Anti-oxidant and anti-inflammatory activities of the various kinds of herbal tea

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ABSTRACT

Objectives : Reactive oxygen species (ROS) are involved in a wide spectrum of diseases including chronic inflammation and cancer. In this study, we investigated the antioxidant activities and anti-inflammatory effects of the extracts from the herbal teas such as Lonicera japonica Thunberg (L. japonica), Chrysanthemum morifolium Ramat (C. morifolium), Mentha arvensis L. (M. arvensis), and P. rhizoma.

Methods : Anti-oxidant activity was evaluated using DPPH radical scavenging assay and Fe2+ chelating assay. And DNA cleavage assay was performed to evaluate an anti-oxidative effect. Anti-inflammatory effect was performed using NO generation assay and western blot in LPS-stimulated RAW264.7 cell line.

Results : L. japonica scavenged DPPH radical by 9.8% at 12.5 µg/ml, 24.8% at 25 µg/ml, 34.3% at 50 µg/ml, 61.1% at 100 µg/ml and 75.8% at 200 µg/ml, respectively. In addition, C. morifolium and M. arvensis removed DPPH radical by 15.6% and 10.4% at 12.5 µg/ml, 34.8% and 22.8% at 25 µg/ml, 66.9% and 43.3% at 50 µg/ml, 87.4% and 69.1% at 100 µg/ml and 92.1% and 73.2% at 200 µg/ml, respectively. However, P. rhizoma did not affect on DPPH radical scavenging. The Fe3+ chelating activity was highest in L. japonica, but lowest in P. rhizoma among the herbal teas. In addition, the extracts from L. japonica, C. morifolium and M. arvensis inhibited oxidative DNA damage via its anti-oxidant activity. In anti-inflammatory effect, the extracts from C. morifolium inhibited NO production. In addition, it suppressed the NF-κB signaling pathway in LPS-stimulated RAW 264.7 cells.

Conclusions : Together, this study indicates that L. japonica, M. arvensis and C. morifolium possess the protective effect against the oxidative DNA damage. Furthermore, C. morifolium exerts an anti-inflammatory effect.

Key words : Herbal teas; Anti-oxidant; Oxidative damage; Anti-inflammation

Introduction

Reactive oxygen species (ROS) produced by cellular aerobic respiration have been regarded as a inducer of oxidative stress including damage of cell matrices such as lipids, proteins and DNA, which is associated with human diseases such as cancer and chronic inflammation1-3). Thus, antioxidant activity can be defined as a suppression of oxidative damage of organic molecule including lipids, proteins, DNA and other molecules4). Antioxidants can be divided to two types: primary antioxidants directly remove the generated ROS and second antioxidants indirectly inhibits the ROS generation by Fenton’s reaction. In generally, herbal teas have been reported to have these two type capacities5).
In addition, ROS have been regarded as a mediator of chronic inflammation by activating proinflammatory cytokines, which has been regarded as a major mechanism for inflammation injury\textsuperscript{6}. Especially, ROS stimulates nitric oxide (NO), one of the inflammation mediators by which inflammatory processes can be provoked or sustained. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation. Therefore, antioxidants can attenuate inflammation\textsuperscript{7}.

Teas have been regarded as the most widely consumed beverages worldwide\textsuperscript{8}. Among teas, herbal teas using the leaves, flowers, seeds, fruits, stems or roots of plant species have been consumed for health care and disease prevention\textsuperscript{9,10} because these contain various active phytochemicals with pharmacological properties such as allergies, insomnia, headaches, anxiety, intestinal disorders, depression, and high blood pressure\textsuperscript{11}. There is growing evidence that herbal teas have several biological effects including anti-cancer, anti-atherogenic, anti-oxidant and anti-microbial activities\textsuperscript{12}. In this study, we evaluated the anti-oxidant and anti-inflammatory capacities of aqueous extracts of Lonicera japonica Thunberg (L. japonica), Chrysanthemum morifolium Ramat (C. morifolium), Mentha arvensis L. (M. arvensis), and P. rhizoma used as a herbal tea.

### Materials and Methods

#### 1. Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) and lipopolysaccharide (LPS) were purchased from Sigma Aldrich Co. (St. Louis, USA). φX-174 RF I plasmid DNA was purchased from New England BioLabs (County Rood Ipswich, MA, USA). Antibodies against IκB-α, p65 and β-actin were purchased from Cell Signaling (Beverly, MA, USA). Cell culture media, Dulbecco's Modified Eagle medium (DMEM)/F-12 1:1 Modified medium (DMEM/F-12) was purchased from Lonza (Walkersville, MD, USA), pNFκ B–Luc cis-Reporter plasmid was purchased from Agilent Technologies (Santa Clara, CA, USA).

#### 2. Sample preparation

Herbal teas, L. japonica, C. morifolium, M. arvensis and P. rhizoma was kindly provided by Bonghwa Alpine Medicinal Plant Expriment Station, Korea. One hundred gram of the herbal teas was extracted with 300 ml of distilled water in 100 °C for 90 min, After 90 min, the extracts were filtered and freeze-dried. The freeze-dried extracts were kept at −80 °C until use.

#### 3. Cell culture and treatment

Mouse macrophage cell line, RAW264.7 cell was purchased Korean Cell Line Bank (Seoul, Korea) and grown in DMEM/F-12 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were maintained at 37 °C under a humidified atmosphere of 5% CO\textsubscript{2}. Aqueous extracts from the herbal tea were dissolved in 1× phosphate–buffered saline (PBS) and treated to cells, 1×PBS was used as a vehicle.

#### 4. DPPH radical scavenging assay

DPPH radical scavenging assay was carried out according to the literature\textsuperscript{13}. Briefly, 760 µl DPPH ethanol solution (300 µM) solution and 40 µl of the extracts were mixed and then incubated at 37 °C for 30 min. After 30 min, the absorbance was measured at 515 nm.

#### 5. Fe\textsuperscript{2+} chelating assay

Fe\textsuperscript{2+} assay was performed according the literature\textsuperscript{13}. The reaction mixture (800 µl) contained 15 µl of FeCl\textsubscript{2} (2 mM), 150 µl of varying concentrations of the extracts and 605 µl distilled water. The mixture was shaken vigorously and left at room temperature for 30 min. After 30 min, 30 µl of ferrozine (5 mM in methanol) was added and mixed. The absorbance of the Fe\textsuperscript{2+}–ferrozine complex was measured at 562 nm.

#### 6. DNA cleavage assay

Conversion of the supercoiled form of plasmid DNA to the open–circular and further linear forms has been used as an index of DNA damage\textsuperscript{15}. The reaction mixtures (25 µl) containing 5 µl of φX-174 RF I plasmid DNA, 10 µl of varying concentrations of the extracts and 5 µl of 1 mM FeSO\textsubscript{4} were incubated at 37 °C for 30 min. After 30 min, 5 µl of a solution containing 50% glycerol (v/v), 40 mM EDTA and 0.05% bromophenol blue was added to stop the reaction and the reaction mixtures were electrophoresed on 1% agarose gel, and the DNA in the gel was visualized and photographed under ultraviolet light after ethidium bromide staining.