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Research report

# Protective effects of a catechin-rich extract on the hippocampal formation and spatial memory in aging rats

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## HIGHLIGHTS

- A green tea extract protected proteins and lipids against aging-induced oxidation.
- The mixture of catechins protected the rat hippocampal-dependent spatial memory.
- Lipofuscin deposition and lysosome number were decreased after extract oral intake.
- Dentate granule cells dendritic trees structure were protected by the catechins.
- Epigallocatechin-3-gallate (EGCG) is not essential for the neuroprotective effects observed herein.

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## ABSTRACT

Green tea (GT) displays strong anti-oxidant and anti-inflammatory properties mostly attributed to (−)-epigallocatechin-3-gallate (EGCG), while experiments focusing on other catechins are scarce. With the present work we intended to analyze the neuroprotective effects of prolonged consumption of a GT extract (GTE) rich in catechins but poor in EGCG and other GT bioactive components that could also afford benefit. The endpoints evaluated were aging-induced biochemical and morphological changes in the rat hippocampal formation (HF) and behavioral alterations. Male Wistar rats aged 12 months were treated with GTE until 19 months of age. This group of animals was compared with control groups aged 19 (C-19M) or 12 months (C-12M). We found that aging increased oxidative markers but GTE consumption protected proteins and lipids against oxidation. The age-associated increase in lipofuscin content and lysosomal volume was also prevented by treatment with GTE. The dendritic arborizations of dentate granule cells of GTE-treated animals presented plastic changes accompanied by an improved spatial learning evaluated with the Morris water maze. Altogether our results demonstrate that the consumption of an extract rich in catechins other than EGCG protected the HF from aging-related declines contributing to improve the redox status and preventing the structural damage observed in old animals, with repercussions on behavioral performance.



## 1. Introduction

The number of individuals aged 65 years and older will increase steeply in Europe and North America leading to a global demographic shift [1,2]. This situation will markedly increase the occurrence of age-related disorders including cancer, diabetes mellitus, cardiovascular and neurodegenerative diseases [3]. Concerning the last disease group, there is a limited ability to treat effectively the associated cognitive decline, which is highly disabling. Over the past years, a great effort has been made to develop strategies that can delay the aging process or even reverse age-related neuronal damage, through life-style and nutritional modifications [4,5]. One of those lines of research is related to dietary antioxidant compounds that are being extensively investigated due to their central role in cell protection from the oxidative and inflammatory events known to be strongly associated with the

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advancement of age [6–8]. Within this scope, there is a growing body of evidence that demonstrates the protective role of dietary components such as the polyphenols [9,10]. As a polyphenol-rich beverage and due to its large consumption worldwide, green tea (GT), an infusion prepared by the brewing of leaves from *Camellia sinensis*, has attracted wide attention. GT is particularly rich in flavonoids mainly catechins, including (−)-epigallocatechin-3-gallate (EGCG), which normally represents approximately 60% of the total of catechins, but also (−)-epigallocatechin, (−)-epicatechin-3-gallate (ECG), (−)-gallocatechin-3-gallate (GCG) and (−)-epicatechin (EC) [11]. GT has been associated, in various experimental settings, with the protection of macromolecules from oxidative damage and modulation of several oxidative stress markers in the brain [11–15].

The pro-oxidant and inflammatory conditions present in age-related neurodegeneration affect multiple neuronal structures, although the lysosomes and mitochondria are particularly affected [16,17]. One of the most important manifestations of oxidative stress-induced damage that occurs within the lysosomal compartment is the formation of lipofuscin [16]. As macromolecules become difficult to eliminate due to oxidative changes that primarily occur during autophagocytic degradation inside lysosomes [18,19], lipofuscin is formed and accumulates within granules, mainly due to the fact that it is undegradable and cannot be removed via exocytosis [20].

In addition to the accumulation of this intralysosomal material, aging cells also display damaged and dysfunctional mitochondria. The increased production of reactive species is probably responsible for a significant part of the macromolecular damage that gradually accumulates and results in mitochondrial malfunction, explaining why these organelles are particularly affected by aging [21,22]. Consequently, the number of defective mitochondria within neurons progressively increases with age [23] and their structure is affected through the increase of organelle volume, loss of cristae and destruction of the inner membrane in some neuronal populations [24].

In this perspective, GT was demonstrated to have favorable effects on neurodegenerative alterations. Previously, we have demonstrated that chronic GT consumption decreased lipid peroxidation and oxidation of proteins, reduced simultaneously the lipofuscin deposit in pyramidal neurons and activated signaling pathways related to antioxidant defenses and neuronal survival [25,26]. However, the knowledge of GT antioxidants mechanisms of action in the brain is still very scarce at the moment. In order to further understand the protective effects of GT polyphenols in the aging process, we aimed to investigate the anti-aging effects of a catechin-rich GT extract (GTE) free of other bioactive components present in the complex GT matrix (e.g. theanine and caffeine) that could also afford neuroprotection [27]. Furthermore, the extract presented only trace amounts of EGCG, the catechin that has been related with most of the protective effects of GT in vivo and in vitro [14,28,29]. This composition allowed testing the involvement of other catechins known to be present in the infusion, although in smaller quantities, in the GT neuroprotective actions previously demonstrated using the same aging rat model [25,26].

In order to pursue our purposes, we focused on the alterations induced by aging on the hippocampal formation (HF), a brain region that undergoes biochemical and structural changes that are known to account for cognitive decline [30]. As such, hippocampal-dependent learning and memory were evaluated and the deposition of lipofuscin pigment and the volumes of mitochondria and lysosomes in CA3 pyramidal cells were quantified. In addition, we quantified markers of oxidative stress such as lipid peroxidation and protein carbonyls in HF tissue. The arborizations of Golgi-impregnated dentate granule cells were also analyzed.

## 2. Materials and methods

### 2.1. Animals and treatments

Thirty male Wistar rats (Charles River Laboratories, Barcelona, Spain), aged 12 months, weighing 624 ± 18 g, were individually housed and maintained under a 12-h light-dark cycle and standard temperature (20–22 °C) with free access to food and a liquid source. Rats were randomly separated into three groups each consisting of 10 rats. Ten rats were treated with a GTE solution (GTE-19M; $n = 10$) as the only liquid source until 19 months of age. The catechin content of the GTE was determined by high performance liquid chromatography according to a procedure previously described in detail [31]. The composition was consistent throughout the experiment and it was as follows: 56% EC, 10% GCG and 31% ECG and, in the remaining, a very small quantity of EGCG, in a total catechin concentration of approximately 200 mg/l. The GTE-treated rats were compared with control groups of 19 month-old (C-19M; $n = 10$) or 12 month-old (C-12M; $n = 10$) rats to provide baseline data. Controls had free access to tap water. During the 7 months of treatment all rats had ad libitum access to standard laboratory animal food (Letica, Barcelona, Spain). Animal weights were recorded weekly and both food and fluids were renewed and ingestion measured every other day. All animals were subjected to behavioral tests during the last 4 weeks of treatment. Thereafter, 5 animals from each group were used for biochemical analyses and the other 5 for morphological studies. Animal procedures followed the European Community guidelines (86/609/EEC) and the Portuguese Act (129/92) for the use of experimental animals.

### 2.2. Biochemical analyses

#### 2.2.1. Tissue preparation for biochemical assays

All chemicals used were of analytical grade. Five rats from each group were deeply anesthetized by intraperitoneal (i.p.) injection of sodium pentobarbital (80 mg/kg) and transcardially perfused with cold saline. The brains were rapidly removed and the HFs were dissected, weighed, codified to allow blind estimations, frozen in liquid nitrogen and stored at −80 °C until further processing. A part of each sample was homogenized (glass-Teflon homogenizer) in ice-cold 50 mM phosphate buffer (pH 7.4) with 0.1% (v/v) Triton X-100. Homogenates were centrifuged at 16,000 × *g* for 10 min at 4 °C and aliquots of the pellet were collected and stored at −80 °C for subsequent quantification of protein carbonyls. The remaining part of the samples was homogenized using the glass-Teflon homogenizer in 10% trichloroacetic acid (TCA), centrifuged (16,000 × *g*, 4 °C, 10 min) and the supernatants were immediately used to measure the degree of lipid peroxidation.

#### 2.2.2. Protein carbonyl levels

Oxidative damage to proteins was determined through spectrophotometric detection of protein hydrazones formed in the reaction of 2,4-dinitrophenylhydrazine (DNPH) with protein carbonyls as described previously [32]. The absorbance of DNPH-treated samples was recorded at 380 nm against blank samples. The results were expressed in nmol of carbonyl groups per mg of protein using a molar extinction coefficient ($\varepsilon$) of $2.20 \times 10^4$ $M^{-1}$ $cm^{-1}$.

#### 2.2.3. Malondialdehyde levels

The extent of lipid peroxidation was evaluated by measuring thiobarbituric acid-reactive substances at 535 nm, as described elsewhere [33]. After precipitation with 10% TCA, samples supernatants were incubated with an equal volume of 1% thiobarbituric acid in a boiling water bath for 30 min. The results were expressed as malondialdehyde (MDA) equivalents per mg of protein ($\varepsilon = 1.56 \times 10^5$ $mM^{-1}$ $cm^{-1}$).

#### 2.2.4. Protein determination

Protein concentration of the crude hippocampal homogenates was performed using bovine serum albumin as standard [34].

### 2.3. Morphological studies

#### 2.3.1. General procedures

Following anesthesia with sodium pentobarbital (80 mg/kg b.w., i.p.), the remaining 5 rats from each group were perfused transcardially with a fixative solution containing 1% *para*-formaldehyde (w/v) and 1% glutaraldehyde (v/v) in 0.12 M phosphate buffer at pH 7.2. After removal of the brains from the skulls, the HFs were isolated, codified to allow blind estimations and immersed in fresh fixative solution.

#### 2.3.2. Golgi impregnation and quantitative analysis of dendritic arborizations of granule cells

After 15 days of fixation, Golgi impregnation was performed according to the method previously described [35], with modifications [36,37]. After processing and storage in the dark for 3–5 days in 1.5% silver nitrate, the tissue blocks were shelled in paraffin and horizontal sections with a nominal thickness of 100 μm were obtained, dehydrated, cleared in terpineol and mounted on slides under Damar resin with no cover slip.

From the lateral (suprapyramidal) blade of the dentate gyrus granular layer, 10 granule cells were sampled and pooled per animal in a single group according to the criteria previously described in detail [37–39]. The dendritic trees of the selected