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L-theanine: A potential multifaceted natural bioactive amide as health supplement



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ABSTRACT

Natural bioactive compounds from plants are of great importance in modern therapeutics, which are used to prepare antibiotics, growth supplements or some other therapeutics. L-theanine is such a bioactive amide amino acid presented in different plants and fungi, especially in tea. Theanine has influential effects on lifestyle associated diseases, such as diabetes, cardiovascular disorders, hypertension, stress relief, tumor suppression, menstruation and liver injury. This amino acid can maintain normal sleep and improve memory function and nullify effect of the neurotoxins. The rate of bioavailability and its medium of ingestion in the body is one of the great concerns for its additional antioxidant properties. Pharmacokinetics of the bioactive compound and its mode of action are described herewith. The biosynthesis and industrial synthesis are also reviewed to promote accelerated production of this bioactive compound in the pharmaceutical industries.

1. Introduction

Bioactive compounds of natural origin have great importance in common days for herbal medicines, antibiotics preparation, health supplements or some other therapeutic uses. Theanine is one of such important non-essential amide bioactive amino acids presented in tea (*Thea sinensis*, Theaceae), *Ilex guayusa* (Aquifoliaceae) and *Boletus badius* (Basidiomycetes). The average contents of L-theanine throughout the tea plants range from 1.2 to 6.2 mg/g fresh weight. Chemically theanine is L- γ -glutamyl ethylamide or 5-N-ethyl-glutamine, with two chimeric forms, which is principally synthesized in the root of tea and accumulated in the leaves that are consumed as beverages. Theanine acts on so many physiological processes of human system in specified and limited doses [1,2].

The different physiological disorders include different lifestyle associated diseases, one of which is diabetes. In India,

diabetes is one of the potential epidemics with more than 62 million peoples currently suffering from this disease. In 2000, India ranked (31.7 million) top in the world with the highest prevalence of diabetes mellitus, followed by China (20.8 million) and United States (17.7 million). The prevalence of diabetes is predicted to double globally from 2000 to 2030 with a maximum increase in India followed by China and United States [3,4].

Apart from diabetes, cardio-vascular diseases and tumor for both malignant and benign are the great challenges in modern medical therapies. World Health Organization (WHO) have targeted 25% reduction of all non-communicable diseases which cause 82% death worldwide, including cardiovascular diseases, cancer, chronic respiratory diseases and diabetes. WHO also targeted 25% decrease in hypertension in young as well as elder. To combat and successfully complete the targets, it is necessary to search a proper potent health supplement and theanine may be one of the potent options in medical science [5].

In modern therapeutics, bioavailability of the bioactive compound is a great concern for preparation of novel antibiotics or health supplements. The high bioavailability and alleviation of many physio-biological complications of human health make theanine suitable for its acceptance as therapeutic and health supplement. It can alleviate many physio-biological complications of human health. Advances in pharmacokinetics, pharmacological and physiological

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functions and biosynthesis of theanine are reviewed and summarized in this article. Industrial production of the bioactive compound has a huge financial prospect to flourish the economics health of a nation [6].

2. Statement of interest

Tea, which is the second most consumed beverage in the world, contains considerable amount of theanine (1.0%–2.5%) in dry weight of leaves, which is consumed regularly through the preparation of green tea. Beneficial bioactive property helps to cure several biological disorders in a cost-effective manner and act as a potential health supplement. Industrial production of theanine has high market value and carries economic importance. The present study emphasized on the economic importance of this bioactive compound and its efficacy against different biological and histopathological as well as psychiatric anomalies. This review suggests the intake of Theanine both as drugs and green tea in considerable doses and quantities respectively in present stressful environment to live healthier and more joyful.

3. Biosynthesis

L-theanine contains molecular formula $C_7H_{14}N_2O_3$ with molecular weight of 174.20 Da and carries melting point of 214–215 °C. The odorless, slightly sweet tasted white crystalline L-theanine is transparent, colorless, water soluble in nature. Theanine biosynthesis in tea starts with the amino acid and alanine presented in higher amount in root, with the enzyme alanine decarboxylate and produces ethylamine mostly found in leaves. Another free amino acid is glutamic acid, mainly found in stem, reacts with ethylamine to produce theanine. Leaves of tea act as sink of theanine although roots are the source of the free non-essential amide amino acid theanine (Table 1) [7].

4. Bioavailability

Theanine especially L-theanine when ingested either in capsulated form or an aqueous solution of tea, is distributed not only in human plasma level but also in the other tissues, and is metabolized in the form of glutamate and ethylamine, because their concentrations in plasma level are increased and found in the urine [8]. Metabolic fate of L-theanine in rat model, after infestation of L-theanine, glutamic acid and ethylamine is found in the plasma and also in urine in dose dependent manner [9,10]. *In vitro*, synthesis of theanine, large amount of glutamic acid and ethylamine are produced after L-theanine

incubation with either glutaminase or γ -glutamyl transpeptidase [11]. Incubation of L-theanine with homogenate from rats' kidney, increased concentration of ethylamine and glutamic acid were observed [9,12], whereas not from small intestine, liver, and brain. In the metabolic aspect, L-theanine is metabolized *in vitro* to equimolar amounts of ethylamine [11]. After intake of 24 h as capsule or tea, the mean excretion of L-theanine refers 47 and 54% of ingested one. Thus, the bioavailability of L-theanine remains in between 47% and 54% [8].

5. Pharmacokinetics of theanine

Theanine is chemically a chiral compound with two isomers, L-form and D-form. In natural production, especially in tea, only L-theanine is found whereas in synthetic both isomers are produced. In physiological responses, there are significant differences between 'L' and 'D' forms.

L-theanine is more readily absorbed than D-theanine. It has been shown that if equal amount of 'L' and 'D' form is given separately to mammals, 'L' form has almost three times more absorption capacity than 'D' form in the plasma level. Also, if these two forms of this bioactive compound are given at a time in equal content, the amount of uptake of L-theanine content is much higher than D-theanine [1].

L-theanine and D-theanine have mutual antagonism when they are administered in the system at a time. 'D' form inhibits absorption of 'L' form in the body [13].

Though these two forms of amino acid have lost from the urinary route, D-theanine excretes more readily than L-theanine, and the rate of excretion is about 10–15 times more in 'D' form than that of L form, while both are consumed either through orally or injected in peritoneum in equal amount that resulted the body becomes readily dehydrated [13,14].

In plasma D-theanine degradation occurs more quickly than L-theanine. Both 'D' and 'L' theanine are partially degraded into ethylamine and glutamic acid, which is catalyzed by a phosphate-independent glutaminase in the kidney [13].

Structurally theanine is similar to the excitatory neurotransmitter glutamate which binds to glutamate receptors with weak interaction in the micromolar range, including the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and kainate receptors, and to a lesser extent, the N-methyl-D-aspartic acid receptor [15–18]. Theanine acts antagonistically of the former two sites [16] and as an agonist of the latter site [19], binds to group I metabotropic glutamate receptors [15,20] and has an inhibitory activity on glutamine transporters which accordingly blocks the reuptake of glutamine and glutamate [18,21,22]. Finally, theanine elicits umami or savory taste results direct binding

Table 1

Amino acids content value of different plant parts ($\mu\text{mol/g}$ fresh tissues \pm SD) of 6 months old seedling of tea.

Organ	Ethylamine	Alanine	Theanine	Glutamic acid	Glutamine	Aspartic acid	Asparagine
Cotyledons	0.55 \pm 0.35	2.26 \pm 0.68	11.38 \pm 0.81	1.79 \pm 0.58	1.42 \pm 0.57	1.39 \pm 0.09	0.28 \pm 0.05
Roots	0.66 \pm 0.12	3.05 \pm 0.16	39.00 \pm 4.74	1.68 \pm 0.32	1.56 \pm 0.21	0.91 \pm 0.13	0.20 \pm 0.02
Leaves	0.88 \pm 0.07	0.55 \pm 0.06	14.96 \pm 1.95	2.3 \pm 0.36	1.00 \pm 0.05	1.77 \pm 0.26	0.44 \pm 0.15
Stems	0.44 \pm 0.02	0.95 \pm 0.17	33.85 \pm 3.68	2.39 \pm 0.59	8.19 \pm 1.14	1.64 \pm 0.75	0.44 \pm 0.02

Fresh weights (g) per organs are as follows: Cotyledons, (1.11 \pm 0.18); roots, (1.05 \pm 0.03); leaves (0.52 \pm 0.08); stems, (0.53 \pm 0.02) [7].

and activates the T1R1 + T1R3 heterodimer or umami (savory) taste receptor [23,24].

6. Mode of action of L-theanine

Theanine is basically non-toxic for all mammalian cells to a certain level of intake and there has been no adverse effect in physiological and histopathological characteristics [1,25]. Theanine in 'L' form has very unique mode of action in the mammalian systems, as mentioned below.

L-theanine increases dopamine and serotonin production in the brain [26] and induces relaxation through the enhancement of alpha wave activity in brain.

L-theanine, as chemotherapeutic agent, competitively inhibits glutamate transport into the infected tumor cells, can also decrease intracellular glutathione (GSH) levels. The efflux of chemotherapeutic agents (*e.g.* doxorubicin, idarubicin, cisplatin, irinotecan) are inhibited by theanine, which causes them to accumulate in tumor cells and to protect normal cells from damage by these drugs through its antioxidant property and maintaining cellular GSH level [22,27–29].

L-theanine can counteract the stimulatory effect of caffeine. In rats, after caffeine administration intravenously with theanine at the same dose, the stimulant effect of caffeine was blunted. Whereas given by normal dose of caffeine with a smaller dose of theanine administration resulted in excitatory effects of caffeine, which suggested a dose specific dual activity of theanine [30].

A dose-dependent hypotensive effect was occurred in spontaneously hypertensive rats injected with L-theanine *in vivo* [31] whereas structurally similar to theanine, glutamine administration made unaltered blood pressure [32].

7. Doses and physiological effects applied for L-theanine in mammals

Functionally L-theanine especially crossing the blood brain barrier, is measurable in a range from 30 min and up to 5 h after ingestion [10,11,33], which is metabolized in the kidneys to glutamate and ethylamine [34]. Recent studies referred anxiolytic effect of single dose of theanine up to 200–250 mg per day and it may be extended up to 1250 mg/day [9,12,35–37]. But there is no such specified dosage of L-theanine suggested for enhanced immune system functions in mammalian systems.

8. Physiological effects of L-theanine

L-theanine has multifaceted physiological effects on human health. A brief account of these effects is mentioned below.

8.1. Neuroprotection

Theanine helps to protect neural damage and improve function by reduction of cerebral infarction by inhibiting different neurological functions like catalase, superoxide dismutase, and succinate dehydrogenase (SDH) activity, thus reduces GSH and oxidative damage of neuronal cells [36–38]. It protects brain from cerebral ischemia [39], and prevents brain injury mediated by glutamate receptor agonist [40] and inhibits cerebral cortical neuron involvement in delayed neuronal death (DND). Neuroprotective role by regulation of group I

mGluR through stimulating the expression of phospholipase C- β 1 (PLC- β 1) and phospholipase C- γ 1 (PLC- γ 1) [20] by L-theanine, resulted in decrease in efficacy of neurotoxin-induced neurotoxicity, oxidative stress and also Parkinson's (PD) and Alzheimer's diseases (AD). DNA fragmentation and nerve cell (SH-SY5Y) apoptosis are also reduced by L-theanine [41–43].

8.2. Hypotensive activity

In adults, L-theanine can serve as a hypotensive agent with anti-stress activity, acting on suppression of cortical neuron excitement, reduction of anxiety and control rising of blood pressure in high stress condition [34]. Theanine showed hypotensive effect on spontaneous hypertensive rat [44,45]. L-theanine showed the hypotensive activity made by the increase of 5-hydroxytryptamine (5-HT) levels through the regulation of peripheral nerves and vascular system. Theanine is accumulated in the animal brain and reduce the levels of brain 5-HT and its metabolite 5-hydroxy indole acetic acid (5HIAA) [46].

8.3. Anti-diabetic effects

Diabetes is principally occurred by the abnormality of zinc metabolism, which is important for protecting heart suffering from diabetic myocardial complications from oxidative stress. Supplements of zinc are very crucial for treatment to prevent cardiac-oxidative damage and delay diabetic cardiomyopathy. Theanine-zinc compound makes a zinc complex and acts as a zinc supplement to maintain hypoglycaemic effect [47,48].

8.4. Anti-fatigue effects

L-theanine causes rise of the concentrations of dopamine (DA) and hepatic glycogen, and reduction of concentrations of 5-HT and serum urea are resulted in the alleviation of physical fatigue. Prolonged theanine treatment reduces 5-HT, serum urea and lactate, as well as increases DA and hepatic glycogen levels [49].

8.5. Anti-tumor effects

First, Theanine inhibits biosynthesis of the glutamate transport protein and intracellular glutathione, resulting in less glutathione doxorubicin conjugate (GS-DOX) of the multi-drug resistance-associated protein-5/GS-X (MRP5/GS-X) pump, and less intercellular transport of doxorubicin (DOX), which is beneficial in effect of anti-tumor drugs [28,50]. L-theanine enhances anti-tumor effects of cisplatin on mouse M5076 cells [9], and it shows significant suppressive effects on tumors with combination of Irinotecan hydrochloride, whereas irinotecan hydrochloride alone has no anti-tumor effect.

Second, L-theanine relieves the toxic side effects induced by some anticancer drugs through reduction of the activity of enzyme glutathione peroxidase and lipid peroxidation levels induced by the drug DOX, results less super oxidative stress [29,35].

And thirdly, theanine derivatives inhibit tumor growth by targeting epidermal growth factor receptor/vascular endothelial growth factor receptor-Akt/nuclear factor-kappa B (EGFR/VEGFR-Akt/NF-kappa B) signaling pathways, which involves

in controlling the survival and proliferation of cells. L-theanine derivatives ethyl 6-fluorocoumarin-3-carboxyl L-theanine (TFC) and ethyl 6-nitrocoumarin-3-carboxyl L-theanine (TNC) can effectively inhibit the cell growth of lung cancer by targeting these pathways [51,52].

8.6. Anti-depressant effects

L-theanine has a convincing effect on anxiety and depression. Intake of L-theanine after weaning prevents mice from stress-induced impairments of hippocampal long-term potentiation (LTP) and improves recognition memory [53]. The antidepressant mechanism of L-theanine involving heart rate reduction and salivary immunoglobulin A (s-IgA) decrease responses to an acute stress task. The reduction in heart rate and s-IgA mediate attenuation of the sympathetic nerve activation or suppression of the excitation of cortical neuron [53–56].

8.7. Anti-oxidant activity

L-theanine can oxidize LDL cholesterol to some extent. Lipid peroxidation marker mediated assay of malondialdehyde inhibit LDL oxidation with theanine *in vitro*, although the effect was weaker than the potent antioxidant effect of green tea polyphenols [57].

8.8. Improving immunity

L-theanine with L-cystine can enhance serum IgG and antigen-specific IgM levels. A combined administration of L-theanine and L-cystine in the human system [58] enhances the immunity to influenza vaccine in elderly persons with low hemoglobin by orally [59] and also alleviates post-gastrectomy inflammation and promotes recovery after surgery during the perioperative period [60].

8.9. Alleviation of liver injury induced by alcohol

Excessive alcohol uptake causes liver injury, increases free radicals and lipid peroxide (LPO) levels, and declines the activity of glutathione peroxidase. To alleviate alcohol-induced liver injuries, theanine increases aldehyde dehydrogenase and alcohol dehydrogenase activity, declines cytochrome P450 CYP 2E1 [61]. L-theanine improves the hepatic indices by raising the activities of catalase, GSH and superoxide dismutase with a reduction of MDA level in the liver. Moreover, administration of L-theanine significantly ameliorates the hepatic function and declines the level of tumor necrosis factor- α in the liver [62,63].

8.10. Protective effects on the cardiovascular system

Consumption of L-theanine improves vascular function and decreases the risk of cardiovascular disease. The protective efficacy of the compound can reduce serum cholesterol [64], rise artery vasodilation and production of nitric oxide [65], and protect brain from cerebral ischemic injury. Alleviation of serum cholesterol levels can reduce the risk of coronary heart disease by the application of L-theanine, which can significantly decrease the levels of abdominal adipose, liver cholesterol, serum neutral fat and cholesterol [64].

8.11. Improvement of memory

L-theanine promotes the maturity of nervous centralis during the neural maturation period, which is beneficial for brain development [11,12], ameliorates learning and memorizing ability by elimination of acquired memory disorders, oxidative stress, and improves the brain α -wave [66]. Cognitive functions can be enhanced by bioactive compound through the rise of brain neurotransmitters such as dopamine, 5-hydroxytryptamine (5-HT), glycine and γ -aminobutyric acid [67,68].

8.12. Reduction of menstrual discomfort

The menstrual discomfort can be reduced by L-theanine considerably. The target population with premenstrual syndrome, have experienced reduction of menstrual discomfort with the intake of L-theanine [69].

8.13. Maintenance of normal sleep

L-theanine helps to maintain normal sleep for better brain development and thus results in better mental and cognitive health. The effect was examined on a target population and considered beneficial for maintenance of normal sleep [69].

9. Side effects of L-theanine

L-theanine in mammalian system is generally well tolerated, and has a high LD₅₀ value (5000 mg/kg) and it is non-mutagenic or non-carcinogenic agent in animal cells or bacteria.

10. Market demand and artificial preparation of L-theanine

Artificial chemical synthesis of L-theanine (purity level \geq 98%) comes from food-grade L-glutamic acid and ethylamine with the solvent system as purified water and ethanol.

The major source of theanine is tea containing 1.0%–2.5% of dry weight of leaves and the average daily demand is 667–1668 mg/day in United States according to Taiyo International, Inc. by JHEIMBACH LLC. To mitigate the huge demand, large scale production is very essential for smooth supply of L-theanine as a food supplement. Though there are some other fungal sources, but the theanine content is very low compared to tea leaves. For large scale production, the most accepted three processes are stated here.

The first production procedure was adopted and patented by Taiyo International, Inc. by JHEIMBACH LLC where biological or green synthesis of the bioactive compound was carried out. It includes a treated organism which is aerobically cultured in a glucose and yeast extract medium. Glutamine, ethylamine, and glutamic acid are derived from raw materials, as well as ammonia produced during the reaction, as impurities. The other compounds, if presented, can be detected by the HPLC. But the disadvantage is the total production system is completely dependent on glutamic acid which is produced by bacterial culture of *Bacillus subtilis* or *Bacillus amyloliquefaciens* and the production is quite low.

The second large and facile production system of L-theanine was as followed: γ -Benzyl glutamate prepared by dissolving in pyridine and trityl chloride (1.28 eq) was added to the solution

[70]. To some successive treatments L-theanine was obtained as white crystals through the crystallization of the residue by the treatment of hot ethanol, and followed by re-crystallization of ethanol–water treatment. Though in this chemical synthesis method we get targeted product in a crystalline form, the production method is very much time consuming.

Another efficient method of industrial production through chemical synthesis process was developed by Zhejiang Tianrui Chemicals Co., Ltd., where the required time is not exhaustive, the whole system is well balanced, and a patent was awarded on this production system.

11. Conclusion and future direction

L-theanine, a non-essential bioactive amino acid, is very much beneficial to sustain in the stressful environment. Simultaneous intake of different medicine and food supplement may reduce the health damage in a considerable limit. The mitigating activity of L-theanine against non-communicable diseases like cancer, cardiovascular disorder, respiratory anomalies, and diabetes may be useful in the current situation. High blood pressure and the others complications can be reduced by using L-theanine in a constant dose as food supplement. Pharmacokinetics, bioavailability and mode of action in mammalian system are discussed in this review. In accordance to the benefits of L-theanine, the industrial production system and the probable effective system is discussed elaborately in this content. The consumption of L-theanine can reduce the chance of application of different drugs which have different critical side effects. Summarily in this review the beneficial role of the bioactive compound serves as a good health supplement in daily consumption of higher combating power for both male and female. In this regards, L-theanine will become a very effective supplement in development of food and drugs in modern days.

Conflict of interest statement

We declare that we have no conflict of interest.

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