Antioxidant and Neuronal Cell Protective Effects of Aqueous Extracts from Lotus Leaf Tea

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Received: JAN. 09, 2011, Revised: MAR. 04, 2012, Accepted: APR. 27, 2012

ABSTRACT

Antioxidant and neuronal cell protective effects of aqueous extract from lotus (Nelumbo nucifera) leaf tea (LLTE) were investigated. The 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging effect, ferric reducing antioxidant power, and malondialdehyde inhibition of LLTE were increased in a dose dependent manner. Intracellular reactive oxygen species accumulation resulting from hydrogen peroxide (H₂O₂) treatment was significantly reduced when LLTE were present in the media compared to PC12 cells treated with H₂O₂ only. In neuronal cell viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT), LLTE showed protective effect against H₂O₂-induced neurotoxicity. In addition, lactate dehydrogenase release into medium was also inhibited by LLTE (7.13-43.89%). Total phenolics of LLTE were 33.16 mg/g and a quercetin was identified as major phenolics (105.93 mg/100g). Therefore, above these data suggest that LLTE including quercetin may be useful in the natural antioxidant substance, and may reduce the risk of neurodegenerative disease.

Key words - Nelumbo nucifera, Quercetin, Antioxidant, Neuronal cell protection

I. INTRODUCTION

Accumulated intracellular H₂O₂ induces the peroxidation of membrane lipids and apoptotic cell death by activation of caspases (Behl et al., 1997; Valencia & Morán, 2004). Alzheimer’s disease (AD) as a neurodegenerative disease is one of the most serious threats to human health in aged societies of developed countries. Many studies have demonstrated that the brains of patients with AD are subjected to an increase of oxidative stress due to free radical damage (Markesbery & Carney, 1999). Many phenolics protect neuronal cells from the oxidative stress induced by ROS or amyloid-β (Aβ) protein, which may be related to the pathogenesis of AD (Kim et al., 2005; Kou et al., 2009). Some phytochemicals from natural plant sources such as fruits and vegetable may reduce the risk of AD because of their antioxidative properties diminishing oxidative insults (Youdim & Joseph, 2001). Epidemiological observation has revealed that the increase of antioxidant uptake is inversely associated with the risk of AD incidence (Crundman & Delaney, 2002). Antioxidants are vital substances which posses the ability to protect the body from damage caused by free radical induced oxidative stress (Ozsöy et al., 2008). There is an increasing interest in natural antioxidants (e.g. polyphenols), present in medicinal and dietary plant, which might help prevent oxidative damage (Silva et al., 2005). Phenolics possess ideal structural chemistry for free radical-scavenging activity, and have been shown to be more effective antioxidant in vitro than vitamin C. A few phenolics have also

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been reported that prevent a decrease in the activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and prevent a significant deletion of glutathione (GSH) (Yu et al., 2009). Therefore, the importance and role of non-nutrient compounds particularly phenolics as natural antioxidants have greatly increased (Hagerman et al., 1998). To find new natural sources of physiological compounds, we studied antioxidant and neuronal cell protective effects of LLTE.

Lotus (Nelumbo nucifera) is a perennial, rhizomatous and aquatic plant, which was distributed throughout Asia. All parts of lotus, including the rhizome, leaf, stem, and seed, have been used as food-stuffs as well as traditional medicines in China and India (Goo et al., 2009). In Korea, lotus leaf, root and seed are usually consumed as a tea, or in braised dishes or soups. According to traditional knowledge, medicinal uses of different part of lotus plants are common in the treatment of diarrhea, tissue inflammation, and haemostasis (Ha et al., 2010). Lotus leaf has been reported to have beneficial effects on antioxidant (Wu et al., 2003), antibacterial (Li & Xu, 2008), anti-hyperlipidemic (La Cour et al., 1995), anti-HIV (Kashiwada et al., 2005), and anti-obesity effects (Ono et al., 2006). Recently, Lin et al. (2008) reported that the flavonoids (catechin, quercetin, quercetin-3-O-glycoside, and kaempferol-3-O-glucoside pyranoside) have been isolated as antioxidant from the leaves.

Although it has already been demonstrated that lotus leaf contain phenolic compounds, little is known about effect of lotus leaf tea on oxidative stress-induced neurotoxicity. Since the protective effect on neuronal cell of LLTE has not previously been reported, the objectives of this study were to determine the antioxidant and protective effect on neuronal cell of LLTE. In addition, active compounds on antioxidant and neuronal cell protection were identified by high performance liquid chromatography (HPLC) analysis.

II. Materials and methods

2.1 Chemicals

Folin-Ciocalteu’s phenol reagent, 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 2,4,6-tripyridyl-S-triazine (TPTZ), trichloroacetic acid (TCA), thiobarbituric acid (TBA), vitamin C, α-tocopherol, catechin, 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES), sodium bicarbonate, penicillin, streptomycin, myricetin, quercetin, kaempferol, ferrous sulfate (FeSO₄), hydrogenperoxide (H₂O₂), dimethyl sulfoxide (DMSO), penicillin, streptomycin, 3-[4,5- dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide (MTT) assay kit, 2’,7’-dichlorofluorescein diacetate (DCF-DA), lactate dehydrogenase (LDH) assay kit, and all solvents used were of analytical grade and purchased from Sigma Chemical Co (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum was obtained from Gibco BRL (Grand Island, NY, USA).

2.2 Extraction from the lotus leaf tea

Lotus (Nelumbo nucifera) leaf tea was purchased from local market in Hamyang of Korea in October 2009. These samples were stored at -20℃ until use. Freeze-dried samples from lotus leaf tea were obtained as follows. Powdered lotus leaf tea (50 g) was suspended and extracted with 500 mL of water at 100℃ for 2 hr. The extracts were filtered through Whatman No. 2 filter paper (Whatman International Limited, Kent, England) and evaporated to dryness. The aqueous extract was concentrated in a vacuum evaporator at 40℃. Water filtrate was frozen and lyophilized. The lyophilized extracts were placed in a glass bottle and stored at -20℃ until used. The lyophilized extracts were re-dissolved in water to a concentration of 1,000 µg/mL.

2.3 ABTS radical scavenging activity

ABTS was dissolved in water to make a concentration of 7 mmol/L. ABTS was produced by reacting the ABTS stock solution with 2.45 mM/L potassium persulfate (final concentration) and allowing