Advanced Research on the Action of Active Substances from Catechins

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Abstract: The efficacy of many of the active components of tea polyphenols has been demonstrated, with the active ingredient epigallocatechin gallate (EGCG) appearing in many human health-related experiments. This article collects the latest research demonstrating the relevance of EGCG and its derivatives to human health. Inhibition of cancer cell proliferation, combination with other active substances, and use of nanoparticles as carriers are the main mechanisms by which EGCG fights tumor cells; different concentrations of EGCG have antioxidant properties with different potencies, and β-lacto globulin is modified by EGCG to have a stronger antioxidant potential. Enzyme-enhanced catechins showed stronger anti-aging activity, and theanine ameliorated the shortened lifespan induced by high doses of EGCG. EGCG resists the development of neurodegenerative diseases by inhibiting changes in the action of proteins. This paper describes the use of EGCG and its derivatives for health promotion and provides a direction for future clinical applications of EGCG.

1. Introduction

Tea is a widely consumed foodstuff worldwide and includes a variety of active ingredients such as tea polyphenols and caffeine, of which catechins are one of the substances that have been studied at high frequency in recent years. According to studies, EGCG has demonstrated a variety of activities, including anti-inflammatory, hypotensive, antioxidant, and more. This article focuses on the anti-cancer, anti-aging, neuroprotective and antioxidant related effects of EGCG, which can be achieved by inhibiting the proliferation of cancer cells, combining with other active substances, and using nanoparticles as carriers; in terms of neuroprotection, EGCG reduces the occurrence of neurological diseases by inhibiting protein expression and so on. In addition, derivatives of EGCG exhibited higher antioxidant activity compared to EGCG. In summary, this paper describes the use of EGCG and its derivatives for health promotion and provides a direction for future clinical applications of EGCG.

2. Anti-cancer effects

Numerous experimental studies have shown that EGCG, a major component of catechins, can prevent the development of cancers such as thyroid, colon, oesophageal and breast cancers. The latest research results show that the anti-cancer effect of EGCG is divided into three main ways: acting alone, combined therapy and using nanoparticles as a vehicle for drug delivery.

2.1 Individual action

Bu L et al. showed that EGCG significantly inhibited the proliferation of papillary thyroid carcinoma cells but did not affect the proliferation of human normal thyroid cells[1].In the study, the effects of EGCG treatment in the papillary thyroid carcinoma cell line TPC-1 and the human normal thyroid cell line Nthy-ori 3-1 were compared and EGCG treatment was found to selectively inhibit the growth of TPC-1 cells but not Nthy-ori 3-1 cells in a time- and dose-dependent manner, as shown in Figure 1. EGCG induced apoptosis and cell cycle arrest in S phase in TPC-1 cells and down-regulated protein expression of cyclin A. EGCG induced autophagic response through inhibition of AKT/mTOR signaling pathway, enhanced EGCG-induced apoptosis and ROS inhibition.

One study demonstrated increased proliferation inhibition and apoptosis in bladder cancer cell lines (5,637 and T24 cells) induced by low concentrations of EGCG [2], as evidenced by increased expression of the apoptosis-related proteins caspase9, caspase3 and BAX. In addition, low doses of EGCG also regulated the expression of autophagy pathway-related proteins (LC3B II and Beclin), which was blocked by PI3K/AKT inhibitors; furthermore, knockdown of ATG5 reversed EGCG-induced apoptosis in 5637 cells, suggesting that EGCG may inhibit bladder cancer through the autophagic pathway.

The study by Zhang Zheyu et al. focused on the effect of EGCG on CRC cell activity and its mechanism of action [3]. In the study, colon cancer cells (HT-29) were subjected to different concentrations of EGCG, using methods such as MTT assays to investigate the underlying mechanisms of EGCG, while they investigated the disordered pathways induced by EGCG treatment at the transcriptional and metabolite levels. Transcriptome analysis revealed that 486 genes were differentially expressed between untreated and EGCG-treated cells. Also, multiple differentially expressed metabolites were identified between untreated and EGCG-treated cells. These altered metabolites were involved in the metabolism of glutathione, glycerophospholipids, starch, sucrose, amino sugars and nucleotide sugars. There was considerable agreement between the results of transcriptomic and metabolomic analyses. The data from the study suggest that the anticancer activity of EGCG on HT-29 cells is mediated by the induction of cell cycle arrest, apoptosis and autophagy and that EGCG regulates the metabolic pathways of cancer cells.

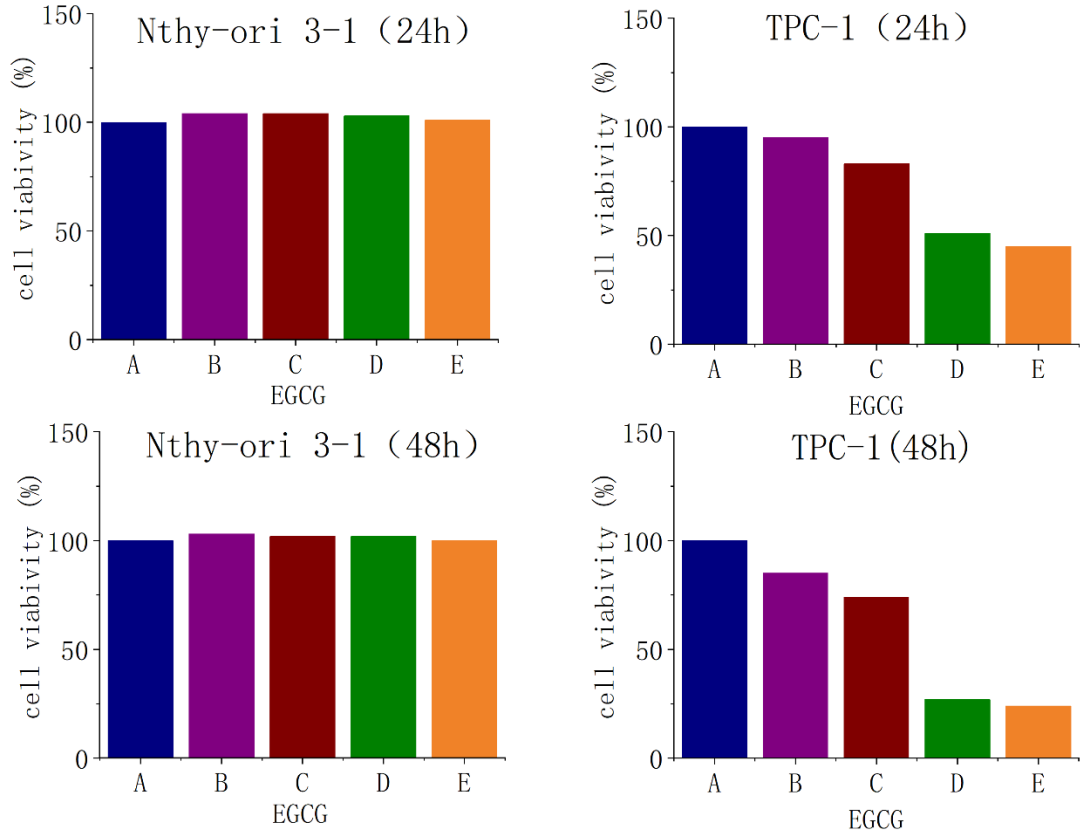

Figure 1. Cell viability of Nthy-ori 3-1cells and TPC-1 cells after EGCG treatments (A, 0μM; B, 5μM; C, 10μM; D, 20μM; E, 40μM) [1].

2.2 Combination therapy

Many studies have shown that the combination of EGCG with other active substances produces a more efficient effect than its own action alone. l. Duan et al. showed that the combination of electrospun fibers with chemotherapy had a synergistic effect in the treatment of colon cancer [4]. This

new study produced amorphous PLGA nets containing different ratios of EGCG and CPT. Drug release experiments showed that the dual drug-loaded meshes had higher release rates than single drug-loaded meshes. In anti-colon cancer assays, these meshes showed significant cytotoxicity against CT-26 cells, with all dual drug-loaded meshes having a combination index value of around 0.5, indicating a strong synergistic effect of EGCG and CPT. These results suggest that dual drug-loaded meshes of EGCG and CPT have potential application in the effective synergistic treatment of colon cancer. In new animal experiments, three colon cancer cells and BALB/c nude mice were used to assess the anti-proliferative effects of EGCG [5]. The results showed that EGCG had a dose-dependent anti-proliferative effect on colon cancer cells with low toxicity to normal colonic epithelial cells. Moreover, the toxic effects of EGCG on colon cancer cells were accompanied by down-regulation of Shh and PI3K/Akt pathways. In addition, EGCG reduced tumour volume and weight without affecting body weight in nude mice and inhibited the activation of Shh and PI3K/AKT pathways in tumour tissues. In conclusion, EGCG inhibited colon tumour growth by downregulating the Shh and PI3K pathways.

The study by Ebrahimifar et al. showed that carboplatin and EGCG have combined anti-esophageal cancer effects [6]. Treatment with carboplatin at IC20, IC25 and IC10 concentrations, combined with similar concentrations of EGCG, synergistically reduced cell viability compared to single treatment with both agents, as shown in Figure 2. Furthermore, in the combination of IC20 and IC25 treatment with both agents, the gene expression ratio of caspases 8 and 9 was significantly increased compared to monotherapy ($p < 0.05$).

In cells treated with the two agents, the Bcl-2 gene expression ratio was decreased compared to the single agent treatment. It was determined that treatment with carboplatin and EGCG promoted cytotoxicity and inhibited cancer progression in EC cells and that combined treatment with low concentrations of carboplatin and EGCG may promote induction of apoptosis and inhibit cell growth. These results confirm the anticancer effects of carboplatin and EGCG and provide a basis for further use of EGCG in the treatment of cancer.

Irinotecan is an alkaloid with antitumour activity, whose application is limited by its low solubility and high toxicity. The study by Wu Wenbing et al. used colorectal cancer cells [7], screened for appropriate concentrations of EGCG and Irinotecan by the CCK8 proliferation assay, and investigated the effects of single and combined administration on tumour cell migration, invasion, DNA damage, cell cycle and autophagy. The results of the study showed that the combination of EGCG and Irinotecan (0.5 mu mol L-) not only inhibited tumour cells more than EGCG or Irinotecan alone, but also prevented tumour cell migration and invasion. EGCG alone did not cause DNA damage in colorectal cancer cells, but its combination with Irinotecan induced cell cycle arrest by inhibiting topoisomerase I, resulting in more extensive DNA damage. EGCG also induced apoptosis by synergistically promoting autophagy with Irinotecan. These findings suggest that the combination of EGCG with Irinotecan may be a promising strategy for the treatment of colorectal cancer.

The combination of gallocatechin gallate and quercetin has been shown to have potent antitumour effects. Studies have investigated the effects of epigallocatechin (EGCG) (150mg) and quercetin (200mg) at different ratios on the proliferation and induction of apoptosis in human colon cancer cells (HCT-116) [8]. The data showed a significant inhibition of colony formation in CRC and in addition, cell cycle analysis revealed that this combination caused cell cycle arrest in the G1 phase at concentrations of 100 g/mL (72.7%) and 150 g/mL (75.25%). The combination of epigallocatechin and quercetin produced anti-proliferative activity against CRC and is promising in replacing conventional chemotherapeutic agents.

In this study, Wei Ran et al. evaluated the effects of EGCG alone or in combination with current chemotherapeutic agents [gemcitabine, 5-flourouracil (5-FU), and doxorubicin] on cell growth in pancreatic [9], colon and lung cancers and the mechanisms involved in the combined action. EGCG reduced cell growth in a concentration- and time-dependent manner reduced the growth of cancer cells and induced apoptosis and cell cycle arrest. In addition, EGCG enhanced the growth inhibitory effects of 5-flourouracil (5-FU), and doxorubicin. EGCG was shown to decrease ERK phosphorylation in a concentration-dependent manner and to sensitize gemcitabine, 5-flourouracil (5-FU), and doxorubicin, thereby further inhibiting ERK phosphorylation in a variety of cancer cell lines.

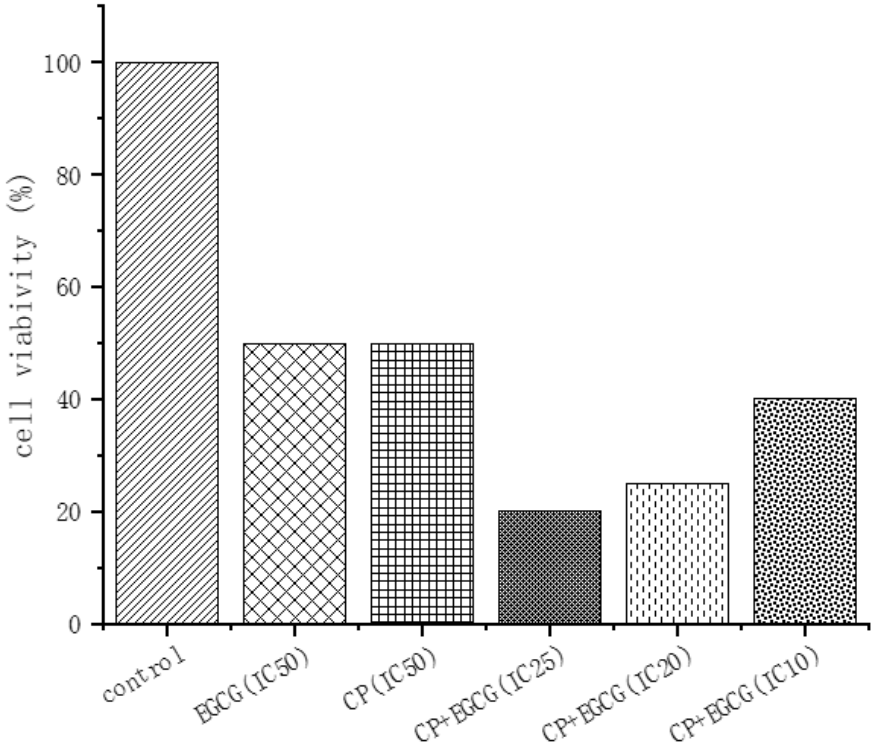

Figure 2. The effect of EGCG and CP treatment alone and in combination on cell viability [6].

2.3 Nanocarriers

The efficacy of EGCG in the prevention and treatment of cancer has been widely reported, however, poor stability and limited bioavailability have hindered the development of EGCG as an effective therapeutic agent. Researchers have begun to select suitable carriers in experiments to improve the stability and efficiency of EGCG action.

The study by Fasolato L. et al. combined innovative nanomaterials and bioactive compounds into a nanoparticle-based system and the study showed synergistic advantages of the nanocomplexes compared to the individual components [10]. In the latest study, a core-shell nanomix SAMN@EGCG, a combination of EGCG and active nanoparticles, emerged. Nano-immobilization on SAMN protects EGCG from degradation and prevents its auto-oxidation, and the nanohybrid is also able to deliver EGCG to cancer cells, showing protein kinase CK2 inhibition and promoting the use of phytochemicals as a valuable alternative for cancer therapy.

EGCG has the potential to impair the viability of breast cancer (TNBC) cells and sensitize them to oestrogen by activating ER-α. However, the mechanism of action of EGCG on TNBC cells remains unclear. CCN5/WISP-2 is a gatekeeper gene that regulates viability, ER-α and stemness in TNBC and other types of cancer. The study by Das Amlan et al. aimed to investigate whether EGCG (free or encapsulated in nanoparticles) enhances its bioavailability and enhances its anticancer effect interacts with CCN5 protein [11]. It was demonstrated that EGCG activates CCN5, inhibits cell viability in vitro through apoptosis, inhibits the ability of TNBC cells to form spheroids by reversing their stemness, and inhibits tumour growth in vivo. Nanoparticles loaded with EGCG were also found to be functionally more active than free EGCG and superior in their ability to inhibit tumours. These studies suggest that EGCG (free or encapsulated) is a novel activator of CCN5 in TNBC cells and is expected to be a future option for the treatment of TNBC with upregulated CCN5 expression.

Photothermal therapy (PTT) in combination with chemotherapy is a promising strategy for breast cancer treatment with great potential to control drug release, reduce multidrug resistance and improve efficacy. However, the challenge lies in achieving deep tissue tumour ablation and NIR controlled drug release. Fang Rangrang et al. developed tumour acidification and near infrared light (NIR) responsive folate (FA) functionalized polydopamine (DPA) nanoparticles (NPs) for doxorubicin (DOX) and epigallocatechin-3-gallate (EGCG) dual delivery [12]. With the help of NIR, the cellular uptake of

DOX-EGCG/DPA-FA NPs was 3-6 times higher compared to the free DOX group and the control group without NIR irradiation. Furthermore, in vivo biodistribution studies showed that DPA-FA NPs enhanced tumour accumulation, penetration and drug retention, with more than half of the mice with breast cancer in the DOX-EGCG/DPA-FA NPs group surviving for more than 70 days. In addition, DOX-EGCG/DPA-FA NPs could enhance apoptosis and necrosis of breast cancer cells by inducing significant inhibition of tumor growth and angiogenesis, thus effectively improving the efficacy of the treatment. DOX-EGCG/DPA-FA NPs may have potential applications as a useful nanoscale carrier for enhancing cancer therapy.

3. Antioxidant Effect

Recent studies have shown that different concentrations of EGCG have different potency in terms of antioxidant activity. A study by Silva E. C. et al evaluated the effect of different concentrations of (+)-catechin or (-)-epigallocatechin gallate (EGCG) on the freezing properties of goat semen. The study treated and froze pools of semen, and after thawing, samples were assessed for kinematics [13], plasma membrane (PMI) and acrosome integrity, morphology and oxidative stress at 0 and 1 h. In experiment 1, at 0h, VSL and VAP were greater at 15μM than at 50 and 100; WOB was lower at 100μM than at 0, 15 and 25; and BCF was higher at 75 and 100μM than at 0. Thus, (+)-catechin or EGCG had a transient inhibitory effect on the kinematics of frozen goat sperm at higher concentrations, whereas EGCG at 100 μM retained PMi. Thus, demonstrating the antioxidant properties of EGCG, which are promising for future frozen sperm.

β -lactoglobulin (β-lg), the predominant protein in bovine whey, was chemically modified by EGCG to create a substance with antioxidant activity [14]. EGCG-modified β-lg was characterized by SDS-PAGE, MALDI-TOF MS and intrinsic fluorescence spectroscopy. The results showed that the EGCG-modified β-lg has great antioxidant potential in scavenging DPPH radicals and chelating ferrous ions. Furthermore, EGCG-modified β-lg showed a protective effect against LDL peroxidation. Taken together, EGCG-modified β-lg may provide significant health benefits as an antioxidant.

The study by Liu Bing et al developed a method for the synthesis of EGCG derivatives. EGCG was lipophilised by esterification to enhance its biological activity [15]. A high conversion rate of EGCG was achieved. The monoesters of the three EGCG derivatives were identified by high performance liquid chromatography-mass spectrometry and the predominant monoester was identified by nuclear magnetic resonance as 4'-O-palmitoyl EGCG. The EGCG derivatives also exhibited good free radical scavenging ability. In lard, the solubility of the EGCG derivatives was 470-fold higher than that of EGCG and they exhibited antioxidant activity. These results suggest that palmitoylated EGCG derivatives can act as lipophilic mediators with antioxidant properties.

4. Anti-aging Mechanism

EGCG not only has anti-cancer effects, but also delays ageing appropriately. In previous studies, EGCG showed a dual effect on nematode lifespan. 200 μM of EGCG maximized the average lifespan of nematodes, while 1000 μM of EGCG significantly shortened it [16]. GA and EGC are the main metabolites of EGCG. To assess the role of GA and EGC in the ageing process of E. elegans, a study by Peng Yuxuan et al. treated adults with different concentrations of GA and EGC and measured their lifespan and found that neither of them extended the lifespan of the worms. Further studies revealed that EC containing multiple phenolic hydroxyl groups did not extend the lifespan of nematodes, while ECG containing both phenolic hydroxyl and ester groups increased lifespan.

The study also showed the effect of theanine and caffeine on EGCG-induced changes in lifespan [16]. Using different concentrations of theanine and caffeine, combined with high doses of EGCG (1000 μM) and low doses of EGCG (200 μM) to treat nematodes, caffeine did not affect the EGCG-induced hormonal effects. However, theanine alleviated the high-dose EGCG-induced shortening of lifespan, while it did not prolong or shorten the low-dose EGCG-induced lifespan.

Previous studies have shown that EGCG-induced lifespan extension in nematodes is associated with ROS production. Whereas this study found a transient increase in ROS formation after 2 days of exposure to high doses of EGCG, a sustained decrease in ROS was observed at 8 days and beyond [16]. The addition of theanine restored ROS levels to age-matched levels. After evaluation it was shown that guanine improved the lifespan of high doses of EGCG by eliminating excess ROS production. However, theanine showed activity in the SOD-3 mutant, ameliorating the high dose EGCG-induced shortening of lifespan, while having no effect on SOD-3: GFP expression. Theanine treatment alone had no effect on the expression of SOD-3 mutants and SOD-3: GFP. Thus, the activity of theanine in ameliorating high-dose EGCG-induced lifespan shortening is due to the elimination of dynamic changes in ROS levels and is not directly related to SOD-3. In conclusion, these results suggest that ester groups play a key role in the anti-aging effects of EGCG. In conclusion, enzyme-added catechins showed stronger anti-aging activity than non-enzyme-added catechins, and theanine ameliorated the high-dose EGCG-induced shortening of life span.

5. Neuroprotective Mechanisms

Epidemiological and animal studies have shown that daily intake of green tea catechins can inhibit cognitive decline and reduce the risk of dementia. The main component of green tea, the catechin EGCG, is thought to cross the blood-brain barrier to reach the brain parenchyma, but EGCG has been found to be more effective in promoting neuronal differentiation than its metabolite EGC, and depending on the permeability of catechins and their degradation products [17], a few cups of green tea, or approximately one bottle of catechins, may reduce age-related cognitive decline. In addition, studies have shown that theanine and arginine have excellent stress-reducing effects, inhibiting the shortening of life span and cognitive decline due to stress. However, the co-presence of caffeine and EGCG largely determines the effects of theanine and arginine. Differences in the molar ratio of CE/TA in green tea have been shown to affect stress reduction and sleep in experimental and clinical studies.

Down's syndrome (DS), is a genetic disorder caused by the presence of all or part of the third copy of chromosome 21. Treatment with EGCG has been reported to improve cognitive performance in animal models and humans, suggesting that EGCG may alleviate the symptoms of DS. Traditionally, EGCG has been associated with the ability to reduce the activity of the bispecific tyrosine phosphorylation-regulated kinase 1A, which is overexpressed in trisomy 21. In their study, Wyganowska - Świątkowska Marzena et al. proposed an alternative way in which EGCG might affect trisomy 21 - namely, by altering the proteolytic activity of the related enzyme activity [18]. The thrombinogen activation system has an effect on the condition of neurons in DS, and its effects are mainly related to the lack of delineation of nerve growth factors. The reduced activity of TPA in the Down's syndrome brain reduces the conversion of plasminogen into plasmin; this produces less pro-NGF to activate NGF. At the same time, plasmin activates pro-MMP-9. Activated MMP-9 inactivates excess NGF, a process controlled by neurospherin. However, degradation of NGF can be simultaneously reduced by EGCG, which inhibits MMP-9, and studies suggest that EGCG may protect NGF by inhibiting MMP-9.

A common thread in the pathogenesis of the neurodegenerative disease NDS is protein misfolding and aggregation. It has been shown that EGCG is able to interact with misfolded proteins such as amyloid β peptide (Aβ), which is associated with Alzheimer's disease (AD), and alpha-synuclein, which is associated with Parkinson's disease (PD) [19]. Although there is considerable evidence supporting the use of EGCG for ND treatment and the potential role of EGCG in NDS is now well described, clinical evidence for its anti-neurodegenerative effects does not exist. Gonçalves, Priscila Baltazar et al. suggest that the inconsistency between evidence from the preclinical phase and human clinical trials may be due to the fact that EGCG has poor pharmacokinetic properties and bioavailability, limiting its effectiveness as a drug lead. However, this could be improved by new technologies, such as nanoparticle-based delivery systems, or by acting in combination with other drugs. In studies EGCG has been shown to reduce the cytotoxicity of amyloid aggregates by binding to oligomers and fibrils and altering their hydrophobic surface exposure. These findings suggest that the use of EGCG could

be seen as a common drug therapy for many NDs targeting protein misfolding. Although the impact of current EGCG studies on clinical practice remains incomplete, the study provides strong evidence and testable hypotheses.

Tau accumulation and duplication are major contributors in the pathogenesis of Alzheimer's disease. Therefore, inhibition of Tau accumulation is a potential therapeutic strategy to ameliorate the disease and EGCG, a phytochemical inhibitor, has shown strong inhibitory potency against Tau aggregation. Sonawane S.K. et al. demonstrated through various biophysical and biochemical analyses that EGCG interacts unstably with full-length Tau at multiple residues, inhibits full-length Tau aggregation and solubilized Tau fibrils and oligomers [20]. The investigators predicted that the higher order structures formed by EGCG are non-toxic to neuroblastoma cells and that EGCG rescues aggregate-mediated toxicity in neuroblastoma cells. The findings suggest that EGCG inhibits Tau aggregation through direct but unstable interactions.

The deposition of Aβ42 aggregates in the form of amyloid plaques is another pathological hallmark of Alzheimer's disease. The ideal route of intervention is to inhibit Aβ42 aggregation. Epigallocatechin gallate EGCG is generally considered to be an inhibitor of a beta aggregation. A study by Park Giovann et al. evaluated experimentally the kinetics of a beta 42 aggregation in the absence and presence of EGCG [21]. It was found that EGCG reduced thioflavin T fluorescence in an EGCG concentration-dependent manner, suggesting that EGCG reduced the amount of a beta 42 fibrils. Furthermore, a higher ratio of EGCG to A beta 42 promoted the aggregation rate of a beta 42, while a lower ratio of EGCG to A beta 42 inhibited the aggregation rate. And electron paramagnetic resonance of spin-labelled a beta 42 aggregates showed that the high ratio of EGCG to A beta 42 resulted in a significant reduction in the amount of a beta 42 fibrils.

6. Conclusion

This article discusses the proven biological effects of various EGCG and its derivatives, which have been shown to increase their anticancer efficacy by inducing apoptosis and cell cycle arrest, combining active substances such as quercetin, and using nanoparticles as carriers to protect them from decomposition; EC containing phenolic hydroxyl groups did not extend the lifespan of nematodes, whereas ECG containing both phenolic hydroxyl and ester groups increased the lifespan. Furthermore, enzyme-added catechins showed stronger anti-aging activity than non-enzyme-added catechins; EGCG was more efficient in promoting neuronal differentiation than its metabolite EGC, reducing the decline in cognitive function. Moreover, EGCG can resist the development of neurodegenerative diseases through mechanisms such as inhibition of MMP-9 expression; different concentrations of EGCG have antioxidant properties with different potencies, β-lg modified by EGCG has great antioxidant potential, and palmitoylated EGCG derivatives can act as lipophilic mediators with antioxidant properties. In summary, many beneficial effects of EGCG have been demonstrated, but most of the experimental data are from in vitro studies, and future research should focus more on the clinical therapeutic effects.

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