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Tea Polyphenolics and their Effect on Neurodegenerative Disorders- A Review

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Review Article

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ABSTRACT

Tea consumption is varying its status from ancient beverage and a lifestyle habit, to a nutraceutical with possible prospective neuroprotective actions beneficial to human health. Quality tea production is beneficial for its effects on human health, which includes controlling of several diseases with its high antioxidant properties. Several evidences suggest that oxidative stress generates reactive oxygen species and causes inflammation to play a role in neurodegenerative diseases, indicative of the importance of radical scavengers and in accordance with the current view that tea polyphenols may have an impact on neurobiological problems in individuals of advanced age. Thus green tea polyphenols may be considered as therapeutic agents in dementia related diseases, and can be serve as neuroprotective agents in neurodegenerative disorders of Parkinson's disease and Alzheimer's disease. So far no single cure has been found however, one supplement that seems to have health benefits in a vast range of areas is tea and tea extract. In this review neuroprotective mechanism of the green tea polyphenols are examined and discussed.

INTRODUCTION

There is rising attention in the world regarding natural product researches as plants are always known to be the traditional source of medicine due to the presence of natural products with high chemical diversity. In natural system plants face a plethora of antagonism and thus evolved myriad of defence mechanisms by which they are able to cope up with various kinds of biotic and abiotic stresses. These large arrays of natural products, in concern here, are referred to as secondary metabolites as they are not directly involved in growth, development and metabolism even though they are derived from primary metabolism. Apart from combating stress, they also function as attractants due to the presence of compounds responsible for conferring bright and attractive colour of fruits and flowers. In addition to their physiological roles in plants, they are also extensively exploited as pharmaceuticals, food additives and cosmetic products. There are three major groups of secondary metabolites namely terpenes, phenolics and N and S containing compound. Terpenes which are being composed of 5-C isopentanoid units are toxins and feeding deterrents to many herbivores. Phenolics synthesized primarily shikimic acid pathway which have several important defensive role in the plants. Members of the third major group which are basically nitrogen (N) and Sulphar(S) containing compounds are synthesized principally from common amino acids [1]. Tea is one of the most popular beverages taken throughout the world is a very good source of important secondary metabolites like monoterpenoids, carotenoids and catechins etc. Monoterpenoids and carotenoids are most important constituents of tea aroma and flavor. The formation of tea aroma is because of the synthesis of volatile monoterpenoids and carotenoids, while catechins are responsible for the beneficial health effects of tea.

Being the second most popular beverage in the world, tea drinking originated in China around 4000–5000 years ago. Today, 3 billion cups of tea are consumed everyday by millions of people all over the world. Now days, it is being cultivated in at least 30 countries around the world. All varieties and cultivars of the tea plant is the member to a single species, *Camellia sinensis* [2], a member of Theaceae family, cultivated across the world inspecially in the tropical and subtropical regions. Tea beverage is an infusion of the dried leaves of the plant. Tea is an evergreen shrub or that can grow to a height of almost 30 feet, but is usually

maintained to a height of 2.5 feet in cultivation. The shrub is heavily branched with dark-green, hairy, oblong, ovate leaves which is being cultivated and preferentially picked up as young shoots. Freshly harvested tea leaves are processed differently in different parts of the world to produce black tea which is 78% of total produce followed by green tea 20% and oolong tea 2% [3]. Though they all are originating from the same plant, but have their unique taste and health benefits arising from the differences in processing [4].

General Description of Tea

Green tea has been widely consumed by the Japanese and Chinese populations for centuries owing to its outstanding medicinal importance. The beneficial effects of green tea are attributed to the presence of polyphenol compounds and their derivatives; a major type of secondary compounds, particularly the catechins, which contributes 30% of the dry weight of green tea leaves [5,6]. To decelerate enzymatic activity of polyphenolic oxidase, a process that essentially converts the polyphenols in their monomeric forms, fresh tea leaves from the plant *Camellia sinensis* are steamed and dried to produce green tea. Black tea, on the other hand, is reproduced by extended fermentation of tea leaves which results in the production of polymeric compounds namely arubigins and theaflavins. Another partially fermented product, the oolong tea, contains a mixture of the monomeric polyphenols [5]. Although all the varieties of tea contain significant amount of caffeine measured from 3% to 6%, it is unaffected by the different processing methods [7].

Green Tea Biochemistry

Green tea is enriched with flavonoids which are the largest group of polyphenols. Flavonoids are generally divided into anthocyanins and glycosylated derivative of anthocyanidin, present in colorful flowers and fruits and anthoxantins which are further divided into several categories including flavones, isoflavones, flavanols, flavans, and flavonols [2] **(Figure 1).**

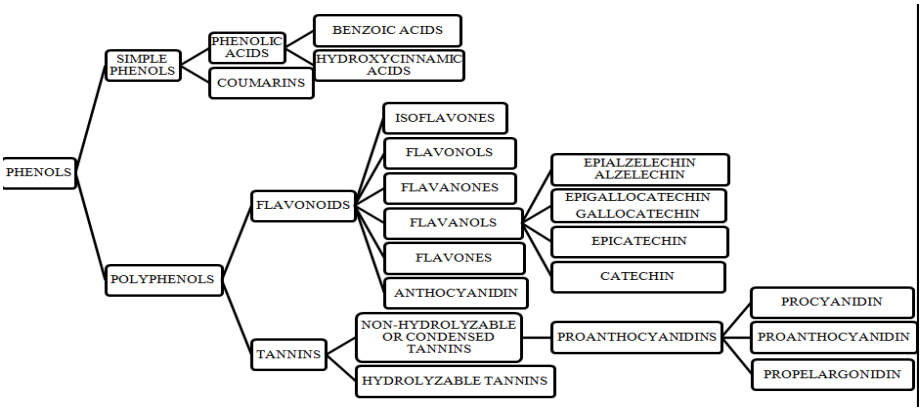

Figure 1. Classification of phenols found in nature.

The natural polyphenols in green tea include epicatechin (EC), (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3- gallate (ECG) **(Figure 2).** Other minor catechins present in tea including (+)-catechin (C), (+)-gallocatechin (GC), (-)-gallocatechingallate (GCG), (-)-catechingallate (CG) [8]. Comparative antioxidant activities among tea catechins has been found to be (-)-epigallocatechin-3-gallate EGCG = (-)-epicatechin-3-gallate (ECG) > (-)-epigallocatechin (EGC) > (-)-epicatechin (EC) [9]. Thus EGCG has the highest concentration followed by EGC, ECG and EC in decreasing order [10]. Green tea catechins have been found to be more efficient as radical scavengers than vitamin E and C [9,11] among them EGCG accounts for more than 10% of the extract dry weight [12]. Most of the health benefits of green tea are mainly due to its antioxidant properties and in scavenging of reactive oxygen species, the ability conferred by polyphenols and catechins [13]. These properties are attributed due to the property of the phenolic hydroxyl groups present in the B-ring in ungalloylatedcatechins (EC and EGC) **(Figure 3)** and in the B- and D-rings of the galloylatedcatechins (ECG and EGCG) [14]. The antioxidant as well as radical scavenging properties are conferred by the presence of the 3,4,5-trihydroxy B-ring [9,15] Metal-chelation property of green tea catechins are also significant contributors to their antioxidant property [16-18].

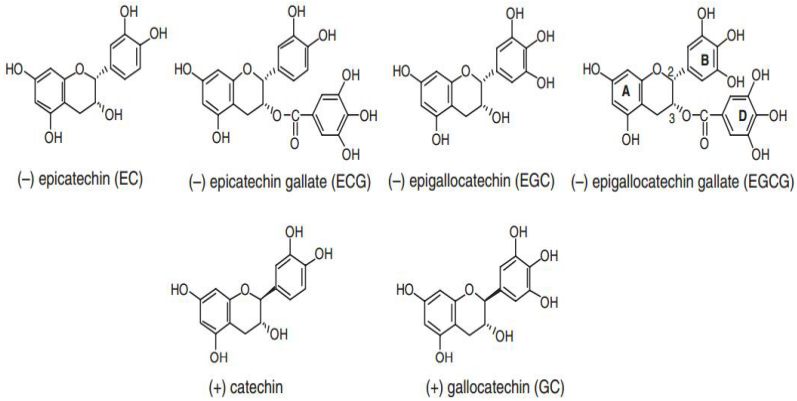

Figure 2. Chemical structure of major catechins present in green tea [124].

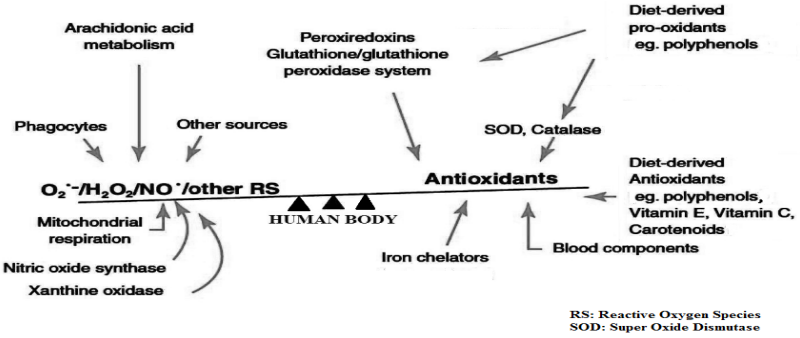

Figure 3. Free radical balancing by free radical scavangers.

Green Tea and Health

The health potential of green tea is known worldwide. Tea especially green tea with rich flavonoid compounds consisting high antioxidant activity through inhibition of oxidative damage of lipoprotein and DNA [19]. Green tea and constituent catechins are evaluated regarding their antioxidant properties against a number of diseases associated with reactive oxygen species (ROS), including cancer, cardiovascular and neurodegenerative diseases. In addition to the cancer preventive properties, green tea and EGCG have been shown to be anti-angiogenic [20,21], anti-mutagenic [22,23], hypo-cholesterolemic [24] and prevents the making of atherosclerotic plaques [25]. Green tea has anti-diabetic effects in animal models which are supported by its insulin resistance [26] as well as its ability in promoting energy expenditure [27] in animal body. Other health benefits of green tea include antimicrobial [28], antiviral [29], anti-aging [30] and anti-inflammatory activities [31]. The results indicate that green tea catechins have a great potential as therapeutic agents [32].

Several epidemiological studies and studies in animal models have proved that green tea protects against various cancers including skin, breast, prostate and lung [33,34]. Animal studies have shown that green tea catechins are effective inhibitors of some potent mutagens like 2-nitropropane [35]. Epidemiological studies have indicated that lower incidence of cancer is highly associated with high intake of green tea [36]. Total antioxidant power of the plasma, can be a good marker of absorption. Benzie et al. [37] showed rapid absorption and increase of green tea catechins in the plasma and urine of healthy individuals after intake of green tea and the antioxidant activity of plasma was also increased. However the in vitro anti-carcinogenic effect of tea antioxidants are unlikely to be realized if tea catechins are inactivated in the gut or are not well absorbed. It was found that EGCG is quite stable in the stomach and small intestine and present in the large intestine after 8 h after a single dose of 50 mg of EGCG administration to rats [38]. Absorbed catechins are bio-transformed in the liver to conjoined metabolites, i.e., glucuronidated methylated, sulfated derivatives.

The growing interest in drinking tea, especially green tea, all over the world, can be connected with the antioxidant activity of the tea polyphenols, fighting the harmful influence of environmentally generated free radicals [39]. Many sources contribute to the production of free radicals. Most reactive oxygen species (ROS) like, superoxide, hydroxyl and peroxyl radicals, come from the endogenous sources as by-products of normal and essential metabolism, like energy production from mitochondria or the detoxification reactions involving the liver cytochrome P-450 enzyme system. These radicals are unstable oxygen compounds with an unpaired electron in the atomic electron shell. Exogenous sources including exposure to cigarette smoke, environmental pollutants such as release from automobiles and industries, excess alcohol consumption, asbestos, exposure to ionizing radiation, and different microbial infections [40-42]. Since all molecules tend to have electron pairs, the radicals react intrusively with other molecules, trapping electrons away from them and cause oxidative damage by destroying their structure through chain reactions. In a healthy organism the chain reaction is interrupted by antioxidants which are known as 'radical traps' [43]. Cellular components like lipid, protein and DNA are among the victims of free radicals. The level of oxidative stress is determined by the balance between the rate of oxidative damage and the rate of its efficiency in repairing. In human body, some defense mechanisms are present to inhibit the oxidation and damage, called 'antioxidant enzyme system' of cell like superoxide dismutase (SOD), catalase and glutathione peroxidase. Thus, there is equilibrium between pro-oxidative and antioxidant process and oxidative stress results in breaking this equilibrium in favour of free radicals [44]. The activity of superoxide dismutase (SOD) and the expression of catalase increased in the serum and aorta respectively [45,46] in addition to decrease in the level of Malondialdehyde, a marker of oxidative stress [47] as a result of green tea intake. Consequently, green tea catechins affect lipid metabolism by different metabolic pathways and helps to prevent the appearance of atherosclerotic plaque, decrease in triglycerides and cholesterol levels [48,49], increase in fat excretion [49]. Thus, long-term feeding of tea polyphenolics may affect lipid metabolism and help to reduce obesity related troubles and simultaneously the risk of associated diseases such as diabetes and coronary diseases may be reduced.

Health potential of tea is highly associated with its antioxidant capacity. Biological antioxidants like catechins possess free radical scavenging properties [50-52], which can scavenge both superoxide and hydroxyl radicals, as well as the 1,1-diphenyl-3-picrylhydrazyl radical (DPPH), peroxyl radicals, nitric oxide ion, carbon-center free radicals, singlet oxygen and lipid free radicals by prevention of the nitration of tyrosine [50,52-56]. Moreover, catechins can help in chelation of metal ions like copper (II) and iron (III) in complexes and prevent the generation of potentially damaging free radicals [57] in much higher efficiency than vitamin E

and C [50,52]. Along with the radical scavenging property of green tea, because of the antioxidant property of tea, it is also related with other mechanisms like depolarization of electrons, making of intramolecular hydrogen bonds [58], rearrangement of the molecular structure [14,59]. These compounds may also prevent oxidative stress by chelation of free copper and iron, which is responsible for catalyzing the formation of reactive oxygen species in vitro [60,61]. Pellegrini et al. [62] compared different beverages in terms of different antioxidant assays and reported FRAP values of 18 mmol Fe2+/L for green tea, 10.09 mmol Fe2+/L for black tea (Aglianico, red). Depending on the mode of extraction of polyphenol from sample, Gramza et al. [63] found higher polyphenol content and antioxidant activity in green tea ethanol extract than green tea aqueous extract.

Oxidative Stress as a Critical Event in the Generation of Alzheimer's Disease

Alzheimer's disease (AD) is an acute neurodegenerative disorder named after its discoverer, German psychiatrist, Alois Alzheimer [64]. Although symptoms differ in individuals, Alzheimer's disease has some common symptoms. Early symptoms are often mistakenly taken to be 'age-related' concerns or manifestations of stress [65]. Symptoms at an early stage including difficulties in remembering recent incidents and as the disease progresses, symptoms like confusion, irritability and aggression, mood fluctuation, trouble with language, and long-term memory loss appear [65,66]. Though the actual cause of the disease is still not comprehensible, several competing hypotheses are proposed. The oldest hypothesis, on which the presently available drug therapies are based on, is the 'Cholinergic Hypothesis' by Francis et al., [67], which proposes that the disease is caused by reduced synthesis of the neurotransmitter acetylcholine.

Based on the Cholinergic Hypothesis, the reduction in availability of acetylcholine, one of the main neurotransmitter responsible for transmitting neuron signal is due to the sudden super activity of acetylcholinesterase (AChE) enzyme which cleaves the acetylcholine into choline and acetate, a frequent phenomenon that occurs during the progression of Alzheimer's disease. Another hypothesis is the amyloid hypothesis which postulates that amyloid β (Aβ) depositions are the primary cause of the disease [68]. Although, Schmitz et al., [69] concluded that the accumulation of amyloid plaques does not relate well with neuron loss. This buttresses the tau protein hypothesis, which postulates the tau protein abnormalities initiate the disease cascade. In this case, hyperphosphorylated tau begins to pair with other threads of tau, thus, they form neurofibrillary tangles inside nerve cell bodies [70] afterward the microtubules disintegrate and simultaneously damaging neuron's transport system [71]. This defect of biochemical signaling between neurons may lead to apoptosis of the cells later [72].

Remedies of Alzheimer's Disease

Since Alzheimer's disease is hard to cure, it is highly important to discover new remedies that delays the onset or reduces the risk of occurrence of the disease. Several plant alkaloids have been proved to be the potent inhibitor of the enzyme. Although tacrine, donepezil, rivastigmine and galanthamine are accepted as existing medication by the Food and Drug Administration in the United States [73] but synthetic drugs have side effects too. Several AChE inhibitors are being studied for the treatment of Alzheimer's disease but until now, no drug of choice for the treatment of this disease has been decided. The search for new AChE inhibitors from different natural products is highly commendable as nature is a great source of biological and chemical diversity. In vivo studies showed that green tea improves impairment of memory, cognitive abilities and reduces Alzheimer's disease. So it will be interesting to see whether green tea rather green tea polyphenols have direct inhibitory effect on AChE or not.

Although there is no epidemiological evidence on human studies regarding the benefit of green tea on progression as well as prevention of Alzheimer's disease, many studies in animal and cell culture models support that EGCG from green tea may target several prospective points associated with deterrence of Alzheimer's disease. It is assumed that histopathologic alterations in Alzheimer's disease are favored by the neurofibrillary tangles and senile plaques formed from amyloid β peptide, have a role in the early onset and progress of Alzheimer's disease [74]. Thus amyloid β peptide is toxic to neurons through several mechanisms like apoptosis, mitochondrial dysfunctionality or through the release of the nuclear transcription factor like NF-κB [75]. Moreover the gathering and toxicity of the amyloid β peptide involved in blocking of trasitional metals [76-79], accumulation of hydrogen peroxide radicals [80] and accumulation of free radical of oxygen [81] that eventually leads to neuronal apoptosis [82]. Accordingly, there are considerable proofs that oxidative stress is a primary and critical event in the progression of Alzheimer's disease [83]. Studies on tissues proved that the levels of malondialdehyde, 4-hydroxynonenal, isoprostane i.e. lipid oxidation products; protein carbonyl, nitrotyrosine like protein oxidation products and rate of DNA oxidation are raised in the brain in Alzheimer's patients [84-89].

Plants Including Tea as a Therapy of Alzheimer's Disease

The protective effect of green tea EGCG may involve its radical scavenging as well as iron chelation activity and antioxidant protective enzymes [90,91]. Over the past decade, intense study has been done on the processing of the amyloid precursor protein (APP) proteolylic study and amyloid β metabolism as possible targets for the prevention of Alzheimer's disease. Choi et al. [92] showed that EGCG defends against β amyloid induced neurotoxicity in cultured hippocampal neuronal cells, a property contributed to its antioxidant properties. Other than that Levites et al. [93] recently showed that EGCG and other green tea catechins help to modulate the processing of APP, through PKC activation pathway to the non amyloidogenic soluble protein APP (sAPP), thus avoid the formation of the neurotoxic β amyloid. It also been shown to inhibit the β-secretase enzyme (BACE1) [94] which is responsible for processing sAPP to β-amyloid, also having a synergistic inhibitory effect on the production of β amyloid **(Figure 4).** Haque et al. [95] concluded that long term oral application of green tea catechins mixed with water improved spatial cognition learning ability of young rats.

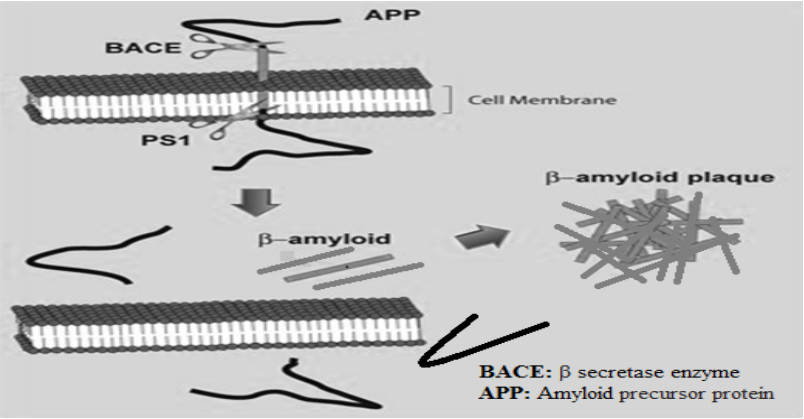

Figure 4. Diagram showing cleavage of APP protein by β secretase enzyme (BACE 1) and formation of β-amyloid plaque.

Another approach as remedies of Alzheimer's is to inhibit AChE by different medicinal plant extracts. Plenty of methods for reducing of AChE inhibitory activity from secondary metabolites of natural resources has been reported based on Ellman's reactions [96]. The TLC based bioautographic method developed by Marston et al. [97], Spectrophotometric study the thin-layer chromatography method [98] and micro-plate assay [98,99] have been reported to be useful in studying AChE inhibiting activities. Direct spectral analysis and studying enzyme kinetics in the presence and absence of the inhibitor can be another rapid approach. Okello et al. [100] carried out an in vitro colorimetric assay from Ellman's reactions [96] and reported that both green tea as well as black tea reduced the activity of AChE in a dose dependent manner IC_{50}value 0.003 ± 0.0004 mg/ml and 0.06 ± 0.005 mg/ml respectively for green tea and black tea. So this study has proven green tea to be more effective than black tea in inhibiting AChE enzyme. This study also revealed that green tea at a final assay concentration of 0.03 mg/ml reduced β-secretase by 27% after 5 min incubation, but after 60 min inhibition reached 38% and no further increase in the activity after 60 min incubation.

Preclinical Studies on EGCG Neuroprotective Mechanism of Action

Green tea has been shown to have significant preventive effect against age-associated neurodegenerative diseases [101]. Alzheimer's disease and Parkinson's disease are two common types of dementia related diseases that have been studied as part of the effort to investigate the effect of green tea on neurodegenerative diseases, as its effects may be relatively exquisite, therefore, it's difficult to measure the benefits of green tea on brain function in healthy people quantitatively. A concern of considerable research has been done to examine the possible effects of green tea in addition to slow down the effects of age related dementia [102]. Alzheimer's disease, the most common form of dementia in later life, where brain cells may have high metabolic rate which in turn ended up in generation of reactive oxidative species. Oxidative damage to neuronal biomolecules and increased aggregation of iron in specific brain areas are considered major pathological measures of Alzheimer's disease [103].There are studies on preventive effects of green tea catechins, in vitro as well as in vivo showed that both green tea extract and EGCG having the potential in reducing mice striatal Acetylcholine depletion as well as in substantial nigra (SN) acetylcholine neuron loss. Catechin containing compounds can be treated as potent radical anti-oxidants and ferric ion chelators. As transition metal ions causes a wide range of neurodegenerative disorders including iron deposition in microglia of SN in Parkinsonism patients [104-106] thus polyphenolics are the main constituents behind the utility of green tea and EGCG against *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) created neurotoxicity [107]. The catechol structural resemblance inhibits [^{3}H] AChE uptake by presynaptic transporters thereby blocking 1-methyl-4-phenylpyridinium (MPP^+) uptake through competition for the vesicular transporter and protects neuronal injury [108]. In vitro studies proved that EGCG resists the activity of the enzyme catechol-O-methyltransferase (COMT) as well as MPP^+ and 6-hydroxydopamine (6-OHDA)-induced neurotoxicity [109,110].

In vitro observations showed that EGCG, also present in green tea helps to prevent amyloid beta peptides (Aβ)-induced neurotoxicity [111], and EC can be used to reduce nascent Aβ fibrils, elongation of the(Aβ) fibrils and destabilization of the formation of assemblies [112] in Alzheimer's disease patients. It can regulate the proteolytic cleaving process of APP under in vivo and in vitro conditions [92] as a result green tea polyphenols might be potent therapeutic agents for Parkinson's disease, as well as for Alzheimer's disease. Long term treatment of mice with EGCG promoted the non-amyloidogenic α-secretase pathway of APP in neuronal cell cultures resulting in a momentous augment in soluble APPα (sAPPα) [92] in the hippocampus. In a recent study, EGCG revealed to be promoted the generation of sAPPα by decreasing Aβ levels and plaques via the non-amyloidogenic α-secretase proteolytic pathway [113,114]. Prophylactic benefits of long term consumption of EGCG studied on rat, found to be effective on spatial cognitive learning impairment caused by Aβ cerebral ventricle infusion [115], as well as in prevention of lipopolysaccharide-mediated neuronal cell death and memory impairment of mice, possibly as a result of reduction of Aβ levels via inhibition of β- and γ-secretases [116,117].

CONCLUSIONS AND FUTURE PERSPECTIVES

Evidences show that effectiveness of green tea polyphenols on neurodegenerative diseases, particularly AD and PD, is

associated with oxidative damage and iron accumulation. However, the properties of green tea and its constituents have not been properly studied in these disorders. Most of the studies are carried out in animal models and cell culture to evaluate the effect of acute and administration of the compounds. EGCG affect the mortality of neuronal cells. The primary role that mitochondria is in oxidative stress causing apoptosis, it may be hypothesised that EGCG-caausing apoptosis might affect mitochondrial targets. This may be a outcome of the blockade of mitochondrial permeability transition pore opening, since EGCG can affect the mitochondrial protein expression, the Bcl-2 family members, like Bax and Bad.

The anti-Parkinsonism neuroprotective drug rasagiline was used to resist the fall in mitochondrial membrane potential and the breaching of mitochondrial voltage dependent anion channel through the increase in Bcl-2 and Bcl-xl by mRNAs and their corresponding proteins [118,119]. Other than that the toxicity of the metabolic product of MPTP, MPP which is also a mitochondrial complex I inhibitor [120,121], induces oxidative stress, iron signaling, and-synuclein expression [122] ,is attenuated by EGCG probably via its metal chelating ability. With this concept a very recent study was published that a iron-responsive element (IRE-type II) within its 5-untranslated region of the AD's APP transcript can be altered by metal chelators [123,124] as EGCG. So we can strongly study the use of brain-accessible iron chelators ac be used to reduce iron concentrations in those brain areas in which it accumulates in neurodegenerative diseases.Future studies in the understanding of the protective effect mechanism of action of green tea polyphenols must concentrate on analyzing the cell targets affected by these compounds and other neuroprotectants.In addition, in vivo studies are needed to check whether EGCG and its metabolites, at sufficient amounts, can be able to reach the brain and can alter the cell signaling pathways after a peripheral injection and can reduce the progression of neurodegenerative disorders. This will be a more particular pathway especially when a multipharmacological action drug cocktail which is to be considered as formulation that can be more effective when used in the more potent treatment for AD and PD.

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