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Review Article
Phytochemistry, Modes of Action and Beneficial Health Applications of Green Tea (*Camellia sinensis*) in Humans and Animals

[1]Muhammad Saeed, [2]Mohamed Ezzat Abd El-Hack, [2]Mahmoud Alagawany, [3,4]Muhammad Naveed, [1]Muhammad Asif Arain, [5]Muhammad Arif, [1]Rab Nawaz Soomro, [6]MohibUllah Kakar, [6]Robina Manzoor, [7]Ruchi Tiwari, [8]Rekha Khandia, [8]Ashok Munjal, [9]Kumaragurubaran Karthik, [10]Kuldeep Dhama, [11]Hafiz Muhammad Nasir Iqbal and [1]Chao Sun

[1]College of Animal Science and Technology, Northwest Agriculture and Forestry University, Yangling, 712100 Shaanxi, China
[2]Poultry Department, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt
[3]Faculty of Pharmacy and Alternative Medicine, Islamia University of Bahawalpur, 63100, Pakistan
[4]Department of Clinical Pharmacy, Scholl of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing Jiangsu Province, 211198, Republic of China
[5]Department of Animal Sciences, University College of Agriculture, University of Sargodha, 40100, Pakistan
[6]Faculty of Marine Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan, 3800, Pakistan
[7]Department of Veterinary Microbiology and Immunology, College of Veterinary Sciences, UP Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalay Evum Go-Anusandhan Sansthan (DUVASU), Mathura, 281 001 Uttar Pradesh, India
[8]Department of Biochemistry and Genetics, Barkatullah University, Bhopal-462 026 (M.P.), India
[9]Central University Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, 600051 Tamil Nadu, India
[10]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, 243 122, Uttar Pradesh, India
[11]School of Engineering and Science, Tecnologico de Monterrey, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N. L., CP 64849, Mexico

Abstract

Green tea is an important herb and its products are extensively used in the traditional Chinese medicinal system through various formulations. Several reports have revealed its beneficial usage and medicinal aspects for various ailments. Consumption of green tea has gained special attention and popularity in various sectors of the modern era of changing lifestyle. This review aimed to extend the current knowledge on the modes of action and beneficial applications of green tea in humans and animals for safeguarding different health issues. The nutritional, immunological, pharmacological and physiological functionalities of green tea are because of the available bioactive components like caffeine, L-theanine, polyphenols/flavonoids and others. It possesses multi-beneficial potential in treating and preventing various disorders of animals and humans, as well as promoting animal (dairy, piggery and goatry) and poultry industry. The present review provides insights on the modes of action and beneficial applications of green tea, which will be useful for nutritionists, pharmacists, physiologists, researchers, veterinarians and animal and poultry producers. Future research emphasis and promotional avenues are needed to explore its potential therapeutic applications for designing appropriate pharmaceuticals, complementary medicines and effective drugs to popularize and propagate its multidimensional health benefits.

Key words: Green tea, *Camellia sinensis*, phytochemistry, modes of action, antioxidant, antimicrobial, anticarcinogenic, anti-stressor, medicinal properties, health benefits

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Corresponding Author: Chao Sun, College of Animal Science and Technology, Northwest Agriculture and Forestry University, Yangling, 712100 Shaanxi, China

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INTRODUCTION

Green tea (*Camellia sinensis*) is a popular herbal plant having multiple beneficial health applications in humans and animals. Owing to the emerging antibiotic resistance, nowadays researchers are searching for alternatives of antibiotics to be used for safeguarding human health, efficiently acting against the pathogenic microorganisms, enhancing immunity and as growth promoters and improving feed efficiency in animals. A great concern on the indiscriminate use of antibiotics in animal and poultry feed owes on behalf of the increasing resistance in bacteria pathogens and the associated public health concerns. Since 2006, the European community has banned the use of antibiotics from promoting growth in animal and poultry feed[1]. Thus, as the replacement of these antibiotics as growth enhancers, the medicinal/herbal plants and their extracts are the most popular options[2-6]. A wider spectrum of many plants such as chicory, moringa, neem, garlic, ginger, kalongi, turmeric, savory, black cumin, *Yuccaschidigera*, mint, sea-buckthorn, etc. has been examined for this purpose[7-15]. Tea is the second most-consumed beverage throughout the world[16]. Green tea (*Camellia sinensis*), a type of Chinese tea, is used as an antioxidant with abundant health benefits and also considered as one of the most well-known beverages in the world, mostly due to its prospective health welfares[17-19]. The concept of using tea was proposed by a Chinese King ShenNung in 2737 BC when by chance some tea leaves were boiled in water and a pleasant fragrance was produced[20].

Leaves of green tea contain antioxidative catechins[21]. Tea catechins have many health benefits such as anti-inflammatory[21,22], antiarthritic[23], anticarcinogenic[24], anti-cancerous[25,26] antimutagenic, antibacterial[27], antiviral[28], antifungal[29], anticoccidial[30], antiprotozoal[31], antiparasitic[32,33], anti-infective[34], hypocholesterolemic[35], resistant to capillary blood congestion[36] and hypolipidemic effects[37,38]. There are various types of tea but the main types comprise of green tea (*Camellia sinensis* L.), white (less processed), oolong or black tea[39,40]. It maintains L-configuration under the natural conditions because it is an acetanilide-group compound[41]. L-theanine was discovered in 1949 in the leaves of green tea. This amino acid is a unique taste constituent of tea, producing a caramel flavor and an attractive aroma and that helps to alleviate tea polyphenols astringency and caffeine bitterness[42]. Technical, safety and toxicological evaluation tests suggested that theanine is a safe and non-toxic photogenic food supplementation. L-theanine was synthesized chemically for the first time from aqueous ethylamine and pyrrolidone carboxylic acid[43,44].

L-theanine is a non-protein amino acid, which is abundant in leaves of *C. sinensis* (green tea)[45]. γ-Glutamylethylamide or L-theanine has been studied as a food additive and functional food in relation to human nutrition and has healthical, pharmacological and biological properties such as anti-cerebral ischemia-reperfusion injury, stress-reducing, antitumor, anti-aging and anti-anxiety activities[46-51]. Also, Cooper[52] stated that dietary supplementation of L-theanine is a feasible way to mitigate Reactive Oxygen Species (ROS)-induced damage.

Several types of research have reported that L-theanine reduces the oxidative damage via decreasing the ROS production, lipid damage and oxidative parameters as well as increasing glutathione concentration. Moreover, L-theanine improves hepatocyte antioxidative capacity via preventing the formation of MDA (malondialdehyde) and restoring/improving the antioxidant activities of enzymes like catalase, reduced glutathione and superoxide dismutase during liver injury *in vivo* and *in vitro*[53,54].

This present review embodies the available information in current time regarding green tea, its beneficial constituents such as L-theanine, EGCG, catechin, etc. and the useful health applications and medicinal values for humans and animals. The article also describes the natural sources, chemical structure and composition, mechanism of actions, beneficial uses of L theanine as feed supplementation and its potential usages in animal and poultry health as well of humans. Most of the previous literature has been studied in human, mice, honey bees and pigs, but information is scanty on the beneficial role of L-theanine among broiler chicken. Multiple beneficial utilities of green tea have been presented for potent use in dairy, piggery, goatry and poultry industry. This review article also presents potential of L-theanine as a natural anti-stressor in poultry diet, particularly in those regions where environmental stress is a significant problem. The overall information presented will be useful for researchers, medical professionals, veterinarians, students/scholars, pharmacists, nutritionists, animal producers, poultry breeders and pharmaceutical industry. It would help to popularize, propagate and promote the multidimensional health benefits of this important molecule. Future research emphasis and exploration of its potential therapeutic applications would pave the way for designing appropriate pharmaceuticals, complementary medicines and effective drugs for safeguarding various health issues.

Ethnopharmacological and ethnomedicinal importance: Tea is the major drink after water. Green tea is a very popular herbal plant enriched with multiple health benefits. The

extracts of green tea have been extensively used as ancient therapeutic agents in traditional Chinese medicinal system. Green Tea is native to China and emerged as a medicinal drink in Shang dynasty (1766-1050 BC). Green tea is the non-fermented leaves of *C. sinensis* in comparison to fermented black tea and semi-fermented oolong tea[55]; hence polyphenols remain in the unoxidised state[56]. After boiling green tea leaves with other herbs, barks or seeds as per requirement of the ailment were given as herbal healing therapy in China. It was the end of Zhou dynasty (1122-256 BC) in Sichuan province when instead of a medicinal decoction with other herbs the green tea alone was used as concentrated liquid as a stimulant. Monks and priest found the green tea preparation helpful in keeping them awakened during long hours of meditation and they declared this as an elixir of life[57].

With ever increasing scientific knowledge, search for safer, potential and novel type of health-related supplements quest, the research scientists are redirecting their interests to explore natural resources i.e. medicinal herbs and plant derived compounds. Many research studies have also proven its remedial power for many ailments, like liver and heart diseases, inflammatory conditions, different metabolic syndromes, cardiovascular risk factors, type II diabetes and obesity and various types of cancers in humans. Consumption of Green Tea has especially gained attention and wide popularity in the modern era of changing lifestyle.

Sources of L-theanine: Tea plant (*C. sinensis* or *Thea sinensis*), member of Theaceae family, is mainly the origin of Asian, African, South American countries with tropical and temperate regions. The majority of this plant is obtained from India, Sri Lanka, China, Japan and depending upon degree of fermentation and processing it can be either black, white, green, yellow or oolong tea[58].

Natural L-theanine is derived from *C. genus*, non-edible mushroom of *Xerocomus badius*, *C. sinensisassamica*, *C. sinensissinensis* which is an amino acid sort in leaves of tea like *C. sasanqua* and *C. japonica*[59]. L-theanine contributes great aroma and particularly it is linked with tea umami taste[60]. L-theanine consists of 50% of tea content of free amino acids. Dry tea contains 1-3% of L-theanine, which further varies according to many factors like geographic zone where tea is cultivated, techniques of production, class of tea, time and type of harvest, etc.[41]. The kind of the tea is essential also regarding L-theanine concentration., *C. sinensisvar. Sinensis* has more theanine in comparison to *C. sinensis* var. *Assamica*[61]. Additionally, the harvested tea at the beginning of summer is reported to be having more theanine comparing with tea harvested in late summer[41].

Synthetic L-theanine (Suntheanine™) is usually obtained as a racemic mixture of D- and L-forms from ethylamine and L-glutamine with the use of glutaminase enzyme[62]. The enzyme glutaminaseis produced by *Bacillus amyloliquefaciens* or *Pseudomonas nitroreducens*, which are non-pathogenic or non-toxic for the human. L-theanine is stable in acidic conditions[63-65].

Chemistry and amino acids profile of green tea: The constituents in green tea which contribute full-bodied sweetness and umami flavor are amino acids. L-theanine represents more than 50% of these amino acids which is unique to tea[66]. The chemical structure of theanine is somewhat like that of glutamine, with its distinct attributes being sweetness, refined and rich flavor. Tea leaves also possess amino acids other than theanine such as alanine, glutamine, arginine, serine and asparagine. The taste of umami of green tea is reported to be attributed to theanine and serine. Kaneko *et al*.[67] claimed that about 70% of the taste of umami in green tea from Japan belongs to theanine amino acid; and also postulated that theanine, theogallin, succinic acid and gallic acid are the main constituents responsible for green tea umami taste. Chaturvedula and Prakash[66] stated that several amino acids like arginine and alanine, in particular, besides caffeine and catechins also could share in the bitterness of green tea.

Horanni and Engelhardt[68] assured that several analytical methods were used to investigate the tea content free of amino acids. The chemical composition of leaves of tea was well documented by Khan[69]. Polyphenols are the main components of tea leaves[55]. The fresh tea leaves possess theophylline (0.02-0.04%), theobromine (0.15-0.20%), caffeine (about 3.50% of dry weight), organic acids (1.50%), lignin (6.5%), theanine (4%) and free amino acids (1-5.5%), chlorophyll (0.5%) and other pigments and a lot of flavoured compounds[56]. Furthermore, a wide variety of other constituents exists, involving, phenolic acids and depsides, alkaloids, flavones, minerals, carbohydrates, enzymes and vitamins[66]. Also, tea contains kaempferol, flavonols, mainly quercetin, myricetin and their glycosides. The most favorable impacts attributed to the green tea are polyphenols, particularly the catechins, which represent 25-35% of the green tea leaves dry weight off[55,70,71].

The dried leaves of green tea contain 92.20% dry matter, 7.80% moisture, 82.40% organic matter, 8.72% ether extract, 18.15% crude protein, 19.32% crude fiber, 36.21% nitrogen free extract, 9.80% total minerals and 3002 kcal kg^{-1} calculated metabolizable energy and amino acids[72]. Karori *et al*.[73] studied that the green tea, consist of volatile oils, alkaloids (caffeine, theophylline and theobromine)

polysaccharides, polyphenols (catechins and flavonoids), lipids, ascorbic acid, amino acids, minerals and other uncharacterized compounds. Polyphenolic compounds including epigallocatechin, epicatechin gallate, epicatechin and epigallocatechin-gallate are collectively referred as catechins and are enriched with versatile health-promoting properties[30].

Green tea plant contains significant levels of amino acids, but theanine is the most plentiful[62], representing almost the half of total amount of amino acids. This amino acid as natural antioxidant plays a major role in preventing tissues from damage induced by free radical and preventing anxiety and induces relaxation by augmenting dopamine as well as serotonin concentrations in cells of the nerve.

Chemical structure of L-theanine: L-theanine chemical structure (N-ethyl-L-glutamine; (2S)-2-ammonio-5-(ethylamino)-5-oxopentanoate) is obtained from proteinogenic L-amino acidglutamic acid[74].

Industrial preparation of theanine: L-theanine is naturally synthesized in the roots of the tea plant and then transferred to the leaves[75]. Many endeavors are being prepared to produce theanine on a large scale. However, most of these remained fruitless, because of high cost, highly complicated processes and low yields[76,77].

The theanine selectivity action to remind MIP was examined by extraction of the phase of solid. While theanine recovery confirmed satisfactory (880.00 g kg^{-1}), the purity of analyte was not confirmed and revealed to be low[78]. Many patents were improved covering the extraction of theanine. Ekamayake and Li[78] could successfully develop a method for the extraction of theanine that prevents using organic solvents. Tea leaves extract in hot water were passed through a column, which was packed by a solid polyamide period. Then, the fractions rich theanine were collected. A method yielding L-theanine polluted by impurities like caffeine, polyphenols as well as saccharides has been described. Baudouin[79] reported another patent through aqueous extracting the black tea, filtration and drying the extract to produce a solid rich in theanine. After this, the extract was dissolved again then the purification process through fractionation by chromatography of cation exchange with DiaionUBK550. The purity of final theanine was relatively low (440 g kg^{-1}), besides the method of production was long and complicated[41].

L-theanine (suntheanine) biosynthesis and mechanism of action: Biosynthesis of theanine in tea plant takes place in the roots, not cotyledons. It is believed to take place as synthetase gene of theanine transcriptions in cotyledons are less comparing with plants and parts[41,59]. Theanine after synthesis is transferred to vines of the plant by phloem then stored in the leaves. After susceptibility to temperature and sunlight, it is hydrolyzed again in the leaves. Ethylamine is used as a portent in the synthesis of catechins. Consequently, catechin level is low where the theanine level is high in the tea, which grows in the climate conditions with a lack of sunlight[41]. Time of the synthesis of L-theanine varies between methods, particularly those that need de-blocking and protection procedures for its reactive groups[80,81].

Previous literature revealed that the theanine acts antagonistically versus the paralysis activated by caffeine. Theanine was found to be quickly absorbed in the intestinal tract. One research study showed that when L-theanine took either 50 or 200 mg was administered, then alpha waves were observed from parietal and occipital regions after 40 min of its administration. In another study, the intensity of alpha waves was determined and detectable after 30 min of L-theanine administration (with a 200 mg dose) showing a significant increase over controls[82]. So the study explored that L-theanine can cross blood brain barrier and has an positive effect over anxiety. It is able to cross blood brain barrier and promote release of GABA, regulating the secretion of dopamine and serotonin levels in the brain, so brain relaxing and improved learning ability can be associated with it[83]. In an acute stress task, reduction in the heart rate and salivary immunoglobulin A, indicates its anti-stressor role[1].

Beneficial applications-an overview: The leaves of green tea have a lot of beneficial properities involving anti-carcinogenic, anti-inflammatory, antioxidative, antimutagenic, hypolipidemic, anti-obesity and antimicrobial influences[21,24,37,26,84]. Other medicinal avenues of green tea are beneficial in treating various disorders in humans and useful role in inhibition of tumorigenesis and angiogenesis, skin/collagen protective role, hepatoprotective role, neuroprotective role and as a memory enhancer. Salient beneficial applications of green tea are presented in Fig. 1. Reports showed that green tea catechins are capable of binding with various minerals, so impacts their concentration and metabolism. Its intake declines the level of iron and zinc; improves the manganese but do not impact the concentration of copper[85-89]. Peng *et al.*[90] observed that L-theanine could elevate the5-hydroxytryptamine (5-HT) levels and depress the levels of corticosterone (CORT), noradrenaline and adrenocorticotropic hormones in rats. Wen *et al.*[91] postulated that dietary supplementation ofL-theanine (400 mg kg^{-1})

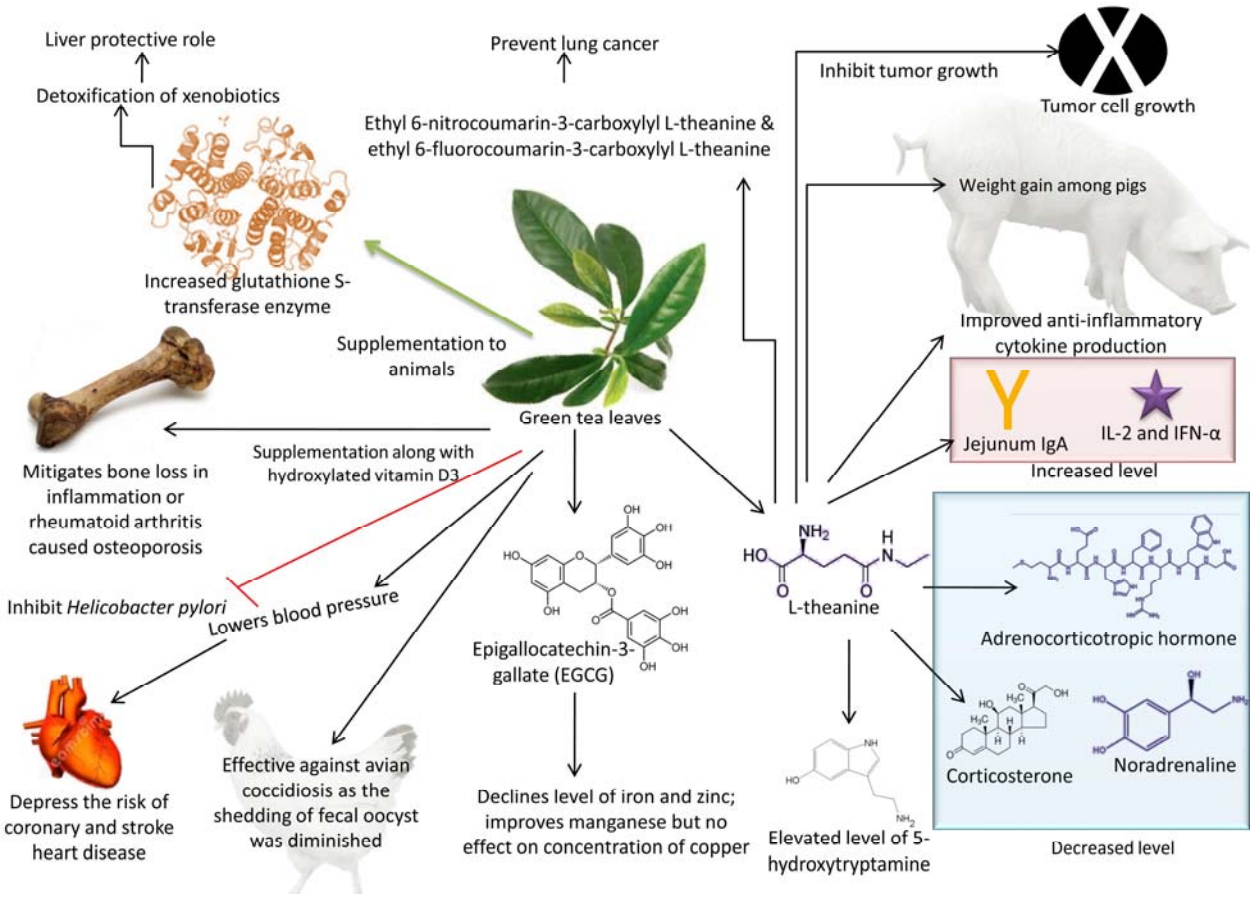

Fig. 1: An overview on the beneficial applications of green tea

increased level of jejunum IgA and serum level of IL-2 and IFN-α in the chickens. Dietary addition of L-theanine to pigs enhanced body weight gain and improved anti-inflammatory cytokine production[92]. Using l-theanine in mice, as a natural antioxidant, revealed neuroprotective effects versus the chronic stress-induced cognitive impairments[92-95].

L-theanine relieves the levels of lipid peroxidation and the activity of glutathione peroxidase enzyme motivated by the DOX drug, resulting in reduced super-oxidative stress[96]. The theanine derivatives inhibit the tumor growth by targeting vascular endothelial growth factor receptor-Akt/epidermal growth factor receptor/nuclear factor-kappa B (EGFR/VEGFR-Akt/NF-αB) signaling pathways that involve significant in control of cell proliferation and survival. The theanine derivatives such asethyl 6-fluorocoumarin-3-carboxylyl L-theanine (TFC) and ethyl 6-nitrocoumarin-3-carboxylyl L-theanine (TNC) prevent the lung cancer cell growth *ex vivo*, *in vivo* and *in vitro* by targeting these pathways[97].

Supplementation with green tea with a dose of 60 mg animal^{-1} day^{-1}, increased glutathione S-transferase enzyme which helps in catalyzing the detoxification of xenobiotics and thereby showed liver protective role[96]. Animals supplemented with green tea polyphenols and hydroxylated vitamin D3, mitigated bone loss in inflammation induced or rheumatoid arthritis caused osteoporosis[30]. On the other hand, green tea extract supplementation in the chicken diet was found effective against avian coccidiosis as the shedding of fecal oocyst was diminished upon tea extract treatment in chickens infected with *Eimeria maxima*[30,32,33]. By reducing the fecal oocystoutput, environmental contamination is also be reduced.

The consumption of green tea has been linked to the prevention of various cancer types, involving colon, lung, mouth, esophagus, small intestine, stomach, kidney, mammary glands and pancreas[98]. Many clinical and epidemiological studies revealed that green tea might decline risk of various chronic diseases[70]. This useful impact has belonged to the existence of high amounts of polyphenols, which act as antioxidants. It has been reported that oxidative stress hinders the maturation of oocyte and supplementation

of green tea catechins improve the reproductive health and fertility possibly due to its strong antioxidant activities[99-101]. Green tea can also the lower pressure of blood[101] and consequently depress the risk of coronary and stroke heart disease[102]. Studies on animals demonstrated that green tea might prevent coronary heart disease development by depressing body weight and blood glucose level[102-104]. However, all these data are depending on middle-aged animals' populations, which nutritional status tends to be more negatively influenced by age-related socioeconomic and biological factors[105]. Results of Sano *et al.*[24] confirmed the inhibitory impacts of green tea against tert-butyl hydroperoxide induced lipid peroxidation and a similar antioxidant impact on the kidney was noticed after oral intake of tea polyphenol EGCG. Using the active oxygen method, the antioxidative properties of individual catechins and crude catechin powder were tested in experiments. Catechins declined the peroxides formation more efficiently than dl-α-tocopherol[106]. The beneficial uses of green tea in treating typhoid and diarrhea were known in Asia since ancient times[107]. Catechins of green tea have an inhibitory impact on the infection by *Helicobacter pylori*[108]. Moreover, Weber *et al.*[28] noticed that green tea catechins inhibit the infection by adenovirus in vitro.

The effect of EGCG was evaluated in Vero-E6 cells and it exhibited more than 90% inhibition in Zika virus entry, when higher concentrations (>100 μM) were used; however pretreatment with EGCG did not affect virus attachment[109]. In present scenario of Zika virus outbreak, such study is of greater relevance. The green tea fruit peel extract has been shown to inhibit angiogensis and tube formation in human umbilical vein endothelial cells (HUVECs). Also it prevents obesity, when given to mice fed with high-fat diets[110]. Concomitantly administered green tea with high fat diets, increases hormone-sensitive lipase, comparative gene identification-58 (CGI-58) and perilipin in mesenteric adipose tissue. Also inflammatory cytokine like TNF-α and other components of proinflammatory signaling including TLR4, MYD88 and TRAF6 are reduced[111]. During chronic inflammation, Th17 lymphocytes produce IL-17 to favor inflammation. Green tea extract inhibits IL-17 synthesis, having impact in reducing the development of rheumatoid arthritis. The extract increases the amount of anti-inflammatory IL10[112]. Fresh green tea extract can chelate Fe metal ion and this might be helping in preventing lipid peroxydation[113]. Anti fungal effects have been demonstrated against *Candida albicans*[114]. In the 10.5 and 1.05 mg L^{-1} concentrations, the green tea extracts were able to reduce the growth of *Listeria monocytogenes*[115].

Technically scientific support and future perspectives: Green tea as a herbal remedy is being consumed since thousands of years owing to its healing and medicinal values of its phytoconstituents. The modern era of scientific advances has revealed several phytochemicals, pharmacokinetics and pharmacodynamics of herbs and phytomedicines along with their experimental, clinical and toxicological based analytical validations. The usage of herbs is nowadays gaining more social awareness, popularity and acceptance for their promising medicinal and multiple beneficial health applications. These have easy accessibility and fewer side effects. Owing to the increasing incidences of chemical drug failures, emerging antimicrobial resistance and adverse side-effects of chemical-based medicines, researchers are now looking forward to the safer and natural alternatives. Added to this, the recent advancements in the field of biotechnology, molecular tools, nanotechnology are together paving the ways to strengthen researches in the areas of herbs and phytomedicines. These help in understanding the structures, screening, isolation, purification, finding active constituents, revealing modes of actions, achieve an optimal yield and come up with value-added products. Such advances pave ways for usage of herbs with high utility as effective pharmaceuticals, drugs and medicines along with 'smart'/ targeted drug delivery systems for enhancing the potential healthical applications. Besides these, regulatory guidelines, quality control, dose standardization, standardization of manufacturing practices, packaging and storage systems need to be appropriately formulated. The desired support of scientific evidences, documentation and validation studies along with good clinical practices is mandatory for a successful implementation and/or launching of drugs/natural products from bench to bedside. Various herbs, phytomedicines and their valuable products are now reaching commercial market with social acceptance and sustainability for safeguarding several health issues and their wider health applications and usages may be seen at optimum levels in near future.

CONCLUSION

Green tea is a popular aromated drink, contains approximately 450 organic compounds. A number of anti-oxidants including vitamin C, vitamin E, lutein and zeaxanthin, minerals like Mg, Na, K, Fe, Ca, Mn; Vitamins like Vitamin C, folic acid, Riboflavin, B6 and at the same time it is zero in trans-fat, poly-unsaturated and saturated fats, cholesterol and sugar. The unique umami taste, the fifth taste after sweet sour, bitter and salty, is imparted by L-theanine. This review discussed the health benefits of green tea

including anti-inflammatory, antioxidant, antimutagenic, antifungal, anticarcinogenic, antimicrobial, antiparasitic and hypolipidemic, anti-obese effects, besides the inhibition of angiogenesis and tumorigenesis and others. It could also decline body pressure and the acute stress state, minimize the cardiovascular diseases, enhance the vascular function and improve immunity and memory and suppress obesity. Indeed now a days it is considered as super food, which must be incorporated in diet. Among its components, L-theanine also individually having important roles in stress busting, memory enhancement and reducing β oxidation and thusa limited and appropriate quantity of green tea could be used in the diets of human, animals and poultry to get improved health.

SIGNIFICANCE STATEMENT

- Green tea and its products are mainly used in the traditional Chinese medicinal system through various preparations. The nutritional, physiological, pharmacological and medicinal properties of green tea are due to its content of some bioactive components such as caffeine, L-theanine and others
- Green tea and its products have many health benefits such as anti-inflammatory, antioxidant, antimutagenic, anticarcinogenic, antimicrobial, antiparasitic and hypolipidemic, besides the inhibition of angiogenesis and tumorigenesis and others
- L-theanine amino acid plays an important role in poultry industry as a natural anti-stressor and growth and productive promoters

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