



Green tea supplementation produces better neuroprotective effects than red and black tea in Alzheimer-like rat model



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ABSTRACT

Green tea from *Camellia sinensis* plays a neuroprotective role in different neurodegenerative conditions, such as memory deficits in Alzheimer disease (AD). However, whether other teas from *Camellia sinensis* present similar neuroprotective effect still is not clear. Here we investigate effects of green, red and black tea supplementation on memory and hippocampus oxidative status in a rat model of Alzheimer-like disease (AD-like). Method: Wistar male rats were supplemented with green, red or black tea during 8 weeks before A β intra-hippocampal injection (2 μ L of A β -25–35, CA1 region). AD and sham rats were submitted to memory tests. After euthanasia, oxidative status in the bilateral hippocampus was quantified. Green and red teas avoid memory deficits in AD rats, but only green tea also avoids oxidative stress and damage in the hippocampus. Green tea was more effective for neuroprotection than red and black teas from the *Camellia sinensis* in the AD rat model.

1. Introduction

The Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by declines in memory and cognition (Jack et al., 2016). AD is a major disease and a socio-economic challenge, with estimation of 100 million cases worldwide by the year 2050 (Liu et al., 2015). There is no current cure for AD and its origin still not elucidated, although some contributing factors have been described. One of them is the accumulation of Amyloid β (A β) plaques in the brain (Jack et al., 2016; Zuo, Hemmelgarn, Chuang, & Best, 2015) leading to synaptic depression, abnormal network activity (Jack et al., 2016) and excessive production of reactive oxygen species (ROS) (Zuo et al., 2015). The high oxidative metabolic activity in the brain and intense production of ROS leads to the generation of free radicals that are involved in chronic neurodegenerative diseases (Ayyappan, Palayyan, & Kozhiparambil Gopalan, 2016). This oxidative stress condition promotes progressive neuronal loss, predominantly by apoptosis (Liu et al., 2015) and contributes to the onset of memory deficits (Liu, Liu, Xiao, & Shi, 2016; Yi, Park, Kim, Kim, & Ryu, 2016). Recognition memory, which is one type of explicit memory (Liu et al., 2016), is important to perform daily activities and depends on the integrity of the hippocampus (Yi et al., 2016). The hippocampus is vulnerable to oxidative stress (Zuo et al., 2015); thus, improving antioxidant defenses may prevent the memory deficits resulting from AD (T. Liu et al., 2016; Yi et al., 2016).

The *Camellia sinensis* (family Theaceae) has strong antioxidant properties (Soung, Wang, Tseng, Fang, & Chang, 2015; Yasmeen & Hasnain, 2015). The antioxidant mechanism is related to the ability of their catechins to sequester free radicals (Yasmeen & Hasnain, 2015). The green tea obtained from *Camellia sinensis* has a neuroprotective role in memory deficits resulting from aging (Flores et al., 2014), ischemia-reperfusion (Schmidt et al., 2014) and AD in transgenic mice (Rezai-Zadeh et al., 2008). Green tea has the potential to work as free radical species scavenger (Okello, McDougall, Kumar, & Seal, 2011; Zuo et al., 2015), most likely due to the presence of polyphenols (Banerjee & Chatterjee, 2015). Polyphenols neutralize the excess of free iron by acting as chelating and leading to suppression of amyloid precursor protein (APP), which plays an important role in AD. The neuroprotective role of *Camellia sinensis* was investigated in other teas processed from the same plant but showing different results. These differences were argued to be a consequence of different amounts of polyphenols, especially catechins (Banerjee & Chatterjee, 2015), between teas (Heber et al., 2014; Soares et al., 2013).

Green tea extract was shown to protect human red blood cells against oxidative stress, but black or white tea extracts did not show the same effect (Gawlik & Czajka, 2007). Both green and black tea increase antioxidant capacity, but green tea is six-fold more potent than black tea (Serafini, Ghiselli, & Ferro-Luzzi, 1996). The presence of epigallocatechin gallate (EGCG) might be the key factor in determining the

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neuroprotective role of green tea in brain diseases and cognitive performance (Xicota, Rodriguez-Morato, Dierssen, & de la Torre, 2015). The processing of *Camellia sinensis* by fermentation results in different teas (Banerjee & Chatterjee, 2015). Green tea is not fermented, whereas red tea is partially fermented and black tea is completely fermented (Okello et al., 2011; Soares et al., 2013). Fermentation influences catechins contents (Banerjee & Chatterjee, 2015) and, therefore, the antioxidant outcomes may differ among different teas. To be best of our knowledge, the neuroprotective potential of different teas obtained from *Camellia sinensis* has not been tested in an AD-like model. Here we investigated the neuroprotective potential of green, red and black teas obtained from *Camellia sinensis* in rats that had an AD-like disease. Our results showed that green tea has a better neuroprotective effect on memory deficits and hippocampal oxidative status than black and red teas.

2. Material and methods

2.1. Animals and experimental design

Two months old male Wistar rats bought from the Federal University of Santa Maria/RS/Brazil Central Vivarium were housed three per cage under controlled light and environmental conditions (12 h light/dark cycle at $23 \pm 2^\circ\text{C}$ and humidity $50 \pm 10\%$) with food and water or tea ad libitum. Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996) and the Local Institution Animal Care and Use Committee (IRB #012015). The rats were randomly assigned to 4 groups: (a) control: not supplemented; (b) supplemented with green tea; (c) supplemented with red tea, and (d) supplemented with black tea. After 8 weeks, sham or AD-like surgeries (see details below) were performed and groups were reorganized ($n = 8\text{--}12/\text{group}$), as follow:

- group 1 — Sham: submitted to the sham surgery receiving saline injection;
- group 2 — Green tea and sham: supplemented with green tea before sham surgery;
- group 3 — Red tea and sham: supplemented with red tea before sham surgery;
- group 4 — Black tea and sham: supplemented with black tea before sham surgery;
- group 5 — AD-like: submitted to surgery with hippocampal Amyloid β injection;
- group 6 — Green tea and AD-like: supplemented with green tea before surgery with hippocampal Amyloid β injection;
- group 7 — Red tea and AD-like: supplemented with red tea before surgery with hippocampal Amyloid β injection;
- group 8 — Black tea and AD-like: supplemented with black tea before surgery with hippocampal Amyloid β injection.

Ten days after surgery, the time necessary for beta-amyloid aggregation and plaque formation (Maurice, 2016), rats were submitted to behavioral tests and euthanized. Biochemical analyses were performed in the bilateral hippocampus to determine levels of reactive oxygen species (ROS), lipid peroxidation by thiobarbituric acid reactive substances (TBARS), and the total antioxidant capacity by ferric reducing/antioxidant power (FRAP). Fig. 1 depicts the experimental design.

2.2. Tea supplementation and chromatography analysis

Rats received green, red or black tea. The teas were acquired all in the same season and the same production batch from the local market (Madrugada Alimentos LTDA/Venâncio Aires/RS/Brazil) as used in previous researches (Martins et al., 2017; Schmidt et al., 2014; Sosa et al., 2015). Teas were prepared daily by mixing 13.33 g of dry tea

extract in one liter of filtered water at 90°C in the concentration of 1333 mg/mL (Martins et al., 2017; Schmidt et al., 2014; Sosa et al., 2015). Teas were administered at ambient temperature ($23 \pm 2^\circ\text{C}$) as a substitute for drinking water, for free consumption. Tea intake volume per box home was monitored daily. To calculate the average daily intake per rat, the total consumption in each box home was divided by the number of rats in each box home. Supplementation continued until the day of euthanasia.

The presences of epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) were determined by high-performance liquid chromatography (HPLC) (see Table 1 for details). HPLC was performed with a Shimadzu Prominence Auto Sampler (YL9100) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu YL9110 reciprocating pumps connected to an YL9101 degasser with an YL9150 integrator, and YL9160 diode array detector. To determine compounds profile the extracts were analyzed using a reversed phase carried out under gradient conditions using Synergi Fusion-RP 80A column ($4.6 \times 250\text{ mm}$). The mobile phase was composed of water (pH = 3): acetonitrile (5:95, v/v) in a gradient mode, until 35 min, in which the mobile phase was 100% acetonitrile. At 38 min water (pH = 3): acetonitrile (5:95, v/v) was used again, in isocratic mode, as a mobile phase, until 50 min. A flow rate of 0.8 mL/min was used and 20 μL of sample were injected. Phenolic compounds were identified and quantified by comparing the retention time and UV-Visible spectral data to known previously injected standards. The chromatography peaks were confirmed by comparing the retention time with those of reference standards and by DAD spectra. Calibration curves were determined for EGC ($y = 101,79x - 10,283$); EC ($y = 91,872x + 7657$); EGCG ($y = 103,5x - 93,211$); ECG ($y = 112,17x - 81,22$). All chromatography operations were carried out at ambient temperature and in triplicate.

2.3. Preparation of amyloid β 25–35

β -Amyloid peptide (25–35) (Sigma Aldrich; product number: A4559) was dissolved in saline (vehicle) at a concentration of 100 μM . Before intrahippocampal injection, the A β was incubated at 37°C during 4 days (in vitro) to induce A β 25–35 aggregation (Ghasemi, Zarifkar, Rastegar, Maghsoudi, & Moosavi, 2014).

2.4. Surgery

The stereotaxic surgeries for intrahippocampal injection of 2 μL A β 25–35 (groups 5–8) or vehicle (groups 1–4) were performed after supplementation. Rats were anesthetized with ketamine and xylazine (i.p. 75 mg/kg and 10 mg/kg, respectively). When the anesthetic plan was confirmed, rats were mounted into a stereotaxic frame and the amon horn 1 (CA1, from *Cornu Ammonis*) region of the dorsal hippocampus was located based on the Paxinos brain atlas (AP — 4.2, LL \pm 3.0, VM — 2.0 mm) (Paxinos, Watson, & Emson, 1980). Bilateral infusions were performed using a Hamilton syringe and an infusion bomb (Limon et al., 2012). After surgery, rats were returned to their cages and monitored during recovery.

2.5. Behavioral control tests

Exploratory and locomotor activities were assessed to ensure injection did not impair such behaviors, 10 days after surgery rats were placed in the left quadrant of a $50 \times 50 \times 39\text{ cm}$ open field (OF) made with wooden painted white, with a frontal glass wall. Black lines were drawn on the floor to divide the arena into 12 equal quadrants.

Crossing and rearing, as measures of locomotor and exploratory activities, respectively, were measured over 5 min (Bonini et al., 2006). Anxiety state was analyzed using an elevated plus maze. Time spent and the total number of entries into the open arms were recorded over 5 min (Pellow, Chopin, File, & Briley, 1985). Nociception, as a measure

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