Title of the article: The Effects of Green Tea Amino Acid L-theanine Consumption on the Ability to Manage Stress and Anxiety Levels: A Systematic Review

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ABSTRACT

The green tea amino acid, L-theanine (L-THE) is associated with several health benefits, including improvements in mood, cognition and a reduction of stress and anxiety-like symptoms. This systematic review evaluated the effect of pure L-THE intake, in the form of orally administered nutritional supplements, on stress responses and anxiety levels in human randomised controlled trials. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, nine peer-reviewed journal articles were identified where L-THE as a supplement was compared to a control. Our findings suggest that supplementation of 200–400 mg/day of L-THE may assist in the reduction of stress and anxiety in people exposed to stressful conditions. Despite this finding, longer-term and larger cohort clinical studies, including those where L-THE is incorporated into the diet regularly, are needed to clinically justify the use of L-THE as a therapeutic agent to reduce stress and anxiety in people exposed to stressful conditions.

Keywords: L-theanine, stress response, anxiety, mental health, green tea, human trials

Abbreviations

L-THE L-Theanine

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT Randomised Controlled Trial

HPA Hypothalamic Pituitary Adrenal

HR Heart Rate

HRV Heart Rate Variability

POMS Profile of Mood States

VAS Visual Analog Scale

STAI State-Trait Anxiety Inventory

BAI Beck's Anxiety Inventory

MAPS Mood, Alertness and Physical Symptoms Questionnaire

DASS Depression Anxiety and Stress Scale

VPT Visual Probe Task

VAMS Visual Analogue Mood Scale

HARS Hamilton Anxiety Rating Scale

sAA salivary Alpha-Amylase (sAA)

s-IgA Salivary Immunoglobulin A

BP Blood Pressure

Introduction

Stress and anxiety are two inter-related conditions that have a substantial impact on individuals, communities and wider society, with 264 million people estimated to be living with anxiety disorders in 2015 [1]. The terms *'stress'* or a *'stressor'* are commonly used to describe physical or psychological responses to a perceived threat to homeostasis, as well as referring to a range of physiological parameters necessary for survival. Additionally, these terms can also characterise an event, or series of events, that cause a response, whether it be positive or negative [2]. In this paper, stress will be used to describe the physiological response that is detrimental to an individual that in turn, elicits a physiological and behavioural response.

Prolonged stress is related to a number of chronic conditions and diseases, including hypertension [3], type 2 diabetes and coronary heart disease [4]. Furthermore, the effects of psychological stress are associated with anxiety-related diseases, including eating disorders [5], irritable bowel syndrome [6] and substance abuse [7]. The evaluation of the stress response, as well as the severity of anxiety, can effectively be divided into two broad categories relating to L-THE; subjective psychological measures using a variety of questionnaires and assessment tools [8-11]; and physiological responses such as

changes in blood pressure (BP), circulating hormones and salivary hormones, heart rate (HR), heart rate variability (HRV) and autonomic nervous system reactivity [12-14]. In recent years, the use of complementary therapies to treat anxiety and stress-related disorders has increased; with mixed evidence surrounding their efficacy [15]. One such approach is the use of green tea, which has a long history of being consumed to enhance relaxation [16].

The consumption of green tea in some of the most densely populated countries in the world, such as China and Japan, accounts for around 20% of global consumption [17]. In recent years the popularity, as well as the significant research interest that green tea has attracted, is typically attributed to its favourable taste as well as numerous proposed health benefits including neuroprotection [18], cholesterol-lowering properties [19-21], strong antioxidant capacity [22] whereby consumption is associated with successful aging [23,24]. Among the many constituents found in green tea such as polyphenols [25], flavonoids [26] and caffeine [27], L-Theanine (L-THE) in particular, has received considerable interest in human trials [28,29].

L-THE was first isolated and identified in 1949 [30] as a water-soluble non-proteinogenic amino acid predominantly found in the tea plant (*Camellia sinensis*), and responsible for the provision of a unique taste similar to the savoury taste sensation that monosodium glutamate produces known as *'umami'* [28,31]. According to the universal nomenclature of the International Union of Pure and Applied Chemistry, L-THE is '*2-amino-4-(ethylcarbamoyl) butyric acid*' and it is referred to by many different names including '*gamma-glutamylethylamide'* and *'gamma-glutamyl-L-ethyl amide'* [32] reflecting the presence of glutamine, a conditionally essential amino acid found as a core unit in its structure [29]. The theanine may occur as a racemic mix of its L- and D- enantiomers that compete for absorption and urinary excretion with D- enantiomer reported to be metabolised at a faster rate while L- being preferentially metabolised by the kidneys [29,33].

Studies in animal models have reported the decline of serum glucose, insulin and urea after the administration of L-THE [34]. Therefore it was proposed that L-THE may be transported into systemic

circulation by sodium-glucose transport protein *1*; a mediator of glucose and galactose absorption [34,35]. Furthermore, kinetic studies indicate that L-THE crosses the blood brain barrier via the amino acid L transport-system as determined by the decrease of other L-system serum amino acids in its presence [36].

The potential health benefits associated with the consumption of L-THE include improvements in emotional status, quality of sleep [29,37], suppression of hypertension [38], and improvements in mood and cognition [39]. Additionally, consumption of L-THE in combination with caffeine promotes antioxidant and anti-inflammatory activity in the brain that may reduce the risk of cognitive impairment [40]. The current evidence on the consumption of L-THE in humans and its effects on stress and anxiety is equivocal. To date, the majority of evidence is based on animal research, which has commonly used pure L-THE, and in combination with other bioactives such as caffeine [41] and catechins that can potentially have synergistic and in some cases potentially antagonistic effects [16,28].

To our knowledge, only one systematic review has investigated the effects of green tea consumption on mood and cognition [39]. Nonetheless, it is important to note that this review evaluated the effects of L-THE as well as a mixture of other tea components (catechins and caffeine) rather than L-THE in its pure form. Therefore, this systematic review aimed to evaluate the effects of L-THE consumption on anxiety levels and the stress response in humans. A secondary aim was to provide insight into the potential mechanism of action that L-THE can pose and its possible therapeutic benefits for reducing stress and anxiety.

Methods

Design

Standardised criteria for conducting and reporting systematic reviews of interventional studies was followed based on the PRISMA 2009 statement and checklist [42]. This systematic review was

registered in an International Prospective Register of Systematic Reviews (PROSPERO); registration number CRD42018104792.

Eligibility criteria

A PICOS (population, intervention, comparator, outcomes and setting) approach was used to structure the systematic review and is as follows:

Population: Human populations of all ages, either healthy or with a diagnosis of anxiety and/or depression were included.

Intervention: Randomised controlled trials (RCTs) or quasi-RCTs that reported the effects of L-THE consumption on anxiety and stress outcomes were included. Trials were required to evaluate orally administered L-THE alone or in combination with conventional therapy versus the same conventional therapy alone. This criterion also included studies where quantifiable levels of L-THE were delivered as part of a supplement or if it was a functional component of a supplement that may also contain other compounds (i.e. food and drinks). Only studies with sample sizes of over 10 participants were included to limit the influence of outliers and allow for a more robust analysis of the results.

Comparator: Using a placebo, active control (i.e. a pharmaceutical product) or other control in single or double-blind human interventions.

Outcomes: Only studies that reported pre- and post-levels of stress and/or anxiety using at least one validated psychological assessment method were included. The primary outcomes of interest included levels of anxiety and/or stress determined using a variety of scales such as the Profile of Mood States (POMS) [8], Visual Analog Scale (VAS) [9], State-Trait Anxiety Inventory (STAI) [10], and Beck's Anxiety Inventory (BAI) [11]. Secondary outcomes were physiological responses including BP, skin temperature, HR, HRV, cortisol and salivary immunoglobulin A (s-IgA) [29].

Setting: Any.

Literature search

Only studies that were published in their entirety in the English language in peer-reviewed journals were included. Article selection was restricted to studies performed on humans (single- and double-blind included) where an L-THE supplement was compared against a control.

Four electronic databases (PubMed, Scopus, Cochrane Library and Web of Science) were searched for articles published from 1990 until October 2018. Articles were not selected prior to 1990 due to the difficulty in synthesis and low commercial availability of L-THE as a supplement prior to this date. All retrieved articles were read in full, and where multiple publications referred to the same study, only the latest publication was used [43]. Searches were re-run prior to submission (March 2019), with only one additional article included [44].

Search terminology

Search terms used were *'theanine'*, *'L-theanine'*, *'L-theanine* AND *stress'*, *'L-theanine* AND *anxiety'*, *'gamma-glutamylethylamide'*, *'gamma-glutamylethylamide* AND *stress'*, *'gamma-glutamylethylamide* AND *anxiety'*, *'gamma-glutamyl-L-ethyl amide'*, *'gamma-glutamyl-L-ethyl amide* AND *stress'*, *'gamma-glutamyl-L-ethyl amide* AND *anxiety'*, *'N^5-ethyl-L-glutamine'*, *'N^5-ethyl-L-glutamine* AND *stress' and 'N^5-ethyl-L-glutamine* AND *anxiety'*. In addition, articles that included L-THE consumption and mood changes were also selected if the mood changes were measured as either stress or anxiety or both.

Study selection and data extraction

Two authors (J.W. and J.E.) independently reviewed the titles and abstracts and extracted data in a predefined table. The following information was extracted: the first author and year of publication; mean age, sample size, experimental intervention (regimen); control intervention (regimen); stress and/or anxiety outcome; and any adverse effects reported.

Risk of bias assessment

Risk of bias in the included studies was independently assessed by two authors (J.W. and J.E.) following the Cochrane guidelines criteria [45]. The Cochrane guidelines assess the risk of bias based on seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, and selective reporting bias. Only articles with a low risk of overall bias were eligible for inclusion and any differences in opinion were resolved through discussion with a third reviewer (N.N.).

Data analysis

Included studies were analysed based on the measures specific to the study design; the intervention conducted; the sample size, age and gender of the participants; and various stress and anxiety outcomes. The results were recorded based on the change in the measures from baseline to post-consumption values and evaluated for effectiveness with the probability value ($p<0.05$) considered as statistically significant. Due to the heterogeneity of the study design, setting, intervention and outcomes, a meta-analysis was not conducted.

Results

Study characteristics

Initial search results identified 261 articles. Following removal of duplicates (n=40), reviews, letters to the editor and articles not written in English (n=177), the number of articles was reduced to 44. Nine articles [38,44,46-52] met the inclusion criteria (Figure 1). Seven studies recruited participants with no known pre-existing mental health issues, while two studies used participants with an existing mental health condition [44,49]. The total number of participants for all included studies was 270 with 134 males and 88 females (Table 1). Two studies were conducted in males only [46,47], and one study did not provide the genders of its 48 participants [38].

Study designs

Eight studies were designed as randomised, double-blind, placebo-controlled trials [38,44,46-49,51,52] while one study used a randomised, single-blind, placebo-controlled design [50]. Five of the studies [46-48,51,52] used a crossover design with all the participants exposed to each of the experimental treatments conditions. The other four studies [38,44,49,50] used a parallel design to compare between an L-THE group and a placebo group. Seven of the studies compared L-THE to placebo while the study by Lu *et al.* [48] compared L-THE against a prescribed anti-anxiety pharmaceutical (Alprazolam; 1mg; Xanax®, Pharmacia and UpJohn Ltd) as well as against placebo. The study by White *et al.* [51] compared L-THE against a placebo in the form of a nutrient drink with (active) and without (placebo).

Treatment duration and doses

In six studies [38,46-48,51,52], treatments were given acutely on the day in which various tests and measurements were conducted. The other three studies were designed to test for chronic effects and involved interventions over periods between seventeen days [50] to eight weeks [44,49]. In the acute single day intervention trials, participants consumed 200 mg/day [46-48,52], while in the chronic trials, participants consumed 400 mg/day of L-THE [49,50], and only one study used 450 and 900 mg/day.

Outcomes

Five studies included investigated the effects of L-THE on stress and anxiety [38,47,50-52] and four studies investigated anxiety outcomes only [44,46,48,49]. The most commonly used scales to assess changes in anxiety symptoms in the included studies was the STAI [38,46-48,50,51], VAS [47,50,52], Hamilton Anxiety Rating Scale (HARS) [44,49], BAI [44,48], and POMS [52]. The Depression Anxiety and Stress Scale (DASS) was used in one study [38]. Physiological data were assessed by HR

[38,46,47], BP [38,52], HRV [47], and skin temperature [52]. Biomarkers were measured in three studies; s-IgA [47], salivary alpha-amylase (sAA) [50] and salivary cortisol [51].

Risk of bias assessment

The risk of bias was assessed for all included studies (Table 2). Overall, a *'Low'* risk of bias was observed across all domains. Some studies received an *'Unclear'* classification due to insufficient information provided in the articles. Only one study received a *'High'* risk classification because this study was single-blinded [50].

L-THE and stress responses

In total, five studies assessed stress responses [38,47,50-52]. In the study by Yoto *et al.* [52], the participants performed a range of physical and mental tasks to test the acute effects of L-THE consumption on the stress response. Following randomisation, the participants were required to complete the POMS and VAS assessments, and baseline physiological measurements were taken before they ingested either L-THE (200 mg), caffeine (100 mg) or a placebo. The participants were then exposed to psychological stress load by auditory oddball target detection task (5 min) and arithmetic mental task test (10 min) twice. This was followed by the cold pressor test (immersion of hand in the slushy ice water) serving as an acute physical test. Participants were also analysed based on the change in BP after the mental tasks while given the placebo: those with BP responses above *('high-response'*) and below the mean ('*low-response'*). The 'high-response' group consuming 200 mg of L-THE had significantly lower systolic (p=0.008) and diastolic BP (p=0.006) in response to the mental tasks, but not the cold pressor test compared to placebo. In the 'low-response' group, there were no treatment effects in systolic or diastolic BP in the measurement periods.

The crossover trial by Kimura *et al.* [47] assessed the effects of L-THE (200 mg) on the stress response (20 min arithmetic task) across four conditions: 1) at the start of the experimental procedure;

2) during the experimental procedure; 3) placebo; and 4) no treatment. The s-IgA and psychological measures (STAI and VAS) were measured post-task while HR and HRV were measured continuously through the experimental sessions. The results indicated a significant interaction between condition and period for s-IgA (p<0.01), and s-IgA levels were higher under the placebo condition compared to the other conditions. In addition, the stress task increased HR under the placebo condition (p<0.05) while the L-THE conditions showed lower HR increments. The results of the HRV measurements revealed that some aspects of the HRV analysis (LF/HF) in the placebo condition were significantly higher (p<0.05) than other three conditions indicating more activation of the sympathetic nervous system during acute stress.

The study by Rogers *et al.* [38] investigated the subjective, behavioural and physiological (BP and HR) effects of L-THE and caffeine supplementation administered alone and/or in combination. All participants received either a capsule of caffeine (250 mg) or placebo and drink containing L-THE (200 mg) or no L-THE. Participants recorded their responses using the Mood, alertness and physical symptoms (MAPS) questionnaire, BP and HR were assessed before and 40 min after the administration of the treatments. The results indicated that the systolic and diastolic BP were higher after the caffeine consumption than any other treatment combinations (p<0.05).

A study by Unno *et al.* [50] examined the effects of L-THE in pharmacy students during a highly stressful period, the first week of pharmacy practice placement. Students were randomly assigned L-THE twice daily (400 mg/day) or a placebo for 17 days, with the STAI performed before and after their placement and sAA measured twice (morning and evening) daily. The results indicated that the sAA was higher in the placebo group (p=0.032) while the L-THE group maintained the baseline observed during the routine activities at the university. Interestingly, the STAI values were not different between the two groups; however, the psychosocial stress was lower in L-THE group (p=0.020) compared to placebo.

The crossover trial by White *et al.* [51] investigated the anti-stress effects of L-THE (200 mg) administered in the form of an L-THE active nutrient beverage commercially available (NeuroBliss®) in comparison to a placebo in 34 healthy participants (15 males, 19 females) [51]. This study carried out a multi-tasking framework as a cognitive stressor and as a cognitive assessment. In total, participants completed the task three times per visit (baseline, during and three hours post-consumption). Stress, mood, fatigue and salivary cortisol were assessed prior to and following the completion of each step of the cognitive stressor. The consumption of L-THE was associated with a decrease in subjective stress responses (p=0.003) and reduced self-rated stress response (p=0.006) measured 1 hr post-administration. No significant differences were reported in the subjective stress response 3 hr after consumption; however, salivary cortisol levels were significantly lower in the L-THE group (p=0.047) in comparison to placebo at this time point.

Effect of L-THE on anxiety

All studies [38,44,46-52] assessed the effects of L-THE on anxiety. The study by Ritsner *et al.* [49] explored the effect of eight weeks of L-THE (400 mg) treatment on patients with schizophrenia or schizoaffective disorders as an add-on to conventional antipsychotic therapy with anxiety levels measured using the HARS. The consumption of L-THE was associated with a reduction in total HARS score (p=0.015) with onset of improvement after the second week, and improvement in five of the HARS items including anxious mood (p=0.003), tension (p=0.021), intellectual (p=0.044), muscular (p=0.012) and sensory somatic complaints (p=0.030) in comparison to placebo group.

Lu *et al.* [48] compared the short term effects between the L-THE (200 mg), Alprazolam (1 mg; Xanax®, Pharmacia and UpJohn Ltd) and placebo on behavioural measures of anxiety in healthy participants. There was no difference between L-THE and placebo on the STAI and BAI scores (All *p's*>0.05); however, L-THE reduced subjective anxiety in comparison with Alprazolam (p<0.05) and placebo (p<0.05) on the *'Troubled'* subscale of the VAMS. Also, VAMS-Tranquil scores were lower

for L-THE group compared to the other two treatments (All p's>0.05). In addition, L-THE intake reduced the total HARS score (p=0.015). Furthermore, the results indicated that neither L-THE nor Alprazolam showed any acute anxiolytic effects under an electric shock model of anticipatory anxiety.

Higashiyama *et al.* [46] investigated the effects of L-THE (200 mg) consumption on attention and reaction time responses in healthy university students with high and low anxiety propensities. The consumption of L-THE resulted in a decrease in HR (p=0.0016) and an improved reaction time response among high anxiety propensity participants compared to placebo. The STAI results indicated time dependant decrement patterns of anxiety; however, no differences were observed between groups, potentially attributing to the participants being familiar with the testing environment. Similarly, Rogers *et al.* [38] assessed anxiety interactions in 48 university students using a visual probing task. The consumption of L-THE slowed overall reaction time on the visual probe task, with social threat sub-task reaction time (p=0.046), whereas no differences were observed in the physical threat reaction task (p=0.100) compared to placebo. The authors did attempt to discuss the potential mechanisms of action and reasons for not seeing anxiety lowering effects which were ascribed to participants being of a healthy mental state.

The study by Kimura *et al.* [47] showed strong interactions between the perception of stress and state anxiety (p<0.01). The STAI scores were higher during the mental arithmetic task period than during the rest periods in the placebo group, but not in the other three conditions (p<0.01). The VAS scores were also lower in both L-THE conditions than under the two control conditions (p<0.01).

In the study by White *et al.* [51] changes in brain alpha oscillatory activity, measured by electroencephalography and using resting-state recordings, were related to the potential anti-stress effects of L-THE supplementation. The results were higher in the L-THE treatment compared to the placebo for the high anxiety trait groups (p=0.019, one-tailed), while no changes were observed in the low anxiety group (p>0.1). It was suggested that the increase in resting alpha activity in the treatment group did not reflect the mechanism that normalises alpha activity in individuals with high anxiety.

Instead, the L-THE treatment enhances alpha oscillatory level activity selectively in individuals that self-reported higher levels of trait anxiety.

The most prolonged (treatment duration) study by Sarris et al. [44] was an eight-week phase II randomised multicentre clinical trial in individuals (n=46) with generalised anxiety disorder. For the first four weeks, participants received 225 mg L-THE twice daily. After that, participants who were considered non-responders (based on HARS score), were prescribed double the original dose. Two measures were used to assess anxiety symptoms (HARS; BAI); however, neither doses of L-THE improved symptoms compared with placebo.

In the study by Unno *et al.* [50], there was an association between STAI and subjective stress in the L-THE (p=0.012), but not in the placebo group. In the study by Yoto *et al.* [52] no differences between the groups were observed for VAS scores; however, the scores were lower in the L-THE group for tension-anxiety sub-scale of the POMS (p=0.004), representing a potential anxiolytic effect.

Discussion

The studies outlined in this systematic review indicate that L-THE supplementation has a potential anti-stress effect and anxiety suppressive properties at levels between 200-400 mg/day. All included articles demonstrate some strengths and indicators of quality, for instance, eight of the nine studies were designed as randomised, double-blind, placebo-controlled trials [38,44,46-49,51,52] while one study used a randomised, single-blind, placebo-controlled design [50]. However, according to the Cochrane assessment of the risk of bias, there was a lack of clarity in some domains of bias present in most studies.

Given the duration of the Ritsner *et al.* [49] study, there appear to be no side effects of taking L-THE (400 mg/day) for 8 weeks. Furthermore, the study by Sarris *et al.* [44] with longer (10 weeks) and higher L-THE intake (900 mg) reported some adverse events in both groups (placebo and L-THE), but with no significant differences between groups. Considering that schizophrenia and schizoaffective

type of disorders are not acute types of mental health conditions, much longer L-THE trials may be required to establish the potential time/concentration side effects**.** In addition, Ritsner *et al.* [49] demonstrated benefits of L-THE through the amelioration of psychotic and anxiety-related symptoms in contrast to the classical dopaminergic theory of psychosis [53].

Consumption of L-THE was also proposed to affect the physiological biomarkers of stress. Elevated sAA is an indicator of autonomic nervous system excitation and was shown to be decreased prior to stress exposure in healthy participants following consumption L-THE [50]. Additionally, a previous study indicated that the consumption of L-THE was associated with improvements in sleep quality [54], which can be regulated through the sAA levels where participants with lower sAA levels had a longer sleep [50]. Once L-THE is consumed, it is absorbed within the intestinal lumen and transported to a range of tissues, including the brain within 10-24 min [55]. Therefore, it can be suggested that the results of the Unno *et al.* [50] study indirectly support the blood-brain-barrier passing of L-THE to illicit its psychological effects [50,55]. Therefore, consumption of L-THE may be an effective method for improvement in sleep quality without the increase in drowsiness.

The L-THE consumption may also suppress excessive glutamatergic tone, which is implicated as a possible co-factor in increasing stress and its related anxiety responses [56,57]. The glutamatergic transmission relies on L-THE-mediated inhibition of neuronal glutamine uptake which, in turn, suppresses the conversion of glutamine to glutamate. Thus, the consequent decrease in glutamate release from the pre-synaptic terminals induced by L-THE represents another plausible mechanism contributing to the anti-stress and anti-anxiety effects (Figure 2) [50]. In addition, another mechanism putatively responsible for the inhibition of glutamatergic transmission is underpinned by the ability of L-THE to bind to the three glutamate receptor subtypes: Ar-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainic acid (KA), thus competing with glutamate [58]. Moreover, in murine models, it has been shown that L-THE intake suppresses the HPA axis and behavioural depression [59], although the same mechanisms are yet to be demonstrated in

humans. Nevertheless, a study by Rogers *et al.* [38] suggested that L-THE may exhibit central monoaminergic and glutaminergic effects suggesting that slowing the reaction time on the visual probe task may also be attributed to the suppression of glutamate [50,58].

Although findings by Unno *et al.* [50] indicated that long-term use of L-THE could provide *'anti-stress'* effects. However, there was no indication this finding was a direct result of the daily intake of L-THE, a higher dose (400 mg), or a potential adaptive effect over several days [50]. Interestingly, in the study by Yoto *et al.* [52], consumption of 200 mg of L-THE reduced subjective stress and BP, but only in the short term. The study by Rogers *et al.* [38] indicated that L-THE could inhibit the caffeine-induced rise in BP, which is accompanied by increased nervousness and considered as a somatic symptom of anxiety. However, the DASS and STAI scores indicated participants were not prone to anxiety or stress. It was further proposed that the initial mental state of the participants might affect the reduction in anxiety caused by L-THE. However, this study speculates that tea is perceived to be more relaxing, partly due to the reason that L-THE reduces alertness/arousal and anxiety [38].

The effectiveness of L-THE in the acute anxiety induced conditions was also compared against the benzodiazepine alprazolam, a common pharmaceutical mediation used for the treatment of anxiety and related disorders [48]. Overall, neither L-THE nor alprazolam treatment resulted in any anxiolytic effects [48]. Despite this outcome, it seems that the intensity of anxiety was too great for L-THE to provide an anxiolytic effect; however, L-THE had a greater effect in the resting state of healthy individuals compared to participants with increased anxiety. Although alprazolam has shown some inconsistencies with reducing anxiety under resting conditions [60], Higashiyama *et al.* [46] highlighted that L-THE (200 mg) displayed positive effects relating to attentional performance as well as reaction time in a cohort prone to high anxiety. This can potentially indicate that L-THE may not be as effective in individuals that are not highly anxious. Furthermore, the results suggest a putative mechanism of action highlighted by the increase in alpha waves as determined by electroencephalogram [61]. This, in turn, supports the previous literature associating the relaxation

properties of L-THE with the increased synthesis of γ-aminobutyric acid, which plays a part in the stress response pertinent to dopamine and serotonin increase in the brain [58,61]. The study by Sarris *et al.* [44] of individuals with generalised anxiety disorder found no benefits using a high dose of L-THE (450-900 mg), establishing that higher levels are potentially not beneficial in this type of a clinical sample. This was the first study to investigate the use of L-THE for the treatment of anxiety and sleep disturbance in generalised anxiety disorder and also the first to administer L-THE at doses higher than 400 mg/day. Although L-THE appeared to improve self-reported sleep satisfaction in participants with generalised anxiety disorder, and insomnia symptoms in a sub-group of individuals with generalised anxiety disorder, this preliminary pilot study found no evidence of any beneficial effects of L-THE over the placebo in measures of anxiety and mood. Additionally, considering this trial used the highest amount of L-THE in the included studies, it appears that understanding how high levels L-THE affects the psychological response is still relatively unexplored.

Lastly, the study by White *et al.* [51] reported a decrease in the stress response post consumption of a nutrient-based drink containing 200 mg L-THE in response to a cognitive stressor task. The results indicated that the anti-stress response is attributed to a decrease in salivary cortisol only in 3 hr post-dosage. The authors reported no differences in cognitive performance, although posterior alpha oscillatory activity was found to be higher in participants exhibiting higher trait anxiety participants but was not associated with the other anti-stress outcomes. This study was limited as the active treatment that contained L-THE (200 mg) contained other ingredients: L-alpha glycerylphosphorylcholine (25 mg), phosphatidylserine (1 mg) and micronized chamomile (10 mg) that were unique to the active treatment and not the placebo. Although the beneficial effects of L-THE were observed after post-consumption, the authors acknowledged that an additional arm of treatment was needed to evaluate consumption of other ingredients in the nutrient drink as they may interact with L-THE absorption.

Thus, in a setting where green tea consumption frequently occurs, as opposed to pure L-THE consumption (4-8 cups/day; 150-200 mg), it is difficult to accurately measure the causative agent for specific physiological and psychological outcomes, as green tea contains a number of polyphenolic compounds (catechins) that might also interfere with the aforementioned health outcomes [16,24]. Indeed, studies have found tea drinkers have lower levels of depressive symptoms [62]; however, it is difficult to identify the lead potential anti-depressive agent due to the other bioactive compounds in green tea such as catechins. It is reported that the consumption of green tea in general or with other constituents may affect the uptake of other compounds, such as vitamin B1 [63], interference of lipid digestion due to green tea catechins [64] as well as the inhibiting effects L-THE shows in the presence of caffeine [29]. Similarly, the presence of L-THE in the blood has previously been shown to decrease the uptake of branched-chain amino acids, such as leucine, isoleucine and valine [36]; however, this mechanism is yet to be elucidated in humans.

Future directions

Given the growing interest and prevalence of green tea bioactives consumption globally [23], future human trials of L-THE consumption should be conducted over a more extended period (ten weeks or longer) with the combination in identifying the therapeutic doses required for different population sub-groups. The effect of L-THE in clinical practice focusing on the type of specific clinical population may, in turn, affect cardiovascular outcomes with potentially beneficial outcomes. Currently, the consumption of L-THE is associated the of a relaxed but alert state, providing the basis of L-THE use as a nutraceutical with stress- and anxiety-reducing properties. Moreover, the use of L-THE with other nutraceuticals (i.e. B-vitamins) may have positive synergistic effects on anxiety, stress, depressive symptoms, and cognition [65,66]. Further, studies should be conducted to ascertain whether L-THE binds to food matrixes before consumption as the health effects of L-THE may vary depending on the type of food and/or compounds in which L-THE has been combined with.

Conclusion

The supplementation of L-THE in its pure form at dosages between 200–400 mg/day may help reduce stress and anxiety acutely in people undergoing acute stressful situations, but there is insufficient evidence to suggest it assists in the reduction of stress levels in people with chronic conditions. However, the results of this study suggest that L-THE taken during times of heightened acute stress or by individuals with a high propensity for anxiety and stress may exhibit beneficial properties via the increased production of alpha waves and decrease of glutamate in the brain.

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References

1. World Health Organization (2017) Depression and Other Common Mental Disorders: Global Health Estimates. World Health Organization, Geneva, Switzerland

2. McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. Brain Res 886 (1-2):172-189

3. Pan Y, Cai W, Cheng Q et al. (2015) Association between anxiety and hypertension: a systematic review and meta-analysis of epidemiological studies. Neuropsych Dis Treat 11:1121-1130

480 4. Hagstrom E, Norlund F, Stebbins A et al. (2018) Psychosocial stress and major cardiovascular
481 events in patients with stable coronary heart disease. J Intern Med 283 (1):83-92
482 5. Fitzsimmons EE, Bardone-Cone AM (2011) Coping and social support as potential moderators of
483 the relation between anxiety and eating disorder symptomatology. Eat Behav 12 (1):21-28
484 6. Myers B, Greenwood-Van Meerveld B (2009) Role of anxiety in the pathophysiology of irritable
485 bowel syndrome: importance of the amygdala. Front Neurosci 3:47
486 7. Durand MV, Barlow DH (2010) Essentials of Abnormal Psychology. Fifth edn. Wadsworth
487 Cengage Learning,
488 8. Spielberger CD (1972) Profile of Mood States. Prof Psychol 3 (4):387-388
489 9. Pfennings L, Cohen L, van der Ploeg H (1995) Preconditions for sensitivity in measuring change:
490 visual analogue scales compared to rating scales in a Likert format. Psychol Rep 77 (2):475-480
491 10. Hedberg AG (1972) State-Trait Anxiety Inventory. Prof Psych 3 (4):389-390
492 11. Dozois DJA, Dobson KS, Ahnberg JL (1998) A Psychometric Evaluation of the Beck Depression
493 Inventory-II. Psychol assessment 10 (2):83-89
494 12. Matthews KA, Woodall KL, Allen MT (1993) Cardiovascular reactivity to stress predicts future
495 blood pressure status. Hypertension 22 (4):479-485
496 13. Gorman JM, Sloan RP (2000) Heart rate variability in depressive and anxiety disorders. Am Heart
497 J 140 (4 Suppl):77-83
498 14. Nater UM, La Marca R, Florin L et al. (2006) Stress-induced changes in human salivary alpha-
499 amylase activity - associations with adrenergic activity. Psychoneuroendocrino 31 (1):49-58
500 15. McCabe D, Lisy K, Lockwood C et al. (2017) The impact of essential fatty acid, B vitamins,
501 vitamin C, magnesium and zinc supplementation on stress levels in women: a systematic review. JBI
502 Database System Rev Implement Rep 15 (2):402-453. doi:10.11124/JBISRIR-2016-002965
503 16. Williams J, Sergi D, McKune AJ et al. (2019) The beneficial health effects of green tea amino acid
504 l-theanine in animal models: Promises and prospects for human trials. Phytother Res 3 (1)

505 17. Liu J, Xing J, Fei Y (2008) Green tea (Camellia sinensis) and cancer prevention: a systematic
506 review of randomized trials and epidemiological studies. Chin Med 3:12
507 18. Katergaris N, Dufficy L, Roach PD et al. (2015) Green Tea Catechins as Neuroprotective Agents:
508 Systematic Review of the Literature in Animal Pre-Clinical Trials. AFTNS Open J 1 (2):48-57
509 19. Bursill CA, Abbey M, Roach PD (2007) A green tea extract lowers plasma cholesterol by inhibiting
510 cholesterol synthesis and upregulating the LDL receptor in the cholesterol-fed rabbit. Atherosclerosis
511 193 (1):86-93
512 20. Bursill CA, Roach PD (2007) A green tea catechin extract upregulates the hepatic low-density
513 lipoprotein receptor in rats. Lipids 42 (7):621-627
514 21. Reto M, Figueira ME, Filipe HM et al. (2007) Chemical composition of green tea (Camellia
515 sinensis) infusions commercialized in Portugal. Plant Foods Hum Nutr 62 (4):139-144
516 22. Vuong QV (2014) Epidemiological evidence linking tea consumption to human health: a review.
517 Crit Rev Food Sci Nutr 54 (4):523-536
518 23. Naumovski N, Foscolou A, D'Cunha NM et al. (2019) The Association between Green and Black
519 Tea Consumption on Successful Aging: A Combined Analysis of the ATTICA and MEDiterranean
520 ISlands (MEDIS) Epidemiological Studies. Molecules 24 (10)
521 24. Crichton GE, Bryan J, Murphy KJ (2013) Dietary antioxidants, cognitive function and dementia--
522 a systematic review. Plant Foods Hum Nutr 68 (3):279-292
523 25. Naumovski N, Blades BL, Roach PD (2015) Food Inhibits the Oral Bioavailability of the Major
524 Green Tea Antioxidant Epigallocatechin Gallate in Humans. Antioxidants 4 (2):373-393
525 26. Yao LH, Jiang YM, Shi J et al. (2004) Flavonoids in food and their health benefits. Plant Foods
526 Hum Nutr 59 (3):113-122
527 27. Nehlig A, Daval JL, Debry G (1992) Caffeine and the central nervous system: mechanisms of
528 action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev 17 (2):139-170

529 28. Vuong QV, Bowyer MC, Roach PD (2011) L-Theanine: properties, synthesis and isolation from
530 tea. J Sci Food Agric 91 (11):1931-1939
531 29. Williams J, Kellett J, Roach PD et al. (2016) L-Theanine as a Functional Food Additive: Its Role
532 in Disease Prevention and Health Promotion. Beverages 2 (13):1-16
533 30. Eschenauer G, Sweet BV (2006) Pharmacology and therapeutic uses of theanine. Am J Health Syst
534 Pharm 63 (1):26, 28-30
535 31. Cooper R (2012) Green tea and theanine: health benefits. Int J Food Sci Nutr 63 Suppl 1:90-97
536 32. Nobre AC, Rao A, Owen GN (2008) L-theanine, a natural constituent in tea, and its effect on
537 mental state. Asia Pac J Clin Nutr 17 Suppl 1:167-168
538 33. Desai MJ, Gill MS, Hsu WH et al. (2005) Pharmacokinetics of theanine enantiomers in rats.
539 Chirality 17 (3):154-162
540 34. Yan Q, Tong H, Tang S et al. (2017) L-Theanine Administration Modulates the Absorption of
541 Dietary Nutrients and Expression of Transporters and Receptors in the Intestinal Mucosa of Rats.
542 Biomed Res Int 2017:9747256
543 35. Scheid L, Ellinger S, Alteheld B et al. (2012) Kinetics of L-Theanine Uptake and Metabolism in
544 Healthy Participants Are Comparable after Ingestion of L-Theanine via Capsules and Green Tea. The
545 Journal of Nutrition 142 (12):2091-2096
546 36. Yokogoshi H, Kobayashi M, Mochizuki M et al. (1998) Effect of theanine, r-glutamylethylamide,
547 on brain monoamines and striatal dopamine release in conscious rats. Neurochemical research 23
548 (5):667-673
549 37. Turkozu D, Sanlier N (2017) L-theanine, unique amino acid of tea, and its metabolism, health
550 effects, and safety. Crit Rev Food Sci Nutr 57 (8):1681-1687
551 38. Rogers PJ, Smith JE, Heatherley SV et al. (2008) Time for tea: mood, blood pressure and cognitive
552 performance effects of caffeine and theanine administered alone and together. Psychopha 195 (4):569-
553 577

554 39. Camfield DA, Stough C, Farrimond J et al. (2014) Acute effects of tea constituents L-theanine,
555 caffeine, and epigallocatechin gallate on cognitive function and mood: a systematic review and meta-
556 analysis. Nutr Rev 72 (8):507-522
557 40. Cicero AFG, Fogacci F, Banach M (2018) Botanicals and phytochemicals active on cognitive
558 decline: The clinical evidence. Pharmacol Res 130:204-212
559 41. Haskell CF, Kennedy DO, Milne AL et al. (2008) The effects of L-theanine, caffeine and their
560 combination on cognition and mood. Biol Psychol 77 (2):113-122
561 42. Moher D, Liberati A, Tetzlaff J et al. (2009) Preferred reporting items for systematic reviews and
562 meta-analyses: the PRISMA statement. J Clin Epidemiol 62 (10):1006-1012
563 43. Clifton PM, Condo D, Keogh JB (2014) Long term weight maintenance after advice to consume
564 low carbohydrate, higher protein diets-a systematic review and meta analysis. NMCD 24 (3):224-235
565 44. Sarris J, Byrne GJ, Cribb L et al. (2018) L-Theanine in the Adjunctive Treatment of Generalised
566 Anxiety Disorder: A Double-Blind, Randomised, Placebo-Controlled Trial. J Psychiat Res 110:31-37
567 45. Higgins JPT, Altman DG, Sterne JAC (2011) Chapter 8: Assessing risk of bias in included studies.
568 In: Higgins J.P.T., S. G (eds) Cochrane Handbook for Systematic Reviews of Interventions Version
569 5.1.0 The Cochrane Collaboration,
570 46. Higashiyama A, Htay HH, Ozeki M et al. (2011) Effects of L-theanine on attention and reaction
571 time response. J Funct Food 3 (3):171-178
572 47. Kimura K, Ozeki M, Juneja LR et al. (2007) L-Theanine reduces psychological and physiological
573 stress responses. Biol Psychol 74 (1):39-45
574 48. Lu K, Gray MA, Oliver C et al. (2004) The acute effects of L-theanine in comparison with
575 alprazolam on anticipatory anxiety in humans. Hum Psychopharm Clin 19 (7):457-465
576 49. Ritsner MS, Miodownik C, Ratner Y et al. (2011) L-theanine relieves positive, activation, and
577 anxiety symptoms in patients with schizophrenia and schizoaffective disorder: an 8-week, randomized,
578 double-blind, placebo-controlled, 2-center study. J Clin Psychiatry 72 (1):34-42

579 50. Unno K, Tanida N, Ishii N et al. (2013) Anti-stress effect of theanine on students during pharmacy
580 practice: positive correlation among salivary alpha-amylase activity, trait anxiety and subjective stress.
581 Pharmacol Biochem Behav 111:128-135
582 51. White DJ, de Klerk S, Woods W et al. (2016) Anti-Stress, Behavioural and
583 Magnetoencephalography Effects of an L-Theanine-Based Nutrient Drink: A Randomised, Double-
584 Blind, Placebo-Controlled, Crossover Trial. Nutrients 8 (1)
585 52. Yoto A, Motoki M, Murao S et al. (2012) Effects of L-theanine or caffeine intake on changes in
586 blood pressure under physical and psychological stresses. J Physiol Anthropol 31:28
587 53. Helton SG, Lohoff FW (2015) Serotonin pathway polymorphisms and the treatment of major
588 depressive disorder and anxiety disorders. Pharmacogenomics 16 (5):541-553
589 54. Lyon MR, Kapoor MP, Juneja LR (2011) The effects of L-theanine (Suntheanine(R)) on objective
590 sleep quality in boys with attention deficit hyperactivity disorder (ADHD): a randomized, double-
591 blind, placebo-controlled clinical trial. Altern Med Rev 16 (4):348-354
592 55. Alteheld B, Scheid L, Stehle P et al. (2012) Kinetics of L-Theanine Uptake and Metabolism in
593 Healthy Participants Are Comparable after Ingestion of L-Theanine via Capsules and Green Tea. J
594 Nutr 142 (12):2091-2096
595 56. Yuen EY, Liu W, Karatsoreos IN et al. (2009) Acute stress enhances glutamatergic transmission
596 in prefrontal cortex and facilitates working memory. Proc Natl Acad Sci U S A 106 (33):14075-14079
597 57. Popoli M, Yan Z, McEwen BS et al. (2011) The stressed synapse: the impact of stress and
598 glucocorticoids on glutamate transmission. Nat Rev Neurosci 13 (1):22-37
599 58. Kakuda T, Nozawa A, Sugimoto A et al. (2002) Inhibition by theanine of binding of [3H]AMPA,
600 [3H]kainate, and [3H]MDL 105,519 to glutamate receptors. Biosci Biotechnol Biochem 66 (12):2683-
601 2686
602 59. Unno K, Iguchi K, Tanida N et al. (2013) Ingestion of theanine, an amino acid in tea, suppresses
603 psychosocial stress in mice. Exp Physiol 98 (1):290-303

60. Baas JM, Grillon C, Bocker KB et al. (2002) Benzodiazepines have no effect on fear-potentiated startle in humans. Psychopha 161 (3):233-247

61. Juneja R, Djong-Chi C, Tsutomu O et al. (1999) L-theanine—a unique amino acid of green tea and its relaxation effect in humans. Trends Food Sci Tech 10 (6-7):199-204

62. Hintikka J, Tolmunen T, Honkalampi K et al. (2005) Daily tea drinking is associated with a low level of depressive symptoms in the Finnish general population. Eur J Epidemiol 20 (4):359-363

63. Wang RS, Kies C (1991) Niacin, thiamin, iron and protein status of humans as affected by the consumption of tea (Camellia sinensis) infusions. Plant Foods Hum Nutr 41 (4):337-353

64. Koo SI, Noh SK (2007) Green tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid-lowering effect. J Nutr Biochem 18 (3):179-183

65. D'Cunha NM, Georgousopoulou EN, Dadigamuwage L et al. (2018) Effect of long-term nutraceutical and dietary supplement use on cognition in the elderly: a 10-year systematic review of randomised controlled trials. Brit J Nutr 119 (3):280-298

66. Barnett H, D'Cunha NM, Geogousopoulou E et al. (2017) Effect of Folate Supplementation on Inflammatory Markers in Individuals Susceptible to Depression: A Systematic Review. ERHM 2:86-100

Figures and Tables

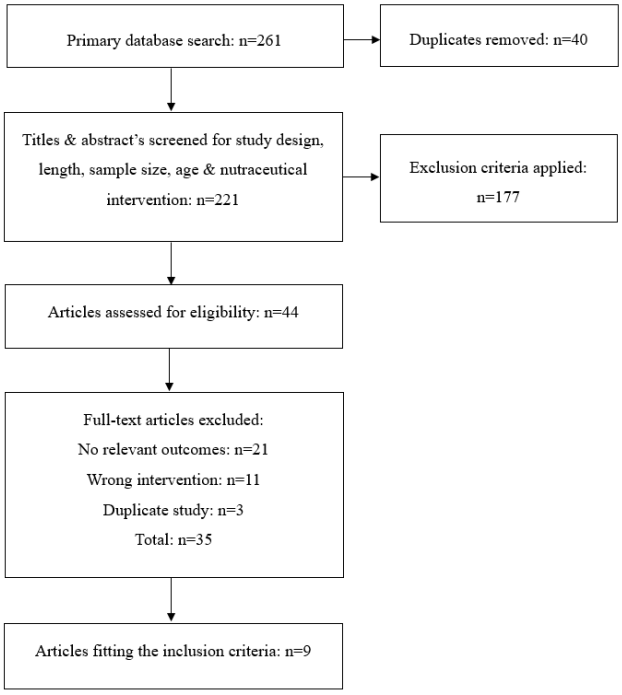

Fig 1 PRISMA flowchart of search strategy and selection of relevant studies.

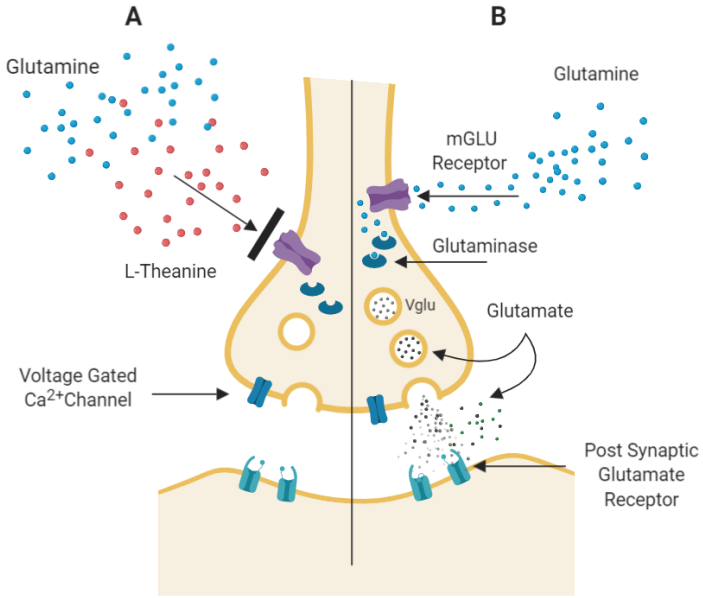

Fig 2 L-THE may reduce the binding of glutamine to glutamate receptors (mGLU) inhibiting the incorporation of extracellular glutamine into the neuron (A). This may prevent the formation of vesicular glutamate (Vglu) decreasing the activation of post synaptic glutamate receptors (B).

636 **Table 1.** Summary of L-THE effects in human clinical trials

Author (Year)	Intervention and dosage	Sample size All (M; F)	Age (y) (Mean±SD)	Study design and duration of intervention	Stress / Anxiety task	Stress / Anxiety outcome	Stress results	Anxiety results
Higashiyama et al. (2011)	L-THE (200mg) in water Placebo	18 (18;0)	19±1	Randomized, placebo-controlled, double-blind intervention. Acute	Visual Attentional Task; Rapid Audio Response Task	STAI; HR	L-THE decreased HR ($p=0.0016$)	L-THE improved attention performance ($p=0.0001$) and reaction time ($p=0.001$) in healthy subjects prone to high anxiety
Kimura et al. (2007)	L-THE (200mg) after initiation L-THE (200mg)– after rest Placebo No treatment	12 (12;0)	21.5±1.4	Randomized, double-blind, placebo-controlled cross over design. Acute	Mental arithmetic task	STAI; VAS; HR; HRV; s-IgA	Placebo increased HR ($p<0.05$), HRV (LF/HF effect of period) ($p<0.05$), and elevated s-IgA ($p<0.01$) compared with L-THE and control	Placebo increased STAI ($p<0.01$) and VAS ($p<0.01$) score compared with L-THE and control
Lu et al. (2004)	L-THE (200mg) Alprazolam (1mg) Placebo	16 (12;4)	26.9±3.4	Randomized, double-blind, placebo-controlled, cross over design. Acute	Relaxed and anticipatory anxiety (AA) condition with electric shock	BAI; BDI-II; VAMS; STAI	NA	VAMS-Tranquil score lower for L-THE compared to placebo and Alprazolam (both p's<0.05). No differences in AA condition
Ritsner et al. (2011)	L-THE (400mg/day) Placebo	40 (9:31)	36.4±11.5	Randomised, double-blind, placebo-controlled design 8 weeks.	Schizophrenia and Schizoaffective disorder patients	HARS	NA	L-THE reduced total HARS score ($p=0.015$)
Rogers et al. (2008)	Caffeine (250mg) + Placebo L-THE (200mg) + Placebo Caffeine (250mg) + L-THE (200mg) Placebo	48	20.5±2.0	Randomised, double blind, placebo-controlled cross over design. Acute	Ratings of mood, anxiety and alertness	MAPS; STAI; DASS; BP; HR, VPT	Caffeine alone increased systolic and diastolic BP compared with all other conditions (all p's<0.05)	L-THE slowed reaction time on the visual probe task social threat sub-task ($p=0.046$)

Sarris et al. (2018)	L-THE (225mg twice daily) Non-responders increased to 450mg twice daily at week 4 Placebo	46 (7;39)	Treatment: 40.7±15.0 Placebo: 32.2±9.3	Randomised, double-blind, placebo-controlled. 10 weeks (8-week trial, 2-week post-observation)	Anxiety, sleep quality and cognition	HARS; BAI; Penn State Worry Questionnaire	NA	No significant improvements in anxiety measures in the treatment group ($p>0.05$)
Unno et al. (2013)	L-THE (200mg twice daily) Placebo	20 (14;6)	22.4±0.2	Randomised, single-blind group comparison. Intervention 1 week prior to stressful condition and 10 days during.	Pharmacy practice task	STAI, VAS, sAA	Psychosocial stress lower in L-THE group ($p=0.020$) sAA lower in the L-THE group ($p=0.032$)	No significant corrections between STAI and sAA for L-THE (all p's>0.05)
White et al. (2016)	L-THE (200mg in a 430ml in a nutrient drink) Placebo	34 (15;19)	26.5±5.0	Randomised, placebo-controlled, double-blind crossover design. Acute	Multitasking framework	STAI, Bond-Ladder and Mood scales.	One hour post-dose subjective stress ($p=0.003$) and self-rated stress ($p=0.006$) levels were lower in L-THE group, and , cortisol was post-dose ($p=0.047$)	Posterior resting alpha activity was higher with L-THE in high trait anxiety group ($p=0.019$, one-tailed)
Yoto et al. (2012)	L-THE (200mg) + Placebo Caffeine (100mg) + Placebo Placebo only	16 (8;8)	22.8±2.1	Randomised, placebo-controlled, cross over design. Acute	Target detection test Arithmetic mental task Cold pressor test	BP, POMS, VAS	Decrease in systolic ($p=0.008$) and diastolic BP ($p=0.006$) after mental task in the high response groups.	Reduced Tension-Anxiety scores in POMS compared with placebo ($p=0.004$)

642 **Table 2.** Summary of risk of bias assessment of included studies

	Higashiyama et al. (2011)	Kimura et al. (2006)	Lu et al. (2004)	Ritsner et al. (2011)	Rogers et al. (2008)	Sarris et al. (2018)	Unno et al. (2013)	Yoto et al. (2012)	White et al. (2016)
Random sequence generation (selection bias)	Unclear	Low	Low	Low	Unclear	Low	Low	Low	Low
Allocation concealment (selection bias)	Low	Low	Low	Low	Low	Low	Low	Unclear	Low
Blinding of participants and personnel (performance bias)	Low	Low	Low	Low	Low	Low	High[†]	Unclear	Low
Blinding of outcome assessment (detection bias) (patient-reported outcomes)	Unclear	Unclear	Unclear	Unclear	Low	Low	High[†]	Unclear	Unclear
Blinding of outcome assessment (detection bias) (Mortality)	Low	Low	Low	Low	Low	Low	Low	Low	Low
Incomplete outcome data addressed (attrition bias)	Low	Low	Low	Low	Low	Low	Low	Low	Low
Selective reporting (reporting bias)	Low	Low	Low	Low	Low	Low	Low	Low	Low

643 [†] This study was single-blinded