30 min before an i.p. injection of pentylenetetrazole	**Anticonvulsant** 1 mg/kg reduced crisis severity 3 mg/kg reduced crisis severity, increased crisis latency and decreased clonus duration	No side effect
	60 Wistar rats	1 mg/kg of CTH, 3 mg/kg of diazepam, and saline (control), i.p.	**EPM** Percentages of entries in open arms increased in both treatment **CDB** CTH decreased the probe burying duration similar to diazepam	
		0.4 mg/kg of CZP, 1 mg/kg of diazepam, and saline (control), i.p.	**CDB** CZP decreased probe burying time compared to saline. CZP had similar activity with DZP but 10-fold greater.	
		CZP on MA-10 mouse Leydig cell mitochondrial preparation in competition experiments against two specific ligands of peripheral-type benzodiazepine receptor	CZP has no affinity to peripheral-type benzodiazepine receptor	

HUMAN

Kerure, Udare, and Vispute, 2022	100 patients with moderate-to-severe acne. Randomised, controlled, multicenter, open-label, trial.	100 mg of CTH and standard of care (SOC) v SOC alone for 84 days, p.o. (SOC: doxycycline, topical adapalene and clindamycin gel)	Serum cortisol levels, perceived stress scale (PSS) scores and Hamilton anxiety rating scale (HAM-A) scores reduced significantly in the CTH group. Inflammatory and non-inflammatory acne lesions and associated symptoms were alleviated in both groups.	Safe and well-tolerated
Phing and Chee, 2019	48 with poor sleep quality (global PSQI ≥ 5). Double-blind, randomised placebo-controlled trial	150 mg of CTH plus 50 mg of L-theanine and skimmed milk (placebo) per day for 4 weeks, p.o.	Sleep latency, sleep disturbances, daytime dysfunction, and depression were improved in week 4 while anxiety and stress levels were improved from week 3 onwards in the supplement group compared to the placebo group.	-
Kim, et al., 2019	48 with poor sleep quality (global PSQI > 5). Double-blind,	300 mg/day of CTH with excipient and excipient (placebo) per day for 4 weeks with 4 weeks washout period in between, p.o.	Total sleeping time, sleep efficiency, sleep latency, and wakefulness after sleep onset were improved. Sleep efficiency in actigraphy was increased.	Well tolerated, did not affect vital signs or haematological profiles.

	randomised placebo-controlled, crossover trial			
Scholey, et al., 2017	160 with poor sleep quality (global PSQI > 5). Double blind, randomised placebo-controlled trial	LZComplex3 (75 mg of CTH, *Zizyphus, Humulus lupulus*, magnesium and Vitamin B6), two times daily for 2 weeks, p.o.	Sleep quality, changes in mood and anxiety, stress reactivity, daytime dysfunction, physical fatigue and cognitive performance showed similar improvements in both supplement and placebo groups.	25 gastrointestinal cases were recorded involving both groups in equal ratio.
Campbell and Neill, 2016	19 with poor sleep quality (Insomnia Severity Index > 15). Double blind, randomized placebo-controlled crossover trial	NightMilk (2.565 ng of melatonin and unknown concentration of CTH) and DayMilk (8.8 pg/mg of melatonin) (placebo) per day for 3 weeks with 1 week washout period in between, p.o.	NightMilk improved insomnia symptoms, sleep efficiency and sleep architecture compared to DayMilk but failed to increase total sleep time.	Not reported

Jacquet, et al., 2015	87 burnout professionals. Double blind, randomised placebo-controlled trial.	75 mg of CTH, 5 mg of extramel, 50 mg of taurine, 50 mg of Siberian ginseng and placebo-excipient twice a day for 12 weeks, p.o.	Very low or low burnout levels were found in treated (34/44) than in placebo (5/41). Both groups showed improvement in quality of life and family and energy, but sleep quality was improved only in the treated group. Depressive symptoms improved better in the treated group than in placebo.	Well tolerated with some gastrointestinal discomforts in both groups
Messaoudi, et al., 2005	42 healthy subjects facing successive mental and physical stress. Double blind, randomised placebo-controlled trial.	400 mg of CTH, 3 times, 12 hours apart and bovine skimmed milk powder as a placebo, p.o.	Stroop test (ST) and combinational assessment of cold pressure test (CPT) and ST increased systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) in both groups but CTH recorded lower percentage change in SBP and DBP compared to placebo. Plasma cortisol concentration decreased, and HR remained stable in CTH group but increased in placebo.	Not reported
In Silico				
Qian, et al., 2021	8 groups (n=12) of 9-week-old, male Kunming	50 and 100 mg/kg of CTH, 2 mg/kg of CZP, ultrapure water, and 5 mg/kg of YYVPL, YFYPEL and YPVEPF,	CTH has 2-fold stronger sleep-enhancing properties compared to CZP. CZP in CTH degraded more slowly than the synthesised CZP CTH produces other sleep-enhancing peptides such as	No potential liver or other organ toxicity was predicted. No sedative

	mice. Structure-function analysis, virtual screening (molecular docking), and statistical optimisation	p.o. and 1 mg/kg of diazepam, i.p. twice per day for 7 days.	YPVEPF and YFYPEL. YPVEPF significantly prolonged the sleep duration and increased the sleep rate. YPVEPF and YFYPEL were able to bind with the residues of $GABA_A$ receptor.	effect compared to diazepam
In Vitro				
Cakir-Kiefer, et al., 2011	Human colon epithelial cancer (Caco-2) cell line	CZP and f91-97 in porcine bile extract (EBS) and an equimolar mixture of taurocholate, cholate, and deoxycholate (BSM).	Hydrolysis of CZP caused higher formation of fragments f91-99, f91-98, and f91-97, Hydrolysis of f91-97 by pancreatic proteases, leading to lower peptide degradation. Absorption of CZP and f91-97 in cells increased significantly in EBS and BSM respectively.	Not related
Kanso, et al., 2014	Thoracic aorta and mesenteric arteries of 12-week-old Wistar Kyoto	0.1 ng - 100 µg/ml of camel (CCTH) and bovine casein tryptic hydrolysate (BCTH)	Dose-dependent relaxation was achieved in both thoracic aorta and mesenteric arteries when treated with CCTH and BCTH compared to the untreated tissue, but the effect was more powerful in CCTH. Relaxation was activated through the nitric oxide-cyclic guanosine monophosphate pathway.	Not related

(WKY) rats	CCTH and BCTH exhibited similar relaxation with a lesser maximal effect in mesenteric arteries compared to thoracic aorta.

p.o.- orally, i.p.-intraperitoneally
- indicates that there was no information given on side effects.

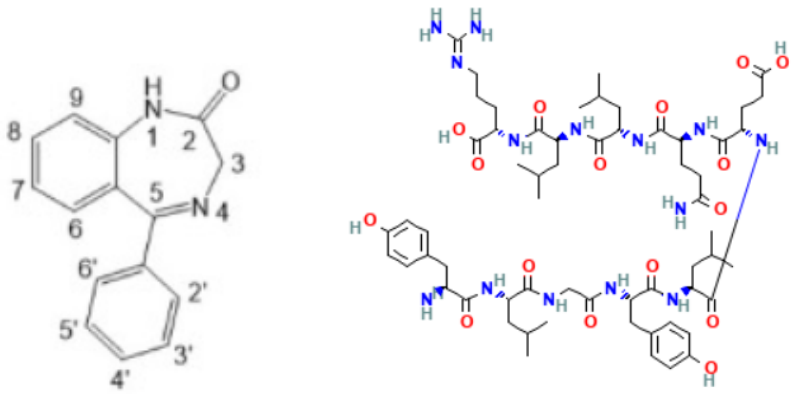
Most researchers used more than one test or a combination of tests, such as EPM and CDB, to study the anxiolytic effect of the natural compound, as this can reduce false-positive results. To study the effect of CTH, a stressful environment was also induced in rats by combining various stressors such as sonorous disturbances, light-dark cycle and a different tilt to the cage every 12 hours (Guedson, et al., 2006).

Digestive enzymes such as pepsin-pancreatin were able to digest milk casein and the digest products could exhibit anxiolytic-like activity (Cakir-Kiefer, et al., 2011) proposed that the anxiolytic activity after oral administration of the CTH may be due to CZP but also to its proteolytic fragments. Tryptic digestion of bovine αS1-casein produces many peptides such as YL, YLG, YLGY, YLGYL and YLGYLEQLLR (α-CZP). Among these, YL, YLG, and α–CZP induce anxiolytic-like activities and are mediated by the activation of serotonin (5-HT1A), dopamine (D1), and $GABA_A$ receptors. They may exhibit anxiolytic-like activity by increasing in the release of endogenous neurotransmitters such as serotonin, dopamine and GABA (Mizushige, et al., 2013).

According to Miclo, et al. (2001), CTH has a strong affinity towards the benzodiazepine site of the $GABA_A$ receptor when tested in the bovine cerebral cortex membrane but only f91-100 or CZP in CTH displayed this property. Although synthetic CZP could compete with flunitrazepam (a benzodiazepine) but was four-fold less potent than the milk-purified peptide. The researcher also stated that CZP in the *in vivo* study demonstrated better anxiolytic activity

compared to the *in vitro* study and the reason behind this difference is unknown (Miclo, et al., 2001).

The CZP has been shown to possess two flexible tyrosine aromatic rings, similar to the classical benzodiazepine aromatic rings. Figure 2.3 shows the chemical structure of benzodiazepine and CZP. Anxiolytic effects have been established due to a strong affinity for $GABA_A$ receptors, the main binding site for benzodiazepine-like drugs (Lecouvey, et al., 1997; Messaoudi, et al., 2009). CTH promoted chloride ion influx in a dose-dependent manner when treated in neuroblastoma cells. This influx was inhibited by bicuculline, a competitive inhibitor of the $GABA_A$ receptor. This shows that the sleep-enhancing feature of CTH is most probably facilitated through GABAergic neurotransmission (dela Peña, et al., 2016).



(a) (b)

Figure 2.3: Chemical structures of benzodiazepine (a) and alpha casozepine (b) (adapted from Loftsson, 2021 and National Center for Biotechnology Information, 2019)

Most of the studies used diazepam as a positive control as diazepam is a known psychotropic drug to control anxiety. The mechanism of action of CZP partially differed from diazepam (dela Peña, et al., 2016; Benoit, et al., 2017). The c-Fos protein is a marker for neural activity. Diazepam induced reduction of c-Fos in both controlled and stressful conditions, unlike CZP, in which the effect was only observed in stressful conditions (Benoit, et al., 2017).

CTH has been widely studied for its sleep improvement compared to CZP although other peptides such as YPVEPF and YFYPEL were shown to possess sleep-enhancing capabilities (Qian, et al., 2021). CTH significantly improved sleep duration (Guesdon, et al., 2006; Kim, et al., 2019), sleep efficiency (Kim, et al., 2019), sleep latency (Kim, et al., 2019, Phing and Chee, 2019), sleep disturbances and daytime dysfunction (Scholey, et al., 2017; Phing and Chee, 2019).

In EEG analysis, CTH was shown to maintain slow wave sleep and increase paradoxical sleep. The result from total sleep, slow wave sleep and paradoxical sleep indicated that the effect of CTH was exerted between 1 to 10 hours after ingestion (Guesdon, et al., 2006). CTH promotes delta waves in pentobarbital-induced sleep, which indicates deep sleep or relaxation, while diazepam increases delta waves and decreases alpha waves during sleep. This indicated that CTH has a sleep-promoting effect rather than sedation as shown in diazepam (dela Peña, et al., 2016). Although CTH could prolong sleep duration, diazepam improved both sleep duration and sleep onset (shortened). This shows that CTH might be lacking some sleep-inducing properties (dela

Peña, et al., 2016).

Tryptic hydrolysate was more potent than peptic and chymotryptic hydrolysate as tryptic hydrolysis could generate more active and/or stable bioactive peptides. CTH was proven to activate endothelial cells and mediate the vasorelaxant property via the NO-cGMP pathway. The activation partly could be due to B2 bradykinin receptor. CTH was a potent vasorelaxant but when a comparison was made between camel and bovine CTH, CTH from camel origin was able to produce more significant relaxant properties. CTH of camel might have a higher affinity towards the cellular target. However, the relaxation effect in mesenteric arteries showed a lesser maximal effect when compared to the aortic ring which could be due to the lower number of B2 bradykinin receptors in the mesenteric arteries (Kanso, et al., 2014).

As for cardiovascular changes, vasorelaxation was achieved in the thoracic aorta of both normotensive and hypertensive rats which was potentially endothelium-dependent and mediated by NO pathway. However, CTH from camel was able to reduce blood pressure transiently but no putative changes were recorded in bovine CTH in 15 days of treatment. These findings show that different sources of CTH might have released different bioactive peptides. Moreover, it was suggested that these peptides should remain active during digestion and absorption in the gastrointestinal tract and reach the cardiovascular system (Kanso, et al., 2016). The milk hydrolysate did not alter heart rate (Kanso, et al., 2016) or blood pressure (Kanso, et al., 2016) although it can maintain those parameters in stressful condition (Messaoudi, et al., 2005).

In order for the anxiolytic peptide to act on the central nervous system, it has to resist hydrolysis by the digestive enzymes and be absorbed by the brush borders of the intestine as well as pass through the blood-brain barrier. Peptidases found in the brush borders could lower the bioavailability of the bioactive peptides (Pauletti, et al., 1997). Intestinal transporters can carry only di- and tripeptides and the possible ways for larger peptides to pass through the intestinal epithelial barrier are endocytic uptake, or paracellular pathway transport across Peyer's patches (Foltz, et al., 2008).

CZP is usually ingested orally and has to go through extreme pH and enzymatic activity of the gastrointestinal tract as well as hepatic first-pass effect which makes the bioavailability four times lesser than the intraperitoneal injection (Benoit, et al., 2017). The main fragment formed from the hydrolysis of CZP by gastrointestinal enzymes is f91-97. The shortened heptapeptide maintains its anxiolytic activity similar to CZP. It was postulated that arginine residue leads to an active structure but additional residues in CZP could impose steric constraints. Thus, the removal of the carboxy-terminal residues may remove this constraint which allows the f91-97 peptide to adopt an active form (Cakir-Kiefer, et al. 2011).

In rodents, the minimum dose of CTH required to elicit an anxiolytic response was 15 mg/kg when oral dosage tested in the range of 5, 15, 30 and 50 mg/kg. Moreover, 15 mg/kg had a similar anxiolytic response to 3 mg/kg of diazepam. Nevertheless, diazepam induced a disinhibition state which can be

related to risk-taking behaviour in the ethological analysis, but CTH did not exhibit such a side effect (Violle, et al., 2006). In rats/mice, 150 mg/kg of dose recorded the longest sleep duration with less and no sedation out of the tested dose range of 75 – 500 mg/kg (dela Peña, et al., 2016). Anxiety was reduced significantly in the elevated plus-maze and in the conditioned defensive burying paradigm at the tested dose of 3 mg/kg by intraperitoneal (i.p.) route (Miclo, et al., 2001).

Current evidence on rodents indicates that CZP and CTH appear to possess anxiolytic effect after acute intraperitoneal injection at a minimum dose of 1 mg/kg (Cakir-Kiefer, et al., 2011; Benoit, et al., 2017; Benoit, et al., 2020) which was similar or comparable to diazepam effect. As for oral administration, 15 mg/kg of CTH was sufficient to promote the anxiolytic effect (Violle, et al., 2006; Guesdon, et al., 2006; Messaoudi, et al., 2009) although there were few studies that used higher dosages (Kanso, et al., 2016; dela Peña, et al., 2016; Yayeh, et al., 2018). Besides, there were not many studies on CZP by oral route, and to date, 0.4 to 4.4 mg/kg has shown effectiveness in contributing to anxiolytic activity (Miclo, et al., 2001; Mizushige, et al., 2013).

In dogs and cats, Calm Canine or Feline diet incorporated two anxiolytic agents, tryptophan and CZP. Tryptophan is a precursor for melatonin and serotonin and able to improve sleep and mood in vulnerable subjects (Silber and Schmitt, 2010). The observed effect could be due to the effects of tryptophan alone, CZP alone, or the combined effect of both. Nevertheless, the difference in tryptophan amount was very minute between the diets while CZP was only

added to the study diet and absent in the control group. Hence, the anxiolytic effects shown in the studies by Miyaji, et al., (2015) and Kato, et al., (2012) at the dosage of 15 mg/kg could be largely due to the presence of CZP.

CZP acts as a putative aid to improving the efficiency of handling and training equines. Routine healthcare procedures can be aversive for horses. Oral administration of 2000 mg of CZP for 5 days improved compliance (McDonnell, Miller and Vaala, 2014). The relaxing peptide was also studied to reduce stress and ease transporting horses when sedation is not a wise option. The result was not as expected probably as only horses that were transported with experienced handlers with no known aversion to loading were chosen to study. Significant results might be obtained if the peptide is tested in animals having anxiety issues or aversion such as in the study conducted by McDonnell, Miller and Vaala (2013). The authors also recommended using weight-sensitive dosage at a maximum level such as 1000 mg/day for equines that weigh less than 500 kg and 2000 mg/day for heavier animals (Ijichi, et al., 2019).

To date, once-daily oral dosing has been found effective in humans as well as animals. In humans, two weeks of treatment (75 mg/kg twice a day with other active ingredients) was insufficient for clinically noticeable results (Scholey, et al., 2017). Thus, the drastic changes could not be observed. Kim, et al. (2019) gave a higher dosage, 300 mg/day and recommended 4 weeks of treatment which was able to exhibit substantial effects. Thus, 150 – 300 mg/day up to 4 weeks or longer were able to give favourable effects, especially on sleep quality parameters, anxiety and depression (Jacquet, et al., 2015; Phing and

Chee, 2019; Kim, et al., 2019).

In a preclinical study, CTH, CZP and its bioactive fragments did not alter spontaneous locomotor activity and motor function (Violle, et al., 2006; Cakir-Kiefer, et al., 2011; Mizushige, et al., 2013). It is apparent that the resistance to anxiety of the CTH-treated rats was not due to the sedative effect of the CTH. Studies showed that CTH has less or no sedation effect (Guedson, et al., 2006; Violle, et al., 2006; dela Peña, et al., 2016) and did not develop tolerance compared to diazepam (Messaoudi, et al., 2009; dela Peña, et al., 2016). Diazepam attained tolerance after repeated treatments (dela Peña, et al., 2016). Moreover, diazepam caused anterograde amnesia and addiction but not in the CTH-treated model (Messaoudi, et al., 2009).

The continued benefits of CTH without tolerance might be due to a slower degree of cellular tolerance at the benzodiazepine site of the $GABA_A$ receptor complex or could be due to unrelated pharmacological factors (Messaoudi, et al., 2009). Messaoudi, et al. (2009) postulated that the action of CTH would be more on alpha 2- and alpha 3-selective benzodiazepine receptors which could give rise to anxiolytic agents with fewer side effects than the present benzodiazepine. Meanwhile, benzodiazepine may act on alpha 1-containing $GABA_A$ receptors which could be a reason for amnesia.

In clinical trials, CTH was well tolerated among the participants. Nevertheless, there were a few adverse events reported in the study conducted by Scholey and researchers (2017). Most of them related to gastrointestinal

disorders such as abdominal pain, dyspepsia, gastritis and gastroenteritis although none of the cases were classified as serious while psychomotor and cognitive functions were not affected. However, a similar number of cases were found in both treatment and placebo groups and could not track the exact aetiological agent as combinational treatment was used (Jacquet, et al., 2015; Scholey, et al., 2017).

The mechanism of how absence of tolerance for CTH and the affinity of its peptides to the $GABA_A$ receptor after repeated administration remains unsolved. Anxiolytic properties of the milk bioactive peptides have been fairly documented in human, laboratory and domestic animals. CTH and its bioactive peptides could improve anxiety and sleep in mankind. Treatment through diet especially to animals is advantageous to pet owners or animal handlers as it is easily achievable with higher compliance. Effective dosage must be investigated to achieve the desired outcome in a timely manner in order for the supplement to be prescribed by medical practitioners widely as nutritional management of stress and anxiety.

2.5.7.2 L-theanine

The amino acid, L-theanine was discovered by Japanese researchers from Kyoto Laboratories in 1949. It can be found in tea plants, especially in green tea which gives the flavour properties (Juneja , et al., 1999) and umami taste but odourless (Chen , et al., 2023). It was also linked to anti-stress or relaxing properties (Juneja, et al., 1999). Since L-theanine is extracted from plants, it is soluble in water and found in nature in the L-enantiomer form with

a molecular weight of 174.198 g/mol (Chen, et al., 2023). Figure 2.4 shows the chemical structure of L-theanine.

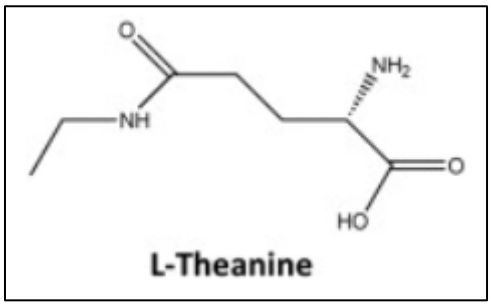


Figure 2.4: The chemical structure of L-theanine (g-ethylamino-L-glutamic acid) (Mu, Zhang, and Jiang, 2015; Chen, et al., 2023)

L-theanine is used globally in the food and pharmaceutical industries as it has been approved by the US FDA as GRAS. There are two ways to obtain L-theanine: chemical synthesis, which is difficult for consumers to accept and cannot be used in the food industry, or isolation, which typically requires labour-intensive, expensive, and time-consuming processes to achieve high purity. L-theanine can also be produced via biological way using bacterial enzymes. The ability to produce L-theanine has been identified in four types of bacterial enzymes: L-glutamine synthetase, γ-glutamylmethylamide synthetase, γ-glutamyltranspeptidase, and L-glutaminase. However, very limited microbial sources are available for L-theanine biosynthesis. Thus, screening and characterising additional microbial sources of L-theanine-producing enzymes is necessary (Mu, Zhang, and Jiang, 2015).

L-theanine has numerous positive health impacts which have been proven in many studies. According to a summary compiled by Bulman, et al., (2023), L-theanine supplementation may improve sleep in numerous sleep

parameters. Administration of 200 mg of L-theanine improves WASO or intermittent awakening followed by refreshed awakening. L-theanine improves sleep quality by modulating the autonomic system: decreasing sympathetic and enhancing parasympathetic activities (Rao, Ozeki and Juneja, 2015).

L-theanine was able to initiate an alpha brain wave pattern that implies a relaxed physical and mental state without drowsiness or impaired motor skills. Besides, it also involves forming the inhibitory neurotransmitter GABA (Mason, 2001; Adhikary and Mandal, 2017) similar to CTH. L-theanine has no known side effects and does not promote sleep as this amino acid does not produce theta waves (Mason, 2001). Additionally, it was discovered that L-theanine did not induce sleep; hence, its ability to increase sleep quality may differ from that of sedatives (Rao, Ozeki and Juneja, 2015).

Table 2.5 depicts the overview of clinical trial results for L-theanine supplementation. L-theanine in the range of 50–655 mg per day may be useful as a supplement, alone or in combination with other bioactive substances, to enhance sleep quality and doses higher than 655 mg may not be beneficial and may be detrimental to overall sleep quality (Bulman et al., 2023). The duration of treatments using L-theanine varied, from 45 min to eight weeks. However, because there were no appreciable changes in haematological tests or biochemical blood tests like liver function, kidney function, or even glucose metabolism, the chronic administration of L-theanine was deemed safe (Hidese, et al., 2017; Baba, et al., 2021). Moreover, in an animal study, the no-observed-adverse-effect-level (NOAEL) for L-theanine is 4000 mg/kg bw/day. No

significant changes were observed related to the treatment at the highest dose tested (Borzelleca, Peters and Hall, 2006).

Besides improving sleep quality (Hidese, et al., 2019; Kunugi, et al., 2019; Moulin, et al., 2024), L-theanine has been shown to reduce stress (Moulin, et al., 2024), anxiety (Hidese, et al., 2017), and depression (Kunugi, et al., 2019). The same supplement could enhance cognitive functions (Baba, et al., 2021; Moulin, et al., 2024; Karunaratne and Dassanayake, 2024) such as improving response latency, error rate, verbal memory and executive functions (Hidese, et al., 2019; Kunugi, et al., 2019).

Although the precise nature of the direct positive association between the benefits of clinical treatment and experimental enhancement of adult neurogenesis remains unclear, it appears that L-theanine can reduce symptoms associated with intact neural network coordination dysfunctions, hence promoting brain health and wellness (Wang, et al., 2022).

L-theanine plays a vital role in boosting immunity to lessen immunosuppression caused by intense exercise and wards off colds and influenza. In addition, L-theanine plays an immunoregulatory role in inflammation, improves intestinal immunity, regulates cytokines and neurotransmitters, tumour immunity, nerve damage alleviation, promotes glutathione synthesis and antioxidant capacity (Chen, et al., 2023).

Table 2.5: The overview of clinical trial outcomes for L-theanine supplementation

Sources	Model/ participants	Dosages	Findings	Safety
Unno, et al., 2013	20 healthy participants with higher traits of anxiety traits (Randomised, placebo-controlled and single-blind)	200 mg/twice a day for 17 days	Lowered the subjective stress level	Not reported
Hidese, et al., 2017	20 patients with major depressive disorder (Open-label and placebo-controlled)	250 mg/day for 8 weeks	Reduced anxiety, improved sleep quality, improved cognitive function such as response latency, error rate, verbal memory and executive function.	Safe as no changes in liver or renal function.
Hidese, et al., 2019	30 healthy individuals without major psychiatric illness (Randomised, placebo-controlled, crossover, and double-blind)	200 mg/day for 4 weeks	Sleep latency, sleep disturbance, and use of sleep score medication improved. Cognitive functions such as verbal fluency and executive function improved.	No significant adverse events.
Sarris, et al., 2019	46 participants with generalised anxiety disorder, non-responsive to antidepressants (Double-blind, randomised; placebo-controlled)	450 mg; 225 mg- 1 capsule twice/day ; 8 weeks (900 mg for those who did not achieve	Sleep quality improved but no changes in insomnia severity index, anxiety symptoms or cognition	No significant adverse events.

		≥35% reduction in anxiety score after week 4)		
Kunugi, et al., 2019	30 healthy participants without psychiatric illness (Randomised, placebo-controlled, and double-blind, crossover)	200 mg/day for 4 weeks	Improved sleep quality, depression score, verbal fluency and executive function.	No significant adverse events.
Evans, et al., 2021	16 healthy individuals with task-induced stress (Randomised, placebo-controlled, and triple-blind, crossover)	200 mg or placebo, single shot	Increased total and frontal alpha power after 3 hours and decreased salivary cortisol after 1 hour compared to placebo.	Safe and well-tolerated as no changes in haematological and biochemical tests.
Baba, et al., 2021	50 middle-aged and older healthy subjects (Randomized Placebo-Controlled)	100.6 mg/ day for 12 weeks	A single dose of L-theanine showed improvement in attention and working memory tasks. After 12 weeks, no significant changes in attentional function.	Safe, as no changes in haematological tests and biochemical tests.
Moulin, et al., 2024	30 healthy adults with moderate stress (Randomised double-blind, placebo-controlled)	200 mg/ twice a day for 4 weeks	Improved sleep quality by decreasing time asleep and light sleep. Decreased perceived stress and	No clinically relevant changes in vital signs, clinical chemistry or

			enhanced cognitive attention. No change in salivary cortisol.	haematological parameters.
Karunaratne and Dassanayake, 2024	24 healthy volunteers but sleep-deprived overnight. (double-blind, placebo-controlled, crossover)	200 mg given 45 min before the traffic-related reaction task	Improved selective visual attention by improving information processing speed and target-distractor discriminability	No adverse events

2.6 Psychological Measures of Poor Sleep

There are a few psychological measures widely used to assess poor sleep quality. Questionnaires that can assess subjective sleep quality are PSQI, the most widely used, with high reliability and validity and other less commonly used in research settings, such as Leeds Sleep Evaluation Questionnaire (LSEQ) and SLEEP-50. Meanwhile, insomnia symptoms can be measured via the insomnia severity index (ISI) and the Athens Insomnia Scale (AIS). On the other hand, daytime functioning due to poor sleep can be assessed by questionnaires such Epworth Sleepiness Scale (ESS) (Fabbri , et al., 2021).

2.6.1 Pittsburgh Sleep Quality Index (PSQI)

PSQI is a psychological measure commonly used to assess sleep quality. The PSQI is a questionnaire that was created by Buysee et al. (1989) with the intention of evaluating the sleep quality of the clinical samples; it is not intended to provide a precise clinical diagnosis of insomnia. However, it can guide

clinicians in their investigation. It provides a standardised and quantitative measure of sleep quality and differentiates between good and poor sleepers. The questionnaire contains 19 items divided into seven components that measure the quality and pattern of sleep during the past 1 month. The seven components that can be measured individually are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Table 2.6 shows the description of PSQI components. The sum of scores is called as global PSQI score, ranging from 0 to 21. The highest score indicates the worst sleep quality. A global PSQI score greater than 5 indicates major difficulties. The questionnaire is attached in Appendix A.

Table 2.6: The description of PSQI components

Components of PSQI	Description
Component 1 Subjective sleep quality	A self-rating of overall sleep quality
Component 2 Sleep latency	Time taken to fall asleep after going to bed
Component 3 Sleep duration	Actual sleep per night in hours
Component 4 Habitual sleep efficiency	Number of hours of actual sleep divided by the total number of hours of being in bed
Component 5 Sleep disturbances	Waking in between sleep due to waking up too early, bathroom use, not being able to breathe comfortably, coughing or snore loudly, feeling too hot or cold, having bad dreams, or having pain
Component 6 Use of sleep medication	Frequency of sleep medication use, either prescribed or over-the-counter, within the past month
Component 7 Daytime dysfunction	Having trouble staying awake or having the enthusiasm to get things done

Adapted from Buysee , et al., 1989

PSQI can be a sensitive, reliable, and valid outcome assessment tool to be used in community-based studies of primary insomnia and able to produce a coherent result (Tsai, et al., 2005; Chen, Kuo and Chueh, 2010). High relationships were found between the PSQI and other sleep quality measures, including the ISI score, certain polysomnography variables, actigraphy, and clinical diagnoses of insomnia (Mollayeva, et al., 2016). In a non-clinical group

of adults that included both younger and older individuals, the global PSQI score was correlated significantly with sleep diary variables (SE, r = -0.562; TST, r = -0.307; WASO, r = 0.262; SL, r = 0.480) and the depression scale (r = 0.305) (Grandner, et al., 2006).

The PSQI has undergone substantial validation across various populations and clinical and non-clinical settings. Due to its widespread use, this questionnaire which was in English has been translated into numerous languages, including Malay (Farah, Saw Yee and Mohd Rasdi, 2019), Korean (Sohn, et al., 2012), Portuguese (João, et al., 2017), Japanese (Doi, et al., 2000), German (Backhaus, et al., 2002), and many more. The PSQI can be used to monitor the results of interventions or treatments targeted at enhancing sleep since it is sensitive to changes in sleep quality over time with strong validity and reliability (Mollayeva, et al., 2016).

2.7 Physiological Measures of Poor Sleep

Polysomnography seems to be the most effective method for studying physiologically measured sleep, which is modest at best. Actigraphy provides a less reliable surrogate measure of sleep. Electroencephalography, electrooculography, electromyography, heart rate, and blood pressure monitoring are other useful tools for studying sleep quality in a population (McCarter, et al., 2022). Cortisol and melatonin are reliable biomarkers that can be analysed in various samples, such as plasma, urine, and saliva, for circadian rhythm studies (Skubic, et al., 2025).

2.7.1 Blood Pressure

Blood pressure is the lateral force that blood exerts on the vessel wall, measured as systolic and diastolic pressure in millimetres of mercury. During ventricular systole, the maximum pressure measured in arteries is known as systolic blood pressure (SBP). Diastolic blood pressure (DBP) is the minimum pressure measured in arteries during ventricular diastole or before the next contraction (Brzezinski, 1990). Raised blood pressure is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg in adults aged 18 and older, as measured by age-standardised prevalence (World Health Organization, 2024).

Studies conducted on persons aged 58 to 60 years found no relationship between blood pressure and sleep duration, but among younger adults, there was an association between short sleep duration and hypertension. Sleep duration was also associated with gender, as women who get less than 5 – 6 hours of sleep per night are more likely to develop hypertension (Dean, et al., 2012).

Meanwhile, the insomnia group with sleep onset problems showed lower baroreflex sensitivity and higher morning systolic and diastolic blood pressure gradients as compared to the good sleepers. This insomnia subtype showed decreased baroreflex sensitivity and weak vagal regulation, suggesting that trouble falling asleep can have a separate impact on cardiovascular function in the morning (Tsai, et al., 2021).

Blood pressure tends to drop by roughly 10–20% when a person falls

asleep as opposed to during the day and this reduction is called a "dip" (Parati, et al., 2013). When the blood pressure reduction or dip is minimal, it may indicate underlying medical conditions such as sleep apnea, hypertension, and elevated cardiovascular risk (Fagard, et al., 2008). A higher risk of SBP and DBP non-dipping was associated with factors such as trouble falling asleep, prolonged WASO, shorter and longer TST, lower SE, and lower REM stage sleep. One potential mechanism by which poor sleep quality was associated with worse cardiovascular outcomes is that clinical symptoms of insomnia and poor sleep quality are associated with nondipping blood pressure (Lyu, et al., 2020).

2.7.2 Heart Rate

Heart rate usually lowers when an individual falls asleep. On the contrary, this decrease might be less noticeable in people with sleep difficulties, which could result in an elevated heart rate at night (Stepanski, et al., 1994).

Individuals who suffer from insomnia frequently have elevated resting heart rates. The resting heart rate was apparently higher in those with all three insomnia symptoms—difficulties initiating sleep, frequent awakenings, and early awakenings several times a week than in those with none of the symptoms (Hauan, Strand and Laugsand, 2018). Heart rate and PSQI score showed a positive correlation ($r = 0.34$), however, this was observed in hypertensive individuals receiving anti-hypertensive medication (Oliveira-Silva, et al., 2020).

Sleep quality alters heart rate variability (HRV). Reduced parasympathetic and elevated stress levels can be indicated by lower HRV,

which is typically the result of poor sleep. Baroreflex sensitivity is the capacity of the body to detect changes in blood pressure and adjust heart rate and vascular resistance accordingly to help maintain stable blood pressure. Baroreflex sensitivity was lower in poor sleepers (Oliveira-Silva, et al., 2020).

2.7.3 Cortisol

Cortisol is a glucocorticoid hormone produced in the adrenal cortex, specifically in the zona fasciculata. Cortisol secretion is controlled by the hypothalamic hormone, corticotropin-releasing hormone (CRH), and the pituitary hormone, adrenocorticotropic hormone (ACTH), in the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol is involved in physical and emotional stress and is thus called a stress hormone (Katsu and Baker, 2021).

Additionally, cortisol plays a role in the regulation of blood pressure and the immune system as well as in the metabolism of carbohydrates, proteins, and fats (Katsu and Baker, 2021). Due to its strong insulin-antagonistic properties, cortisol may worsen glucose intolerance, increase fasting glucose levels, and ultimately cause diabetes mellitus (Schernthaner-Reiter, et al., 2021).

Laboratory stress-induced sleep deprivation in healthy individuals may cause heightened autonomic nervous system or cortisol responses (Messa, et al., 2024). Diagnosis and severity of insomnia are related to the overactivation of the HPA axis and the hypersecretion of cortisol (Richardson and Roth, 2001). Chronic insomnia symptoms are linked to a steeper morning rise in cortisol (Abell, et al., 2016), over the first 30–45 min). It has been shown that cortisol is

a reliable marker of HPA activity (Backhaus, et al., 2002).

Higher salivary cortisol levels upon waking were substantially associated with anxiety disorder, but no correlations were found between anxiety state and salivary cortisol levels in the evening (Vreeburg, et al., 2010). Cortisol found in saliva is free (not bound) and biologically active. The transfer of cortisol from blood to saliva happens no more than 2 to 3 minutes (Bozovic, Racic, and Ivkovic, 2013), and salivary cortisol levels are unaffected by salivary flow rate or salivary enzymes (Vining and McGinley, 1987). Moreover, the salivary cortisol is a more convenient approach for analysis than plasma cortisol as it does not generate external stress as if in plasma cortisol when phlebotomy is required. The saliva samples can be collected at home and across a longer period as well as at specific time points (Aardal-Eriksson, Karlberg, and Holm, 1998; Hellhammer, Wüst and Kudielka, 2009).

HPLC analytical method is preferred to quantify cortisol in saliva because of its advantages, such as specificity, accuracy, rapidity, precision, and ease of automation. Any material that can dissolve in a liquid can be used to separate, identify, and quantify the compounds present in it (Bhardwaj, Dwivedia and Agarwala, 2015).

HPLC involves mobile and stationary phases. The principle is the sample is injected into a column of porous material which acts as a stationary phase and liquid or solvent is pumped at higher pressure through the column which acts as a mobile phase. Separation can be done based on the affinity of the solute that

adsorbs on the stationary phase. In general, HPLC system consists of solvent reservoirs that store the mobile phase, a pump that provides a continuous flow of the solvent from the reservoir, an injector that allows the sample containing analytes to be entered into the stream, a column where the mobile phase encounters the stationary phase which aids in analytes separation with pore size from 0.2 to 5 μm and finally data acquisition controls all parameters of the instrument including temperature, mobile phase composition and flow rate (Kazakevich and Lobrutto, 2007).

Depending on the application, a variety of commercially accessible HPLC detectors are available, such as ultraviolet (UV), fluorescence and electrochemical. The detector for HPLC is selected based on the chemical nature of the analytes, the limit of detection, compatibility with HPLC, and the detector's availability and cost. The most popular HPLC detector in use today is the UV-visible absorbance detector because a lot of compounds of interest absorb in the UV or visible region within the wavelength of 190–600 nm. Beer's Law determines the fraction of light transmitted through the detector cell, which in turn determines sample concentration from the absorbance value. UV detectors come in three varieties: photodiode array and variable detectors, which rely on one or more wavelengths produced by a broad spectrum lamp, and fixed wavelength detectors, which rely on specific wavelengths (Swartz, 2010).

A chromatogram is generated as separation output in a standard HPLC system. Each specific analyte in the chromatogram is denoted by a peak. The distance of the peak from the injection point expressed in time is called retention

time. The retention time depends on the flow rate of the mobile phase (Kazakevich and Lobrutto, 2007).

Since the invention of the technique, adsorbents with high polar surfaces such as porous silica, called packing materials have been used together with a non-polar mobile phase. The introduction of chemically modified hydrophobic surfaces replaces the stationary phase or the packing material from polar to non-polar while mobile phase as an analyte carrier becomes polar. The relative polarity of the stationary and mobile phase appears to be “reversed” and thus coined as reversed-phase liquid chromatography. The earlier mode was called as normal phase instead (Kazakevich and Lobrutto, 2007).

2.7.4 Electroencephalography

The human brain is an organ with a complex structure and produces electrical signals called brain waves. Brain waves are produced due to the changes in electrical signals in the brain. The waves can be categorised based on frequency bands and referred to as alpha, beta, theta, gamma, and delta. The electrical activities of the brain can be captured by various sensors placed on different regions of the head. The method is called electroencephalography (EEG) and also a popular method for assessing sleep quality and alertness (Su, Xavier, and Kuan, 2023).

The brain waves lie in a specific frequency band referred to as alpha, beta, theta, gamma and delta ranging from 0.1 Hz to more than 100 Hz and Figure 2.5 shows the frequency for each type of brain waves. They are closely

associated with different states of the brain, including consciousness and sleep (Chauhan and Preetam, 2016; Siddiqui, Srivastava and Saeed, 2016; Gao, et al., 2020). The balance of brain wave patterns can be disrupted by various factors including stress, sleep deprivation, some medications, and others.

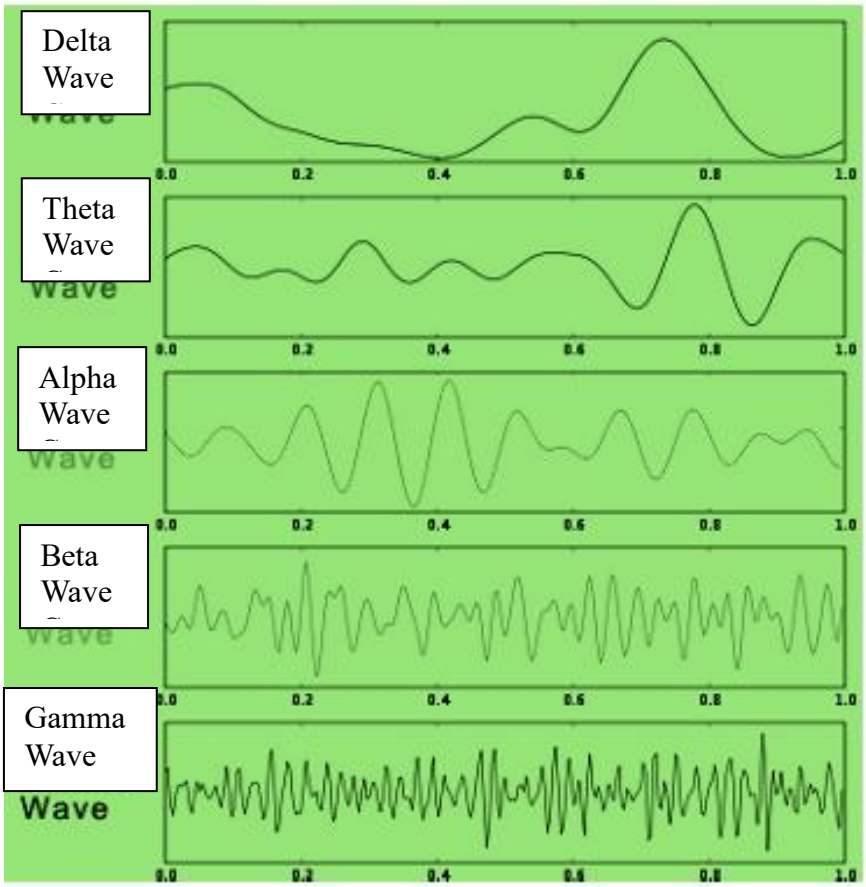


Figure 2.5: The frequency of each type of brain waves (Roy, et al., 2022).

A restful night's sleep progresses through several stages, which are dictated by neuronal activity in the brain, muscles, and eyes. Thus, three basic metrics are used to study sleep. Gross brain activity, muscle tone and eye

movement are measured by EEG, electromyography, and electrooculography, respectively. According to the American Academy of Sleep Medicine, sleep is categorised into five stages. They are wake (W), non-rapid eye movement (NREM) and rapid eye movement (REM). NREM is further divided into three stages namely N1, N2 and N3. When an individual falls asleep, he or she moves from the NREM stage to the REM stage. An individual goes through each stage four to six times at night, taking an average of ninety minutes on each cycle. In the first cycle, REM sleep is brief; later in the night, greater REM periods and a reduction in deep sleep duration (NREM) take place. Alterations in normal sleep architecture may indicate certain sleep disorders.

Figure 2.6 shows the sleep analysis by measuring the activity of brain, eye, and chin muscles. During light NREM sleep, sleep spindles are detected in the brain activity. During deeper NREM sleep, referred as slow-wave sleep (SWS), chin muscles start to relax and the curves representing brain activity get slow. REM occurs 90 to 120 minutes and during this period, the eyes move quickly and the muscles are extremely relaxed, often associated with vivid dreams. During REM sleep, muscle activity is the lowest, except for the extraocular muscles and diaphragm, brain activity gets faster, and the eyes start making rapid movements (James, Joechner and Muehlroth, 2020). Despite only a small portion of sleep, it is crucial for cognition and physiological homeostasis. Table 2.7 summarises the EEG recordings, physiological changes, and clinical significance associated with each sleep stage.

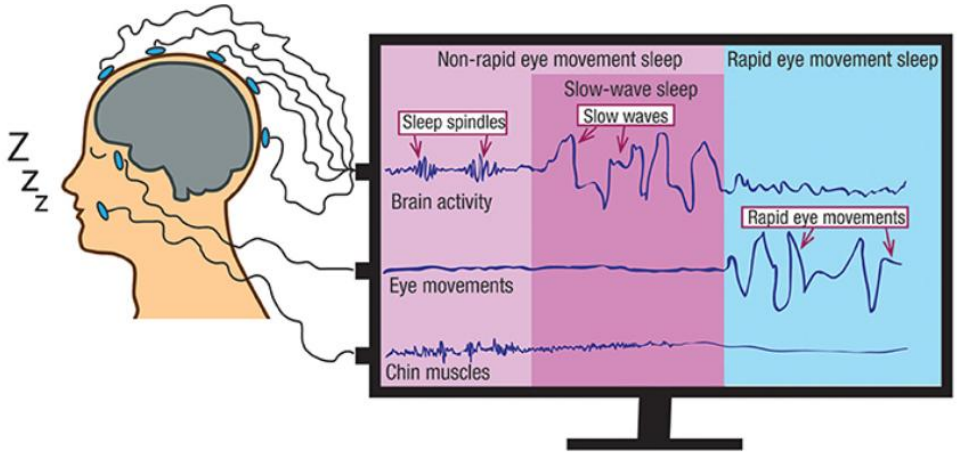


Figure 2.6: The sleep analysis by measuring the activity of neurons, eye and chin muscles (James, Joechner and Muehlroth, 2020)

Table 2.7: EEG recordings, physiological changes and clinical significance associated with each sleep stage

Stage	EEG Recording	Physiological changes	Clinical significance
W	alpha waves are seen during quiet/relaxed wakefulness eye-open - beta waves predominate. Eye-close - Alpha waves become the predominant pattern as individuals become drowsy	Skeletal muscle movement contraction as well as eye blinking and rapid movement	
N1 (transitional/light sleep)- 5%	theta waves - low voltage 50% of the alpha waves are replaced with beta activity called as low-amplitude mixed-frequency (LAMF) activity	Skeletal muscle tone is present but less and heart rate becomes regular, shallow breathing occurs and blood pressure drops.	
N2 (intermediate/spindle sleep)- 45%	predominantly theta activity followed by sleep spindles and K complexes (long delta waves)	Decrease in heart rate, breathing and body temperature and no eye movement.	memory consolidation and teeth-grinding
N3 (Deepest sleep)- 25%	delta waves - lowest frequency, highest amplitude known as slow-wave sleep (SWS)	Slowest heart and breathing rate, no eye movement and fully relaxed body. The body repairs and regrows tissues, builds bone and muscle and strengthens the immune system.	Associated with cognitive performance, sleepwalking and bedwetting
REM - 25%	beta waves - similar to during wakefulness	Atonic skeletal muscles, increased brain activity, active eye movement and diaphragmatic muscles	dreaming, nightmares, and penile/clitoral tumescence

(Malhotra and Avidan, 2014; Della Monica, et al., 2018; Patel, et al., 2022)

Figure 2.7 shows the changes in sleep stages as humans age. According to Ohayon, et al., 2004, age and the percentage of SWS had a substantial negative correlation. In adults, TST, sleep efficiency, percentage of SWS and REM sleep significantly decreased with age, while sleep latency, percentage of stage 1 sleep, stage 2 sleep, and WASO significantly increased with age (Ohayon, et al., 2004). Individuals who were depressed spent more time in REM sleep stage and the time between sleep onset and the start of the first REM period was also short (Steiger and Pawlowski, 2019).

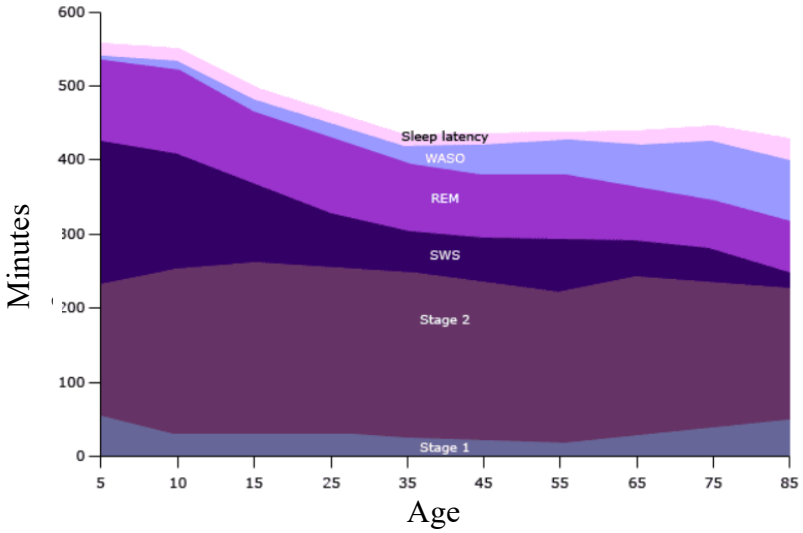


Figure 2.7: The age-related trends for stage 1 sleep (N1), stage 2 sleep (N2), SWS (N3), REM sleep, WASO, and sleep latency (in minutes) (adapted from Ohayon, et al., 2004).

Meanwhile, individuals affected with insomnia had slightly different sleep stages when compared with good sleep quality. Insomniac subjects had wake-like REM sleep and were emotionally more negative (Feige, et al., 2018). Spending less energy inhibiting information process during sleep could improve subjective sleep quality. However, insomnia subjects invested more energy into inhibiting information processing during N3 sleep stage but less in N2 sleep stages compared to subjects with good sleep quality. This finding may possibly

indicate that there are differences in threshold and the susceptibility of sleep stages to stimulation between insomniac subjects and good sleepers during sleep monitoring (Ceklic and Bastien, 2015).

2.7.4.1 Alpha Wave

The alpha wave falls in the 8-13 Hz range and is found in all ages. Primarily, it can be detected in adults who are awake but in a relaxed condition (Kumar and Bhuvaneswari, 2012). It originates from the parietooccipital region bilaterally. A normal alpha rhythm is synchronous and symmetrical over the cerebral hemispheres (Malhotra and Avidan, 2014).

It acts as a bridge between the subconscious and conscious mind and induces the secretion of serotonin. Serotonin contributes to relaxation and pain relief (Kumar and Bhuvaneswari, 2012). A higher level of alpha wave activity is found in the frontal and occipital regions of the brain. As mental stress levels increase, the power of alpha in the frontal area decreases (Chandra, et al., 2017). There have been reports of elevated alpha power in subjects practising different kinds of meditation, primarily over frontal areas (Fell, Axmacher and Haupt, 2010).

2.7.4.2 Theta Wave

Theta waves fall in the range from 4 to 8 Hz. Theta activity originates in the central vertex regions. These brainwaves are related to subconscious activities and are observed in meditation and relaxation (Kumar and Bhuvaneswari, 2012). Although there is no amplitude criterion for this wave, it

is the most common EEG sleep frequency (Malhotra and Avidan, 2014).

It induces the production of hormones such as human growth hormones, serotonin and cortisol. Thus, it may contribute to relaxation, pain relief, memory and learning (Kumar and Bhuvaneswari, 2012). Overall, the awakening theta power in insomnia patients was much higher than in healthy individuals (Siddiqui, Srivastava and Saeed, 2016).

2.7.4.3 Beta Wave

Beta waves can be detected at 13 – 30 Hz and are concerned with behaviour and sensory (Kumar and Bhuvaneswari, 2012). Beta activity originates from the frontal and central part of the brain but can be found diffuse manner. It can be detected during a wakeful state yet is more prominent during drowsiness, reduced during deeper sleep, and reemerges during the REM stage of sleep (Malhotra and Avidan, 2014).

Beta waves induce cortisol production and interface with memory and learning. These waves are detected in a conscious state such as talking, problem-solving, and decision-making (Kumar and Bhuvaneswari, 2012). Stress brought on by anxiety, excitement, and tension produces a strong beta band. Nonetheless, different regions of the brain exhibit distinct behaviours in beta waves (Sulaiman, et al., 2009). In the awake EEG analysis, the normalised power of beta activity for normal cases was found in the higher range but was in the quite lower range in insomnia cases (Siddiqui, Srivastava and Saeed, 2016).

2.7.4.4 Delta Wave

Delta waves have a frequency range between 0.1 and 4 Hz and are the slowest brain waves with the highest amplitude. These waves are observed in deep sleep (Kumar and Bhuvaneswari, 2012). Delta activity originates in the frontal region of the brain (Malhotra and Avidan, 2014). It induces growth hormone. When an adult is awake, a delta wave is aberrant, but it is usual to observe in infants with the dominant rhythm (Kumar and Bhuvaneswari, 2012).

According to the EEG study, the normalised power of delta activity in the awakening state was shown to be in the higher range for normal cases, but in the slightly lower range for insomnia cases (Siddiqui, Srivastava and Saeed, 2016). A reduction in delta brain waves has been linked to a reduction in homeostatic sleep pressure and an increased desire to sleep (Gao, et al., 2020).

2.7.4.5 Gamma Wave

The frequency of gamma waves falls in the range of 30 – 100 Hz and is the fastest. However, the range can vary substantially between 20 and 200 Hz and it is difficult to measure with the current technology. This type of brain wave is observed during the integration of sensory inputs and hyperalertness (Fell, Axmacher and Haupt, 2010; Kumar and Bhuvaneswari, 2012).

Studies indicate that individuals with cognitive impairments or learning disabilities might not generate as many gamma waves. Meditation practitioners possessed high gamma activity, which could be due to cortical restricting and

learning processes (Fell, Axmacher and Haupt, 2010).

CHAPTER 3

METHODOLOGY

3.1 Overview of Methodology

Figure 3.1 shows an overview of the methodology. The study has been divided into three stages. Firstly, the sample population was screened for sleep quality. Subsequently, those with poor sleep quality were recruited into the intervention to study the effectiveness of CTH and L-theanine. Additionally, a systematic literature review was conducted on CZP and CTH.

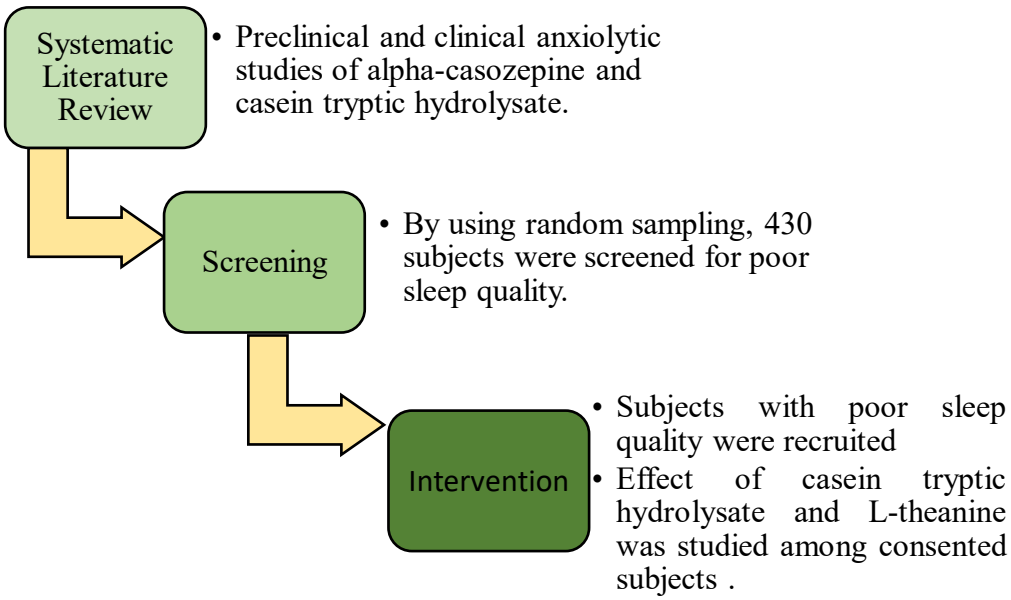


Figure 3.1: Flow chart on the overview of the methodology

3.2 Systematic Literature Review

3.2.1 Overview

An extensive search was carried out in the following electronic databases: Scopus, PubMed, Springer Link, and SAGE. The bibliographic sources were selected using the following keywords alone or in combination: “α-casozepine”, “alpha-casozepine”, “casein tryptic hydrolysate”, “tryptic hydrolysate of αS1-casein”, “Lactium”, “Zylkene”, “anxiolytic activity”, and “anxiety”. A total of 158 bibliographic sources, ranging from the year 2001 to June 2023 were utilised. Figure 3.2 shows the preferred reporting items for the systematic review and meta-analyses (PRISMA) framework for this study.

3.2.2 Inclusion Criteria

All original articles indexed in the above electronic databases were included. For clinical studies: scientific publications which included individuals of all ages, women and men treated with CTH and CZP or as an add-therapy and when the effect of CTH and CZP was evaluated in the human subjects. For preclinical studies, the following criteria were used: scientific publications from rodents, horses, ponies, cats and dogs as well as *in vitro* studies in which the bioactive peptides were administered alone or in combination with other active ingredients by any route of administration via oral (p.o.) and intraperitoneal (i.p.) and that the effect of bioactive peptides was evaluated in the model studied.

3.2.3 Exclusion Criteria

Bibliographic sources referring to the purification of peptides and

peptides from whey were excluded.

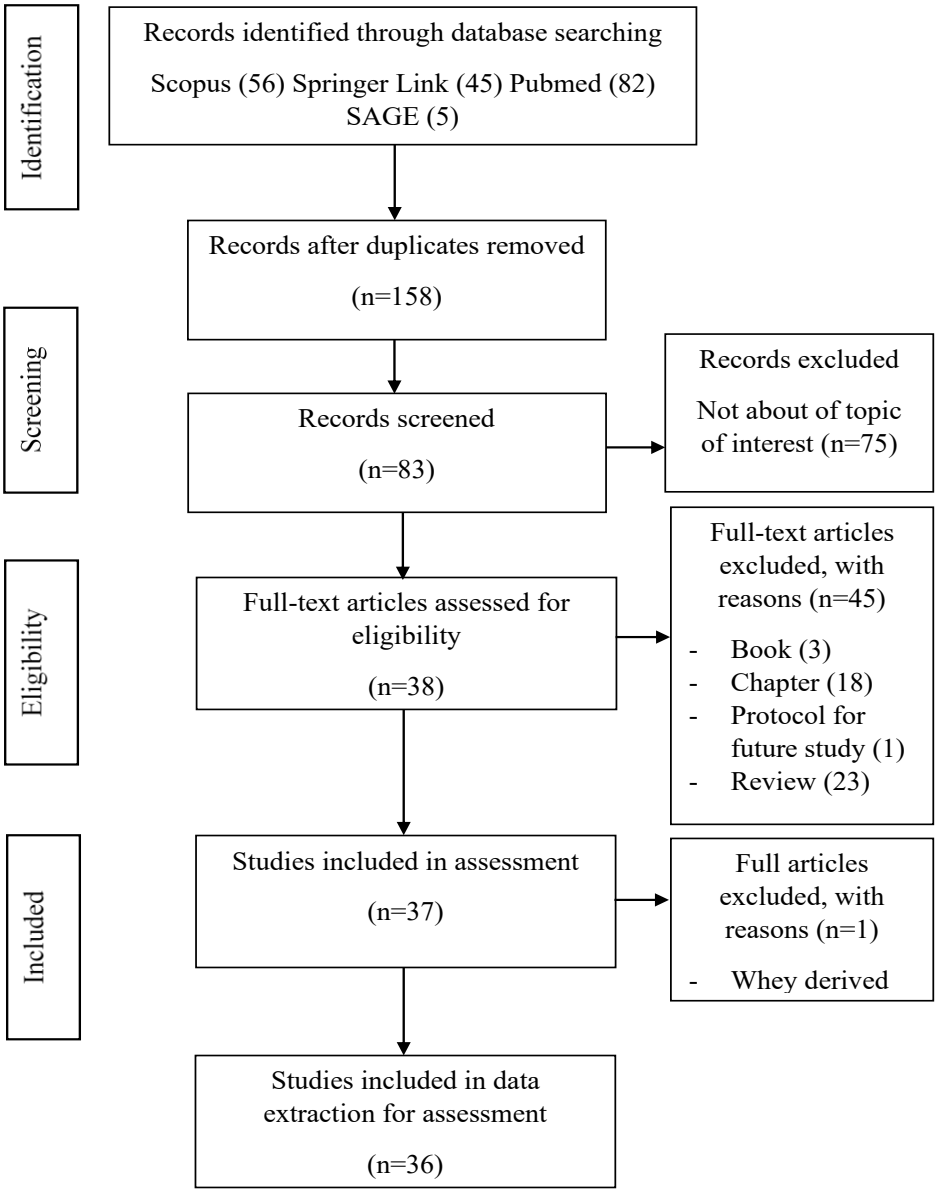


Figure 3.2: The flow chart of the findings and inclusion criteria used for the review process based on PRISMA guidelines

3.3 Screening for Poor Sleep Quality

3.3.1 Sampling

The screening for sleep quality was conducted among academic staff, both men and women aged 21 and above working at UTAR Kampar Campus, Malaysia. Prior to the screening, ethical approval was obtained from the UTAR Scientific and Ethical Review Committee (U/SERC/66/2017) for a year from August 2017 till August 2018.

The academic staff number was non-stationary throughout the data collection period, but it was 632 at the beginning of the study in 2018. The inclusion criterion of this cross-sectional study was all the teaching staff in the Kampar campus, but deans and deputy deans were excluded as they were involved more in the administrative work than teaching.

3.3.2 Sample Size Calculation

The sample size was calculated based on the prevalence of poor sleep quality with symptoms of insomnia among adults in Malaysia (Zailinawati, et al., 2008). A total of 430 randomly chosen staff were approached during the working days of the teaching semester. A random table number was used to select random samples. Written informed consents were obtained from all the selected subjects in accordance with the Declaration of Helsinki and Malaysian Good Clinical Practice Guidelines.

Below is the random sampling size calculation for screening (Suresh and Chandrashekara, 2012).

$$N = (Z_{\alpha/2})^2 \, P(1-P)*D / E^2$$

$$= \frac{(1.96)^2 \times 0.338 \times (1-0.338) \times 1}{(0.05)^2}$$

$$= \frac{3.8416 \times 0.2238}{(0.05)^2}$$

$$= 343.900 \approx 344$$

Where P is the prevalence or proportion of event of interest for the study (Zailinawati, et al., 2008), E is the Precision (or margin of error). Generally, E will be 5-10% and Zα/2 is normal deviate for two-tailed alternative hypothesis at a level of significance (α = 0.05). D is the design effect that reflects the sampling design used in the survey type of study. 1 is for simple random sampling.

Final adjusted sample size, allowing the non-response rate of 10-20%, the adjusted sample size was 344/ (1-0.20) = 430.

3.3.3 Questionnaire

The quality of sleep of the academic staff was measured by the validated English version of the PSQI questionnaire. The questionnaire is validated in healthy, clinical groups as well across many cultures and languages, including multi-ethnic Asian populations (Koh, et al., 2015; Mollayeva, et al., 2016).

This self-rating instrument contains 19 questions, which are combined to form seven component scores. They are subjective sleep quality (C1), sleep latency (C2), sleep duration (C3), habitual sleep efficiency (C4), sleep disturbances (C5), use of sleeping medications (C6), and daytime dysfunction (C7). These components of the PSQI are the standardised versions of areas routinely assessed in clinical interviews of patients with sleep complaints. Each item is weighted on a zero to three-interval scale. The seven component scores were then totalled up to produce a global PSQI score (maximum 21 points). The global PSQI score was used to distinguish between poor-quality sleep (a score of six or higher) and good-quality sleep (a score of five or lower). A global PSQI score of more than five yielded a diagnostic sensitivity of 89.6% and a specificity of 86.5% for the diagnosis of primary insomnia or clinically relevant pathological sleep (Buysse, et al., 1989).

A consent form with demographic questions was attached to the PSQI questionnaire. Basic demographic profiles such as age, gender, ethnicity, professional position and education status were recorded. The questionnaires were distributed via email. Those who did not respond through email were approached directly.

3.3.4 Statistical Analysis

Values are expressed in means with standard deviations for continuous variables and numbers (percentages) for categorical variables. IBM SPSS Statistics for Windows software version 21.0 was used to analyse the data. χ^2 statistical test was used to assess the association between the categorical

variables and the Pearson correlation test between two continuous variables. Student-t test was used to compare the means of two continuous variables, and a one-way ANOVA test was used for more than two groups. Statistical significance was set at $P < 0.05$. Tukey's post hoc test was conducted to further identify the significance between the groups.

3.4 Intervention of CTH and L-theanine on Poor Sleep Quality and other Variables

3.4.1 Sample Size Calculation

By using G*Power software (3.1.9.7 version, Franz Faul, University of Kiel, Kiel, Germany) (Faul, et al., 2007), it was estimated that a total of 30 participants would be required with an anticipated non-compliance or drop-out rate of 33%, yielding 80% power to detect an effect (Cohen's d = 1.3099) based on previously published data (Scholey, et al., 2017) with a two-tailed t-test at a 0.05 significance level. In total, 42 subjects out of 143 affected respondents with poor sleep quality (global PSQI > 5) agreed to participate in the intervention study.

3.4.2 Investigational Product

The test product or supplement contained 150 mg CTH and 50 mg L-theanine in the same capsule while the placebo was a low-fat milk powder, encapsulated similar to the supplement. Figure 3.3 shows the capsules of the placebo and supplement. Both supplement and placebo capsules were provided by LiveLife Biosciences AG, Zurich, Switzerland. The capsules were labelled as VC6500A (placebo) and VC65003 (supplement) and the content information of the capsules was revealed by a manager from LiveLife Sdn. Bhd., Malaysia

after completion of the study period.

Figure 3.3: The capsules of the placebo and supplement containing CTH and L-theanine

3.4.3 Study Design

This clinical trial was a randomised double-blind, placebo-controlled and cross-over study of nine weeks, including four weeks of treatment (supplement or placebo) and a week of washout period (Stevens, et al., 2017) between the treatments, as shown in Figure 3.4. The study was designed so that it could fall during the teaching weeks, as all the academic staff were assumed to have similar work stress. The study was also approved by the UTAR Scientific and Ethical Review Committee, and the earlier ethical approval was extended (U/SERC/66/2018).

3.4.3.1 Inclusion Criteria

Subjects who scored more than 5 in global PSQI in the screening stage, which indicates poor sleep quality (Backhaus, et al., 2002), and could adhere to the appointment schedules were invited to participate in the study. Written informed consent was obtained from all the selected subjects in accordance with the Declaration of Helsinki and Malaysian Good Clinical Practice Guidelines. All the subjects completed a questionnaire about their medical history.

3.4.3.2 Exclusion Criteria

Those who were pregnant, taking sleeping pills or any supplements to improve their sleep quality within the past month, consuming more than 10 cups of green tea, coffee, or tea in a day, are lactose intolerant, have a myocardial infarction or liver and kidney disorders were excluded from the study.

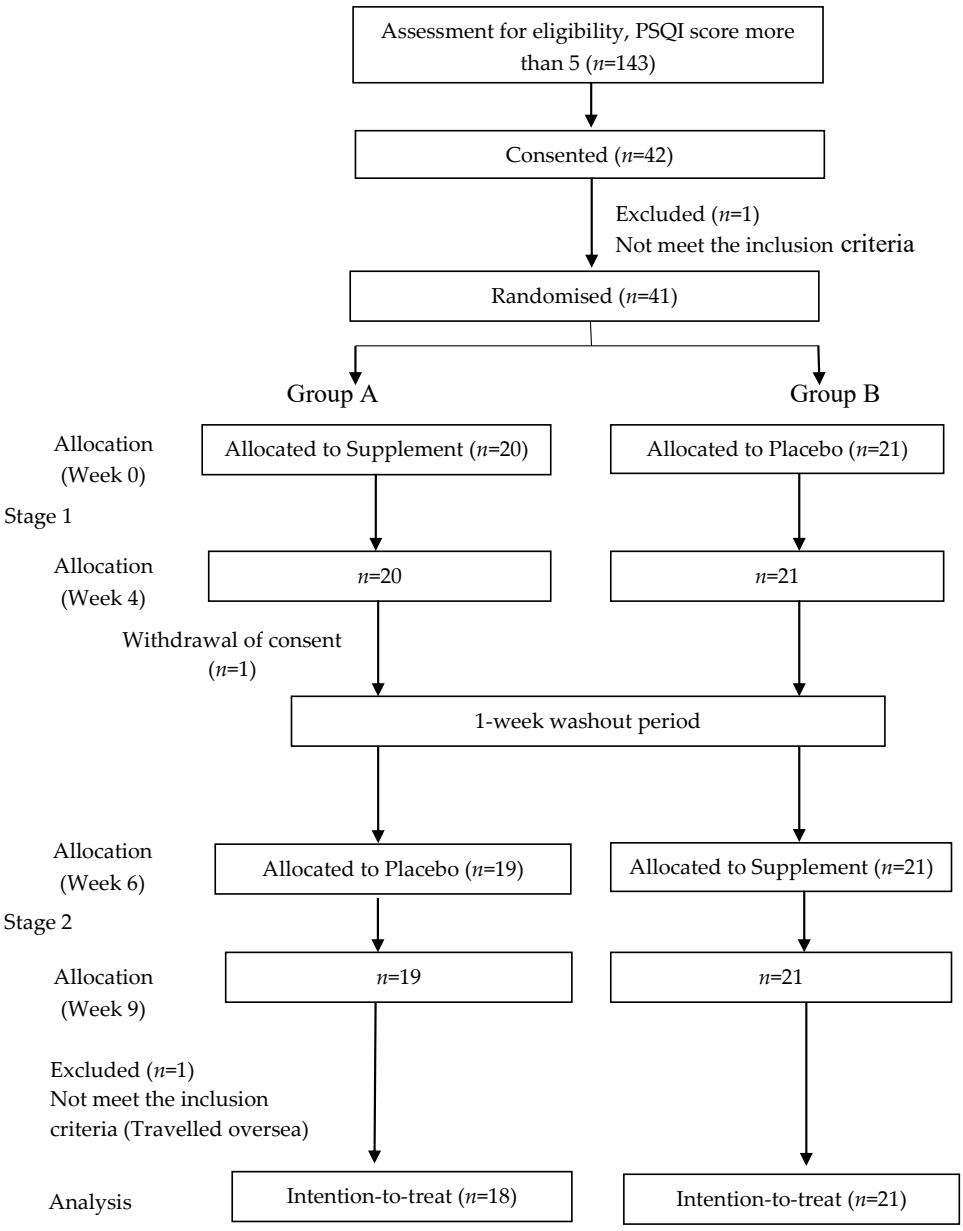


Figure 3.4: The study design for the intervention

One participant was withdrawn before the commencement of the study as she became pregnant and another participant in Group A completed stage 1 but withdrew his consent during the washout period. During stage 2 of the same group, one participant was suspended as she travelled overseas during the study period. The participants were instructed to take one capsule a day an hour before sleep and their compliance for the study was checked verbally. Those who consumed fewer than 80% of the capsules would be considered non-eligible. As shown in Figure 3.5, PSQI score, blood pressure, heart rate, and awake EEG were recorded and saliva samples for cortisol analysis were collected before and after each treatment (baseline, week four, week six and week nine).

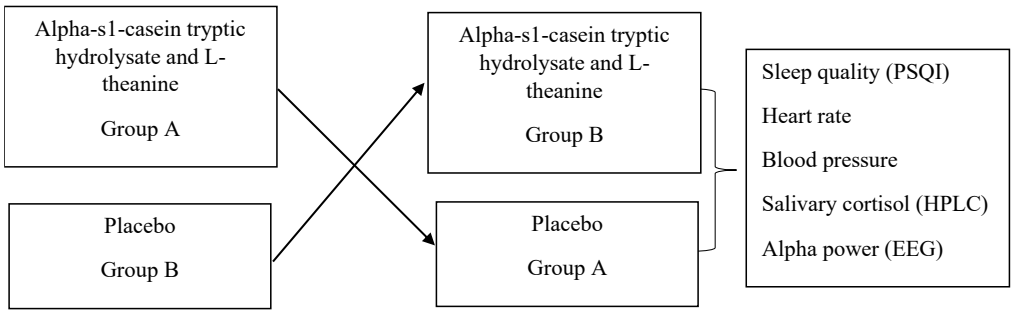


Figure 3.5: The variables analysed following treatment and placebo

3.4.4 Sleep Quality Measurement using PSQI

The participants were required to fill out the PSQI questionnaire (Buysse, et al., 1989) before and after the treatment of each stage to assess sleep quality. Nineteen discrete items of the questionnaire that produced seven individual component scores were recorded. The sum of scores for these seven components would generate a total score of subjective sleep quality.

3.4.5 Blood Pressure and Heart Rate Measurements

Blood pressure and heart rate of the participants were measured by using the blood pressure monitor Microlife BP3AG1 (Microlife AG, Widnau, Switzerland) and an Omron Heart Scan HCG-801 (Omron Healthcare, Kyoto, Japan), respectively, before and after each treatment.

3.4.6 Salivary Cortisol Analysis

3.4.6.1 Chemicals and Reagents

The following chemicals were obtained from the respective manufacturers: cortisol (11-α, 17-α, 21-trihydroxy, α-4 pregnene 3,20-dione, hydrocortisone; MW 362.5; purity 98%) from Nacalai Tesque, Kyoto, Japan; 6-α methylprednisolone (MW 374.5; purity 98%), which was used as an internal standard (IS), from Tokyo Chemical Industry, Tokyo, Japan; methanol HPLC grade and n-hexane HPLC grade (98%) from Rank Synergy (Synerlab), Kuala Lumpur, Malaysia; diethyl ether analytical grade (99.8%) from Ansansi Gyeonggi-do, South Korea; acetonitrile HPLC grade (99.9%) and acetone HPLC grade (99.8%) from QRec (Asia), Rawang, Malaysia.

3.4.6.2 Sample Collection

The Salivette® Cortisol (Sarstedt AG & Co., Nümbrecht, Germany) sampling device was used to collect saliva samples, as it is designed to achieve precise analytical values from small volumes and samples with a very low level of salivary cortisol, an indicator of chronic insomnia (Elio, et al., 2009). Participants were required to collect saliva within 30 to 45 min after awakening. A simple mouth rinse with water for at least 10 min was suggested before

collecting the sample. The participants were not allowed to eat, drink, brush their teeth, smoke or use tobacco products, use any mouthwash or engage in exercise or similar physical activity before collecting the sample. The saliva–cotton sample (about 2 mL obtained by chewing the cotton swab for 2 min) was directly spat without force or inducement into the internal vial of the double-chamber tube, capped and stored at 4 °C until transported to the laboratory. The samples were centrifuged at 2000 *g* for 10 min at room temperature to remove any particulates and the supernatant was aliquoted and stored at −80 °C for later analysis.

3.4.6.3 Solid Phase Extraction

When ready to analyse, the aliquots were thawed and centrifuged again at 2000 *g* for 10 min at room temperature to break down mucins and obtain a clear fluid. The supernatants were collected and poured into freshly labelled tubes. The saliva samples were extracted using the solid phase extraction (SPE) technique modified from Elio, et al. (2009). SPE Discovery DSC-18 columns (1 mL, Supelco, Bellefonte, PA, USA) were used for saliva sample clean-up and enrichment.

Prior to extraction, 500 µL of centrifuged saliva sample or cortisol standard (10, 20, 40, 60, 80, and 100 nmol/L) were spiked with 5 µL of IS (final concentration: 53 nmol/L). Before adding the sample, the SPE columns were equilibrated with 3 mL of methanol followed by 1.5 mL of deionised water. After sample introduction, the columns were washed sequentially with 0.25 mL of deionized water, 0.5 mL of acetone: water mixture (1:4, v/v), and 1 mL of

hexane. Finally, 1.5 mL of diethyl ether was used to elute the sample. Each eluted sample was dried in a vacuum chamber and consecutively resuspended with 100 µL of HPLC mobile phase before injection. Figure 3.6 shows the solid phase extraction of the samples.

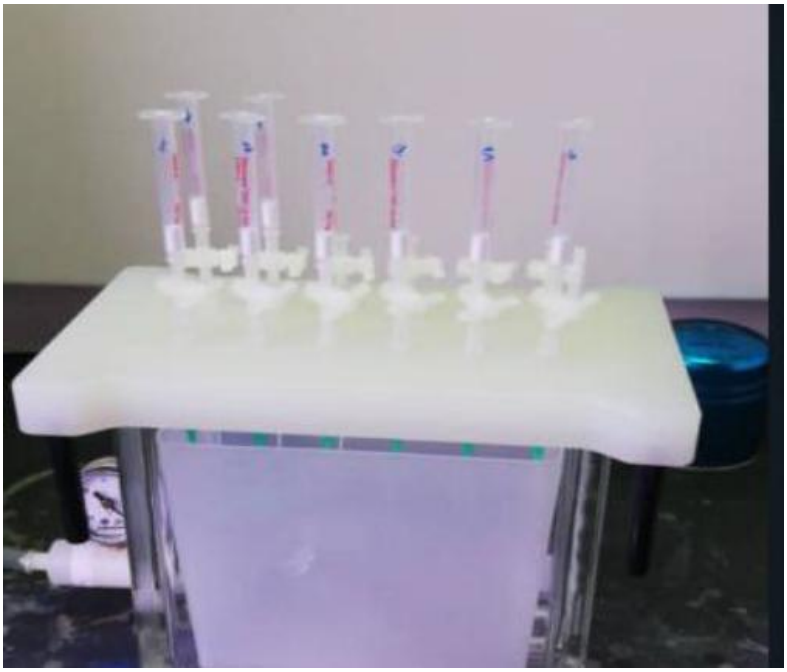
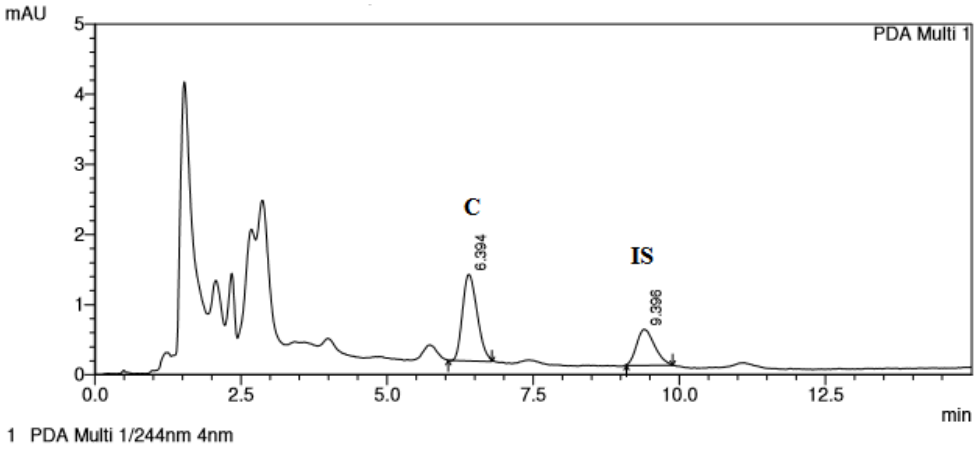


Figure 3.6: Solid phase extraction of the samples

3.4.6.4 High-Performance Liquid Chromatography Analysis

The HPLC equipment (Shimadzu, Kyoto, Japan) comprised the solvent delivery module CBM-20A, the photo-diode array detector SPD-M20A, and the column oven CTO-10AS VP. The analytical column used was a stainless-steel pentafluorophenylpropyl silica column (15 cm × 2.1 mm, 5 µm) (Discovery, HS-F5, Supelco, Bellefonte, PA, USA). An isocratic elution using acetonitrile and water (27% and 73%) as the mobile phase was chosen to separate the analytes. The injection volume was 20 µL. The mobile phase flow rate was 0.25 mL/min and the column temperature was kept at 35 °C.

Figure 3.7 shows the chromatogram of SPE extracted for cortisol (C) samples added with methylprednisolone (IS) using a UV detector. The absorbance was measured at 244 nm. The chromatogram was acquired using the LCsolution software, Version 1.0.0.1 (Shimadzu, Kyoto, Japan). Each sample analysis was completed in 15 min. Cortisol and IS were detected at 6.3th min and 9.3th min (±5%), respectively. The standard calibration curve was plotted by using the peak height ratio of cortisol (standard) against IS and was used to calculate the amount of cortisol present in saliva samples (Elio, et al., 2009). The ranges of the calibration curves for cortisol standard were 10.0 – 100.0 nmol/L ($y= 0.0531x+ 0.551$; $r^2= 0.985$) as shown in Figure 3.8.

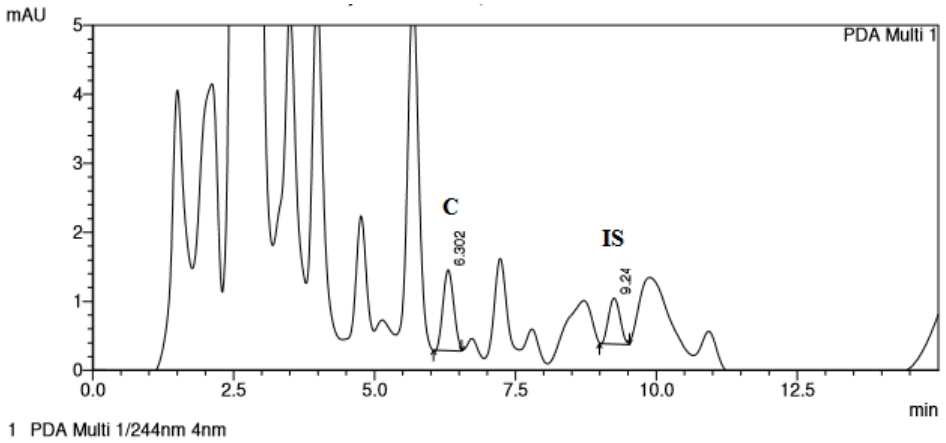


PeakTable

PDA Ch1 244nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	6.394	21599	1235	66.142
2	9.396	11056	517	33.858
Total		32655	1751	100.000

A



PeakTable

PDA Ch1 244nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	6.302	15454	1170	62.067
2	9.244	9445	665	37.933
Total		24899	1835	100.000

B

Figure 3.7: The chromatogram of SPE extracted for cortisol (C) samples added with methylprednisolone (IS) using a UV detector

A: Standard solution containing 40 nmol/L, C and 53 nmol/L IS and B: Morning saliva sample of a participant with XX nmol/L C and 53 nmol/L IS. Separation conditions: pentafluorophenylpropyl silica column, isocratic elution with 27% of acetonitrile and 73% of water at 35 °C, flow rate 0.25 mL/min, injection volume: 20 µL, UV absorbance at 244 nm wavelength

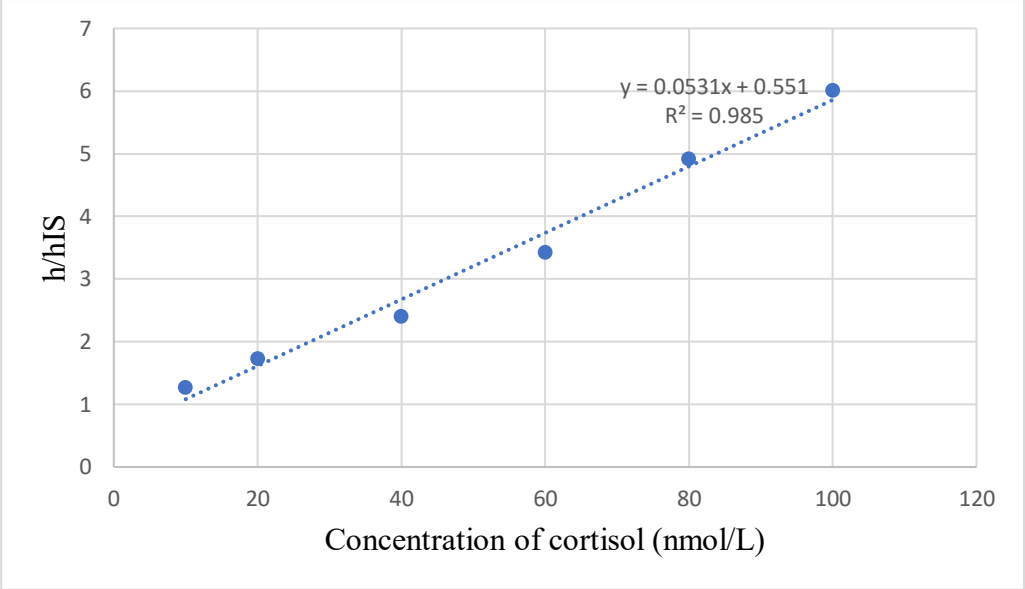


Figure 3.8: The linear dependence of the ratio of the peak height of cortisol (standard) to peak height of methylprednisolone (IS)

3.4.7 Electroencephalography Analysis

An Emotiv EPOC EEG wireless headset (Emotiv Systems Inc., San Francisco, CA, USA) was used to record EEG signals of participants at the same time that other data were collected in a quiet setting. Figure 3.9 shows Emotiv EPOC EEG wireless headset. The signals (with eyes open) were obtained over three min from 14 active (AF3, AF4, F3, F4, F7, F8, FC5, FC6, P7, P8, T7, T8, O1, and O2) and two reference (CMS and DRL) electrodes. The active electrodes were placed at different locations on the scalp, as shown in Figure 3.10, to detect the electrical activity of neurons. The signal from each electrode was sampled at a frequency of 128 Hz and converted to digital form with the aid of a built-in 16-bit analogue-to-digital converter.

Figure 3.9: Emotiv EPOC EEG wireless headset (Emotiv Systems Inc., San Francisco, CA, USA) (Kawala-Janik, et al., 2013)

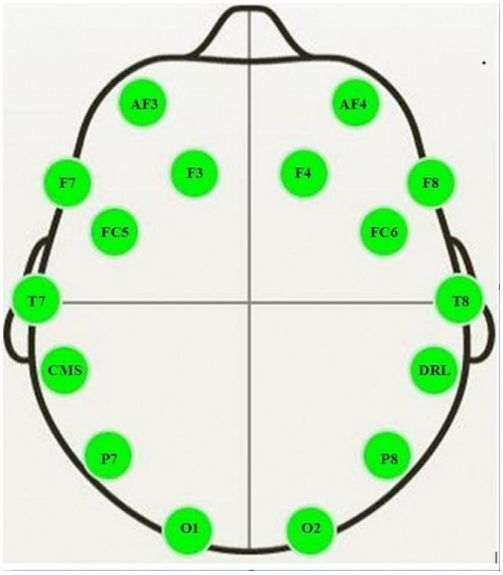


Figure 3.10: The locations of the active electrodes (AF3, AF4, F3, F4, F7, F8, FC5, FC6, P7, P8, T7, T8, O1 and O2) and reference electrodes (CMS and DRL) on the scalp for Emotiv EPOC headset (Kawala-Janik, et al., 2013)

The recorded raw data were pre-processed with the help of EEGLAB software (Delorme and Makeig, 2004). The data was loaded in the software and the linear Finite Impulse Response (FIR) filter was used to bandpass the data between 1 and 60 Hz band decomposition. Next, the resulting data was plotted

for visual inspection and the artefacted portion of the data was eliminated using the eegplot function.

The clean EEG signal was decomposed into different frequency bands and power spectral analysis was carried out for the alpha band. The spectral analysis was performed using the spectopo function provided in the MATLAB software version r2020a signal processing toolbox associated with EEGLAB (with a resolution of 1 Hz) to measure absolute power density (μV2/Hz). For the spectral analysis, the input data had to be stationary. Therefore, the EEG data was divided into brief epochs with 1000 ms each, thus in this small interval of time the data was considered stationary to perform the analysis. All the channels were used to calculate total alpha power, while frontal alpha power was measured from the electrodes AF3, AF4, F3, F4, F7, F8, FC5, and FC6 and the average power was recorded. Due to artefacts and inconsistent EEG data, two participants were excluded from the study during the pre-processing.

3.4.8 Statistical Analysis

IBM SPSS Statistics for Windows, Version 26 (IBM Corp, Armonk, NY, USA) was used for data analysis. As for the interventional study, a paired t-test was used to compare the results before and after the treatments (supplement or placebo) for each variable. Comparisons between the parameters of the supplement and placebo groups were conducted using the Student's t-test. All tests were two-tailed, and the level of statistical significance was set at $P < 0.05$.

CHAPTER 4

RESULTS

4.1 Analyses of Screening Study

Out of 430 randomly chosen academic staff, 344 (80%) responded in this cross-sectional study. Table 4.1 shows the demographic data and the PSQI scores and sub-scores. The sample taken consisted of 154 (44.8%) male and 190 (55.2%) female staff. They were in the age groups of 23–75 years with a mean age of 38.0 ± 9.1 years. For the ethnicity, most of them were Chinese (61.6%). The rest of the ethnicity categories such as Malay, Indians and others were grouped as non-Chinese. Most of the subjects were lecturers and specialists (59.0%) and belonged to the Faculty of Business and Finance (32.8%).

In the screening, 42.7% of academic staff were affected by poor sleep quality with the global PSQI scores of more than 5. On average, the mean sleeping duration for the academic staff was 6.68 ± 1.24 hours and 38.95 % or 134 subjects had a sleep duration of less than 7 hours. Overall, age was not correlated with the global PSQI scores (r = - 0.042, $P = 0.437$). Neither gender, age group, ethnicity, professional position nor faculty had a mean difference in terms of global PSQI scores except for education status.

Table 4.1: The demographic data and the PSQI scores and sub-scores

					PSQI Component Score						
	N	**Age (Mean ± SD)**	**Global PSQI Score (Mean ± SD)**	**TSH (Mean ± SD)**	**C1 (Mean ± SD)**	**C2 (Mean ± SD)**	**C3 (Mean ± SD)**	**C4 (Mean ± SD)**	**C5 (Mean ± SD)**	**C6 (Mean ± SD)**	**C7 (Mean ± SD)**
Overall	344	38.0 ± 9.1	5.4 ±3.03	6.68 ± 1.24	1.06 ± 0.82	0.92 ± 0.91	0.97 ± 0.73	0.54 ± 0.99	1.08 ± 0.49	0.12 ± 0.50	0.79 ± 0.73
Gender											
Male	154 44.8%	40.20 ± 10.52	5.30 ± 3.02	6.69 ± 1.33	0.95 ± 0.74	0.89 ± 0.93	0.99 ± 0.72	0.49 ± 1.00	1.03 ± 0.49	0.17 ± 0.57	0.78 ± 0.73
Female	190 55.2%	36.20 ± 7.28	5.49 ± 3.04	6.67 ± 1.16	1.15 ± 0.86	0.95 ± 0.89	0.95 ± 0.74	0.57 ± 0.98	1.12 ± 0.49	0.08 ± 0.44	0.79 ± 0.73
P Value		<0.001***	0.551	0.903	0.027*	0.522	0.605	0.454	0.098	0.108	0.897
Age											
40 and below	249 72.4%	33.53 ± 3.48	5.45 ± 3.08	6.72 ± 1.22	1.10 ± 0.83	0.94 ± 0.91 %	0.94 ± 0.72	0.59 ± 1.00	1.08 ± 0.50	0.06 ± 0.31	0.77 ± 0.73

41 and above	95 27.6%	49.69 ± 8.83	5.29 ± 2.90	6.57 ± 1.28	0.97 ± 0.78	0.88 ± 0.90	1.04 ± 0.74	0.40 ± 0.93	1.08 ± 0.48	0.27 ± 0.79	0.82 ± 0.73
P Value		-	0.671	0.333	0.193	0.612	0.270	0.098	0.948	0.012*	0.570
Ethnicity											
Non-Chinese	132 38.4%	36.88 ± 8.39	5.54 ± 3.02	6.58 ± 1.37	1.07 ± 0.82	0.94 ± 0.93	1.06 ± 0.80	0.64 ± 1.06	1.07 ± 0.53	0.16 ± 0.62	0.70 ± 0.67
Chinese	212 61.6%	38.69 ± 9.44	5.33 ± 3.03	6.75 ± 1.15	1.06 ± 0.81	0.92 ± 0.89	0.92 ± 0.68	0.47 ± 0.93	1.09 ± 0.47	0.09 ± 0.41	0.83 ± 0.76
P Value		0.072	0.527	0.222	0.898	0.809	0.071	0.127	0.696	0.287	0.097
Education Status											
Ph.D. Holders	101 29.4%	40.39 ± 10.29	4.90 ± 2.85	6.75 ± 1.14	0.88 ± 0.79	0.76 ± 0.84	0.91 ± 0.66	0.50 ± 0.98	1.04 ± 0.51	0.13 ± 0.54	0.68 ± 0.73
Non-Ph.D. Holders	243 70.6%	37.00 ± 8.35	5.62 ± 3.07	6.65 ± 1.28	1.14 ± 0.81	0.99 ± 0.93	1.00 ± 0.75	0.56 ± 0.99	1.10 ± 0.49	0.12 ± 0.48	0.83 ± 0.72
P Value	0.140	0.02*	0.045*	0.504	0.008**	0.032*	0.325	0.605	0.312	0.821	0.095

Professional Position											
Tutors and Assistant Lecturers	36 10.5%	33.22 ± 6.39	5.28 ± 2.92	6.78 ± 1.22	1.17 ± 0.85	0.78 ± 0.83	0.92 ± 0.84	1.05 ± 0.58	1.06 ± 0.58	0.06 ± 0.23	0.78 ± 0.68
Lecturers and Specialists	203 59.0%	37.21 ± 8.21	5.71 ± 3.10	6.65 ± 1.24	1.13 ± 0.81	1.03 ± 0.94	1.00 ± 0.73	1.11 ± 0.47	1.11 ± 0.47	0.13 ± 0.52	0.85 ± 0.75
Senior Lecturers	11 3.2%	44.18 ± 11.64	4.82 ± 3.52	6.41 ± 1.99	1.00 ± 0.77	1.00 ± 1.10	1.00 ± 0.89	0.91 ± 0.30	0.91 ± 0.30	0.00 ± 0.00	0.55 ± 0.52
Assistant Professors	83 24.1%	39.25 ± 8.61	4.78 ± 2.75	6.77 ± 1.11	0.92 ± 0.81	0.73 ± 0.78	0.92 ± 0.65	1.04 ± 0.53	1.04 ± 0.53	0.06 ± 0.36	0.70 ± 0.73
Associate Professors and Professors	11 3.2%	52.45 ± 14.22	5.55 ± 3.17	6.64 ± 1.36	0.64 ± 0.67	0.82 ± 1.08	1.00 ± 0.77	1.09 ± 0.54	1.09 ± 0.54	0.63 ± 1.21	0.45 ± 0.52
P Value		<0.001***	0.194	0.854	0.104	0.110	0.905	0.500	0.563	0.006**	0.175
Faculty											
Science	59 17.2%	39.22 ± 8.69	5.58 ± 3.00	6.51 ± 1.13	1.12 ± 0.77	0.86 ± 0.88	1.02 ± 0.63	0.51 ± 0.92	1.10 ± 0.52	0.03 ± 0.18	0.93 ± 0.78
Engineering	32	42.66	5.47 ± 3.56	6.52 ±	0.94 ±	0.88 ±	1.09 ±	0.47 ±	0.94 ±	0.34 ±	0.81 ±

and Green Technology	9.3%	±13.36		1.34	0.95	1.01	0.82	0.92	0.50	0.90	0.78
Business and Finance	113 32.8%	37.52 ± 7.46	5.86 ± 3.14	6.69 ± 1.41	1.10 ± 0.77	1.07 ± 1.02	0.96 ± 0.77	0.67 ± 1.06	1.15 ± 0.47	0.13 ± 0.49	0.80 ± 0.71
Arts and Social Science	48 14.0 %	35.33 ± 8.04	4.85 ± 2.99	6.81 ± 1.17	0.98 ± 0.86	1.02 ± 0.86	0.90 ± 0.75	0.52 ± 0.99	1.13 ± 0.53	0.02 ± 0.14	0.75 ± 0.81
Information and Communication Technology	31 9.0%	38.87 ± 8.69	5.03 ± 2.77	6.71 ± 0.96	0.94 ± 0.77	0.90 ± 0.70	0.97 ± 0.66	0.39 ± 0.92	1.00 ± 0.37	0.13 ± 0.56	0.71 ± 0.69
Institute of Chinese Studies	8 2.3%	43.75 ± 7.48	5.75 ± 1.58	6.56 ± 0.73	1.25 ± 0.46	0.75 ± 0.71	1.13 ± 0.35	0.75 ± 1.39	1.13 ± 0.35	0.00 ± 0.00	0.75 ± 0.46
Centre for Foundation Studies	53 15.4%	35.87 ± 9.58	4.89 ± 2.76	6.83 ± 1.17	1.11 ± 0.91	0.66 ± 0.76	0.91 ± 0.77	0.40 ± 0.84	1.00 ± 0.55	0.15 ± 0.60	0.66 ± 0.65
P Value		0.002**	0.375	0.776	0.796	0.194	0.876	0.610	0.258	0.095	0.613

Total sleeping hours (TSH), subjective sleep quality (C1), sleep latency (C2), sleep duration (C3), habitual sleep efficiency (C4), sleep disturbances (C5), use of sleeping medications (C6) and daytime dysfunction (C7); significance at *P* value * indicates <0.05, ** indicates <0.01, *** indicates <0.001. Independent T-test was used to compare the means of the two groups and a one-way ANOVA test was used for more than two groups.

Table 4.2 shows the prevalence of poor sleep quality and its association with the demographic categories. Table 4.2 shows none of the demographic categories were associated with the prevalence of sleep quality. However, certain components in the questionnaires showed significant results. The females had poorer subjective sleep quality (Component 1) than the males (P = 0.027) although there was no significant difference in the total sleeping hours (TSH) between the genders. The females were significantly younger than the male staff ($P < 0.001$).

There were no statistically significant differences between the age groups for most of the components except for Component 6 where the 41 and above age group took sleep medication more frequent than the younger age group. On the other hand, a significant mean difference was shown in the professional position category for the same component. Associate professors and professors tended to consume more sleep medication than the other positions such as tutors and assistant lecturers (P = 0.006), lecturers and specialists (P = 0.010), senior lecturers (P = 0.022), and assistant professors (P = 0.003). This could be due to age differences. Academic staff holding the position as associate professors and professors were significantly older than tutors and assistant lecturers ($P < 0.001$), lecturers, senior lecturers, and specialists ($P < 0.001$) and assistant professors ($P < 0.001$). The sleep medication consumption was also in line with the prevalence of poor sleep quality among the associate professors and professors which was 54.5%, way higher than the overall poor sleep quality prevalence.

In this study, the academic staff were divided into Doctor of Philosophy (Ph.D.) holders and non-Ph.D. holders who are with the degree of bachelor and/or master. Overall, the global PSQI score was significantly higher in non-Ph.D. holders (P = 0.045). Non-Ph.D. holders had poorer subjective sleep quality (P = 0.008) and sleep latency (P = 0.032) compared to Ph.D. holders. The prevalence of poor sleep quality among the non-Ph.D holders was 45.3%, higher than the overall prevalence of poor sleep quality. These findings show that non-Ph.D. holders were affected by poor sleep quality although they were significantly younger (P = 0.02) than the Ph.D. holders.

Table 4.2: Prevalence of poor sleep quality and its demographic associations

	Good Sleep Quality	Poor Sleep Quality	$\chi 2$	*P* Value
Overall	197 57.3%	147 42.7%		_
Gender				
Male	91 59.1%	63 40.9%	0.379	0.584
Female	106 55.8%	84 44.2%		
Age Category				
40 and below	144 57.8%	105 42.2%	0.117	0.412
41 and above	53 55.8%	42 44.2%		
Ethnicity				
Non-Chinese	77 58.3%	55 41.7%	0.099	0.823
Chinese	120 56.6%	92 43.4%		
Education Status				
Ph.D. Holders	64 63.4%	37 36.6%	2.173	0.152
Non-Ph.D. Holders	133 54.7%	110 45.3%		
Professional Position				
Tutors and Assistant Lecturers	18 50.0%	18 50.0%		
Lecturers and Specialist	112 55.2%	91 44.8%		
Senior Lecturers	7 63.6%	4 36.4%	4.696	0.320
Assistant Professors	55 66.3%	28 33.7%		
Associate Professors and Professors	5 45.5%	6 54.5%		

Faculty				
Science	35 59.3%	24 40.7%		
Engineering and Green Technology	19 59.4%	13 40.6%		
Business and Finance	57 50.4%	56 49.6%		
Arts and Social Science	30 62.5%	18 37.5%	4.443	0.617
Information and Communication Technology	21 67.7%	10 32.3%		
Institute of Chinese Studies	4 50.0%	4 50.0%		
Centre for Foundation Studies	31 58.5%	22 41.5%		

$\chi 2$ statistical test was used to assess the association between the categorical variables. Statistical significance was set at $P < 0.05$.

4.2 Analyses on Double-Blind, Randomised Placebo-Controlled Crossover Trial

The data collection was conducted from June 2017 to September 2018. A total of 20 male (51.3%) and 19 female (48.7%) participants were enrolled in the trial. Table 4.3 shows the age and baseline characteristics of the study participants. No side effects or adverse events were reported from any of the participants throughout the study period. This was based on the self-reporting method. All of them achieved an 80% compliance rate (consumed 80% or more of the capsule wither supplement or placebo for each stage).

Table 4.3: The age and baseline characteristics of the study participants

Variables	Mean ± SD (n= 39)	Range
Age (years)	37.3 ± 8.4	26-65
Systolic blood pressure (mmHg)	117.92 ± 13.33	91-144
Diastolic blood pressure (mmHg)	75.82 ± 11.86	51-102
Heart rate (beats per min)	77.54 ± 8.26	63-100

Table 4.4 shows the crossover variables after the supplement or placebo administration. Based on the global PSQI score, supplement-receiving groups had significant improvements ($P < 0.001$) compared to placebo in both stages. Although the placebo-receiving group in Stage 1 showed a significant decrease in the global PSQI score by 1.38 ($P = 0.026$), this is likely caused by a placebo effect. The number of hours of actual sleep was significantly improved only in supplement-receiving groups, where they were able to sleep 55 and 37 min longer ($P = 0.003$ and $P = 0.002$) than before the treatment.

In individual component analysis, the supplement effect over placebo was seen in components 3 and 4, sleep duration and sleep habitual efficiency whereby only supplement-receiving groups showed significant improvements. Subjective sleep quality (component 1) and sleep latency (component 3) showed significant improvements in both placebo and supplement-receiving groups in Stage 1 whereas only the supplement supplement-receiving group was significantly improved in Stage 2. Although the placebo marked a significant effect, the scores were not greater than the supplement-receiving group. A significant reduction of daytime dysfunction (component 7) was only observed in the supplement-receiving group in Stage 2 (P <0.001). Component 6 was not included as none of the participants took sleeping medications.

Neither blood pressure (systolic and diastolic) nor heart rate showed any significant changes between supplement and placebo-receiving groups. As for salivary cortisol levels, neither the supplement nor placebo-receiving group marked any significant differences in Stage 1.

Meanwhile, more favourable results were obtained in Stage 2, whereby the placebo-receiving group showed no significant difference while supplement-receiving group recorded a significant decrease in cortisol level by 34.6% (P = 0.007). Based on EEG analysis, the total alpha power as well as frontal alpha power were significantly (P = 0.031, 0.037) increased only in the supplement-receiving group in Stage 1.

Table 4.4: Crossover variables after the supplement or placebo administration

Primary Outcomes	Group	Treatments	Pre Mean ± SD	Post Mean ± SD	*P* -Value
			Global PSQI Score		
Stage 1	Group A	Supplement	9.00 ± 2.27	5.83 ± 1.54	<0.001 ***
	Group B	Placebo	7.90 ± 1.37	6.52 ± 2.40	0.026*
Stage 2	Group A	Placebo	5.56 ± 2.33	5.67 ± 2.40	0.834
	Group B	Supplement	6.76 ± 2.39	4.95 ± 2.48	<0.001***
			Total Sleeping Time (h)		
Stage 1	Group A	Supplement	5.28 ± 1.08	6.19 ± 0.69	0.003 **
	Group B	Placebo	5.71 ± 1.02	6.21 ± 1.27	0.061
Stage 2	Group A	Placebo	5.97 ± 0.95	6.11 ± 0.95	0.514
	Group B	Supplement	6.10 ± 1.28	6.71 ± 1.35	0.002 **
			Component 1 Subjective Sleep Quality		
Stage 1	Group A	Supplement	1.72 ± 0.46	1.11 ± 0.58	0.004**
	Group B	Placebo	1.57 ± 0.60	1.24 ± 0.70	0.049*
Stage 2	Group A	Placebo	1.17 ± 0.62	1.06 ± 0.64	0.430
	Group B	Supplement	1.38 ± 0.50	1.05 ± 0.74	0.016*
			Component 2 Sleep Latency		
Stage 1	Group A	Supplement	1.67 ± 0.97	1.00 ± 0.77	0.004**
	Group B	Placebo	1.48 ± 0.68	1.10 ± 0.83	0.029*
Stage 2	Group A	Placebo	0.94 ± 1.06	0.94 ± 1.00	1.000
	Group B	Supplement	1.24 ± 0.94	0.90 ± 0.94	0.005**
			Component 3 Sleep Duration		
Stage 1	Group A	Supplement	1.89 ± 0.68	1.22 ± 0.43	0.001**
	Group B	Placebo	1.52 ± 0.97	1.24 ± 0.89	0.137
Stage 2	Group A	Placebo	1.28 ± 0.75	1.39 ± 0.70	0.495
	Group B	Supplement	1.33 ± 0.97	0.95 ± 0.80	0.008**
			Component 4 Sleep Habitual Efficiency		
Stage 1	Group A	Supplement	1.06 ± 1.00	0.17 ± 0.38	0.003**
	Group B	Placebo	0.67 ± 0.73	0.52 ± 0.87	0.576
Stage 2	Group A	Placebo	0.33 ± 0.49	0.44 ± 0.62	0.542
	Group B	Supplement	0.62 ± 0.86	0.33 ± 0.66	0.030*
			Component 5 Sleep Disturbances		
Stage 1	Group A	Supplement	1.56 ± 0.51	1.39 ± 0.70	0.269
	Group B	Placebo	1.48 ± 0.51	1.33 ± 0.48	0.329
Stage 2	Group A	Placebo	1.17 ± 0.62	1.06 ± 0.54	0.495
	Group B	Supplement	1.10 ± 0.30	1.05 ± 0.50	0.666

Component 7 Daytime Dysfunction					
Stage 1	Group A	Supplement	1.11 ± 0.58	1.00 ± 0.49	0.331
	Group B	Placebo	1.14 ± 0.48	1.10 ± 0.77	0.789
Stage 2	Group A	Placebo	0.72 ± 0.57	0.78 ± 0.73	0.668
	Group B	Supplement	1.14 ± 0.73	0.67 ± 0.58	<0.001***
Systolic Blood Pressure (mmHg)					
Stage 1	Group A	Supplement	118.61 ± 12.50	121.56 ± 12.24	0.330
	Group B	Placebo	117.33 ± 14.30	112.52 ± 19.07	0.117
Stage 2	Group A	Placebo	123.11 ± 14.44	119.17 ± 11.95	0.077
	Group B	Supplement	116.62 ± 12.99	115.24 ± 13.51	0.366
Diastolic Blood Pressure (mmHg)					
Stage 1	Group A	Supplement	75.78 ± 10.23	75.61 ± 7.89	0.933
	Group B	Placebo	75.86 ± 13.35	74.05 ± 11.23	0.289
Stage 2	Group A	Placebo	77.39 ± 10.39	75.28 ±7.37	0.172
	Group B	Supplement	74.90 ± 12.63	73.90 ± 10.93	0.492
Heart Rate (beats per min)					
Stage 1	Group A	Supplement	76.39 ± 7.37	75.06 ± 7.63	0.451
	Group B	Placebo	78.52 ± 9.60	81.00 ± 8.68	0.210
Stage 2	Group A	Placebo	76.72 ± 9.14	75.33 ± 7.84	0.503
	Group B	Supplement	78.90 ± 11.56	78.86 ± 11.93	0.985
Salivary Cortisol Concentration (nmol/L)					
Stage 1	Group A	Supplement	33.85 ± 14.42	32.85 ± 18.35	0.871
	Group B	Placebo	27.70 ± 10.41	27.33 ± 14.46	0.893
Stage 2	Group A	Placebo	31.25 ± 21.70	31.82 ± 21.96	0.927
	Group B	Supplement	21.57 ± 12.92	14.11 ± 9.44	0.007**
Total Alpha Power (uV^2/Hz)					
Stage 1	Group A	Supplement	4.83 ± 5.31	14.95 ± 21.50	0.031*
	Group B	Placebo	7.79 ± 11.48	8.75 ± 11.86	0.831
Stage 2	Group A	Placebo	10.22 ± 14.74	9.04 ± 12.63	0.785
	Group B	Supplement	8.75 ± 8.73	11.86 ± 12.69	0.337
Frontal Alpha Power (uV^2/Hz)					
Stage 1	Group A	Supplement	3.53 ± 4.63	20.05 ± 34.28	0.037*
	Group B	Placebo	6.02 ± 6.33	7.82 ± 9.73	0.381
Stage 2	Group A	Placebo	11.40 ± 15.06	10.67 ± 15.77	0.866
	Group B	Supplement	8.53 ± 9.87	14.43 ± 15.96	0.088

A paired t-test was used to compare between the before and after the treatments. All the tests were two-tailed, and the level of statistical significance was set at P <0.05. Significance at P value * indicates < 0.05, ** indicates < 0.01, *** indicates < 0.001 (P > 0.05).

Table 4.5 shows the crossover summed up variables after the supplement or placebo administration. Overall, the supplement effects were much more convincing in this type of analysis. As for PSQI analysis, the global score and all the components except for sleep disturbances showed significant improvement ($P < 0.001$) for the supplement-receiving group. A significant placebo effect was noticeable only for the subjective sleep quality component ($P = 0.037$).

No significant changes were recorded for systolic and diastolic blood pressures, heart rate, and cortisol levels in neither supplement nor placebo-receiving groups except systolic blood pressure was slightly dropped in the placebo-receiving group ($P = 0.021$). However, for the EEG analysis, both total alpha power and frontal alpha power were significantly improved in the supplement-receiving group ($P = 0.022$ and 0.008) compared to the placebo group.

Table 4.5: Crossover summed up variables after the supplement or placebo administration

Variables	Pre Mean ± SD	Post Mean ± SD	Paired t-Test *P* Value	Pre Mean ± SD	Post Mean ± SD	Paired t-Test *P* - Value	Value Changes ± SD		Student t-Test *P* Value
	Supplement			Placebo			Supplement	Placebo	
Global PSQI Score	7.79 ± 2.57	5.40 ± 2.12	< 0.001***	6.82 ± 2.20	6.13 ± 2.41	0.096	2.44 ± 1.94	0.69 ± 2.54	0.001**
Total Sleeping Hours	5.72 ± 1.25	6.47 ± 1.11	< 0.001***	5.83 ± 0.98	6.16 ± 1.12	0.053	-0.76 ± 0.96	-0.33 ± 1.03	0.063
C1 Subjective Sleep Quality	1.54 ± 0.51	1.08 ± 0.66	< 0.001***	1.38 ± 0.63	1.15 ± 0.67	0.037 *	0.46 ± 0.68	0.23 ± 0.67	0.135
C2 Sleep Latency	1.43 ± 0.97	0.948 ± 0.86	< 0.001***	1.23 ± 0.90	1.03 ± 0.90	0.088	0.49 ± 0.68	0.21 ± 0.73	0.083
C3 Sleep Duration	1.59 ± 0.88	1.08 ± 0.66	< 0.001***	1.41 ± 0.79	1.33 ± 0.80	0.421	0.51 ± 0.64	0.10 ± 0.79	0.014*
C4 Habitual Sleep Efficiency	0.82 ± 0.94	0.26 ± 0.55	< 0.001***	0.51 ± 0.64	0.49 ± 0.76	0.872	0.56 ± 0.88	0.03 ± 0.99	0.013*

C5 Sleep Disturbances	1.31 ± 0.47	1.21 ± 0.61	0.253	1.33 ± 0.58	1.21 ± 0.52	0.230	0.10 ± 0.55	0.13 ± 0.66	0.852
C7 Daytime Dysfunction	1.13 ± 0.66	0.82 ± 0.56	0.001**	0.95 ± 0.56	0.95 ± 0.76	1.000	0.31 ± 0.52	0.00 ± 0.69	0.029*
Systolic Blood Pressure (mmHg)	117.54 ± 12.64	118.15 ± 13.17	0.701	120.00 ± 14.47	115.59 ± 16.33	0.021*	-0.62 ± 9.94	4.41 ± 11.44	0.042*
Diastolic Blood Pressure (mmHg)	75.31 ± 11.45	74.69 ± 9.56	0.601	76.56 ± 11.95	74.61 ± 9.54	0.088	0.62 ± 7.28	1.95 ± 6.94	0.410
Heart Rate (beats per min)	77.74 ± 9.51	77.10 ± 10.23	0.687	77.69 ± 9.31	78.38 ± 8.68	0.626	0.64 ± 9.87	-0.69 ± 8.80	0.531
Cortisol (nmol/L)	27.24 ± 14.81	22.76 ± 16.94	0.158	29.34 ± 16.46	29.40 ± 18.19	0.984	4.48 ± 19.43	-0.62 ± 19.56	0.307
Total Alpha Power (uV^2/Hz)	6.95 ± 7.53	13.28 ± 17.11	0.022*	8.91 ±12.95	8.68 ±11.25	0.920	-6.33 ± 16.03	0.23 ± 14.11	0.066
Frontal Alpha Power (uV^2/Hz)	6.23 ± 8.21	17.01 ± 25.78	0.008**	8.49 ± 11.37	9.13 ± 12.75	0.777	-10.78 ± 23.25	-0.63 ± 13.49	0.070

A paired t-test was used to compare the before and after treatments and a Student t-test was used to compare between the treatments. All the tests

were two-tailed, and the level of statistical significance was set at $P < 0.05$. Significance at P value * indicates <0.05, ** indicates <0.01, *** indicates <0.001 ($P > 0.05$).

It is evident from both analyses (Tables 4.4 and 4.5) that the sleep duration and habitual sleep efficiency were significantly improved in the supplement-receiving group. Thus, this supplement increased the total sleeping time by a mean of 45 min and the time spent in bed asleep without any adverse event being reported from the participants. Meanwhile, Component 6 (use of sleep medications) was not reported as none of the participants were taking sleep medications.

CHAPTER 5

DISCUSSION

5.1 Screening for Poor Sleep Quality

PSQI can be a sensitive, reliable, and valid outcome assessment tool to be used in community-based studies of primary insomnia and able to produce a coherent result (Tsai, et al., 2005; Chen, Kuo and Chueh, 2010). The prevalence of insomnia among workers who are not on alternating shifts has been estimated to be 5–45 % (Doi, 2005). The prevalence of poor sleep quality among the general population worldwide is 30-35 % (Morin, et al., 2015). The prevalence of insomnia or poor sleep quality was 15 % in China (Cao, et al., 2017), 39.4 % in Hong Kong (Wong and Fielding, 2011) and 33.8 % in Malaysia (Zailinawati, et al., 2008). These studies demonstrate how sleep problems are common across many populations and geographical areas, highlighting the necessity of specialised interventions to promote sleep health in both the general and working populations.

However, the prevalence rate could vary due to confounding factors such as sampling method, sleep quality measures, and response rate. The average sleep duration or TSH recorded in this study was 6.68 hours. The TSH among the staff was almost similar to the findings among academic staff of a university in Indonesia, with an average sleep duration of 6.7 hours (Yogisutanti, et al., 2012). The sleeping duration of this study was also similar or less than Australia

but longer than in the USA as the population in the USA had less than 6 hours of sleep (Adams, et al., 2017; Luckhaupt, 2012). However, sleep problem is considered an endemic problem in Australia (Adams, et al., 2017).

The percentage of subjects in this study who slept less than 7 hours was 38.95%, similar to the studies conducted in the UK and the US. Out of 48,990 working adults (42.6 ± 12.9 years old) in the UK, 41.6% had less than 7 hours of sleep (Weston, et al., 2024). Among the 20,851 US participants 18 years old and above, 39.45% slept less than 7 hours (Sullivan and Ordiah, 2018). Weston, et al. (2024) also found that working hours and sleep duration had an inverse relationship. The prevalence of poor sleep quality, 42.7% is extremely comparable to a survey of Singaporean employees across a range of industries, which found 42.5% of them to have poor sleep quality with the same global PSQI score criterion (PSQI >5) (Visvalingam, et al., 2020).

The result of this study is consistent with a meta-analysis of the prevalence of insomnia in China, where no gender difference was reported (Cao, et al., 2017). A study by Yoshioka and colleagues (2012) in Japan showed that the significant difference in the prevalence of insomnia between the genders was wiped out when the family characteristics and paid work were adjusted.

The global PSQI score and TSH did not substantially differ by gender and were not correlated with age, which is similar to the work of Ishibashi and Shimura (2020). In the study conducted by Ishibashi and Shimura (2020) among Japanese employees comprising different sectors, neither gender (1835 males

with global PSQI score of 6.36: 1062 females with global PSQI score of 6.44) nor age (18-76 years old) showed a statistically significant difference in term of the global PSQI score.

Although the TSH was not significantly different between males and females in this study, the subjective sleep quality was lower in females suggesting that females were not satisfied with their sleep experience or desired more sleep. Irrespective of age, females probably need a longer sleep duration (8 hours) than males (7.37 hours). The sleep deficit recorded was around 28.8 minutes in females (Tonetti, Fabbri and Natale, 2008; Anderson and Horne, 2008). A leading British expert on sleep stated that women need 20 minutes longer TSH than men due to their brain complexity and multitasking ability (Elkins, 2019). Desiring more sleep may indicate the need for time out not a sleep deficit. Anderson and Horne (2008) also revealed that sleep insufficiency was related more to stressful life than daytime sleepiness.

In the US, both males and females aged 18 and above showed a linear relationship between sleep duration and the number of poor mental health days ($\beta = -1.06$). In contrast, female had a stronger correlation (male $\beta = -0.84$, female $\beta = -1.27$) with the frequency of mental health symptoms even with additional sleep time (Sullivan and Ordiah, 2018). Moreover, depressive symptoms tend to occur with higher component scores of subjective sleep quality in females. Nevertheless, sleep quality can be independently associated with depressive symptoms in both males and females (Slaven, et al., 2011).

Contrary to the typical belief that older folks have more sleeping problems, this study showed that there is no association between age group and prevalence of poor sleep quality or no correlation between age and global PSQI score, although it is inconclusive, as only 15 staff in total, or 4.4 % were in the elderly group aged 60 and above. Cao, et al. (2017) showed that the prevalence of insomnia was even higher in younger adults (age < 43.7) comparatively. This could be due to long working hours, career stress, and overuse of computers and smartphones. In a study conducted among university academic staff in Japan, age and years of teaching experience were negatively correlated with insomnia and anxiety, implying junior staff were affected by mental health and sleep (Kataoka, et al., 2014). Generally, academic staff were found to be fatigued even at the beginning of their working day and the fatigue level was negatively correlated with sleep duration (Yogisutanti, et al., 2012).

Besides, the usage of sleep medication was significantly higher in the age group of 41 and above. Elderly people are usually more accessible to health professionals and insist on sleeping medications as a relief for sleeping problems although there are similar effective alternative treatments like behavioural intervention (Olfson, King and Schoenbaum, 2015). There were no statistically significant differences between the age or age groups for the rest of the sleep components in this study. This is similar to a meta-analysis conducted involving 3,577 subjects aged 5 to 102 years, total sleep duration and sleep efficiency were significantly decreased with age while sleep latency and WASO were significantly increased with age. However, according to the researchers, the TSH did not vary with five years of age or older; instead, it seemed to be tied to

environmental influences rather than biological changes (Ohayon, et al., 2004). Age seemed to be associated with a slight increase in sleep onset but it was a subtle change. Substantial difference was observed only when extremely young individuals were compared to elderly individuals. There was an overall increase in sleep latency of less than 10 minutes between the ages of 20 and 80 (Ohayon, et al., 2004).

In Malaysian public universities, Ph.D. holders accounted for 37% of their total academic staff while private universities and colleges were only able to achieve 13% of academic staff with Ph.D. in 2014 (Ministry of Education, 2019). According to the Malaysia National Higher Education Action Plan, at least 75 % of lecturers in the research universities and 60 % in other universities are expected to have Ph.D. qualifications (Ministry of Higher Education, 2011). In this study, only 29.4 % of the samples were Ph.D. holders in UTAR Kampar Campus. Thus, most academic staff have to pursue their Ph.D., especially on a part-time basis in private institutions including UTAR to sustain their career. This may contribute to more occupational stress.

When education status was compared within the same profession in an organisation, staff with lower paper qualifications or educational degrees had a higher risk of sleep disturbances (Chen, Kuo and Chueh, 2010). This explains why the global PSQI score in non-Ph.D. holders was significantly higher than the Ph.D. holders and the same trend was observed in subjective sleep quality as well as in sleep latency. The mean age of the former group was also significantly lower than the latter (Table 4.1) and this is not surprising as newly joined UTAR

academic staff especially those involved in teaching bachelor or advanced degrees have to start pursuing their Ph.D. studies within two years of commencement of work.

In another study, poor sleep quality was strongly associated with younger age and lower academic attainment in white-collar workers (Noordin and Jusoff, 2009). Moreover, job satisfaction of academic staff in higher institutions in Malaysia was at a moderate level and this was due to their position in the institution as well as their salary and age (Braido, et al., 2021). Education used to be provided as a public good by nonprofit organisations with unambiguous social goals and no commercial pressure. Nonetheless, this industry has developed into a worldwide business with quasi-companies operating in a very competitive market (Safdar, et al., 2020). Organisations are set to accomplish specific targets, and human resources propel them toward achieving those targets.

Poor sleep quality might affect both work and personal or family life. More than one-fifth of the academic staff in two leading Malaysian public universities (22.1 % for Universiti Malaya and 23.3 % for Universiti Sains Malaysia) were found to have occupational stress. Non-Malays were more stressful than Malays in the study. The most work-related stressor was found to be career development (salary and rewards), followed by research and teaching (Ismail and Noor, 2016). Research has been an essential component to be fulfilled by each academic staff to get promoted. However, research including publication not only needs funding, but it is also a protracted process and one

might get to work more than the standard 8 hours to achieve the expected work performance. There could be other contributing factors that may affect their sleep quality in these subjects such as common medical conditions and other prescribed medications. People with medical conditions such as asthma (Braido, et al., 2021), hypertension (Doi, Minowa and Tango, 2003), and gastroesophageal reflux disease (Vela, et al., 2014) were found to have poor sleep quality. Certain common medications such as beta-blockers, diuretics, corticosteroids, and histamine blockers, as well as some sleep medications, may affect one's sleep in the long run (Honkus, et al., 2003).

Self-reported poor sleep quality has been associated with physiological dysregulation leading to elevated multisystem biological risk including cardiovascular system, glucose and lipid metabolism, HPA system and so on. These findings were independent of factors such as sociodemographic, self-evaluated health, chronic health conditions as well as depression (Carroll, et al., 2015b). However, many respondents were not aware of their poor sleep quality until they participated in the screening. Simple lifestyle changes like exercising, controlling diet, reducing emotional stress, and having a good sleep environment may pose marked changes in sleep quality as well as the consequences of sleep problems (Chen, Kuo and Chueh, 2010). Thus, awareness about poor sleep quality and its associated pathological disorders should be raised.

5.2 Effect of CTH and L-theanine on Poor Sleep Quality among Academic Staff

Given that hyperarousal may be exacerbated by many factors such as work or financial stress, this study aimed to investigate whether four weeks of

oral daily intake of the supplement would improve various sleep factors in academic staff suffering from poor sleep quality. Numerous studies were conducted either on CTH (Saint-Hilaire, et al., 2009; Kim, et al., 2019) or L-theanine (Juneja, et al., 1999; Kimura, et al., 2007; Hidese, et al., 2019; Evans, et al., 2021), or with other supplement combinations (Scholey, et al., 2017), and they showed mixed effects. To our knowledge, this is the first clinical trial that studied the possible effect of the combination of CTH and L-theanine on alpha power and salivary cortisol concentration besides sleep quality. In addition, most of the intervention was conducted on clinical patients (Giuseppe, et al., 2017) or healthy participants (Messaoudi, et al., 2005; Hidese, et al., 2019; Evans, et al., 2021). Thus, this study may resemble a general population with a degree of variability in sleep patterns.

In this study, 150 mg of CTH and 50 mg of L-theanine showed significant improvements, especially in sleep duration and sleep habitual efficiency. These changes were observed only in supplement-receiving groups. Subjective sleep quality and sleep latency components significantly improved in both stages of the supplement-receiving groups, despite a placebo effect in one of the stages. Our results were comparable to a trial using CTH alone (211.21 mg/day) for the Japanese insomniac participants whereby the most profound improvement was observed in subjective sleep quality after two weeks of treatment. The sleep latency and daytime dysfunction were decreased after 4 weeks while the sleep disturbances only improved after 35 days. There was also a significant difference in the placebo group although CTH-treated participants marked more significant changes (up to $P < 0.005$) (Saint-Hilaire, et al., 2009).

In another study using a sleep diary as a sleep subjective measurement, the total sleeping time, sleep latency, and sleep efficiency were improved significantly in the CTH treatment group (300 mg/day), while the placebo group showed a worsening in sleep quality (Kim, et al., 2019).

The PSQI findings from the present study were consistent with the study conducted among a healthy population in Japan using L-theanine at a dosage of 200 mg/day for 4 weeks. It improved PSQI, depression, and anxiety scores compared to the placebo group. Some of the components of the PSQI scores, such as sleep latency, sleep disturbances, and the use of sleep medication decreased significantly compared to the placebo. Additionally, L-theanine administration did improve cognitive functions, particularly verbal fluency and the executive function of the subjects (Hidese, et al., 2019).

Another clinical trial using the supplement and the same dosage as this study was evaluated on Malaysian subjects with sleep disorders. A profound effect was observed in the intervention group in terms of sleep latency, sleep disturbances, and daytime dysfunction; furthermore, stress, anxiety and depression scores decreased significantly relative to the placebo group (Phing and Chee, 2019).

A very recent clinical trial reported the effect of chronic usage of the supplement combination at a higher dosage which was CTH at 300 mg/day and L-theanine at 204.12 mg/day for 8 weeks in 40 participants with sleep disturbances. The double-blind placebo-controlled study reported that TSH,

SOL, SE and WASO were significantly improved compared to the placebo group (Lim, et al., 2024).

As for L-theanine, Kunugi, et al. (2019) reported that supplementation at 200 mg/day for 4 weeks not only improved cognitive function in healthy adults but also the global PSQI score, sleep latency, sleep disturbance, and use of sleep medication. In the meantime, L-theanine did not reduce the anxiety symptoms in generalised anxiety disorders who were non-responsive to antidepressants. Nevertheless, L-theanine improved their sleep quality in the treated group although the ISI did not show any significant changes. Moreover, the placebo-receiving group with anxiety disorder reported notable sleep disturbances as an adverse event. It is also to be noted that the study participants who had serious anxiety issues were treated with unusually high dosages of L-theanine (450/900 mg per day). Thus, the study showed that L-theanine was able to improve sleep satisfaction and could be used to treat mild insomnia (Sarris, et al., 2019).

Studies reported that there were placebo effects compared with treatments of CTH in terms of global PSQI score in Kim, et al. (2019) and the combination of CTH and L-theanine; lower sleep latency and habitual sleep efficiency in Phing and Chee (2019) and global PSQI score and ISI in Lim, et al. (2024). The placebo effect can occur in any component of PSQI since there was no specific trend. Thus, the placebo effect was not critically affecting the results compared to the supplement-receiving group, which was more consistent with those published studies.

Messaoudi, et al. (2005) showed that in the healthy subjects treated with 400 mg of CTH three times 12 hours apart, their systolic and diastolic blood pressures recorded significantly lower percentage changes in the Stroop test (testing for cognitive function), while only systolic blood pressure showed a significant lower percentage change in a cold pressure test (cardiovascular response due to stress) compared to the placebo group. However, this was an acute effect of the supplement, unlike 4 weeks of treatment in this study.

Heart rate did not show any significant difference in this study in both analyses. Patients with chronic insomnia had significantly higher heart rates at night, but in the morning, their heart rates did not alter from baseline. Additionally, the insomniac group's pulse rate was found to be noticeably greater during the performance challenge. These findings support that the heart rate of insomniacs (Stepanski, et al., 1994) and healthy individuals (Messaoudi, et al., 2005) changed in response to ad hoc stress. The CTH treatment in Messaoudi, et al. (2005) did not affect the heart rate as it remained stable, which is in agreement with our study.

Phing and Chee (2019) reported that there was a placebo effect whereby the diastolic blood pressure was significantly lower in both the placebo and the supplement-treated groups. In contrast, this study revealed a placebo effect only for systolic blood pressure in the crossover summed-up analysis. Oliveira-Silva , et al. (2020) reported that systolic blood pressure, diastolic blood pressure, and heart rate were not significantly different between the poor and good sleeper groups.

Meanwhile, salivary cortisol level was associated with long-term sleep problems where chronic insomnia symptoms predicted an abrupt increase of cortisol in the morning (Abell, et al., 2016). CTH with a dosage of 150 mg/day for 14 days was able to reduce salivary cortisol levels as well as stressful symptoms in healthy subjects (Yeganehzad, et al., 2018). CTH was also able to reduce blood cortisol concentration in another study. However, this was a short-term effect with a considerably higher dosage of CTH (three doses of 400 mg) that was given to healthy males who were subjected to mental and physical stress and studied within 36 hours (Messaoudi, et al., 2005).

In contrast, the administration of L-theanine did not decrease salivary cortisol levels in the healthy population even given at a higher dosage, 200 mg/day for four weeks (Hidese, et al., 2019) and 200 mg twice a day for four weeks (Moulin, et al., 2024), but it did improve sleep quality, anxiety, depression, and cognitive functions (Hidese, et al., 2019; Moulin, et al., 2024). It has been postulated that changes in cortisol levels are transient (Hidese, et al., 2019), whereby a study with a single dose of 200 mg L-theanine showed a significant decrease of salivary cortisol post-1 hour of treatment in a moderately stress-induced healthy population compared to the placebo group (Evans, et al., 2021).

In addition, the wide variation in changes in salivary cortisol among participants who supplemented could be the reason for the lack of statistical significance in morning salivary cortisol between groups. Besides the sampling

time, salivary cortisol readings can be affected by several factors, including strenuous exercise, food consumption, light exposure, and the previous day or upcoming stressful events or difficulties. Strenuous or intense exercise, carbohydrate rich food, exposure to bright or blue light, and both acute and chronic stress may cause increased in cortisol levels (Hansen, Garde and Persson, 2008; Katsu and Baker, 2021).

In this study, salivary cortisol concentration decreased significantly in line with the daytime dysfunction of the supplement-receiving group (B) (Table 4.4). Daytime dysfunction can be associated with less enthusiasm to perform tasks (Pal, et al., 2004). The salivary concentration of cortisol in the morning may range from 10.2–27.3 nmol/L for normal adults (Laudat, et al., 1988). Based on the cross-over summed up analysis, cortisol levels decreased and fell into the normal range, although the change was not significant in the supplement-receiving group. In contrast, cortisol levels remained higher than the normal range in the placebo group. The autonomic nervous system and HPA axis activation is expected due to daily stress reactions in today's modern lifestyle. L-theanine may integrate into the brain and help to reset the stress response to a baseline state (Unno, et al., 2013).

The EEG results in this study suggested that the participants treated with the supplement had greater total and frontal alpha power. The brain activity of nine insomnia patients was compared with 16 normal people using EEG analysis in the awake stage. Lower alpha activity was found in insomnia (Siddiqui, Srivastava and Saeed, 2016) and stressed subjects (Chandra, et al., 2017)

compared to healthy subjects. Subjective sleepiness is negatively correlated with the total alpha power of the cortical region in awake EEG (Strijkstra, et al., 2003). When medical doctors had an average of 2.2 hours of sleep duration during on-call duty, the relative alpha power against the total EEG spectral power was reduced significantly in the frontal region and as a total due to sleep deprivation (Su, Xavier and Kuan, 2023).

Increased alpha power indicates augmentation of arousal. This will contribute to better performance and increased alertness, which could have been affected if a person had a lack of sleep regardless of whether they were suffering from sleep-disordered breathing (Wong, et al., 2008). Frontal alpha power was associated with creative cognition, indicative of heightened internal awareness (Fink, Schwab and Papousek, 2011). A single dose of 200 mg of L-theanine has been shown to increase total and frontal alpha power after 3 hours of intake in a stress-induced healthy population, and this reflects its calming effect (Evans, et al., 2021).

CTH was believed to promote sleep through anxiolytic effects via activation of serotonin (5-HT_{1A}), dopamine (D_1), and $GABA_A$ receptors, which release serotonin, dopamine, and GABA (Mizushige, et al., 2013). The peptide from CTH, CZP was also shown to reduce stress by influencing the autonomic nervous system (Makawey, et al., 2020). These results are corroborated by a subsequent study by Qian, et al. (2024), which found that CTH might reverse the behavioural effects of chronic stress, such as anxiety and insomnia, through the serotonergic and GABAergic systems. The ERK/CREB-BDNF-TrκB

signaling pathway, the HPA response, and the reduction of inflammation were also included in this modulation. CTH also increases delta waves during sleep, which is an indication of deep sleep and relaxation (dela Peña, et al., 2016).

Findings indicate that L-theanine may be beneficial for enhancing SOL, alleviating daytime dysfunction, and improving overall sleep quality. Due to a lack of study of L-theanine alone for the management of sleep disturbances is not well known. Therefore, the exact mechanism by which L-theanine promotes sleep has not been determined (Bulman, et al., 2025). However, similar to CTH, L-theanine was able to influence neurotransmitters, including dopamine, serotonin, glutamate, glutamine, and GABA, which are related to anxiolytic and stress-alleviating properties (Ota, et al., 2015; Saeed, et al., 2017; Bulman, et al., 2025). Relaxation and stress reduction with L-theanine was reflected through lowering blood pressure, heart rate, enhanced alpha brain waves and cortisol reduction (Kimura, et al., 2007; Evans, et al., 2021).

CTH alone with a dosage of 300 mg/day showed better efficacy when 62.5% of the outpatients who came for psychiatric consultation affected with poor sleep quality were able to engender significant clinical responses compared to 45% of patients who took CTH with standard treatments such as antidepressants and mood stabilisers (Giuseppe, et al., 2017). CTH produced double the anxiolytic and pro-hypnotic effects amongst the outpatients. This could be due to the side effects imposed by the standard treatment. For example, serotonergic antidepressants could cause sexual dysfunctions and nausea, which may add more stress and subsequently affect the sleep quality. However, this

study was neither randomised nor double-blinded. Nevertheless, the absence of adverse events or side effects of CTH can improve compliance among participants compared to standard drugs in open-label studies like this (Giuseppe, et al., 2017). In a recent study, CTH promoted sleep better than the synthetic decapeptide, CZP, as CTH has slower degradation and might have other sleep-enhancing properties (Qian, et al., 2021). Meanwhile, studies on L-theanine revealed that the anxiolytic effect can be achieved with a single dose of up to 200–250 mg per day without any sedative effect (Adhikary and Mandal, 2017).

Similar to many other studies, there were no reported adverse events, indicating the safety or tolerability of four weeks of this supplement intake. A very recent clinical trial studying the supplement at a higher dosage (300 mg /day of CTH and 204.12 mg/day for 8 weeks) did not report any adverse events. In terms of aspartate aminotransferase and alanine transaminase levels in the blood, it did not show any significant difference between the treated and placebo group (Lim, et al., 2024). These are liver enzymes that are usually elevated in liver dysfunction or tissue damage, indicating that the supplement did not cause liver toxicity at the tested dosage.

CHAPTER 6

CONCLUSION

Systematic literature review shows that anxiolytic properties of the milk bioactive peptides have been fairly documented in human, laboratory, and domestic animals. CTH and its bioactive peptides could improve anxiety and sleep. CTH is well tolerated in both clinical and preclinical studies with negligible adverse effects. Effective dosage must be investigated to achieve the desired outcome in a timely manner in order for the supplement to be prescribed by medical practitioners widely as nutritional management of stress and anxiety-related diseases.

Meanwhile, poor sleep quality in this study is more prevalent (42.7%) than the previous study conducted in the country. The academic staff slept an average of 6.68 hours, while 38.95% of them slept for fewer than 7 hours. Lack of quality sleep not only can affect health and social life but also workplace functioning. In the future, poor sleep quality could be an economic burden directly from health issues and indirectly like reduced productivity and work absenteeism. Good sleep is not a current priority of our nation, yet it has been linked to our key national health priority, metabolic syndromes such as diabetes, obesity and cardiovascular disease. It is necessary to study the possible risk factors associated with academic staff's sleep quality and compare them with other institutions to reach an apposite conclusion. Awareness and measures to control the prevalence of poor sleep quality should be instigated. Healthcare

providers should screen regularly for sleep difficulties in the population due to their high incidence.

The intervention part of the study was performed among the randomly screened poor sleepers and recruited in a randomised, placebo-controlled, double-blind crossover trial to explore the efficacy of a daily supplement containing 150 mg of CTH and 50 mg of L-theanine for 4 weeks. The regimen significantly improved global PSQI score, sleep latency, sleep duration, sleep habitual efficiency, daytime dysfunction, and alpha power in the supplement-receiving groups. The results suggest that the supplement can mitigate poor sleep quality and may also have an anxiolytic effect. Nonetheless, the present report may pave the way for future investigation amongst specific populations like the elderly, adolescents, pregnant or lactating women, or patients with comorbidities with different dosages, especially higher dosages for L-theanine and in comparison to the individual supplement (CTH alone and L-theanine alone).

6.1 Limitations

In the screening, the majority of the respondents were Chinese and had limited generalisability as only one university was involved. Besides, the analysis was conducted at the univariable level without controlling for potential confounders such as mental health and pre-existing sleep disorders. Nevertheless, this study does have several strengths. Randomised sampling and random assignment of participants was used and the study instrument, PSQI is highly validated for measuring sleep quality. However, using this questionnaire

might have recall bias as it required recording symptoms experienced in the past one month. To date, this study is the first to use the PSQI in studying sleep quality among academic staff in Malaysia. Thus, it provides new findings regarding academic staff in the university, a population that has been less studied in the literature.

On the other hand, the clinical trial was conducted in a real-world setting and might be influenced by biases such as the consumption of beverages other than tea and coffee, diet, and stress. The study was also carried out on a small population of 39 people, which may make it subject to type II errors, although the sample size had been estimated according to the effect size from the previous study in patients with poor sleep quality (Scholey, et al., 2017). Furthermore, the study might not represent the general population of Malaysia and its wide range of social backgrounds, as it was conducted among the academic staff of an institute.

6.2 Future Studies

The findings of this screening of poor sleep prevalence are preliminary, yet they can act as a basis for broader and empirical research. Health status, work performance, workload pressure and teaching experiences may act as confounding factors for sleep quality and thus can be studied in the future. As a recommendation, it is necessary to find out possible risk factors associated with the sleep quality of academic staff and compare them with other institutions to reach an apposite conclusion.

Meanwhile, four weeks of intervention with a minimal dosage might not be sufficient to produce a significant response. Furthermore, data from this study support the translational value of these nutraceutical ingredients considering their apparent benefits. Further studies with extended washout periods with various dosages in comparison with a standard treatment are warranted to make the supplementary treatment approach more valid considering there was a lack of adverse events. An essential next step in the study of the supplement's potential would be the confirmation of its safety and tolerability over an extended period of time among persons with poor sleep quality.

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APPENDIX A

1 Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. **Please answer all questions.**

1. During the past month, what time have you usually gone to bed at night? _____
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night? _____
3. During the past month, what time have you usually gotten up in the morning? _____
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.) _____

5. During the past month, how often have you had trouble sleeping because you…	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe:				

6. During the past month, how often have you taken medicine to help you sleep (prescribed or “over the counter”)?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				

SCORING INSTRUCTIONS FOR THE PITTSBURGH SLEEP QUALITY INDEX:

The Pittsburgh Sleep Quality Index (PSQI) contains 19 self-rated questions and 5 questions rated by the bed partner or roommate (if one is available). Only self-rated questions are included in the scoring. The 19 self-rated items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of '1 0" indicates no difficulty, while a score of "3 [11] indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points, "0" indicating no difficulty and "21 " indicating severe difficulties in all areas. Scoring proceeds as follows:

Component 1: Subjective sleep quality

Examine question #6, and assign scores as follows:

Response	Component 1 score
"Very good"	0
"Fairly good"	1
"Fairly bad"	2
"Very bad"	3

Component 1 score:_____

Component 2: Sleep latency

1. Examine question #2, and assign scores as follows:

Response	Score
S15 minutes	0
16-30 minutes	1
31-60 minutes	2
> 60 minutes	3

Question #2 score: _____

2. Examine question #5a, and assign scores as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Question #5a score: _____

3. Add #2 score and #5a score

Sum of #2 and #5a: _____

4. Assign component 2 score as follows:

Sum of #2 and #5a	Component 2 score
0	0
1-2	1
3-4	2
5-6	3

Component 2 score:_____

Component 3: Sleep duration

Examine question #4, and assign as follows:

Response	Component 3 score
> 7 hours	0
6-7 hours	1
5-6 hours	2
< 5 hours	3

Component 3 score:_____

Component 4: Habitual sleep efficiency

1. Write the number of hours slept (question #4) here:_____
2. Calculate the number of hours spent in bed:

Getting up time (question #3): _____Bedtime (question #1):_____
Number of hours spent in bed:_____

3. Calculate habitual sleep efficiency as follows:

(Number of hours slept/Number of hours spent in bed) X 100 =
Habitual sleep efficiency (%) (_____/_____) x 100 = %

4. Assign component 4 score as follows:

Component 4

Habitual sleep efficiency %	score
> 85%	0
75-84%	1
65-74%	2
< 65%	3

Component 4 score:_____

Component 5: Step disturbances

1. Examine questions #5b-5j, and assign for each question as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

5b score: _____
5c score: _____
5d score: _____
5e score: _____
5f score: _____
5g score: _____
5h score: _____
5i score. _____
5j score: _____

2. Add the scores for questions #5b-5j:

Sum of #5b-5j: _____

3. Assign component 5 score as follows:

Sum of #5b-5j	Component 5 score
0	0
1-9	1
10-18-4	2
19-27	3

Component 5 score:_____

Component 6: Use of sleeping medication

Examine question #7 and assign scores as follows:

Response	Component 6 score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score:_____

Component 7: Daytime dysfunction

1. Examine question #8, and assign as follows:

Response	Score
Never	0
Once or twice	1
Once or twice each week	2
Three or more times each week	3
Question #8 score:	_____

2. Examine question #9, and assign scores as follows:

Response	Score
No problem at all	0
Only a very slight problem	1
Somewhat of a problem	2
A very big problem	3
Question #'9 score:	_____

3. Add the scores for question #8 and #9:

Sum of '8 and '9: _____

4. Assign component 7 score as follows:

Sum of #8 and #9	Component 7 score
0	0
1-2	1
3-4	2
5-6	3

Component 7 score:_____

Global PSQI Score
Add the seven component scores together:
Global PSOI Score:_____

APPENDIX B

List of Publication

(a) Thiagarajah, K., Chee, H.P. and Sit, N.W., 2022. Effect of Alpha-S1-Casein Tryptic Hydrolysate and L-Theanine on Poor Sleep Quality: A Double Blind, Randomized Placebo-Controlled Crossover Trial. *Nutrients*, 14(3), p.652.

(b) Thiagarajah, K., Sit, N.W. and Chee, H.P., 2023. Prevalence of Sleep Quality among Academic Staff of a Private University in Malaysia. *Malaysian Journal of Medicine & Health Sciences*, 19(6), pp.28-34.