Chapter

IMPACT OF GREEN TEA (*CAMELLIA SINENSIS* L.) CONSUMPTION IN DIABETES MELLITUS-INDUCED NEURODEGENERATION

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ABSTRACT

The medicinal properties of tea (*Camellia sinensis* L.) have a long and interesting history, dating back to many centuries ago. Green tea has aroused considerable interest in recent years, being nowadays one of the most studied types of teas. Green tea is a complex mixture of thousands of chemical compounds, including proteins and free amino acids, polysaccharides, vitamins, organic acids, methylxanthines, and polyphenols. Catechins, caffeine and L-theanine are often reported as the main phytochemicals responsible for green tea's health benefits, namely by its antioxidant, hypoglycemic, and neuroprotective properties.

Diabetes mellitus (DM) is the most common metabolic disease and its incidence is dramatically rising. In addition, DM is associated to a high risk of developing neurodegenerative diseases, since the brain is particularly susceptible to glucose fluctuations and hyperglycaemia-induced oxidative stress. Throughout this chapter we will discuss the phytochemical composition and bioactivities of green tea, especially antioxidant, antidiabetic, and neuroprotective activities. The potential beneficial effects of green tea consumption on DM and how it can be used to reduce the severe brain damage induced by this disease will be emphasized.

INTRODUCTION

The tea plant, *Camellia sinensis* (L.), has been extensively used in traditional medicine and ancient cultures to prevent and treat several diseases [1, 2]. The origins of tea are mythological. The "Father of Tea", Eisai, said: "Tea is a miraculous medicine for the maintenance of health. Tea has an extraordinary power to prolong life." [2]. Tea is the infusion prepared by using *C. sinensis* leaves and is one of the most widely consumed beverages in the world [3]. The sensorial properties and stimulating effects, with potential health benefits make this a very popular drink [1, 4, 5]. Green tea is the most studied type of tea and, like white tea, has been reported to have a beneficial effect in cardiovascular diseases, cancer and reproduction [1, 6-9]. In recent years, the interest regarding the potential benefits for health of green tea intake has grown [1] due to the interest in alternative medicinal strategies. Indeed, scientific studies of this beverage and its constituents have been underway for less than three decades. Several of those studies have shown a significant association between green tea consumption and reduced rate of cardiovascular and metabolic diseases, cancer, neurodegenerative diseases, and others [1, 10]. Moreover, it is known that phenolic compounds, namely catechins, and other phytochemicals, such as methylxanthines and L-theanine, are responsible for the medicinal effects of this beverage [1, 6, 7].

Diabetes mellitus (DM) is one of the greatest threats to modern global health and is one of the most prevalent chronic diseases in western societies. It was estimated that about 300 million of people will develop DM in 2025 [11]. This is a metabolic disorder that may result from absolute deficiency of insulin, insulin resistance, or both [12]. It can be classified in two major forms: Type 1 Diabetes Mellitus (T1DM) or Type 2 Diabetes Mellitus (T2DM). T1DM results from the autoimmune destruction of the pancreatic beta cells

and T2DM is characterized by impaired insulin secretion and increased insulin resistance. Although these types of DM result from different actions, the hyperglycemic state is a common feature responsible for changes in the structure and function of several cells, tissues and organs. The brain is no exception. Indeed, high blood glucose levels are implicated in the development of cerebrovascular disease and other neurological comorbidities, such as cognitive dysfunction and dementia [13, 14]. Diabetic individuals are reported to have a higher risk of cognitive decline and neurodegeneration [15]. Some studies report that individuals with T2DM have an accelerated cognitive decline associated with a greater increase in the volume of brain ventricles [16]. Furthermore, various studies demonstrated the connection between T2DM and Alzheimer's disease (AD) [17], though the exact mechanisms by which DM affects the health of the brain remain unclear. This is of extreme relevance since DM is one of the major causes of dead in the world. In addition, neurodegenerative diseases are major medical and social challenges that modern societies face. Thus, the association of DM with an increased probability of its development must be carefully discussed. In the last years it has been discussed that new ways to reduce the damage caused by DM may arise through a modification in lifestyle, particularly by changes in diet. There is a large interest in finding an effective therapy for DM, particularly to DM-associated neurodegeneration, and tea seems to be a good candidate [18]. Green tea and its phytochemicals have gain attention from different research groups due to its interesting antidiabetic, neuroprotective and antioxidant properties [19-21]. Throughout this chapter we will discuss the phytochemical composition of green tea, as well as its beneficial effects in DM and the neurodegenerative processes caused by this metabolic disease.

ORIGIN AND PRODUCTION OF GREEN TEA

C. sinensis, commonly known as the tea plant, is an evergreen shrub of the Theaceae family, native to Southeast China [22]. Nowadays, tea is cultivated in over thirty countries across the world [2], including one single place in Europe - S. Miguel Island (Azores Archipelago, Portugal) [23]. Tea is the most ancient and widely consumed beverage in the world, with a per capita consumption of approximately 120 mL/day [24]. Notably, it has been used in traditional medicine for centuries due to its several claimed health benefits [1, 4, 20] which include the prevention and treatment of some diseases, including DM [1, 2].

Tea is the infusion prepared from the leaves or buds of the *C. sinensis*. Tea plant can originate four main types of tea, usually classified in three categories: unfermented (white and green teas), semi-fermented (oolong tea) and fully fermented (black tea) forms. This classification is based on the differences that occur in the collection and manufacture processes, resulting in different chemical compositions [25]. Upon harvesting, the leaves suffer an enzymatic oxidation process, commonly called "fermentation" [4, 20, 26]. This process occurs with exposure to air by a reaction, which involves the enzyme polyphenol oxidase (PO). As expected, according to the level of "fermentation", the types of tea have different chemical compositions (phenolic profiles) and organoleptic properties (appearances and tastes). To produce green tea, the fresh leaves are harvested and quickly steamed to inactivate PO, preventing the oxidation of catechins, and then rolled and dried [1, 2] (Figure 1). Thus, its composition is very similar to that of *C. sinensis* leaves.

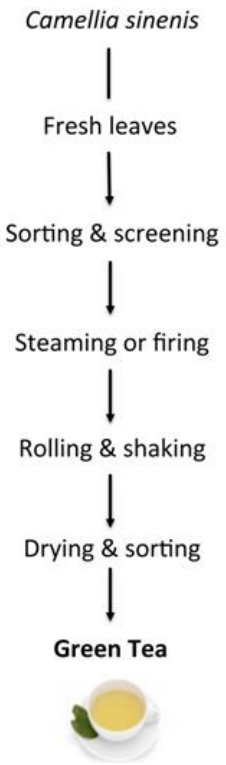


Figure 1. Schematic representation of green tea processing. In the production of green tea, the leaves are harvested and then quickly steamed or fired, to inactivate polyphenol oxidase (PO) and prevent oxidation, before drying.

CHEMICAL COMPOSITION OF GREEN TEA

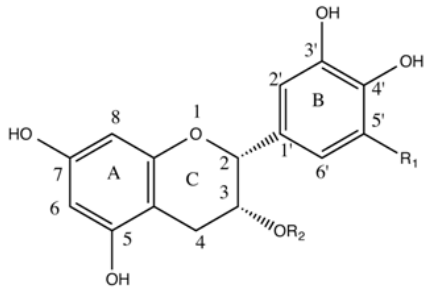
Green tea is generally prepared in a proportion of 1 g of tea leaves per 100 mL of boiling water. This preparation guarantees that the green tea has a very complex chemical composition, containing polyphenols (e.g. catechins and their derivatives), methylxanthines (e.g. caffeine, theophylline and theobromine), free amino acids (e.g. L-theanine), minerals and trace elements, organic acids, lipids, and other components [4, 20, 26]. However, the chemical composition of green tea varies according to the preparation since the tea phytochemicals are susceptible to extraction conditions (e.g. solvents, temperatures, times of extraction) [27, 28] In addition, several other factors such as geographical origin, climate, growing conditions, harvesting practices, and manufacturing processes [20, 29] can alter the chemical and organoleptic properties of the tea.

Green Tea Polyphenols

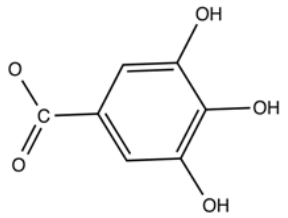
Polyphenols seem to be the most abundant and active group present in tea leaves. In addition, several studies highlight that they are responsible for much of the health benefits attributed to green tea [30, 31]. These phytocomponents are relatively abundant [1, 2]. In fact, 200 mL of green tea might contain up to 200 mg of polyphenols [32]. Catechins (or flavan-3-ols) constitute the most abundant class of phenolic compounds found in unfermented teas. The major catechins found in green tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin 3-gallate (EGCG) [1, 7, 20]. These compounds have a very high antioxidant power [31, 33]. The health benefits attributed to catechins are mainly due to its chemical structure. The major catechins are composed of three rings (two aromatic rigs, A and B, linked to a dihydropyran heterocyclic ring, C) and are characterized by the presence of several hydroxyl groups [34] (Figure 2). Their chemical differences are due to the presence of different groups attached to those rings [4, 34]. EC contains an ortho-di-hydroxyl group in the B ring (at carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3); EGC has a three

hydroxyl groups at carbons 3', 4', and 5' on the B-ring, while ECG has a gallate moiety esterified at carbon 3 of the C-ring and EGCG contains both the three hydroxyl groups at carbons 3', 4', and 5' on the B-ring and a gallate moiety esterified at carbon 3 on the C-ring.

The concentration of catechins is different in each type of tea. It has been shown that green and white teas have the higher levels of catechins [4, 29]. EGCG is considered the most abundant and active, and has been extensively studied [2, 35]. In addition, phenolic acids, flavonols, and/or flavones and their derivatives were also consistently found in green tea extracts [6, 23, 36]. The oxidation process of catechins, catalyzed by PO, results in the formation of theaflavins and thearubigins [29]. Theaflavins are composed by a bicyclic benzotropolone ring, and result from the dimerization of catechins. Thearubigins have oligopolymeric structures and can result of the hydroxylation of theaflavins. Interestingly, these compounds are less soluble and are responsible for black and oolong teas bitter taste and dark color [2, 36, 37].



Main catechin structure



Gallate group

	R1	R2
EC	H	H
EGC	OH	H
ECG	H	Gallate group
EGCG	OH	Gallate group

Figure 2. Chemical structure of the main green tea catechins. The major catechins are composed by two aromatic rings (A and B) and a dihydropyran heterocyclic ring (C). The (-)-epicatechin (EC) is constituted by an ortho-di-hydroxyl group in the B ring (at

carbons 3' and 4') and a hydroxyl group in the C ring (at carbon 3). Its ester derivative, (-)-epicatechin 3-gallate (ECG), differs in this structure by possessing an additional gallate moiety esterified in the C ring, at carbon 3. On the other hand, (-)-epigallocatechin (EGC) contains three hydroxyl groups on the B ring (at carbons 3', 4' and 5') and its ester derivative (-)-epigallocatechin 3-gallate (EGCG) additionally possesses an esterified gallate at the carbon 3 of the C ring.

The redox properties of polyphenols, which are associated with their chemical structures, are in the basis of the reported antioxidant properties of tea. The relationship between the content of pyrogallol and hydroxyl groups, and the presence of galloyl moieties, may be involved in the superoxide anion and hydroxyl radicals scavenging ability, respectively [1, 38]. Moreover, the number and position of the hydroxyl groups influence the antioxidant ability of flavonoids [34]. However, higher total phenolic component may not always directly represent a greater antioxidant capacity, since different phenolic profiles can yield different responses [39]. Fermented teas have a lower catechins concentration comparatively to unfermented teas but black and oolong tea have also considerable antioxidant properties, such as hydroxyl radical scavenging and nitric oxide suppressing [29]. Several studies suggest that tea catechins are effective scavengers of reactive oxygen species (ROS) [40, 41], and this is of extreme relevance for the control of oxidative stress (OS). It is know that OS is implicated in the establishment and progression of DM and its associated co-morbidities [42]. In addition, OS induces neuronal death, suggesting that it may be in the origin of DM-induced neurodegeneration [17]. Thus, there is a growing interest in the possible benefits of green tea against the neurodegeneration reported in DM individuals.

Besides the phenolic compounds, there are other phytochemicals that may contribute to the improvement of health by green tea, such as caffeine, theophylline, L-theanine, among others.

Tea Methylxanthines

Caffeine (Figure 3) is the main methylxanthine present in teas (1.0-3.5%) [29]. Theobromine and theophylline are other important methylxanthines present in tea (Figure 3).

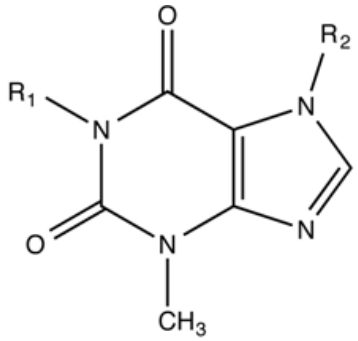
Caffeine is one of the most consumed psychoactive substances in the world [43], mainly due to its stimulant properties. Tea leaves are a major

source of dietary caffeine. Due to its chemical stability, the oxidation process does not affect its levels in tea [44]. However, the caffeine content in the various types of tea is not completely consensual. Different extraction conditions and distinct analytical methods may explain the controversy between absolute values. Some authors reported that fermented teas present greater caffeine content than green tea [29]. However, a recent study conducted by our research group showed that white tea can contain a higher concentration of caffeine than green tea [7]. Interestingly, some authors proposed that the lowest caffeine content in green tea contributes to its beneficial health properties [45], but this subject remains under debate.

Pharmacokinetic studies have shown that humans easily absorb caffeine and approximately 100% of bioavailability is achieved when taken by oral route. This methylxanthine is absorbed in the stomach and small intestine within 45 minutes after intake and reaches a maximum concentration in blood after 15-120 minutes [46]. After being absorbed, caffeine is distributed to various tissues, and is reported to stimulate the central nervous system (CNS). It acts through stimulation of adenosine receptors and competitively inhibits the action of adenosine in the cells, which results in an increased release of hormones such as norepinephrine, dopamine and serotonin [47]. Moderate levels of caffeine are reported to have some benefits to health. For example, caffeine seems to be a likely candidate against memory loss [48], and has a notorious neuroprotective potential [49]. Of note, the consumption of caffeine-containing beverages, in particular tea, is associated with a lower risk of developing T2DM [19]. Caffeine, in moderate doses, is beneficial to health due to antioxidant effects. It is know that antioxidants may reduce the amount of ROS, decreasing insulin resistance and beta cell dysfunction. In addition, a study in rats showed that this methylxanthine can interact with glucose transporters in adipocytes and act as an antagonist of adenosine receptors [50]. Others have also reported that caffeine has an antioxidant role, protecting against cellular damage, by decreasing lipid peroxidation [51, 52]. For instance, it was recently shown that moderate consumption of caffeine appears to be safe to the metabolic functioning of human Sertoli cells, and male fertility in general [53]. However, when caffeine is consumed in excess, it may lead to various deleterious health effects [54], such as coronary heart disease, reproductive disorders, and psychiatric disturbances [47]. Nevertheless, data on the role of caffeine on tea-associated health benefits remain largely unknown and more studies are needed.

Tea Amino Acids

L-Theanine (Figure 4) is non-proteinogenic amino acid that was first isolated from green tea leaves in 1940s by Sakato [55]. This free amino acid usually constitutes about 1-3% of the dry weight of tea, but this percentage may vary according to growing location and method of cultivation, tea grade, variety, processing and collection time [55]. For instance, reduced sunlight during tea growing has been shown to induce higher concentrations of L-theanine and lower contents of catechins [55]. Moreover, tea variety is also important; for example, *C. sinensis* var. *sinensis* is known to contain higher concentrations of L-theanine than C. *sinensis* var. *assamica* [55]. Nevertheless, green, oolong and black teas are reported to contain similar levels of L-theanine [56].



	R1	R2
Caffeine	CH_3	CH_3
Theophylline	CH_3	H
Theobromine	H	CH_3

Figure 3. Chemical structure of the three methylxanthines present in tea. Caffeine (1,3,7-trimethylpurine-2,6-dione), theophylline (1,3-dimethylpurine-2,6-dione) and theobromine (3,7-dimethylpurine-2,6-dione). They are all purine derivatives, with three methyl groups at positions 1, 3 and 7or two methyl groups at positions 1 and 3 or 3 and 7.

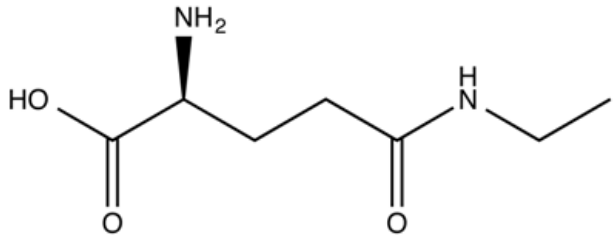


Figure 4. Chemical structure of L-theanine (N-ethyl-L-glutamine). This is an amino acid analogue of L-glutamate and L-glutamine that has an ethyl group at the amide nitrogen.

Some health benefits have been reported to this amino acid. For instance, it can be considered as a relaxing agent with antioxidant and neuroprotective effects [57, 58]. Metabolically, it is easily absorbed from the gastrointestinal tract and peak plasma concentrations are detected 30 minutes after administration [58]. In addition, it is partially transported to the brain via leucine preferring transporter system and can cross the blood brain-barrier (BBB) having protective and preventive effects on neuronal cell death [59]. Nevertheless, its pharmacology and modes of action remain relatively unknown.

Besides L-theanine, other amino acids can also be found in this beverage: L-glutamic acid, L-arginine, L-aspartic acid, L-glutamine, L-serine, L-tyrosine, L-alanine, L-asparagine, L-lysine and L-valine [60].

GREEN TEA AND DIABETES MELLITUS

DM is considered one of leading causes of morbidity and mortality worldwide. It is described as a metabolic disorder with several long-term complications that result from physiological and morphological alterations in tissues and organs throughout the organism [20, 61]. Hyperglycaemia is a hallmark of this disease as a consequence of impaired insulin secretion, insulin resistance, or both. Consequently, body glucose metabolism becomes deregulated. Some risk factors related to lifestyle, such as overweight and unhealthy diet, may contribute to development of this disease. Furthermore, due to the complexity of this disease, it has been established an intermediate state called prediabetes. It is characterized by elevated blood glucose levels,

though not high enough to be considered DM [12]. This prodromal stage is increasing among young people and is known as a major risk for the development of T2DM. T2DM is the most common type of DM, accounting for up to 90-95% of all cases diagnosed [12]. Age, obesity, cardiovascular diseases, and lack of physical activity are some conditions that increase the risk of developing T2DM [62] and thus promote prediabetes. Noteworthy, the clinical symptoms are frequently detected only in an advanced phase of the disease, allowing the progression of functional changes in cells and tissues that may not be reverted.

DM is incurable but there are many available strategies for its treatment. However, the side effects and the loss of effectiveness of some treatments are issues that must be take into consideration when discussing the therapy to this disease. In recent years, it has been encouraged the search of more efficient and cost-effective alternatives, recurring to dietary and lifestyle changes. In that search, natural products have arisen has a possible strategy.

In vitro Studies and Animal Models

Functional foods and nutraceuticals have been targets of great interest in the field of Food Science in order to complement or even replace current therapies. Green tea has been valued around the world due to its medicinal properties, and is being widely studied. Many health benefits have been attributed to tea [6-8, 20, 26] and green tea phytochemicals play an important role in contributing to the overall human health.

Green tea is an excellent source of potent antioxidants, being EGCG its main and well known antioxidant phytochemical. Animal studies and *in vitro* studies report elucidative data illustrating that green tea can be a very effective treatment to DM. Traditionally, green tea has been used to control glucose levels. *In vitro* studies in H4IIE rat hepatoma cells treated with EGCG, the major catechin of green tea, showed that EGCG is insulinomimetic, decreasing not only the production of glucose by these cells but also the expression of genes that control gluconeogenesis [63]. Similarly, Wolfram and collaborators [64] studied the influence of EGCG on glucose and lipid metabolism-related genes in the same cells. It was reported that this catechin reduced expression of genes involved in fatty acids synthesis, downregulated genes involved in gluconeogenesis, and increased genes involved in glycolysis and glucose transporter 1 (GLUT 1) [64]. Others suggested that catechins, mainly EGCG, and theaflavins help to prevent hyperglycaemia by enhancing insulin activity

and possibly by preventing damage in pancreatic beta-cells [65]. Moreover, in isolated pancreatic islet cells culture, the addition of EGCG improved the survival rate [66] and protected against cytokine-induced damage to the pancreatic beta cell line RINm5F [67]. The summary of the *in vitro* studies reported herein is presented in Table 1.

Table 1. Summary of the main effects of green tea and its bioactive components, as reported in *in vitro* and *in vivo* studies, to DM and neurodegeneration

		Green tea / Bioactive Component Tested	Main Remarks
***In vitro* Studies**	H4IIE rat hepatoma cells [63, 64]	EGCG	↓ Glucose production [63] ↓ Expression of genes that control gluconeogenesis [63, 64] ↓ Expression of genes involved in fatty acid synthesis [64] ↑ Genes involved in glycolysis [64] ↑ GLUT 1 [64]
	Fat cells [65]	EGCG (and theaflavins)	↑ Insulin activity [65] ↑Hepatoprotection [65]
	Pancreatic islets [66]	EGCG	↑ Survival rate [66]
	RINm5F cell line [67]		Protection against cytokine-induced damage [67]
	Hippocampal neuronal cells [68, 69]		↓ Development of dementia and neurodegenerative diseases [68] ↑ Neuronal viability [68]
***In vivo* Studies**	Rat model [70-79]	Green tea	↓ Blood glucose [70, 71] ↑ Biochemical and histopathological status [70] ↑ GSH levels [70] ↑ Glucose tolerance [71] ↓ Lipid peroxidation [71] ↑ Antioxidant potential [71] Prevent striatal dopamine depletion [75] ↓ Loss of substantia nigra dopaminergic neuron [75]

		Green tea / Bioactive Component Tested	Main Remarks
		EGCG	↑ Glucose tolerance [72] ↑ Glucose-stimulated insulin secretion [72] Preservation of islets of Langerhans structure [72] Pro-oxidant effects [73, 74] ↓ OS [76] ↑ Antioxidant defenses [76] ↑ Spatial cognition and learn ability [77] ↓ Cerebral amyloidosis [78]
		EC, EGC, ECG	↑ Redox status [79] ↓ Structural damage [79]

Legend: EGCG – (-)-epigallocatechin 3-gallate; EGC – (-)-epigallocatechin; ECG – (-)-epicatechin-3-gallate; EC – (-)-epicatechin; OS – Oxidative stress; GLUT1 – Glucose transporter 1; GSH – Reduced glutathione; ↓ - Decrease; ↑ - Increase/Improve.

Of note, some of the effects of tea components observed *in vitro* have, to some extent, been also reported *in vivo*. It has been reported that green tea reduces blood glucose levels and improves glucose metabolism in diabetic rats [80, 81]. Hyperglycaemia has been associated to oxidative stress (OS) and OS is involved in the development and progression of DM [82] illustrating a cyclic link between these events. It has been reported that DM-related hyperglycaemia amplify OS [83] by increasing the production of free radicals and/or by declining the antioxidant defenses [42]. Notably, OS is reported as a hallmark present in the early (prediabetic state) and late phase of DM [84]. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually lead to cell death [85]. Glucose oxidation, non-enzymatic glycation of proteins, oxidative degradation of glycated proteins and the mitochondrial respiratory system form free radicals in diabetic individuals [42]. Moreover, it has been shown that reactive oxygen species (ROS) are produced in various tissues under diabetic conditions [86]. Thus, antioxidants that scavenge ROS may be of great interest to prevent the onset and /or the progression of diseases that are associated with oxidative unbalance.

The antidiabetic and antioxidant activities of green tea were reported in several animal models studies (summarized in Table 1). For instance, a study performed in streptozotocin (STZ)-induced diabetic rats with hepatic injury

showed that the treatment with green tea during 8 weeks was able to reduce the blood glucose level and improve the biochemical and histopathological status of these rats [70]. In addition, green tea consumption was able to increase the reduced glutathione (GSH) levels [70]. This was an important data since GSH is the major endogenous antioxidant produced by the cells, thus playing an important role in the neutralization of free radicals and ROS [70]. Thus, the daily consumption of green tea improved the antioxidant status of rats with STZ-induced DM. In another study, alloxan was used to induce a diabetic state in rats [71]. Alloxan is a glucose analogue, which accumulates in pancreatic beta cells and inhibits the secretion of insulin [87]. This substance generates ROS by a redox reaction with diluric acid, in the presence of glutathione. The auto-oxidation of dialuric acid generates free radicals. Notably, the continuous treatment with an aqueous solution of green tea polyphenols (500 mg per kg of body weight) was able to increase the glucose tolerance in normal rats, at 60 minutes [71]. In addition, a dose level of 100 mg per kg of body weight of green tea polyphenols reduced the serum glucose level in alloxan diabetic rats [71]. The lipid peroxidation was decreased and the antioxidant potential was improved, namely by improvements in superoxide dismutase and glutathione levels [71]. Diets supplemented with EGCG during 10 weeks in *db/db* mice, a model of obesity, diabetes and dyslipidemia, showed that this catechin improved glucose tolerance, increased glucose-stimulated insulin secretion and preserved the islets of Langerhans structure [72]. Very recently, our research group has reported that daily consumption of white tea improves glucose tolerance and insulin sensitivity in STZ-prediabetic rats [9, 18]. Moreover, tea consumption altered the glycolytic profile, improved oxidative status, and increased the antioxidant power of cerebral cortex of prediabetic rats [18]. In addition, this type of tea was also able to improve cardiovascular metabolic state of prediabetic rats [9]. Green and white teas are very similar, with respect to processing, although it has been reported that green tea presents lower levels of antioxidants than white tea [7]. Certainly, both types of tea cause similar effects. In this context, the dietary supplementation with the major green and white teas catechins can be a nutritional strategy in the prevention and treatment of DM.

Several works have reported that green tea phenolic compounds, namely catechins, are potent antioxidant agents, scavenging ROS [41] and metal chelators [88]. As previously referred, the chemical structure of tea catechins is associated with its antioxidant properties [34]. It has been suggested that content of pyrogallol and hydroxyl groups influences the superoxide anion radical scavenging ability [1]. Furthermore, the presence of galloyl moieties

improves the ability to quench hydroxyl radicals [1]. Several structures appear to be important for these antioxidant activities of tea polyphenols, including the ortho-3',4'-dihydroxyl (catechol) group in the B-ring, that promotes the formation of a stable phenoxyl radical due to effective electron delocalization [89] or the 3',4',5'-trihydroxyl group in the B-ring, a gallate group esterified at the 3 position of the C-ring, and hydroxyl groups at the 5 and 7 positions of the A-ring [90].

Although the antioxidant capacity of green tea polyphenols has been reported, recent studies highlight that when polyphenols are present in high concentrations they can also exert pro-oxidant effects. Tea catechins also possess the ability to generate ROS due to their instability and undergo auto-oxidative reactions, in typical cell culture conditions [91]. Moreover, the stability of EGCG is dependent on the total concentration of catechins, the pH of the system, the presence of oxygen, and the temperature of the incubation [91]. All these effects have been tested and reported in animal models. A study conducted by Yun and collaborators [74] showed that an intraperitoneal treatment with EGCG (5 mg per kg per day) during 4-days, impaired the beta-cell response to high glucose in the diabetic rats. On the other hand, treatment of CF-1 mice with a single oral dose of 1500 mg per kg EGCG reduced the animals' survival by 85% and the administration of daily doses of 500 and 750 mg per kg decreased survival by 20% and 75%, respectively [73].

Epidemiological and Interventional Clinical Studies

The probability of developing DM depends on several factors, including the lifestyle behavior. Of note, an unbalanced diet highly increases the risk for developing this disease. Thus, the link between nutrition and DM has been clearly suggested and is well established [92]. Green tea has received special consideration due to the beneficial effects of its phytochemicals to health. In fact, this type of tea appears as a good antidiabetic agent. However, in studies in humans, the conclusions are not so elucidative as *in vitro* and *in vivo* studies. Some studies in humans suggest that regular consumption of green tea contributes to a protection against the development of DM [93-97]. Other studies report that the consumption of this beverage has no association with the disease [98]. These studies are very important but there are some drawbacks that must be considered. For example, the studies discussed herein were performed in very different populations, which greatly vary in terms of age, countries and lifestyles. Moreover, green tea is generally prepared by

using 1 g of tea leaves per 100 ml of boiling water but differences can arise from preparation and the protocol for consumption, as well as metabolization. Moreover, the differences between the animal species subjected to research and humans may also hamper the correct interpretation, extrapolation and practical application of the results and conclusions.

A retrospective cohort study conducted by Iso and collaborators [96] evaluated the relationship between green tea consumption and the risk for T2DM. The authors concluded that people that drink 6 or more cups of green tea per day are less likely to develop T2DM than those who drink less than one cup of this beverage per week [96]. A previous cross-sectional study showed an unclear association between green tea consumption and glucose tolerance [97]. This study evaluated 3224 Japanese men and concluded that impaired fasting glucose was less frequent in those who consumed more green tea. Nevertheless, the association between green tea consumption and glucose tolerance is not clear. Another cohort study of middle-aged and older women reported that women who consumed 4 or more cups of tea per day had a 30% lower risk of developing T2DM [95]. In a double-blind randomized study of decaffeinated green tea extract performed in adults with T2DM, during 3 months, the data showed that there were no significant effects on glucose levels in adults with T2DM at the end of the treatment [98]. In addition, the intake of green tea extract containing 300 mg EGCG, during 12 weeks by healthy volunteers, showed a reduction of plasma glucose and insulin [94]. Rizvi and collaborators [93] also reported that EGCG may protect against the development of long-term complications that arise from DM, reducing OS in the erythrocytes. An overview of human studies focused on the effects of green tea consumption on DM is provided in Table 2.

GREEN TEA AND NEURODEGENERATION

The mammalian brain depends upon glucose as one of its main source of energy. Thus, it is expectable that glucose dysfunction promoted by DM, namely T2DM, may be responsible for brain damage and/or dysfunction [20, 99]. In fact, DM is implicated in the development of cerebrovascular disease and other neurological comorbidities, such as cognitive dysfunction and dementia [13]. Moreover, it was reported that the risk of cognitive decline and neurodegeneration are increased at an early stage, before the onset of disease [15] though the role for glucose dysfunction in these events remains largely unknown. Pathological alterations in CNS are also associated to both types of

DM [14]. Notably, it has long been discussed that some brain areas are more vulnerable to deregulation of glucose metabolism. For example, cortical neurons and astrocytes are reported to be more susceptible than cells from striatum or hippocampus [100]. Furthermore, the cerebral cortex is particularly sensitive to deregulated metabolism [101], since it is quite vulnerable to OS due to its high consumption of oxygen, the abundance of easily oxidizable fatty acids, and the relative low presence of antioxidant defenses [102]. Interestingly, the cerebral cortex is greatly affected by AD, and several studies have demonstrated that AD and DM are connected [17]. In addition, OS is reported as an important factor that contributes to aging processes and neurodegeneration [102].

In vitro studies, animal data and human epidemiological studies provided compelling evidence that drinking tea may have pharmacological benefits in the protection of the brain. These findings are described below. *In vitro* and animal studies are summarized in Table 1, whereas human studies are summarized in Table 2.

In vitro Studies and Animal Models

Green tea and its bioactive compounds have biological and pharmacological activities very relevant to human health. So, consumption of green tea may be an advantage in the protection of the brain, particularly against metabolic diseases. Many studies have shown that tea consumption is inversely correlated with the incidence of dementia and neurodegenerative diseases [68, 69, 107, 108] and it decreases the prevalence of cognitive impairment [77, 109, 110]. In recent years, many research groups have been trying to understand the molecular mechanisms by which green tea acts and protects the brain. Besides its antioxidant activity, neuron viability can also be improved by the modulation of signal transduction pathways, cell survival/death genes, and mitochondrial function [68]. Several authors consider the main catechin, EGCG, as one of the greatest natural antioxidants and the most pharmacologically active compound in the unfermented teas [4, 20, 26]. Several of the neuroprotective activities of tea are associated with this powerful catechin. EGCG is reported to cross the BBB and thus, it can easily reach the brain parenchyma [111] exerting its effects. The neuroprotective effect of tea polyphenols was shown in animal models of neurological disorders, through improving age-related cognitive decline and protecting against cerebral ischemia/reperfusion injuries [112]. A study performed in a

mice model of Parkinson's disease (PD) induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), showed that green tea extract or isolated EGCG were able to prevent striatal dopamine depletion and the loss of substantia nigra dopaminergic neuron [75]. Another study in Wistar rats showed that continuous administration of EGCG (2 mg per kg of body weight) for 30 days was able to improve rats' antioxidant defenses, ameliorating the age-induced OS levels in their brains [76]. A long-term administration of green tea catechins or EGCG showed to improve spatial cognition and learning ability in rats [77]. Similarly, it was also able to reduce cerebral amyloidosis in AD transgenic mice [78]. Recently, with the aim of studying other bioactive compounds of green tea, some authors investigated the anti-aging effects of a catechin-rich green tea extract, free of caffeine and L-theanine, and low content of EGCG [79]. The results showed that EC, EGC and ECG are effective protectors of proteins and lipids against oxidative changes related to aging [79]. Thus, this demonstrates that other catechins are able to protect against oxidative damage.

Table 2. Epidemiological studies regarding green tea consumption or tea phytochemicals, and its effects to DM and neurodegeneration

Type of Study	Population Studied	Tested components / Amount	Main Remarks	Citation
Retrospective cohort	17413 Japan adults	≥ 6 cups green tea / day	↓ Risk of incident T2DM	[96]
Cross-sectional	3224 Japanese men	≥ 5 cups green tea /day	↓ Glucose tolerance	[97]
Cohort	38018 women	≥ 4 cups green tea /day	↓ 30% risk of developing T2DM	[95]
Double-blind randomized	49 subjects	375 mg tea (150 mg green tea catechins + 75 mg black tea theaflavins + 150 mg other tea polyphenols /day)	No effects	[98]
Case-control	23 healthy males	Green tea extract (of which 300 mg was EGCG)	↓ Plasma glucose and insulin	[94]
Case-control	31 T2DM subjects	EGCG, EGC, ECG, EC (each 10^{-5}-10^{-8} mol/L)	↓ OS in erythrocytes	[93]
Cross-sectional	1003 Ederly Japanese subjects	≥ 2 cups green tea /day	↓ Cognitive deficits	[103]

Type of Study	Population Studied	Tested components / Amount	Main Remarks	Citation
Case- control	557 subjects	≥ 2 cups green tea /day	↓ Risk of developing PD	[104]
Prospective cohort	25000 Finnish adults	≥ 3 cups green tea /day	↓ Risk of developing PD	[105]
Double-blind randomized	24 subjects	L-theanine (250 mg) + caffeine (150 mg)	↑ Cognitive performance	[106]

Legend: T2DM – Type 2 diabetes mellitus; EGCG – (-)-epigallocatechin 3-gallate; EGC – (-)-epigallocatechin; ECG – (-)-epicatechin-3-gallate; EC – (-)-epicatechin; OS – Oxidative stress; PD – Parkinson diseases; ↓ - Decrease; ↑ - Increase/Improve

In vitro findings reported that neuronal cell death caused by the neurotoxins 6-hydroxydopamine (6-OHDA), and amyloid beta can be prevented by green tea catechins [69, 113]. Notably, green tea catechins are reported to interfere in the modulation of several protein kinase-signaling pathways modulating cellular functions. For example, the neuroprotective action exerted by EGCG may be due to its involvement with protein kinase C (PKC) [68]. This protein is involved in the regulation of cell survival, apoptosis, long-term potentiation, and consolidation of different types of memory [68]. EGCG is able to activate PKC, illustrating that this mechanism may be responsible for most of the neuroprotective capacity of the main tea catechin [68, 113]. The mitogen-activated protein kinases (MAPK), phosphatidylinositide 3′-OH kinase/AKT and protein kinase A signaling cascades, are other pathways reported to be activated by the action of green tea catechins [114]. These pathways may exert neuronal protection functions and are essential for neuronal differentiation and survival [115].

The pathological development of AD begins with the abnormal processing of the amyloid precursor protein (APP), leading to the excessive accumulation of the amyloid beta (Aβ) peptide in the brain, and consequent formation of senile plaques. *In vitro* observations show that EGCG inhibits OS and neurotoxicity [69], and EC reduces the formation of Aβ-fibril formation [116] illustrating possible mechanisms by which these catechins exert their protective effective against AD. Moreover, the proteolytic processing can be regulated APP *in vivo* and *in vitro* by EGCG [117], suggesting that green tea polyphenols might be potentially promising therapeutic agents for neurodegenerative diseases.

L-theanine is the major free amino acid found in green tea and has been reported as antioxidant and neuroprotective against PD-related neurotoxicants and may be clinically useful for preventing PD symptoms [108].

Epidemiological and Interventional Clinical Studies

Human epidemiological studies suggest that the pharmacological benefits of tea consumption may help to protect the brain. A cross-sectional study in Japan explored the association between consumption of green tea and cognitive function in elderly Japanese subjects. It was reported that the consumption of two or more cups per day (approximately 100 mL per cup) of green tea is associated with lower prevalence of cognitive impairment [103]. In a case-control study in the United States, it was found that subjects who consumed two or more cups of tea per day have a decreased risk of developing PD [104]. Likewise, a prospective cohort study with more than 25.000 Finnish adults aged 25–74 years found that drinking three or more cups (200 mL per cup) of tea was associated with a reduced risk of PD [105]. Therefore, is extremely important well-designed controlled studies to assess the probable reduction of developing neurodegenerative diseases for those who drink green tea. Undoubtedly, the biological effects of green tea consumption may benefit subjects with neurodegenerative diseases, but more studies are necessary to investigate the effectiveness of green tea in humans. Moreover, the different mechanisms of its neuroprotective function must be unveil to better understand the potential benefits and risks associated with tea drinking.

The major free amino acid found in green tea, L-theanine, has been a focus of attention in the last years. This amino acid has the ability to pass through BBB, and remains in the brain for, at least 5 hours, after administration [118]. Moreover, L-theanine may influence the secretion and function of neurotransmitters in the CNS [119]. It possesses antioxidant [108, 120] and neuroprotective properties [57, 58]. It also improves memory function [121] and prevents memory impairment induced by cerebral ischemia [122]. A study involving thirty-five individuals evaluated L-theanine effect on mental state [123]. The results showed that L-theanine has a significant effect on the general state of mental alertness or arousal. In addition, this amino acid has relaxing capacity without inducing drowsiness [123]. Besides L-theanine, other bioactive compounds, such as caffeine, are also present in considerable amounts in green tea and may play a key role for the beneficial health effects reported for this beverage [4, 7]. A double-blind randomized study [106] evaluated the acute cognitive and mood effects of L-theanine, caffeine, and both in combination. L-theanine combined with caffeine provides better cognitive outcomes than in isolated form, but the mechanisms underlying the effects are not known [106].

All of these studies show that green tea and its bioactive compounds are associated with reduced risk of DM and consequently reduced risk of neurodegeneration. However, the mechanisms of action remain largely unknown. In sum, green tea consumption and/or the administration of its phytochemical compounds ameliorates glucose metabolism, improving insulin sensitivity and decreasing insulin resistance. Furthermore, green tea is able to protect the brain by preventing cognitive impairment, neuronal loss, neurodegeneration and dementia. Nevertheless, all these effects have not yet been sufficiently evaluated. Furthermore, *in vivo* studies are needed to clarify whether green tea and its bioactive compounds, reach the brain, at sufficient concentrations and with enough bioactivity to promote the effects reported *in vitro*. Noteworthy, *in vitro* and animals' studies consume higher doses of tea than those consumed by humans, because the experimental conditions are generally optimized for the evaluation of a protective effect. Moreover, it is believed that tea's consumption benefits are due to the synergistic action of several compounds. Finally, based on these observations, green tea consumption is recommended to the normal population.

CONCLUSION

DM is a pandemic disease, affecting an enormous number of people worldwide. There are also several comorbidities associated with this disease that highly increase its complexity. Compelling evidence illustrates that DM affects brain function, being hyperglycaemia and OS key mediators of DM-induced neurodegeneration. Green tea has been used in traditional medicine to prevent and treat several health problems and diseases, including DM. Currently, this pleasant, economical and popular beverage appears as a potential functional food product that contains several nutraceuticals, such as catechins, caffeine and L-theanine with documented health benefits, namely antidiabetic, antioxidant and neuroprotective properties. However, more studies are necessary to explain the molecular mechanisms involved in the protective effects of green tea and its phytochemicals. DM and associated brain damage can be prevented by modification of lifestyle, particularly by alterations in diet. Thus, green tea consumption is proposed herein as an excellent dietary habit to diabetic individuals and can protect the brain from DM-related neurodegeneration.

LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AD	Alzheimer's disease
APP	Amyloid precursor protein
Aβ	Amyloid beta
BBB	Blood brain-barrier
CNS	Central Nervous System
DM	Diabetes mellitus
EC	(-)-epicatechin
ECG	(-)-epicatechin-3-gallate
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin 3-gallate
GLUT1	Glucose transporter 1
GSH	Reduced glutathione
MPTP	*N* -metil-4-fenil-1,2,3, 6-tetra-hidropiridina
OS	Oxidative stress
PD	Parkinson's disease
PKC	Protein kinase C
PO	Polyphenol oxidase
ROS	Reactive oxygen species
STZ	Streptozotocin
T1DM –	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

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