Food Chemistry 242 (2018) 601–610

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# L-Theanine: An astounding sui generis integrant in tea

Eshita Sharma[a,b], Robin Joshi[a], Ashu Gulati[a,*]

[a] CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India
[b] Department of Molecular Biology and Biochemistry, Guru Nanak Dev University, Amritsar, Punjab, India

ARTICLE INFO

*Keywords:*
L-Th
Umami taste
Production approaches
Quantitation methods
Health benefits

ABSTRACT

L-theanine (L-Th), a non-protein amino acid present in tea, is a valuable nutraceutical product with unique health benefits and used as an additive in food industry. L-Th enhances the umami taste but its use is limited due to its inadequate production. Different extraction approaches from tea shoots, chemical synthesis to microbial transformation have been tried to meet its demand. *In vitro*, *in vivo* as well as clinical studies have shown its positive effect in regulating CNS disorders. L-Th has become choice ingredient in CNS active products due to its anti-stress and neuroprotective role in dementias particularly in retrogression of Alzheimer's. L-Th biochemically modulates various anti-neoplastic agents by increasing their bioavailability in tumour cells. The review, is an effort to condense the recent research on L-Th highlighting its biological resource, plausible role in tea plant, production approaches, its physiological role on human health and future prospects.

## 1. Introduction

Changes in lifestyle, dietary habits and increased life expectancy has led to increased prevalence of lifestyle diseases such as obesity, diabetes, hypertension and hyper-cholestemia. The growth of global nutraceuticals market in last two decades has documented an increase in the Annual Average Growth Rate from 7.3% in 1990–2002 to 14.7% in 2002–2010 (Frost, 2011) and is expected to reach US $250 billion by 2018 (Stirling & Kruh, 2015). More than 500 research articles on L-Th and nearly 300 review articles on tea have reported beneficial effects of L-Th on CNS and emphasized on its commercial production (Lardner, 2014; Williams et al., 2016). Substantial information on L-Th is available from different sources in public domain, but comprehensive information does not exist at one place. This review, therefore, focuses to provide comprehensive information on the source of L-Th, its role in the tea plant, production and detection approaches, biochemistry and pharmacological properties along with future research perspectives.

## 2. Occurrence, absorption and metabolism of l-theanine

L-Th, not only enhances the flavour and quality of infused tea, but also has important physiological role in tea plant. L-Th is chemically 2-amino-4-(ethylcarbamoyl)-butyric acid, a chiral compound that exists in L-(S) enantiomeric form in nature. L-Th is synthesized from glutamic acid and ethylamine by enzyme theanine synthetase in roots of the tea plants, which is then translocated to apical bud and subtending three leaves (Fig. 1). L-Th accumulates in the developing shoots which are also the principal site for polyphenol synthesis (Walter, Puangrat, & Philip, 1986). On exposure to sunlight, L-Th gets hydrolysed to its precursors and liberates glutamine and ethylamine which serve as precursor for catechin synthesis. In tea plant, the role of L-Th is to detoxify ammonia absorbed by roots and convert it to other nitrogenous compounds. L-Th serves as a reservoir for nitrogen and an initiator for skeletal carbon compounds during germination (Deng, Ogita, & Ashihara, 2010). Accumulation followed by catabolism of L-Th and other nitrogenous compounds during slow division of meristematic tissues in young tea plant indicate its role in developmental physiology

*Abbreviations:* AIDA, alanine decarboxylase; ALT, alanine transaminase; 5′-AMP, adenosine monophosphate; L-Th, L-theanine; ADHD, attention deficit hyperactivity disorder; ADA, adriamycin; AD, Alzheimer disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ATP, adenosine triphosphate; cAMP, cyclic-adenosine monophosphate level; CNS, central nervous system; CRF, corticotrophin releasing factor; DNA, deoxyribonucleic acid; DOX, doxorubicin; EGCG, epigallocatechin gallate; GOGAT, glutamine oxoglutarate aminotransferase; GDH, glutamate dehydrogenase; GGT, γ-glutamyl transpeptidase; GS, glutamine synthetase; GMAS, γ-glutamylmethylamide synthetase; GABA, γ-aminobutyric acid; 5′-IMP, inosine monophosphate; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; HPLC, high performance liquid chromatography; HPTLC, high performance thin layer chromatography; MAE, microwave-assisted extraction; NE, norepinephrin; NMDA, *N*-Methyl-D-aspartic acid or *N*-Methyl-D-aspartate; OPA, o-phthalialdehyde; OFC, orbito-frontal cortex; PDA, photodiode array detectors; PITC, phenylisothiocyanate; P-gp, P-glycoproteins; SWE, sub-critical water extraction; SUMO, small-ubiquitin related modifier; SFE, supercritical fluid extraction; SHR, spontaneously hypertensive rats; TS, theanine synthetase; ThYD, theanine hydrolase; TLC, thin layer chromatography; UAE, ultrasound-assisted extraction; UHPE, ultrahigh pressure extraction; WKR, Wistar Kyoto rats

* Corresponding author.
*E-mail address:* ashugulati@ihbt.res.in (A. Gulati).

http://dx.doi.org/10.1016/j.foodchem.2017.09.046
Received 27 December 2016; Received in revised form 6 September 2017; Accepted 11 September 2017
Available online 12 September 2017
0308-8146/ 

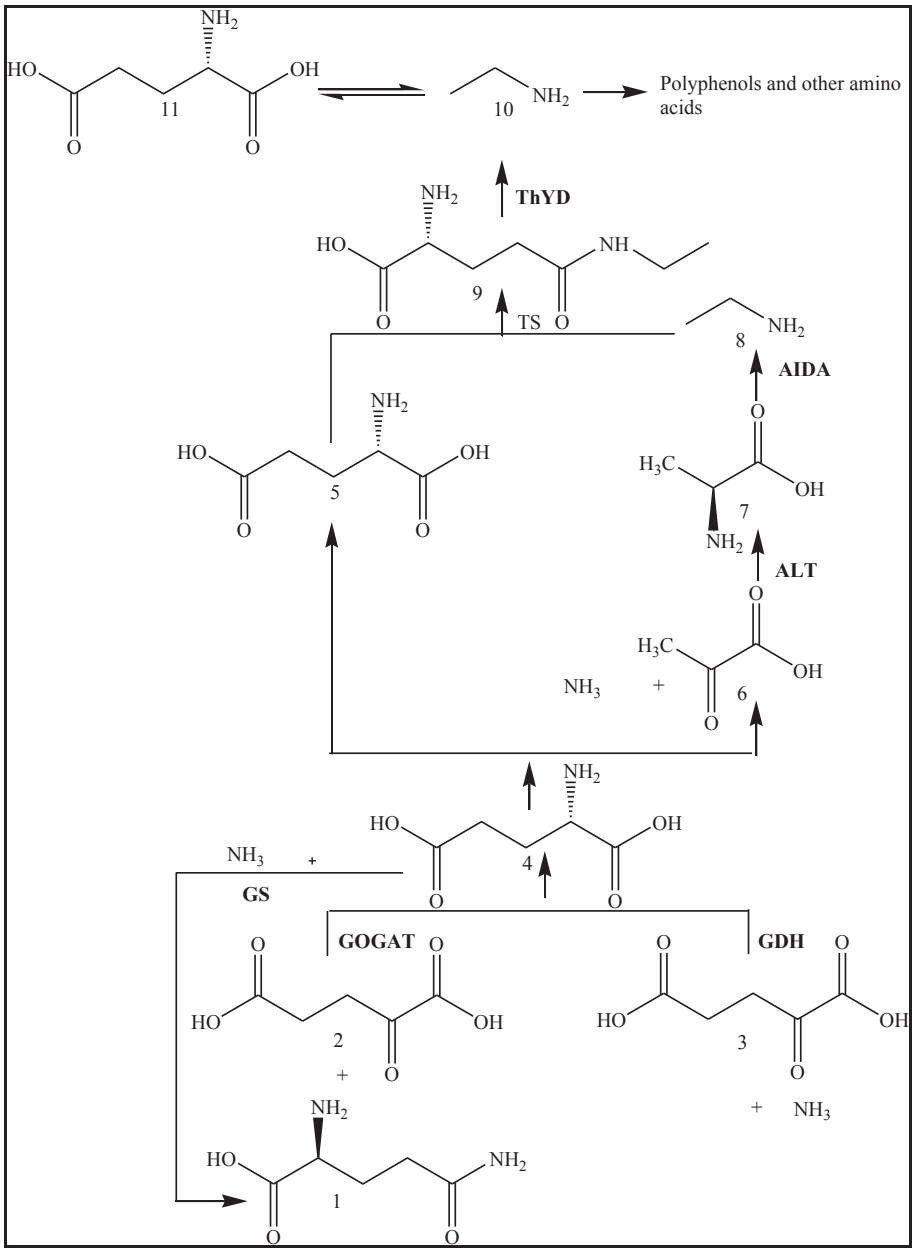


**Fig. 1.** Bio-synthetic pathway of L-theanine in *Camellia sinensis*. 1. Glutamine, 2,3. Oxoglutarate, 4,5. Glutamate, 6. Pyruvate, 7. L-alanine, 8. Ethylamine, 9. Theanine, 10. Ethylamine, 11. Glutamate. GS: Gluamine synthetase; GOGAT: Glutamine oxoglutarate aminotransferase; GDH: Glutamate dehydrogenase; ALT: Alanine transaminase; AIDA: Alanine decarboxylase; TS: Theanine synthetase; ThYD: Theanine hydrolase. The figure is based on the information provided in Shi et al., 2011 with permission.

of the plant (Walter et al., 1986). The content of L-Th varies with the type of tea cultivar, manufacture process i.e., green and black teas and infusion preparation method. A standard 200 mL cup of tea (500 mg of green and black tea) brewed at 80 °C for 2 min contains 7.9 and 24.2 mg of L-Th in green and black tea, respectively. Type of manufacture and packaging influences L-Th content in a cup in the order: tea bags (24.6 mg) > black tea (24.4 mg) > white tea (11.5 mg) > speciality black tea (10.9 mg) > green tea (7.9 mg). Addition of semi-skimmed milk during tea infusion preparation significantly lowers L-Th content in a tea cup compared to cup infused with skimmed or full fat milk. Addition of sugar during tea infusion preparation, however, did not significantly affect L-Th content. Higher content of L-Th was found in the cup prepared with vigorous agitation (Keenan, Mike, Jones, Rogers, & Priestley, 2011). Once tea is consumed, L-Th gets rapidly absorbed through microvilli via intestinal epithelial cells from where, it is transported to the brain tissues as it crosses the blood brain barrier. In brain tissues, L-Th concentration reaches to maximum in 5 h and effects metabolism and secretion of various neurotransmitters within 30 min. L-Th catabolises to ethylamine and glutamic acid by amide hydrolysis in kidneys through phosphate independent glutaminase pathway excreting ethylamine into urine and glutamic acid was converted into glutamyl peptides via γ-glutamyl transferase reaction *in vivo* (Tsuge, Sano, Hayakawa, Kakuda, & Unno, 2003).

## 3. Biochemistry behind the "umami" taste

"Umami" means delicious, savoury, broth-like or meaty flavour and has been accepted as the fifth taste in addition to sweet, salt, sour and bitter. L-Th along with 5′-IMP produced from 5′-AMP during tea processing impart umami flavour to teas. In oral cavity, various G-protein-coupled receptors (GPCRs) like truncated type 1 and 4 metabotropic glutamate receptors contain heterodimer T1R1 and T1R3 subreceptors which respond specifically to umami stimuli (Pin, Galvez, & Prezeau, 2003). Interaction of heterodimer taste receptors T1R1 and T1R3 with G receptors modulates cAMP and stimulates phosphokinase C which causes channel modulation in CNS and membrane depolarization. The decrease in cAMP results in closure of cyclic nucleotide-gated channels releasing neurotransmitters like serotonin and norepinephrin via gap

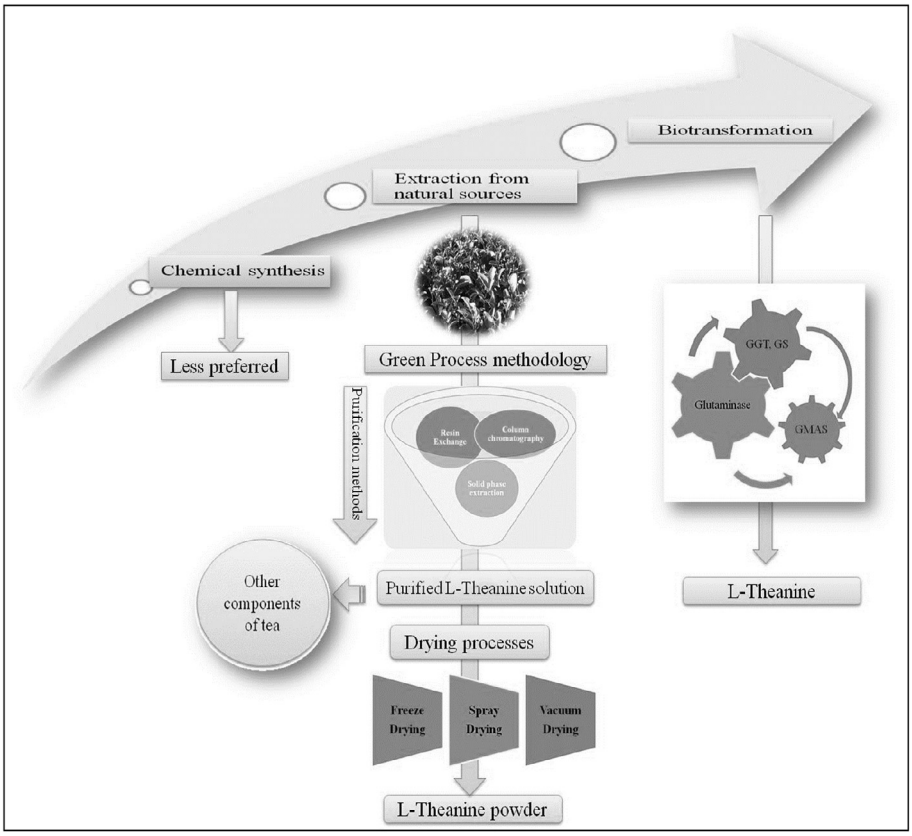


**Fig. 2.** Summarisation of different approaches for L-theanine production i.e., chemical synthesis, isolation from tea and microbial biotransformation.

junction hemi-channels which transmit gustatory signals from CNS. In brain, primary and secondary taste cortex regions constitute anterior insula, adjoining frontal operculum and OFC respectively, and are involved in perception of umami taste. For neuronal taste representation and oral stimuli, neurons in primary region respond to glutamate taste specifically and activate OFCs. The intensity of combined stimuli produced by OFC activation and pregenual cingulated cortex neurons with glutamate gives a pleasant umami savoury taste (Narukawa, Toda, Nakagita, Hayashi, & Misaka, 2014).

## 4. Methods for l-theanine production

Different approaches were tried by researchers in the past for L-Th extraction and production (Fig. 2). However, most of the methods were promising at lab scale only.

### 4.1. Extraction of l-theanine from tea

L-Th constitutes about 7–21 mg/g of dry weight in tea leaves and its extraction from tea leaves in considerable amount is a challenging task. A green process involving hot water (70–100 °C) extraction of L-Th from green tea leaves contained other water soluble constituents of tea viz. caffeine and phenolics, which were difficult to separate. Though with the use of resin, nano- and ultra-filtration, yield up to 88% could be achieved. The process was tedious, time consuming and uneconomical for commercial production (Ekamayake & Li, 2007). Preparative HPLC for isolation of L-Th from green tea extract was tried by Zhang, Chen, Huang, and Shi (2004). Low yield and recovery of L-Th made these protocols commercially non-viable. Baudouin (2010) used cation exchange resin Diaion UBK 550 to yield pure L-Th from aqueous black tea extract. Lower yield and purity of 44% of L-Th with longer purification procedure were the main disadvantages of this method. L-Th extraction using other novel techniques including MAE, UAE, SFE, UHPE and SWE were optimized for solvent volume and flow properties, pre-leaching time, strict temperature, pressure regimes etc. Although, these novel techniques swamped the demerits of natural extraction methods by being fast with lesser purification steps, high yield and purity with appreciable recovery rates and limited use of solvents. However, overall maintenance, higher cost and negative impact of extraction solvents on human health made these techniques unacceptable for commercial production. An efficient, reliable, faster, cost effective, high yielding with good product purification method is still a far cry.

### 4.2. Chemical synthesis

Chemical syntheses provide simple and cost effective approach for large scale theanine production (Gu, Jiang, & Wang, 2004; Lichtenstein, 1942). Major drawback of these methods is production of racemic mixture of D-and L-Th which are very difficult to separate. Moreover, synthetic products are less preferred because of the use of chemicals with reported health hazards. Resistance of consumers to “non-natural products” made these synthetic methods less acceptable for scaled-up L-Th production (Wan, Zhang, & Li, 2009).

### 4.3. Biotransformation using glutaminase, GGT,GS and GMAS

Microbial bio-transformation has many advantages like stereo-selectiveness, high specificity, short reaction time, higher yield and recyclability over other production techniques. Researchers are today focused on increasing production by using immobilization of enzymes that can make the process more cost effective. A detailed information on biotransformation studies undertaken by different researchers for theanine production are given in Table 1. In 1998, Taiyo Kagaku Limited, Japan won 'Food Research Ingredient Award' for production of L-Th on industrial scale employing microbial enzymes (Juneja, Chu, Okubo, Nagato, & Yokogoshi, 1999). The enzymes which have been found to have potential as biocatalysts for theanine production are glutaminase (Nandakumar, Yoshimune, Wakayama, & Moriguchi,

**Table 1**
Various enzymes involved in biotransformation of L-theanine from different sources.

| Enzyme | Source | γ-Glutamyl donor | Donor conc (mM) | Acceptor | Acceptor (mM) | Reaction Conditions | Conversion (%) | References |
|---|---|---|---|---|---|---|---|---|
| *GGT* | E. coli | GAME | 100 | Ethylamine | 1000 | pH 10,45 °C, 8 h | 95 | Zhang et al. (2010) |
| | Bacillus subtilis GGT.SK11.004 | L-Glutamine | 20 | Ethylamine | 50 | pH 10, 37 °C, 4 h | 94 | Shuai, Zhang, Jiang, and Mu (2010) |
| | Recombinant *E. coli* GGT | L-Glutamine | 267 | Ethylamine | 2000 | pH 10.5,37 °C, 24 h | 80 | Wang et al. (2011) |
| | Bacillus subtilis GGT | GZC | 48 | Ethylamine | 1500 | pH 9, 37 °C, 3 h | 63.8 | Wang et al. (2012) |
| | Recombinant *E. coli* GGT | γ-GpNA | 5 | Ethylamine | 50 | pH 9, 37 °C, 6 h | 93 | Zhang et al. (2013) |
| | Recombinant *B. licheniformis* GGT | L-Glutamine | 80 | Ethylamine | 600 | pH 9, 37 °C, 4 h | > 84 | Bindal and Gupta (2014) |
| *Glutaminase* | Pseudomonas nitroreducens IFO 12694 | L- Glutamine | 0.7 | Ethylamine or Methylamine | 1.5 | pH 9,30 °C, 7 h | – | Tachiki et al. (1998) |
| | Recombinant B. licheniformis | L- Glutamine | 50 | Tris-HCl | 100 | pH 7.5, 30 °C, 6 h | – | Sinsuwan, Yongsawatdigul, Chumseng, and Yamabhai (2012) |
| | Pseudomonas nitroreducens | L- Glutamine | 300 | Ethylamine | 1500 | pH 10, 37 °C, 5 h | – | Pu et al. (2013) |
| *GS* | Pseudomonas taetrolens Y-30 | Sodium glutamate | 200 | Ethylamine | 1200 | pH 7, 30 °C, 48 h | – | Yamamoto et al. (2006) |
| *GMAS* | Methylovorus mays No.9 | Sodium glutamate | 200 | Ethylamine | 300 | pH 7, 30 °C, 10 min | – | Yamamoto et al. (2008) |

GGT: γ-Glutamyl transpeptidase; GS: Glutamine synthetase; GMAS: γ-glutamylmethylamide synthetase; GAME: L-glutamic acid γ-methylester; GZC: Glutamate zinc complex; HCl: Hydrochloride; γ-GpNA: L-γ-Glutamyl-p-nitroanilide; *E. coli*: Escherichia *coli*.

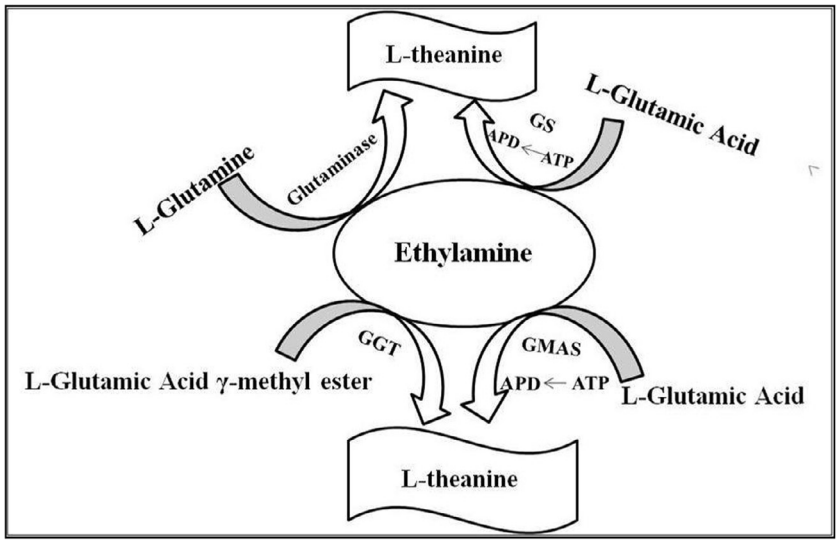


**Fig. 3.** Outline of biotransformation for L-theanine production using different microbial enzymes.

2003), GGT (Zhang, Zheng, Jiao, Liu, & Zhao, 2010), GS (Yamamoto, Wakayama, & Tachiki, 2006) and GMAS (Yamamoto, Wakayama, & Tachiki, 2008) (Fig. 3). The enzyme glutaminase is present in prokaryotes and eukaryotes with applications in food and pharmaceutical industries (Nandakumar et al., 2003). The primary function of glutaminase is to catalyse the glutamine to glutamate which is directly linked with asparagine for redox balance in cancer cells especially in acute lymphocytic leukaemia. Transferring reaction of *Pseudomonas nitroreducens* (*P. nitroreducens)* was catalysed using methylamine and ethylamine as acceptor molecules forming L-Th, stability and production of enzyme was enhanced by immobilizing *P. nitroreducens* IFO 12694 with Carrageenan as support. Glutaminase at pH 7.0–8.0 with ethylamine (1.5 M) and glutamine (0.7 M) as substrates gave 47 g/L of L-Th (Tachiki et al., 1998). GGT is another key enzyme, catalyses glutathione metabolism and transfer of γ-glutamyl group of donor to acceptor amino acids and peptides. In plants, there is no evidence of existence of glutamyl cycle, however, GGT acts as a nitrogen source in *E. coli* and as nitrogenous nutrition during limiting nutrient conditions in *Bacillus*. Broad substrate specificity for various γ-glutamyl acceptors and non requirement of ATP for its transferase activity are advantageous for utilizing bacterial GGT in various biotechnological applications (Verma, Gupta, & Goel, 2015). L-Th has been produced using GGT from microorganisms including *E. coli* Novablue (Hung, Lo, Hsu, Chen, & Lin, 2008), *B. subtilis* SK 11.004 (Shuai, Zhang, Mu, & Jiang, 2011) and *B. licheniformis* (Bindal & Gupta, 2014). Enhanced L-Th production was achieved from conversion ratio of 69.5 to 95% using modified glutamyl donor, L-glutamic acid γ-methylester (GAME, 300 mM) and ethylamine (3 M) with immobilized GGT (0.1 g/

**Table 2**
Random clinical studies supporting health benefits of L-theanine.

| Participants | Age | Study design | Formulation | Key conclusion | Study |
|---|---|---|---|---|---|
| *Anti-anxiety and relaxing effects* | | | | | |
| 50 Females into 2 groups, high and low anxiety | 18–22 y | MAS | 50 or 200 mg of L-Th, measurement of brain waves after 30 min of administration | L-Th promotes generation of α waves in humans and produce relaxation in brain | Juneja et al. (1999) |
| 16 Healthy participants, 12 males, 4 females | Males-24.8 ± 5.4 y Females-29.0 ± 1.4 y | Double blind, placebo-controlled STAI design, repeated measures Latin square design | Placebo, L-Th (200 mg), alprazolam (1 mg) | L-Th produced relaxing effect on behavioural measures of anxiety on healthy volunteers, alprazolam didn't produce any anxiolytic effect | Lu et al. (2004) |
| 55 Male sprague rats | – | 5 Randomly selected groups with 11 rats in each group | Saline (Control), L-Th (10 mg/kg), midazolam (1.5 mg/kg) flumazenil (3 mg/kg) + L-Th (10 mg/kg) midazolam (1.5 mg/kg) + L-Th (10 mg/kg) | L-Th alone or in combination with flumazenil did not produce any antianxiety effect but in combination with midazolam produce antianxiety effect | Heese et al. (2009) |
| 60 Patients, DSM- IV schizophre nia, 12 women and 18 men, 5% married, 76.7% single, 18.3% divorced | 36.5 y | 8-Week, double blind, randomize d, placebo controlled study, psychology scores measured by PANSS 3-dimensional mode l, anxiety by HARS scale, CANTAB for neurological function. | 400 mg L-Th + psychotic drug | L-Th amerolation effect was observed from second to sixth week, L-Th of negative and depressive symptoms, general functioning, extra pyramidal effects during study | Ritsner et al. (2011) |
| *Physiological effects* | | | | | |
| Male WHR and SHR | – | WHR and SHR | 0, 500, 1000, 1500, 2000 mg/kg of L-Th intraperitoneally to SHR and WHR | Antihypertensive effect was observed in SHR not in WHR and rapidly after L-Th administration. Glutamine administration didn't change blood pressure and heart rate, significant decrease in brain 5-HIAA level was observed in both SHR and WHR | Yokogoshi et al. (1995) |
| 12 Male undergraduate students, | 20–25 y | Double blind, placebo controlled STAI design, repeated measures monitoring effect, physiological indices of stress (heart beat and IgA conc.) under high and low stress conditions. | 200 mg L-Th + placebo | Physiological indices of stress under high-stress conditions compared with placebo, statically no significant levels reported, reduction in self-reported anxiety. | Kimura et al. (2007) |
| Over 100 boys, diagnosed with ADHD | 8–12 y | A randomized, double-blind, placebo-controlled trial, wrist actinography, paediatric sleep treatment | 100 mg of L-Th or placebo for 6 weeks | 400 mg of L-Th is safe and capable of improving some aspects of sleep quality in tests suffering from ADHD | Lyon et al. (2011) |
| 16 Healthy volunteers, Students, 8 men and 8 women. | 22.8 ± 2.1 y | Cross over, randomized, placebo controlled design, 3-way cross over for stress load task. | L-Th (200 mg + placebo, caffeine (100 mg + placebo), only placebo | L-Th and caffeine inhibit blood pressure elevation induced by physiological stress. | Yoto et al. (2012) |
| Male ddY mice, n = 24 divided into 4 groups, 20 min task | 4 weeks | Physiological indices of stress (heart beat and IgA conc.) under high and low stress conditions. Confrontational housing, weight of adrenal gland, measurement of corticosterone and ACTH under stress conditions. | 5–100 μg/mL of L-Th, 200 mg L-Th + placebo | L-Th relieves psychosocial stress through modulation of hypothalamic–pituitaryadrenal axis activity. | Unno et al. (2013) |
| *Neurotransmitters and Neuroprotection* | | | | | |
| Young wistar rats (approx 100 g) with 6 rats in each group, temperature 24 °C. | – | 3 Dose dependent experiments | Saline or L-Th at 100, 200, 400, 800 mg/100 g of body weight after 2 h of administration | L-Th reduced serotonin synthesis and increased serotonin degradation in brain | Yokogashi et al. (1998a) |
| Young Wistar rats (approx 100 g) with 6 rats in each group, temperature 24 °C. | – | 3 Different dose dependent experiments | Saline or L-Th at 100, 200, 400, 800 mg/100 g of body weight after 2 h of administration. | L-Th might affect metabolism or release of monoamine and dopamin e neurotransmitters | Yokogashi et al. (1998c) |
| Male Wistar rats, 8 rats in each group | – | Effect of L-Th and glutamate blocker L-trans-2,4 PDC on neurotransmitters and other | Microinjection of L-Th or L-trans-2,4 PDC | L-trans-2,4-PDC caused excitatory neurotransmi ssion and L-theanine caused inhibitory neurotransmiss ion via | Yamada et al. (2005) |

*(continued on next page)*

**Table 2** *(continued)*

| Participants | Age | Study design | Formulation | Key conclusion | Study |
|---|---|---|---|---|---|
| | | amino acids was investigated | | glycine receptors | |
| Crude synaptosomal from whole brain of male wistar rats | 8–10 weeks | Neuronal culture, determination of [$^3$H] Glutamine transport in cultured cells, L-theanine [$^3$H] binding capacity | – | L-Th inhibits the glutamate transport in neurons and astroglia in rat brain | Kakuda et al. (2008) |
| 8 Young Male Wistar rats | – | Neurotransmitter concentration was measured by using brain micro dialysis | L-Th injected (0.2 μmol/2 μL), each antagonist perfusate (2 μL/perfusate). | Theanine effects the concentration of neuro transmitters in the brain striatum interstitium in conscious rats | Yamada et al. (2009) |
| Human neuroblastoma cells | – | Gel mobility shift assay, lipid per oxidation, carbonyl protein and total glutathione estimation. | – | L-Th prevents oxidative damage to neuronal cells and may be useful in prevention and treatment of Alzheimer disease | Jo et al. (2011) |
| Male Wistar rats | – | Two designed experiments, experiment one infarct size determination and neurological tests, experiment 2 intrastraitial injection of L-Th. | L-Th 1 and 4 mg/kg, intraperitoneal ly at 3,12 and 24 h | L-Th exerts neuroprotective action in *in vivo* rat model | Zukhurova et al. (2013) |
| Male Wistar rats, 6 animals in 6 each group, | – | Morris water maze test, rota rod activity, open field test, forced swim test, biochemical and histopathological studies. | L-Th (100 mg/g body weight) orally before 3-nitropropionoic acid treatment | L-Th has neuroprotective action against 3-NP neurotoxicity | Thangarajan, Deivasigamani, Natarajan, Krishnan, and Mohanan (2014) |
| 14 Healthy adults, 7 men and 7 women | 31.0 ± 7.0 y | A randomized, double blind, placebo controlled trial, PPI test | 0, 200, 400 and 600 mg of L-Th for 4 adults, 0 and 50 mg L-Th for 4 adults | L-Th effects sensorimotor gating in healthy humans | Ota et al. (2014) |
| *Anti-cancer activities* | | | | | |
| Tumour bearing mice, Ehrlich carcinoma cells, 7–8 mice per group | – | Effect of L-Th with adriamycin on tumour cells. | L-Th 11, 13, 15 and 17 days after tumour inoculation | L-Th acts as bio modulator prevents efflux of adriamycin from tumour cells without side effects of adriamycin, normalization of lipid peroxide levels | Sadzuka et al. (1996) |
| P388 leukaemia cells transplan ted onto backs of BDF1 mice | – | Effect of L-Th and idriamycin on tumour activity | Idriamycin (0.25 mg/kg/day for 4 days) on 7, 9, 11 & 13 day, L-theanine (10 mg/kg/day for 4 days) on 8, 10, 12 and 14 day. | L-Th in combination with idriamycin have anti-tumour activity | Sadzuka, Sugiyama, Suzuki, and Sonobe (2000) |
| Male C57BL/6 and BDF1 mice, M5076 ovarian sarcoma cells | 5 weeks | Effect of L-Th in glutamate and glutathione levels to clarify mechanism of interaction of L-Th with DOX | DOX (2.0 mg/kg/day for 4 days), L-Th (10 mg/kg/day for 4 days) | L-Th interferes with adverse reaction caused by DOX during oxidative stress, maintains levels of glutathione and glutamate in normal cells | Sugiyama and Sadzuka (2004) |
| White rabbits, A549 lung adreno-carcinoma cell lines and K562 erythroblastoid leukaemia cell lines. | – | Investigation of role of L-Th in lung cancer and leukaemia cells | L-Th orally 100 mg/mL/kg for 3 days | L-Th caused suppression *in vivo* and *ex vivo* growth of lung cancer A549 and K562 cell lines on dose and time dependent manner | Liu et al. (2009) |
| Male BDF1 mice | – | Study of effect of DOX (antineo plastic and side effect cardiac damage) and L-Th on acute cardiac injury | DOX (10–20 mg/kg/ day for 4 days), L-Th (10 mg/kg/day for 4 days), combination of L-Th and DOX (2.5–10 mg/kg/day for 4 days). | L-Th protects against cardiac injury caused by DOX by enhancing the activity of cardiac markers creatine kinase and lactate dehydrogenase | Nagai et al. (2013) |
| SD rats, cell lines human lung cancer A549 and NCI-H460, human leukaemia K562, LLC, MRC-5, peripheral blood lymphocytes. | 6 weeks | Synthetically produced theanine derivatives, *In vivo* and *ex vivo* growth assays, flow cytometry, migration assay, EMSA, subcutaneous tumour model | TFC or TNC orally 80 mg/ml/kg for 16 h | Theanine derivatives significantly inhibit lung cancer cell migration and growth of lung cancer and leukaemia cell lines and suppression of LLC and A549 tumour growth without toxicity | Zhang et al. (2014) |

L-Th: L-theanine; MAS: Manifest anxiety scale; STAI: The state-trait anxiety inventory; DSM: Diagnostic and statistical manual of mental disorders; PANSS: Positive and negative syndrome scale; HARS: Hamilton anxiety rating scale; CANTAB: Cambridge neuropsychological test automated battery; WHR: Wistar hypertensive rats; SHR: Male spontaneously hypertensive rats; 5-HIAA: 5-Hydroxyindole acetic acid; IgA: Salivary immunoglobulin A; ADHD: Attention deficit hyperactivity disorder; ddY: Deutschland, Denken, and Yoken; ACTH: Adrenocorticotro pic hormone; L-trans-2,4 PDC: L-trans-pyrrolidine-2,4-dicarboxylic acids; PPI: Prepulse inhibition; LLC: Lewis lung cancer; MRC-5: Human embryonic lung fibroblast; SD rats: *Sprague Dawley rats;* DOX: Doxorubicin; EMSA: Electrophoretic mobility shift assay; TFC: Ethyl 6-fluorocoumarin-3-carboxylyl L-theanine, TNC: Ethyl 6-nitrocoumarin-3-carboxylyl L-theanine.

ml) by optimizing molar ratio (1:10) of substrates (Zhang et al., 2010). Further, increased L-Th production could be achieved using recombinant GGT following fusion techniques including glutathione S-transferase (GST) and small-ubiquitin related modifier (SUMO). L-Th (41 g/L) was produced from 0.267 mM glutamine and 2 M ethylamine after 24 h reaction by SUMO fusion system (Wang et al., 2011). Advantages of using GGT for producing L-Th are better L-Th conversion rate, shorter reaction time, non requirement of ATP as compared to other enzymatic methods. However, higher concentration of ethylamine is required for L-Th conversion using GGT (Zhang et al., 2010) which may sometimes inhibit GGT activity. The autotranspeptization of substrates (glutamic acid and ethylamine) was restrained by formation of L-glutamine Zn (II) complex in which Zn blocked free α-amino group and carboxyl group, to substitute L-glutamine as substrate, thereby increasing L-Th yield by 16.9% (Wang et al., 2012). GS regulates nitrogen metabolism in plants by fixing and catalysing ATP-dependent fixation of ammonium ions with δ-carboxyl group of glutamate to form glutamine. GS from *P. taetrolens* Y-30 was purified, characterised and optimized which showed 7% higher ligation activity towards substrate ethylamine as compared to ammonia. Further, recombinant *P. taetrolens* Y-30 showed 30-fold higher enzyme activity compared to original bacterial *P. taetrolens* Y-30 (Yamamoto et al., 2006). However, requirement of continuous supply of ATP and difficulty in controlling pH of the reaction mixture were the main drawbacks of this process. Low reactivity of *P. taetrolens* GS for ethylamine was overcome by GMAS which catalyzes γ-glutamylmethylamide biosynthesis from L-glutamate and methylamine with the hydrolysis of ATP and showed much higher ligation activity of γ-glutamyl group towards ethylamine. *Methylovorus mays* No. 9 showed 100-fold activity for ethylamine, producing approximately 600 mM of L-Th with 100% yield based on glucose consumption (Yamamoto et al., 2008). In spite of huge potential in biotransformation protocols for L-Th, a well characterized efficient enzyme for biotransformation is still an industrial requirement. Structure activity relationship using efficient substrate molecules can also be applied for improving enzyme activity with site directed mutagenesis for better outcomes.

## 5. Analytical methods for l-theanine estimation

Initially, L-Th was detected by simple paper chromatography (Cartwright, Roberts, & Wood, 1954) which was followed by TLC (Walter et al., 1986). Later with development of rapid separation methods HPTLC and HPLC are now mostly employed for detection and quantification. HPTLC method has been used for rapid quantitative determination of L-Th using 1-butanol: acetone: acetic acid: water as mobile phase (Kumar, Gulati, & Sharma, 2015). HPLC quantification of L-Th along with other amino acids with pre or post column derivatization reagents OPA, PITC and fluorescence or PDA detectors was employed (Baptista, Lima, Paiva, Andrade, & Alves, 2012). Low stability of amino acid derivatives, special column clean-up procedures, pre sample preparation were the main short comings of these methods. To overcome these demerits, methods without pre-column derivatization have also been developed (Rana, Singh, & Gulati, 2015). HPLC methods are quick, simple, reliable, however, detection of L-Th by PDA and fluorescence have disadvantage of low detection limit with uncharacterized UV spectrum of all amino acids. Other analytical techniques employed for L-Th quantitation were capillary electrophoresis and micellar electro-kinetic chromatography (Chen et al., 2003). Capillary electrophoresis is faster, sensitive, cost effective, uses smaller amount of sample and solvents, generates low levels of residues and does not require any specialized cleanup protocols compared to HPLC, but low reliability, due to lesser precision and sample stacking make determination in unknown samples difficult (Bizzotto, Meinhart, Ballus, Ghiselli, & Godoy, 2013).

## 6. Health benefits of l-theanine

Psychological stress is the main cause of depression, mood swings, immune and age-related disorders, cardiovascular diseases and different types of cancer (Rogers, Smith, Heatherley, & Pleydell-Pearce, 2008). Increased health awareness and stressed life-style has made natural health supplements/additives as preferred choice of people over synthetic products. L-Th was certified as Generally Recognized as Safe (GRAS) by United States Food and Drug Administration (US FDA) and used in food industry as a flavour modifier. Suntheanine™ by Taiyo Kagaku Ltd, Japan is a successful L-Th nutraceutical product in capsules (Juneja et al., 1999). Another product, miniCHILL® with functional component Relarian™- an aqueous mixture of theanine, valerian root extract, 5-HT and GABA, is reported to increase mental focus while providing relaxation during task performance. Proloftin™ contains L-Th along with other amino acids (Benjamin, 2009). A detailed information on health benefits of L-Th is given in Table 2.

### 6.1. *As neuroprotective and its interaction with neurotransmitters*

Epidemiological studies have shown that consumers of tea are significantly less prone to stroke incidences due to neuroprotective effects of L-Th. *In vitro* administration of L-Th in lateral ventricle with induced ischemia in hippocampal CA1 area of gerbils significantly decreased ischemic neuronal death in a dose dependent manner (Kakuda et al., 2000). Neuroprotective effects of L-Th in modulation of glutamic acid in brain might be through antagonistic role of glutamate receptors (NMDA, AMPA and kainate) on cortical neuron (Kakuda, Nozawa, Sugimoto, & Nino, 2002). Jo et al. (2011) reported neuroprotective effects of L-Th on amyloid beta ($A\beta_{1-42}$)-induced neurotoxicity in AD via inhibition of reactive oxygen species (ROS) and signal generated in p38/ERK1/2, NF-κB pathways, helped in recovering glutathione levels in Aβ-induced toxic cells in a dose dependent manner. An *in vivo* study based on administration of L-Th at different time intervals (3, 12, 24 h) after perfusion in rat model reported reduction in the size of brain infarct at 3 and 12 h while no reduction in brain infarct was found at 24 h (Zukhurova et al., 2013). Physiological states of brain are modulated by release and inhibition of neurotransmitters, alter emotions by interacting with various neurotransmitters viz. 5-HT, GABA, dopamine, norepinephrin. L-Th administration to rats for 3 months caused degradation in synthesis of serotonin, 5-HIAA and increased tryptophan levels in cerebral cortex (Yokogashi, Mochizuki, & Saitoh, 1998a). Excess GABA concentration plays an important role in various neurological disorders viz. anxiety, depression and insomnia, while dysfunction of GABA-nergic receptors cause mood and depression disorders. The structural similarity of L-Th with glutamic acid enables its action on GABA receptors which regulates the concentration of certain neurotransmitters including dopamine, serotonin, glycine and GABA, promote nerve growth factors and modulate functions of CNS. L-Th effects the brain development in infant rats via increasing levels of GABA which is an inhibitory neurotransmitter for mature brain function (Yamada et al., 2007). L-Th inhibits glutamate uptake by blocking glutamate receptors in hippocampus, hypothalamus and striatum in brain (Kakuda et al., 2002), mediating the increase in dopamine and GABA concentration while decreasing norepinephrin levels (Yokogoshi, Kobayashi, Mochizuki, & Terashima, 1998). In an *in vivo* study, comparison of the effect of a glutamate transport blocker, L-*trans*-pyrrolidine-2,4-dicarboxylic acids and L-Th on glutamate transport for the release of dopamine and other amino acids (aspartic acid, glutamic acid, glycine) showed an increase in glutamic acid and fall in aspartic acid concentration by both perfusions. An increased dopamine concentration via glycine receptors was speculated for L-Th (Yamada, Terashima, Okubo, Juneja, & Yokogoshi, 2005). Co-injection of glycine receptor antagonist, strychnine reduced theanine-induced these changes in dopamine (Yamada et al., 2009). L-Th when given to healthy humans in a dose dependent manner (0, 200, 400, 600 mg) decreased

prepulse inhibition (PPI) at 200–400 mg of L-Th, however, significant increase in PPI was found at 600 mg (Ota et al., 2014).

### 6.2. Anxiolytic and relaxing effects

In India, 69.8% of anxiolytic patients suffer from panic anxiety disorders with co-morbid primary depression (Trivedi & Gupta, 2010). Alterations in serotonin and GABA levels have been considered as molecular targets for various anxiety disorders. L-Th acts as anxiolytic agent by potentiating GABA receptors which produce relaxation (Juneja et al., 1999). Suitable doses of L-Th inducing anxiolytic effects need to be established for human. A double blind placebo controlled test using human volunteers was first conducted to compare the anxiolytic effects of L-Th with alprazolam, benzodiazepine and induced anxiety conditions. However, the results demonstrated no effects by alprazolam, benzodiazepines and L-Th to reduce anxiolytic states compared to placebo (Lu et al., 2004). A twofold study was conducted on Male Sprague-Dawley rats concluded that the synergic effects of L-Th in combination with midazolam helped in modulation of CNS by increasing anxiolysis and decreasing motor, basic and fine movements. L-Th and flumazenil did not modulate $GABA_A$ benzodiazepine receptor site as no significant difference was found between control, L-Th and flumazenil groups (Heese et al., 2009). Juneja et al. (1999) reported that L-Th is also responsible for generation of α-waves in brain which produce relaxation. An increase in alpha electric band was observed within 60 min in a double blind, placebo controlled trial on healthy male university students when given L-Th (200 mg) in a visual attention and rapid audio tasks in repeated measurement design (Higashiyama, Htay, Ozeki, Juneja, & Kapoor, 2011). Another randomized, double blind placebo controlled study revealed that L-Th (400 mg) induced quality sleep with less nocturnal motor activity along with less wake fullness after onset of sleep in ADHD children (Lyon, Kapoor, & Juneja, 2011).

### 6.3. Physiological and psychological effects

In an *in vivo* study on hypertensive SHR and WKR, significant decrease in systolic blood pressure was found in SHR at higher doses of L-Th (1500–2000 mg/kg). Further, concentration of 5-HIAA in brain recorded decrease in both SHR and WKR on treatment with L-Th in comparison with glutamine. However, the study was unable to explain why a higher concentration of L-Th was required for antihypertensive effect and restriction of such effects to SHRs (Yokogoshi et al., 1995). Similarly, L-Th was found to reduce blood pressure in SHR when compared with glutamic acid in an *in vivo* experiment (Yokogashi & Kobayashi, 1998b). Effect of L-Th on cognitive function, psychological and anti-stress activity in mice under chronic induced stress during confrontational housing showed L-Th (20 μg/ml, 5–6 mg/kg) suppressed cerebral atrophy, learning impairment, behavioural depression and oxidative damage of cerebral DNA in confronted mice. L-Th produced psychosocial effects by suppressing adverse adulterations of hypothalamic–pituitaryadrenal axis (HPA-axis) activity during chronic stress (Unno et al., 2013). A randomized, single dose study on healthy volunteers in an acute stress challenge concluded that L-Th (200 mg/100 ml) exerted anti-stress effects during the challenge (Kimura, Ozeki, Juneja, & Ohira, 2007). Another cross over, randomized, placebo controlled design with healthy volunteers when given either L-Th (200 mg/250 ml) + placebo, caffeine (200 mg/250 ml) + placebo orally for 7 days concluded that L-Th was more efficient than caffeine in inhibiting blood pressure elevation and reducing anxiety during mental task (Yoto, Mao, Sato, & Yokogoshi, 2012).

### 6.4. Cognitive performance and mood

Only a few studies have focused individual and combinational effects of caffeine and L-Th on mood and cognitive performance (Owen, Parnell, Bruin, & Rycroft, 2008; Rogers et al., 2008). A randomized, double blind, balanced cross over study on twenty-four volunteers concluded that L-Th (250 mg) and caffeine (150 mg) improved performance in simple reaction time, working numeric memory and sentence verification accuracy. A different pharmacological profile of tea beverages containing caffeine and L-Th is expected compared to coffee beverages containing caffeine alone (Haskell, Kenedy, Milne, Wesnes, & Scholey, 2008). Reports have shown that individually caffeine and L-Th have antagonistic effects with caffeine increasing blood pressure, alertness, nervousness and anxiety in susceptible individuals whereas L-Th has opposite effects. In a seven days trial, when participants were administered L-Th (100 mg) + caffeine (50 mg) showed synergistic effects of caffeine and L-Th on cognitive performance with improved accuracies on attention switching tasks and reduction in distraction (Owen et al., 2008). In another study conducted on tea and coffee consumers endorsed the statement 'alerts me more' and 'interferes with my sleep' for coffee while 'relaxes me more' for tea containing caffeine and L-Th (Rogers et al., 2008). Investigations on neurocognitive effects of L-Th, caffeine and EGCG individually or in combination showed that L-Th and caffeine when in combination produce higher attentional switching accuracy, lower uni- or multisensory attentional outcomes in volunteers compared to EGCG, which was reported to have higher calming effect (Camfield, Stough, Farrimond, & Scholey, 2014). More randomized, controlled and robust clinical trials are required to establish the mechanistic role of L-Th in cognitive performance activities, stress conditions, multisensory outcomes and mood elevations. Further studies on L-Th with tea catechins in combination or alone and without caffeine could be useful in understanding its role in enhancing cognitive performance.

### 6.5. Role in cancer therapy

Several ecological and laboratory studies suggest that eating healthy and plant based diet is beneficial for reducing cancer diseases. P-gp is one of the ATP binding cassette (ABC) transporters which are capable of effluxing various toxic material out of cell including chemotherapeutic drugs thereby, causing multi drug resistance. It was, therefore, thought to include a chemical modulator of P-gp which could be co-administered with anticancer drug to decrease its efflux from the cell (Xue & Liang, 2012). L-Th acts by competitively binding on to glutamate and NMDA receptors, reducing glutamate uptake by tumour cells, thereby, lowering efflux of drug from tumour cells. L-Th enhanced bioavailability of anti-cancer drugs (ADA, DOX) in tumour cells in *in vivo* (Nagai & Konishi, 2013; Sadzuka, Sugiyama, Miyagishima, Nozawa, & Hirota, 1996; Zhang et al., 2014), *ex vivo* and *in vitro* models (Liu, Duan, Luan, Yagasaki, & Zhang, 2009). Recently, theanine derivatives (methyl coumarin-3-carboxylyl L-Th, ethyl coumarin-3-carboxylyl L-Th, ethyl 6-flourocoumarin-3-carboxylyl) were found to be more effective in inhibiting human and mouse lung cancers (Zhang et al., 2014).

## 7. Future research

The available information on research conducted on L-Th provides insight into its diverse health benefits. The role of L-Th in enhancing cognitive performance and mood elevation under different conditional stress tasks could serve as a base for designing in-depth studies related to onset and progression of cognitive disorders particularly in elders. Effect of L-Th on autonomous nervous system as well as CNS under different stresses requires in-depth investigations. A natural nutraceutical formulation containing L-Th with other plant extracts having sleep induction properties could be helpful to solve different sleep related disorders in general public in near future. In cancer therapy, further research may be warranting on its role as an adjuvant with anticancer agents and in other anticancer applications including skin cancers in combination with catechins and other neoplastic agents as

nano-formulations. Biochemical modulation either for enhancement of pharmacological properties or in reducing toxicity of antitumour agents with L-Th could contribute in more effective chemotherapy. The near future will be an interesting time for L-Th scale up research scenario and many new advancements of L-Th in food, nutraceutical and pharmaceutical industries.

## Acknowledgements

The authors acknowledge the Director, CSIR-Institute of Himalayan Bioresource Technology for providing laboratory facilities. Eshita Sharma acknowledges financial support received under the CSIR Network Project 'Plant-Microbe-Soil Interactions'. IHBT Publication No. 4079.

## References

Baptista, J., Lima, E., Paiva, L., Andrade, A. L., & Alves, M. G. (2012). Comparison of Azorean tea theanine to teas from other origins by HPLC/DAD/FD. Effect of fermentation, drying temperature, drying time and shoot maturity. *Food Chemistry, 132*, 2181–2187.

Baudouin, S. X. (2010). Process for purifying theanine. European Patent. EP 2144868.

Benjamin, S. (2009). Weeks, Formulations of dietary supplements and herbal extracts for relaxation and anxiolytic action: Relarian™. Medical Science Monitor, 15, RA256-262.

Bindal, S., & Gupta, R. (2014). L-Th synthesis using γ-glutamyl transpeptidase from *Bacillus licheniformis* ER-15. *Journal of Agricultural and Food Chemistry, 62*, 9151–9159.

Bizzotto, C. S., Meinhart, A. D., Ballus, C. A., Ghiselli, G., & Godoy, H. T. (2013). Comparison of capillary electrophoresis and high performance liquid chromatography methods for caffeine determination in decaffeinated coffee. *Food Science and Technology, 33*(1), 186–191.

Camfield, D. A., Stough, C., Farrimond, J., & Scholey, A. B. (2014). Acute effects of tea constituents L-Th, caffeine, and epigallocatechin gallate on cognitive function and mood: a systematic review and meta-analysis. *Nutrition Reviews, 72*, 507–522.

Cartwright, R. A., Roberts, E. A. H., & Wood, D. J. (1954). Theanine, an amino-acid N-ethyl amide present in tea. *Journal of Agricultural and Food Chemistry, 5*, 593.

Chen, C. N., Liang, C. M., Lai, J. R., Tsai, Y. J., Tsay, J. S., & Lin, J. K. (2003). Capillary electrophoretic determination of theanine, caffeine, and catechins in fresh tea leaves and oolong tea and their effects on rat neurosphere adhesion and migration. *Journal of Agricultural and Food Chemistry, 51*, 7495–7503.

Deng, W. W., Ogita, S., & Ashihara, H. (2010). Distribution and biosynthesis of theanine in Theaceae plants. *Plant Physiology and Biochemistry, 48*, 70–72.

Ekamayake, A., & Li, J. J. (2007). Process for enriching extracts of natural theanine. US Patent 689910.

Frost & Sullivan. (2011). Global Nutraceutical Industry: Investing in healthy living. FICCI.

Gu, H. N., Jiang, Y. X., & Wang, J. (2004). A practical synthesis of ethyl L-glutamine. *Organic Preparations and Procedures International, 36*, 182–185.

Haskell, C. F., Kenedy, D. O., Milne, A. L., Wesnes, K. A., & Scholey, A. B. (2008). Behavioural effects of caffeine and theanine alone and in combination. *Journal of Psychophamacology, 19*(Suppl.), A45.

Heese, T., Jenkinson, J., Love, C., Milam, R., Perkins, L., Adams, C., ... Ceremuga, E. (2009). Anxiolytic effects of L-Th- a component of green tea – when combined with midazolam, in the male sprague-dawley rat. *American Association of Nurse Anesthetists Journal, 77*(6), 445–449.

Higashiyama, A., Htay, H. H., Ozeki, M., Juneja, L. R., & Kapoor, M. P. (2011). Effects of L-Theanine on attention and reaction time response. *Journal of Functional Foods, 3*, 171–178.

Hung, C. P., Lo, H. F., Hsu, W. H., Chen, S. C., & Lin, L. L. (2008). Immobilization of *Escherichia coli* novablue γ-glutamyltranspeptidase in Ca-alginate-k-carrageenan beads. *Applied Biochemistry and Biotechnology, 150*, 157–170.

Jo, M., Park, M. H., Young, C. D., Yuk, D. Y., Lee, Y. M., Lee, J. M., Jeong, J. H., Oh, K. W., Lee, M. S., Han, S. B., & Hong, J. T. (2011). Neuroprotective effect of L-Theanine on Aβ-induced neurotoxicity through anti-oxidative mechanisms in SK-N-SH and SK-N-MC cells. Biomolecules & Therapeutics, 19(3), 288–295.

Juneja, L. R., Chu, D. C., Okubo, T., Nagato, Y. & Yokogoshi, H. (1999). L-Theanine – a unique amino acid of green tea and its relaxation effect in humans. Trends in Food Science & Technology, 10, 199–204.

Kakuda, T., Hinoi, E., Abe, A., Nozawa, A., Ogura, M., & Yoneda, Y. (2008). Theanine, an ingredient of green tea, inhibits [3H]glutamine transport in neurons and astroglia in rat brain. *Journal of Neuroscience Research, 86*, 1846–1856.

Kakuda, T., Nozawa, A., Sugimoto, A., & Nino, H. (2002). Inhibition by theanine of binding of [3H]AMPA, [3H]Kainate and [ᵀN]MDL 105,519 to glutamate receptors. *Bioscience, Biotechnology and Biochemistry, 66*, 2683–2686.

Kakuda, T., Yanase, H., Utsunomiya, K., Nozawa, A., Unno, T., & Kataoka, K. (2000). Protective effect of γ-glutamylethylamide (theanine) on ischemic delayed neuronal death in gerbils. *Neuroscience Letters, 289*, 189–192.

Keenan, E. K., Mike, D. A., Jones, P. F. S., Rogers, P. J., & Priestley, C. M. (2011). How much theanine in a cup of tea? Effects of tea type and method of preparation. *Food Chemistry, 125*(2) 588–59.

Kimura, K., Ozeki, M., Juneja, L. R., & Ohira, H. (2007). L-Theanine reduces psychological and physiological stress responses. *Biological Psychiatry, 74*, 39–45.

Kumar, D., Gulati, A., & Sharma, U. (2016). Determination of theanine and catechin in Camellia sinensis (Kangra Tea) leaves by HPTLC and NMR techniques. *Food Analytical Methods, 9*, 1666–1674.

Lardner, L. A. (2014). Neurobiological effects of the green tea constituent theanine and its potential role in the treatment of psychiatric and neurodegenerative disorders. *Nutritional Neuroscience, 17*(4), 145–155.

Lichtenstein, N. (1942). Preparation of γ-alkylamides of glutamic acid. *Journal of the American Chemical Society, 64*, 1021–1022.

Liu, Q., Duan, H., Luan, J., Yagasaki, K., & Zhang, G. (2009). Effects of theanine on growth of human lung cancer and leukemia cells as well as migration and invasion of human lung cancer cells. *Cytotechnology, 59*, 211–217.

Lu, K., Marcus, A. G., Oliver, C., Liley, D. T., Harrison, B. J., Bartholomeusz, C. F., ... Nathan, P. J. (2004). The acute effects of L-Theanine in comparison with alprazolam on anticipatory anxiety in humans. *Human Psychopharmacology Clinical and Experimental, 19*, 457–465.

Lyon, M. R., Kapoor, M. P., & Juneja, L. R. (2011). The effects of L-Th (Suntheanine®) on objective sleep quality in boys with Attention Deficit Hyperactivity Disorder (ADHD): a randomized, double-blind, placebo-controlled clinical trial. *Alternative Medicine Review, 16*, 348–354.

Nagai, K., & Konishi, H. (2013). Protection of theanine against doxorubicin-induced acute cardiac toxicity. *Biomedicine & Preventive Nutrition, 3*, 197–199.

Nandakumar, R., Yoshimune, K., Wakayama, M., & Moriguchi, M. (2003). Microbial glutaminase: biochemistry, molecular approaches and applications in the food industry. *Journal of Molecular Catalysis B Enzymatic, 23*, 87–100.

Narukawa, M., Toda, Y., Nakagita, T., Hayashi, Y., & Misaka, T. (2014). L-Theanine elicits umami taste via the T1R1 + T1R3 umami taste receptor. *Amino Acids, 46*, 1583–1587.

Ota, M., Wakabayashi, C., Matsuo, J., Kinoshita, Y., Hori, H., Hattori, K., ... Kunugi, H. (2014). Effect of L-Th on sensorimotor gating in healthy human subjects. *Psychiatry and Clinical Neurosciences, 68*, 337–343.

Owen, G. N., Parnell, H., Bruin, E. A., & Rycroft, J. A. (2008). The combined effects of L-Th and caffeine on cognitive performance and mood. *Nutrition Neuroscience, 11*, 193–198.

Pin, J.P., Galvez, T., & Prezeau, L. (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacology & Therapeutics, 98, 325–354.

Pu, H., Wang, Q., Zhu, F., Cao, X., Xin, Y., & Luo, L. (2013). Cloning, expression of glutaminase from *Pseudomonas nitroreducens* and application to theanine synthesis. *Biocatalysis and Biotransformation, 31*, 1–7.

Rana, A., Singh, H. P., & Gulati, A. (2015). Concurrent analysis of theanine, caffeine, and catechins using hydrophobic selective C12 stationary phase. Journal of Liquid Chromatography & Related Technologies, 38, 709–715.

Ritsner, M. S., Miodownik, C., Ratner, Y., Tatyana, S., Mar, M., & Pintov, L. (2011). L-Theanine relieves postive, activation, and anxiety symptoms, in patients with schizophrenia and shizoaffective disorder, An 8 week, randomized, double blind, placebo controlled, 2-center study. *Journal of Clinical Psychiatry, 72*, 34–42.

Rogers, P. J., Smith, J. E., Heatherley, S. V., & Pleydell-Pearce, C. W. (2008). Time for tea: mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together. *Psychopharmacology (Berl), 195*, 569–577.

Sadzuka, Y., Sugiyama, T., Miyagishima, A., Nozawa, Y., & Hirota, S. (1996). The effects of theanine, as a novel biochemical modulator, on the antitumor activity of adriamycin. *Cancer Letters, 105*, 203–209.

Sadzuka, Y., Sugiyama, T., Suzuki, T., & Sonobe, T. (2000). Improvement of idarubicin induced antitumor activity and bone marrow suppression by theanine, a component of tea. *Cancer Letters, 158*, 119–124.

Shi, C.-Y., Yang, H., Wei, C.-L., Yu, O., Zhang, Z.-Z., Jiang, C.-J., ... Wan, X. C. (2011). Deep sequencing of the *Camellia sinensis* transcriptome revealed candidate genes for major metabolic pathways of tea-specific compounds. *BMC Genomics, 12*, 131.

Shuai, Y., Zhang, T., Jiang, B., & Mu, W. (2010). Development of efficient enzymatic production of theanine by γ-glutamyltranspeptidase from a newly isolated strain of *Bacillus subtilis*, SK11.004. *Journal of the Science of Food and Agriculture, 59*, 2563–2567.

Shuai, Y., Zhang, T., Mu, W., & Jiang, B. (2011). Purification and characterization of γ-glutamyltranspeptidase from *Bacillus subtilis* SK11.004. *Journal of Agricultural and Food Chemistry, 59*, 6233–6238.

Sinsuwan, S., Yongsawatdigul, J., Chumseng, S., & Yamabhai, M. (2012). Efficient expression and purification of recombinant glutaminase from *Bacillus licheniformis* (GlsA) in *Escherichia coli*. *Protein Expression and Purification, 83*, 52–58.

Stirling, C., & Kruh, W. (2015). Nutraceuticals: The future of intelligent food. https://www.kpmg.com/ID/en/industry/CM/Documents/neutraceuticals-the-future-of-intelligent-food.pdf.

Sugiyama, T., & Sadzuka, Y. (2004). Theanine, a specific glutamate derivative in green tea, reduces the adverse reactions of doxorubicin by changing the glutathione level. *Cancer Letters, 212*, 177–184.

Tachiki, T., Yamada, T., Mizuno, K., Ueda, M., Shiode, J. I., & Fukami, H. (1998). *Γ* -Glutamyl transfer reactions by glutaminase from *Pseudomonas nitroreducens* IFO 12694 and their application for the syntheses of theanine and γ -glutamylmethylamide. *Bioscience, Biotechnology, and Biochemistry, 62*, 1279–1283.

Thangarajan, S., Deivasigamani, A., Natarajan, S. S., Krishnan, P., & Mohanan, S. K. (2014). Neuroprotective activity of L-theanine on 3-nitropropionic acid-induced neurotoxicity in rat striatum. *International Journal of Neuroscience, 124*, 673–684.

Trivedi, J. K., & Gupta, P. K. (2010). An overview of Indian research in anxiety disorders. *Indian Journal of Psychiatry, 52*, S210–S218.

Tsuge, H., Sano, S., Hayakawa, T., Kakuda, T., & Unno, T. (2003). Theanine, gamma-

glutamylethylamide, is metabolized by renal phosphate-independent glutaminase. *Biochimica et Biophysica Acta, 1620*, 47–53.
Unno, K., Iguchi, K., Tanida, N., Fujitani, K., Takamori, N., Yamamoto, H., ... Hoshino, M. (2013). Ingestion of theanine, an amino acid in tea, suppresses psychosocial stress in mice. *Experimental Physiology, 98*, 290–303.
Verma, V. V., Gupta, R., & Goel, M. (2015). Phylogenetic and evolutionary analysis of functional divergence among Gamma glutamyl transpeptidase (GGT) subfamilies. *Biology Direct, 10*, 49.
Walter, F., Puangrat, Y., & Philip, H. C. (1986). Investigation of the presence and significance of Theanine in the tea plant. *Journal of Agricultural and Food Chemistry, 31* S27-534.
Wan, X., Zhang, Z., & Li, D. (2009). Chemistry and biological properties of theanine in Tea and Tea Products, pp. 255–274. Ho, C. T., Lin, J. K., and Shahidi, F., Eds., CRC Press, Boca Raton.
Wang, Q., Min, C., Zhu, F., Xin, Y., Zhang, S., & Luo, L. (2011). Production of bioactive γ-glutamyltranspeptidase in *Escherichia coli* using SUMO fusion partner and application of the recombinant enzyme to L-Theanine synthesis. *Current Microbiology, 62*, 1535–1541.
Wang, H. Q., Yao, Z., Zhou, Z., Sun, Y., Wei, Ping., & Ouyang, P. (2012). Enzymatic synthesis of theanine with L-glutamine-Zn(II) complexes. *Biotechnology and Bioprocess Engineering, 17*, 1135–1139.
Williams, J., Kellett, J., Roach, P. D., McKune, A., Mellor, D., & Thomas, J. (2016). L-Theanine as a functional food additive: Its role in disease prevention and health promotion. *Beverages, 2*, 13. http://dx.doi.org/10.3390/beverages2020013.
Xue, X., & Liang, X. J. (2012). Overcoming drug efflux-based multi drug resistance in cancer with nanotechnology. *Chinese Journal of Cancer, 31*, 100–109.
Yamada, T., Terashima, T., Kawano, S., Furuno, R., Okubo, T., & Juneja, L. R. (2007). Theanine, γ-glutamylethylamide, increases neurotransmission concentrations and neurotrophin mRNA levels in the brain during lactation. *Life Science, 81*, 1247–1255.
Yamada, T., Terashima, T., Kawano, S., Furuno, R., Okubo, T., & Juneja, L. R. (2009). Theanine, γ-glutamylethylamide, a unique amino acid in tea leaves, modulates neurotransmitter concentrations in the brain striatum interstitium in conscious rats. *Amino Acids, 36*, 21–27.
Yamada, T., Terashima, T., Okubo, T., Juneja, L. R., & Yokogoshi, H. (2005). Effects of theanine, γ-glutamylethylamide, on neurotransmitter release and its relationship with glutamic acid neurotransmission. *Nutritional Neuroscience, 8*, 219–226.
Yamamoto, S., Wakayama, M., & Tachiki, T. (2006). Cloning and expression of *Pseudomonas taetrolens* Y-30 gene encoding glutamine synthetase: an enzyme available for theanine production by coupled fermentation with energy transfer. *Bioscience, Biotechnology, and Biochemistry, 70*, 500–507.
Yamamoto, S., Wakayama, M., & Tachiki, T. (2008). Cloning and expression of *Methylovorus mays* No. 9 gene encoding γ-glutamylmethylamide synthetase: an enzyme usable in theanine formation by coupling with the alcoholic fermentation system of baker's yeast. *Bioscience, Biotechnology, and Biochemistry, 72*, 101–109.
Yokogoshi, H., Kato, Y., Sagesaka, Y., Matsuura, T., Kakuda, T., & Takeuchi, N. (1995). Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Bioscience, Biotechnology, and Biochemistry, 59*, 615–618.
Yokogoshi, H., Kobayashi, M., Mochizuki, M., & Terashima, T. (1998). Effect of theanine, γ-glutamyethylamide, on brain monoamines and striatal dopamine release in conscious rats. *Neurochemical Research, 23*, 667–673.
Yoto, A., Mao, M., Sato, M., & Yokogoshi, H. (2012). Effects of L-Theanine or caffeine intake on changes in blood pressure under physical and psychological stresses. *Journal of Physiological Anthropology, 31*, 28.
Zhang, Y., Chen, B., Huang, Z., & Shi, Z. (2004). Preparative isolation and purification of L-theanine by HPLC. *Journal of Liquid Chromatography & Related Technologies, 27*, 875–884.
Zhang, G., Ye, X., Ji, D., Zhang, H., Sun, F., Shang, C., ... Wan, X. (2014). Inhibition of lung tumor growth by targeting EGFR/VEGFR-Akt/NF-κB pathways with novel theanine derivatives. *Oncotarget, 5*, 8528–8543.
Zhang, F., Zheng, Q. Z., Jiao, Q. C., Liu, J. Z., & Zhao, G. H. (2010). Synthesis of theanine from glutamic acid γ-methyl ester and ethylamine catalyzed by *Escherichia coli* having γ-glutamyl transpeptidase activity. *Biotechnology Letters, 32*, 1147–1150.
Zukhurova, M., Prosvirnina, M., Daineko, A., Simanenkova, A., Petrishchev, N., Sonin, D., ... Vlasov, T. (2013). L-Theanine administration results in neuroprotection and prevents glutamate receptor agonist-mediated injury in the rat model of cerebral ischemia-reperfusion. *Phytotherapy Research, 27*, 1282–1287.