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Original Article

Exploring *in vitro* neurobiological effects and high-pressure liquid chromatography-assisted quantitation of chlorogenic acid in 18 Turkish coffee brands

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ABSTRACT

The hydroalcoholic extracts of the Turkish traditional coffee samples from 18 commercial brands were tested for their neurobiological effects through enzyme inhibition based on enzyme-linked immunosorbance microtiter assays against acetylcholinesterase, butyrylcholinesterase, and tyrosinase, linked to Alzheimer's and Parkinson's diseases. The extracts were also subjected to several antioxidant test systems to define their antiradical, metal-chelation capacity, and reducing power. Total phenol and flavonoid contents in the extracts were delineated by spectrophotometric methods, while chlorogenic acid in the coffee samples was quantified by high-pressure liquid chromatography. The extracts displayed low to moderate inhibition (from 2.13 ± 0.01% to 36.12 ± 1.07% at 200 μg/mL) against the tested enzymes, whereas they had notable 2,2′-diphenyl-1-picrylhydrazyl radical scavenging activity up to 56.15 ± 2.03% at 200 μg/mL. The extracts exerted a remarkable ferric-reducing antioxidant power values, while chlorogenic acid was found to range between 0.288 ± 0.005% and 2.335 ± 0.010%.

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1. Introduction

Drinking Turkish coffee is an important part of the culture and indoor/outdoor social activities in Turkey. Coffee was introduced to Turks approximately five centuries ago. During the Ottoman period, an Ottoman governor to Yemen who was brought back to Istanbul introduced the coffee beans to the Ottoman capital in 1543 (http://www.turkish-coffee.org/turkish_coffee.htm). Turkish coffee is also famous and consumed widely in the same style in the Balkan and Middle East countries. It is traditionally prepared from the seeds of *Coffea arabica* L. (Rubiaceae) using a special type of narrow-topped small boiling pot called a *cezve*.

Neurodegenerative diseases are among the deadly disorders affecting elderly population. In particular, Alzheimer's (AD) and Parkinson's disease (PD) have been found to have a multifactorial and progressive nature and no complete cure is yet available for either disease. Therefore, intensive research is being conducted on finding new drug candidates of natural or synthetic origins for the treatment of AD and PD, whose pathogeneses are still mostly unclear. The cholinergic hypothesis has been the most accepted theory for AD, whereby a marked deficit in level of acetylcholine (ACh) has been shown in the AD patients. Thus, inhibition of the enzyme acetylcholinesterase (AChE), which can break down ACh in the brain, has been a widely used treatment strategy against AD [1]. Relevantly, butyrylcholinesterase (BChE; also known as pseudocholinesterase or plasma cholinesterase) is a nonspecific cholinesterase that has ability to hydrolyze many different choline esters including ACh and, therefore, inhibition of BChE is also important for AD treatment [2]. Since dementia is also associated with PD, cholinesterase inhibitors are also of interest for PD treatment [3]. Moreover, neuromelanoma associated with enzymes such as tyrosinase (TYR) and tyrosine hydroxylase has been suggested to play a role in increased susceptibility to both PD and melanoma [4]. Hence, inhibition of TYR has emerged as a possible new strategy towards PD.

In our enduring research on finding new cholinesterase and TYR inhibitors of natural origin, we have recently investigated neuroprotective properties of a number of traditional herbal coffees consumed in Turkey such as terebinth coffee as well as some other herbal coffees prepared from the seeds of carob, black cumin, date, and tumble thistle [5,6]. In the current study, our target was to evaluate neurobiological effects of the hydroalcoholic extracts (ethanol, 80%) prepared from 18 commercial brands of Turkish coffee sold in Turkey and northern Cyprus. For this purpose, cholinesterase and TYR inhibitory activity of the extracts was tested using enzyme-linked immunosorbance microtiter assays. As oxidative stress has been stated to contribute to neurodegeneration [7], antioxidant activity of the coffee extracts was tested using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and *N*,*N*-dimethyl-*p*-phenylendiamine (DMPD) radical scavenging activity, metal-chelation capacity, ferric-reducing antioxidant power (FRAP), and phosphomolibdenum-reducing antioxidant power (PRAP) assays. Total phenol and flavonoid contents in the extracts were calculated spectrophotometrically. In addition, the extracts were subjected to high-pressure liquid chromatography (HPLC) analysis for quantification of chlorogenic acid, a phenolic acid widely found in coffee beans.

2. Methods

2.1. Coffee samples

The medium-roasted type of Turkish coffee samples belonging to 18 commercial brands (Kaffka, Shazili, Alkan, Hiscafe, Madenci, Asli, Arif, Con, Hisar, Mehmet Efendi, Oza, Oza light, Dibek, Action, Nescafe Falci, Ozerlat, Cezbeli, and Ulker) were purchased from supermarkets in Turkey or northern Cyprus during 2011.

2.2. Extraction procedure

Each coffee sample was weighed precisely in a digital balance (Shimadzu, Kyoto, Japan) and, then, extracted with ethanol (80%) at room temperature over 3 days by shaking occasionally. The hydroalcoholic phases were filtered through a regular filter paper and evaporated *in vacuo* until dryness to give the crude coffee ethanol extracts.

2.3. AChE and BChE inhibitory activity assays

AChE and BChE inhibitory activity of the extracts was measured by slightly modified spectrophotometric method of Ellman et al [8]. Electric eel AChE (Type-VI-S, EC 3.1.1.7; Sigma, St Louis, MO, USA) and horse serum BChE (EC 3.1.1.8; Sigma, St. Louis, MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma) were employed as substrates of the reaction, respectively. 5,5′-Dithio-bis(2-nitrobenzoic) acid (Sigma) was used for the measurement of the anticholinesterase activity. All reagents and conditions were as described in our previous publication [5]. Hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of 5,5′-dithio-bis(2-nitrobenzoic) acid with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (VersaMax; Molecular Devices, Sunnyvale, CA, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software (Softmax Moleculer Devices, Downingtown, USA). Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank (ethanol in phosphate buffer, pH = 8) using the formula:

$$(E - S)/E \times 100, \quad (1)$$

where *E* is the activity of enzyme without test sample and *S* is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine (Sigma) was used as the reference.