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Antioxidant and Nicotine Degradation Effects of Medicinal Herbs

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Abstract

Antioxidant activity and nicotine degradation activity (NDA) of 21 medicinal herbs were determined by using a 1,1-diphenol-2-picrylhydrazyl (DPPH) method and a PLC/PRF5 human liver cell line method, respectively, to develop an anti-smoking aid. The highest and lowest antioxidant activities represented by IC_{50} value were 30 μg/mL of *Eugenia caryophyllus* and 3,270 μg/mL of *Panax ginseng* C. A. Meyer, respectively. Antioxidant activity of *Eugenia caryophyllus* was equal to 38.0±1.2 mg VCEAC(vitamin C equivalent antioxidant capacity)/g herb. The highest and lowest NDA values were 1.81 of *Astrgalus membranaceus* Bunge and 1.01 of *Raphani seed* and *Lespedeza tomentosa* Sieb, respectively. Eleven medicinal herbs with high antioxidant activity and/or NDA were selected to make an herbal tea. The herbal tea had high antioxidant activity (50 μg/mL IC_{50} and 22.4±1.4 mgVCEAC/g) and NDA (1.243). The medicinal herb tea could help smokers quitting smoking by degrading and exhausting nicotine accumulated in body and removing reactive oxygen species.

Key words: quit smoking, antioxidant, nicotine degradation activity, cotinine, medicinal herb

INTRODUCTION

The incidence of smoking among Korean male adults was approximately 74.2% for several years, and the smoking rate is highly increasing (1,2). Despite several efforts to reduce the number of people who begin smoking and to assist smokers quitting, smoking accounted for a relatively high death rate among Korean males (3). The deleterious health effects of smoking is severe and extensive including impaired lung capacity, lung cancer, phlegm, cough, difficulty in breathing, bronchial trouble and vesicular emphysema. Also, smoking causes many kinds of heart disease and impairs the effectiveness drugs for treating heart disease.

Cigarette smoking produces about 4,000 toxic chemicals such as tar, nicotine, and reactive oxygen species (ROS) (4). Tar is the concentrated, thick, and dark brown liquid substance formed when the cigarette smoke is released. Nicotine, a highly addictive alkaloid, occurs at concentrations of about 2 mg per a cigarette, as is the reason why most of smokers failed to quit smoking (5,6). Nicotine also has deleterious mental effects: antihypnotic effects at low concentrations and nervously stable effects at high concentrations. ROS formed by cigarette smoking are associated with the oxidative damage of proteins, lipids and nucleic acids, and accelerating age-related diseases such as cancer, diabetes, joint inflammation and hardening of the arteries (4,7). Nicotine absorbed by the body is metabolized primarily by the liver and, to a small extent, in the lung and kidney. It is converted by α-hydroxylation mainly to cotinine and excreted in urine (8, 9). Compared to the half-life of nicotine (2 hrs), cotinine has a half-life of about 19 hrs and can provide a reliable indication on the recent smoking history (10,11). The concentration of cotinine in urine has been measured by gas chromatography (GC) (12), high performance liquid chromatography (HPLC) (13), radio-immunoassay (RIA) (14) and direct barbituric acid (DBA) (15). GC and HPLC methods produce reproducible and sensitive results but these were inconvenient and expensive when many samples are necessary to be measured. The RIA method, requiring radioactive isotopes, is not safe and can cause health concerning problems. However, the DBA method can measure many samples quickly and simply, and the measured levels are well correlated with daily cigarette consumption (16), so it was used in this study.

It is suggested that consumption of high concentrations of antioxidants may be related with reducing the oxidative damage from smoking and related diseases, and helping ease the withdrawal symptoms (17). Many of herbal remedies, which have been used as foods or medicinal purpose for a long time, are safe and reported to have potent antioxidant activities.

However, NDA of medicinal herbs and a tea has not been reported in literature. One strategy to incorporate medicinal herbs as an aid of quitting smoking is to select

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medicinal herbs containing high antioxidant and NDA, and to maximize those activities through a medicinal herb tea. The objectives of this study were to measure the antioxidant activity of medicinal herbs by DPPH method and NDA by PLC/PRF5 human liver cells and to determine the antioxidant activity of a tea of selected medicinal herbs.

MATERIALS AND METHODS

Materials

DPPH(1,1-diphenol-2-picrylhydrazyl), (-)nicotine, (-) cotinine, L-ascorbic acid, barbituric acid, and sodium acetate were purchased from Sigma Chemicals, USA. Chloramine T and sodium metasulfite were purchased from Aldrich Chemicals, USA. Twenty-one medicinal herbs, which were selected based on the efficacy documented in the oriental medicine book, Bangyakhappeun (18), were obtained from local markets (Kook Il Co. Ltd., Korea) (Table 1).

Sample preparation

The medicinal herbs were cut into small pieces and 10 g of each sample were put into 500 mL 80% methanol. They were extracted in a shaker for 24 hrs, and filtered with Toyo No.1 filter paper. The filtered extract was concentrated using a rotary vacuum evaporator (Eyela NE1S, Japan). The extract was resolubilized to a final volume of 10 mL with 80% methanol for the antioxidant activity test. The extracts were stored at -20°C in the dark and thawed just before use. The sample extracts for the cell culture were dissolved in 10 mL PBS (phosphate-buffered saline), and (-)nicotine was prepared from 10mM stock in PBS. These were stored in a dark cold room (4°C) and used within 1 week.

Preparation of a medicinal herb tea

Oriental medicine herbs were chosen based on the high antioxidant activity and NDA. The selected herbs were mixed, ground and extracted as described above.

Measurement of antioxidant activity

The antioxidant activity of the sample was measured by DPPH radical method of Brand-Williams et al. (19). The absorbance at 517 nm was monitored in the present of different concentrations of the sample extracts. The control, 80% methanol instead of the sample solution, was carried out to determine the absorbance of DPPH before interacting with the sample extracts. The antioxidant activity was expressed as IC_{50}, the amount of medicinal herbs necessary to decrease the initial DPPH absorbance by 50%. L-Ascorbic acid was used as a standard for the measuring antioxidant activity, so the antioxidant activity of the sample was indexed to a percentage of L-ascorbic acid. The antioxidant activity was also expressed as mg VCEAC/g medicinal herbs (VCEAC; vitamin C equivalent antioxidant capacity) (20).

Table 1. Antioxidant and nicotine degradation activities of 21 medicinal herbs

No.	Korean name	Scientific name	Plant part	IC_{50} (μg/mL)[1)]	NDA[2)]
1	Gamcho	*Glycyrrhiza uralensis* Fisch	Radix	$69^{cd,5)}$	1.10^{c}
2	Kangwhal	*Ostericum koreanum* (Maximowicz) Kitagawa	Radix	86^{e}	1.60^{k}
3	Gungang	*Zingiber officinale* Roscoe	Rhizoma	84^{de}	1.50^{j}
4	Goboon	*Angelica tenuissima* Nkai	Rhizoma	369^{jk}	1.04^{b}
5	Kilkyoung	*Platycodon grandiflorum* (Jacq.) A. Dc.	Radix	921^{m}	1.13^{d}
6	Nabokja	*Raphani seeu*	Seed	346^{i}	1.01^{a}
7	Makmundong	*Liriope platyphylla* Wang *et* Tang	Tuber	2880^{o}	1.12^{d}
8	Baggi	*Angelica dahurioa*	Radix	355^{ij}	1.20^{e}
9	Sandugeun	*Lespedeza tomentosa* Sieb	Radix	308^{h}	1.01^{a}
10	Sansaja	*Crataegi fructus*	Fructus	51^{b}	1.75^{l}
11	Sambakcho	*Saururus chinensis* (Lour Baill)	Leaves	54^{bc}	1.24^{g}
12	Seungma	*Cimicifuga heracleifolia* Komarov	Rhizoma	64^{bc}	1.39^{i}
13	Aloe	*Aloe arborescens*	leaves	380^{k}	1.05^{b}
14	Ogapi	*Acathopanax sessiliflorus* (Ruprecht *et* Maximowicz) Seemenn	Cortex	54^{bc}	1.23^{fg}
15	Insam	*Panax ginseng* C. A. Meyer	Radix	3270^{p}	1.30^{h}
16	Jeonho	*Anthriscus sylvestris* Hoffmann	Radix	731^{l}	1.60^{k}
17	Junghang	*Eugenia caryophyllus*	Cortex	30^{a}	1.22^{f}
18	Jinpi	*Aurantii nobilis*	Preicarpium	125^{g}	1.23^{fg}
19	Taksa	*Alisma canaliculatum* All. Br. *et* Bouche	Rhizoma	104^{f}	1.10^{c}
20	Hasuo	*Pleuropterus multiflorus* Turcz.	Rhizoid	2280^{n}	1.28^{h}
21	Hanggi	*Astrgalus membranaceus* Bunge	Radix	744^{l}	1.81^{m}

[1)] IC_{50}: The amount of medicinal herbs necessary to decrease the initial DPPH absorbance by 50%.
[2)] NDA: Nicotine degradation activity was measured by the absorbance ratio at 490 nm of the beginning time to that of 1 hour after treatment of medicinal herbs.
[3)] Different superscripts are significantly different among medicinal herbs ($p<0.05$).

Measuring nicotine degradation activity (NDA)

PLC/PRF5 human liver cell line was obtained from a Korean cell line bank (Seoul, Korea). The PLC/PRF5 stock cells were fractionated equally at the 100% confluence stage and further cultured. The effects of each medicinal herb and a combination of herbs on nicotine degradation were measured by the following method. The pre-cultured cells were washed and cultured for 24 hours in a 3 mL solution containing 10 μL nicotine stock solution. Ten μL of each medicinal herb and a combination of selected medicinal herb extracts was added to the above culture solution and incubated. To optimize the incubation time of NDA, the absorbances of sample mixtures were measured at 490 nm for 60 min and 120 min by UV-VIS spectrophotometer (Shimazu UV1601PC, Japan). NDA was measured by the absorbance ratio at 490nm of the beginning time to that of 1 hour or 2 hours after treatment of medicinal herbs.

Statistical analysis

The data were analyzed statistically by MANOVA and correlation using StatView™ (BrainPower Inc., Calabasa, CA). A p-value ($p < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Antioxidant activities of medicinal herbs

Antioxidant activities of twenty-one medicinal herbs are shown in Table 1. There were significant differences among some tested samples ($p < 0.05$). The highest and lowest antioxidant activities represented by IC_{50} values were 30 μg/mL of *Eugenia caryophyllus* and 3,270 μg/mL of *Panax ginseng* C. A. Meyer, respectively. The medicinal herbs with IC_{50} values of less than 150 μg/mL were *Eugenia caryophyllus* (30 μg/mL), *Crataegi fructus* (51 μg/mL), *Acathopanax sessiliflorus* (Ruprecht *et* Maximowicz) Seemenn (54 μg/mL), *Saururus chinensis* (Lour Baill) (54 μg/mL), *Cimicifuga heracleifolia* Komarov (64 μg/mL), *Glycyrrhiza uralensis* Fisch (69 μg/mL), *Zingiber officinale* Roscoe (84 μg/mL), *Ostericum koreanum* (Maximowicz) Kitagawa (86 μg/mL), *Alisma canaliculatum* All. Br. *et* Bouche (104 μg/mL), and *Aurantii nobilis* (125 μg/mL). VCEAC (Vitamin C equivalent antioxidant capacity) values of ten selected medicinal herbs are shown in Fig. 1. *Eugenia caryophyllus* (No. 17) had the highest VCEAC value of 38.0±1.2 mg/g among selected samples. The VCEAC values of *Glycyrrhiza uralensis* (No. 1), *Ostericum koreanum* (No. 2), *Zingiber officinale* (No. 3), *Crataegi fructus* (No. 10), *Saururus chinensis* (No. 11), *Acathopanax sessiliflorus* (No. 14), *Eugenia caryophyllus* (No. 17), *Aurantii nobilis* (No. 18) and *Alisma canaliculatum* (No. 19) were 16.4, 13.2, 13.5, 22.0, 20.8, 15.3, 21.0, 38.0, 9.3, and 11.1 mg/g, respectively. Choi et al. (21) reported the antioxidant activities of 95 edible and medicinal plants in aqueous or 75% ethanol extraction. *Astragalus membranaceus*, which showed high antioxidant activity in Choi et al. (21) study, gave low antioxidant activity (744 μg/mL of IC_{50}) in this study.

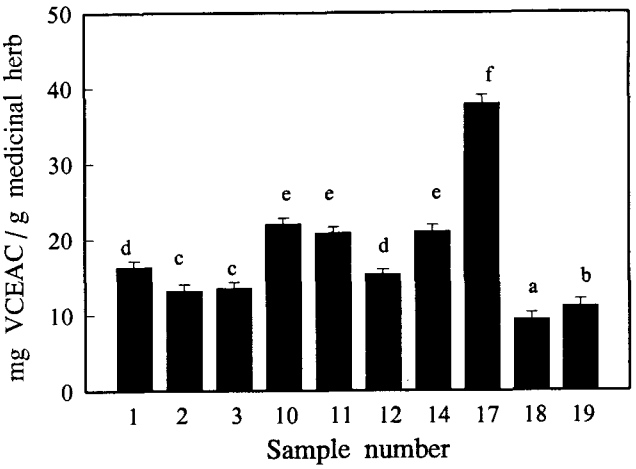

Fig. 1. Vitamin C equivalent antioxidative capacity (VCEAC) of medicinal herb extracts.
The numbers of medicinal herbs are the same that those in Table 1. Different letters are significantly different among medicinal herbs ($p < 0.05$).

Nicotine degradation activity (NDA) of medicinal herbs

To determine the optimum treatment time for NDA test, treatment time effects of 7 selected medicinal herbs were studied (Fig. 2). *Crataegi fructus, Astrgalus membranaceus, Anthriscus sylvestris* and *Pleuropterus multiflorus* showed high NDA in order from high to low for 60 min after treatment but the order changed into *Anthriscus sylvestris, Crataegi fructus, Pleuropterus multiflorus*

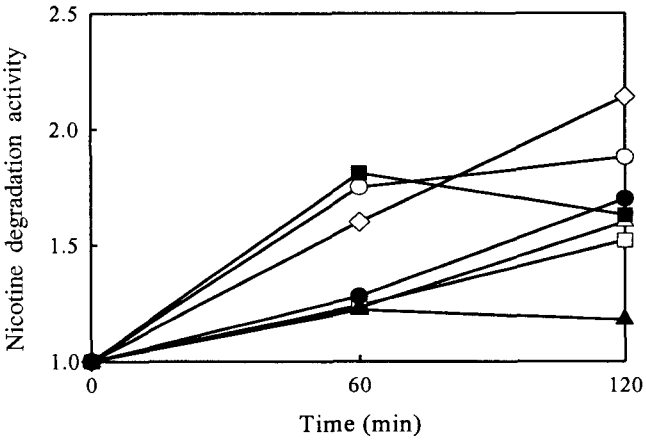

Fig. 2. Effect of time on nicotine degradation in PLC/PRF5 cells treated with medicinal herbs.
No. 10 (○), No. 11 (□), No. 14 (△), No. 16 (◇), No. 17 (▲), No. 20 (●), No. 21 (■).

and *Astrgalus membranaceus* for 120 min. These changes seemed to come from the death of cells due to the apoptosis activity of medicinal herbs (22). Kyung and Lee (23) showed that cotinine formation rate from nicotine was influenced by addition of green tea extract and testing methods. Cotinine concentration was increased with a limited time of 30 min in direct-mixing method and 60 min in cell culture, respectively. The effect of green tea extract with ginseng extract on the cotinine formation was approximately 1.5 times faster than only green tea extract did (23). Each medicinal herb showed the different NDA activity depending on measuring time. Sixty min was chosen as treatment time for further experiment.

NDA values of medicinal herbs determined using PLC/PRF5 cells for 60 min treatment are shown in Table 1. There were significant differences in NDA among some medicinal herbs ($p<0.05$). The highest and lowest NDA were 1.81 and 1.01 absorbance ratio of treatment/control at 490 nm. Among 13 medicinal herbs showing higher NDA of over 1.20, eight medicinal herbs had high antioxidant activity with IC_{50} values of less than 150 μg/mL. However, some medicinal herbs, including *Astrgalus membranaceus, Panax ginseng, Anthriscus sylvestris*, and *Angelica dahurioa* showed high NDA values and low antioxidant activities. *Ostericum koreanum, Zingiber officinale, Angelica tenuissima, Platycodon grandiflorum, Raphani seed, Angelica dahurioa, Lespedeza tomentosa, Crataegi fractus, Cimicifuga heracleifolia, Aloe arborescences*, and *Aurantii nobilis* showed high correlation coefficient ($R^2=0.84$) between antioxidant activities and NDA values while the others showed low correlation coefficient ($R^2=0.27$). This result suggests that antioxidant activities of medicinal herbs may not be a critical factor for NDA but, to certain extent, play a role in nicotine degradation.

Effect of the medicinal herb tea on antioxidant activity and NDA

Eleven medicinal herbs were selected based on the high antioxidant activities and NDA values, including *Eugenia caryophyllus, Acathopanax sessiliflorus, Crataegi fructus, Glycyrrhiza uralensis, Aurantii nobilis, Raphani seed, Pleuropterus multiflorus, Angelica tenuissima, Platycodon grandiflorum, Aloe arborescences*, and *Anthriscus sylvestris*. The medicinal tea of selected 11 medicinal herbs was made not only to optimize synergistic effects of antioxidant and NDA activity but also to minimize malefical and harmful health effects which might come from several herbs. The medicinal herb tea showed 50.6 μg/mL IC_{50}, 22.4 ± 1.4 mgVCEAC/g, and 1.24 NDA value. This herbal tea could be helpful to degrade and exhaust nicotine accumulated in body and reduce free radicals. More comprehensive intake test in human body should be studied to confirm the effects of a combination mixture of herbs as an anti-smoking aid.

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