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Antioxidant and Anti-stress Activities of Aqueous Extract from Medicinal Herbal Plants.

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ABSTRACT

Recently, the extracts or bioactive components of medicinal plants are being extensively explored for antioxidant activities in several cellular systems. However, the mechanisms involved in its pharmacological properties are still not elucidated. This study was aimed to evaluate antioxidant and anti-stress activities of aqueous extract from medicinal herbal plants by *in vitro* and *in vivo* study. In the present study, DPPH radical scavenging activities of lemongrass and peppermint extracts were significantly higher than lavender extract in an *in vitro*. In our *in vivo* study, medicinal plant extract, especially lemongrass markedly increased the GSH content in liver, compared to stress group. Antioxidant enzyme activities were improved in lemongrass-treated group. The expressions of plasma IL-6 and TNF-α were markedly reduced in the lemongrass or betaine-treated groups compared to the stress group. The plasma cortisol level was markedly reduced by 1.55-fold in the lemongrass-treated group and by 1.48-fold in the betaine-treated group, compared to the stress group. The 5-HT and 5-HIAA levels were significantly increased in the lemongrass and/or lavender-treated group compared to the stress group. These results suggest that medicinal herbal plant extracts could provide antioxidant and anti-stress effects by acting as an efficient scavenger against several free radicals.

Keywords: Aqueous extracts, Antioxidant activity, Anti-stress, Medicinal herbal plants

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INTRODUCTION

Stress is a common phenomenon that is experienced by every individual. Stress is involved in the pathogenesis of a several diseases that includes mental disorders such as depression, anxiety, and immunosuppression, endocrine disorders including diabetes mellitus, hypertension, male impotence, cognitive dysfunction and peptic ulcer [1]. Several previous studies indicated that alterations in the hypothalamic-pituitary-adrenal (HPA) axis system were characteristic of depression as evidenced by increased releases of corticotrophin-releasing factor (CRF) and cortisol [2, 3].

Recently, little research has been done on the secretion of the adrenal androgen dehydroepiandrosterone (DHEA) and its more stable sulfate ester DHEA-sulfate (DHEAS). DHEAS is the major secretory steroid of the adrenal gland and the most abundant steroid hormone in the human body [4]. Especially, the levels of DHEA(S) and cortisol have been observed to become dissociated under conditions of chronic medical illness including immunodeficiency syndrome (AIDS), rheumatoid arthritis and type 2 diabetes mellitus [5-7].

Oxidative stress is implicated in the stress response and in the pathogenesis of neurologic and psychiatric diseases [8]. Recently, about 80% of the world population relies on traditional medicines for their primary health needs, and most of this therapy involves the use of aqueous extract of plants [9]. Peppermint (*Mentha piperita* L.) is one among the most popular herbal teas. Several previous reports have been indicated that peppermint has many health beneficial effects including antioxidant capacity, antitumor, antiallergenic, antiviral activity and anti-inflammatory actions [10-14]. The tea made from lemongrass (*Cymbopogon citratus*) leaves is also popularly used as antispasmodic, anti-inflammatory, diuretic and sedative [15]. Moreover, lavender (*Lavandula* L.) essential oil contains abundant esters and it has the main action of relaxation on the nerve system [16].

It has been reported that the extracts or bioactive components of these medicinal herbal plants are being extensively explored for antioxidant activities in several cellular systems.[17] However, the mechanisms involved in its pharmacological properties of these plants are still not elucidated. Therefore, this study was aimed to evaluate *in vitro* antioxidant capacities and *in vivo* anti-stress activities of aqueous extract from medicinal herbal plants.

MATERIALS AND METHODS

Reagents

5,5-Dimethyl-1-pyrroline N-oxide (DMPO), 2,2-azobis (2-amidinopropane) hydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), (4-pyridyl-1-oxide)-N-tert-butylnitrone, and the enzymes catalase (CAT), glutathione (GSH), glutathione peroxidise (GPx), and glutathione-S-transferase (GST) were obtained from Sigma Chemical Co. (St Louis, MO, USA), and superoxide dismutase (SOD) kits were obtained from Fluka Co. (FLUKA, Buchs, Switzerland). ELISA assays to detect IL-6 and TNF-α were from Endogen (Woburn, MA, USA). All other reagents were of the highest grade available commercially.

Preparation of the medicinal herbal plants extract

Three kinds of medicinal herbal plants were obtained from local markets (Chungju, Korea). The following 3 herbal plants were studied: lemongrass, lavender and peppermint. The fresh edible tissues of each plant were washed with water. Each plant sample was prepared by boiling in water for 10 min with a ratio of plant to distilled water at 1:20 (w/v). After the extract was filtered under suction through Whatman No. 1 filter paper, the crude extract was concentrated by a rotary evaporator under reduced pressure at low temperature down to 40 mL. The extract was freeze dried, and stored at -20°C for analysis of antioxidant and anti-stress effects.

Free radical scavenging activities

DPPH radical scavenging activity

DPPH radical scavenging activity was measured with the use of the method described by Nanjo *et al*. [18] Briefly, 60 µL of each extracts at various concentrations were added to 60 µL of DPPH (60 µM) in a methanol solution. After the solution was mixed vigorously for 10 sec, it was then transferred into a 100 µL Teflon capillary tube, and the scavenging activity of each extracts with regard to DPPH radicals was measured with the use of an ESR spectrometer (JEOL Ltd., Tokyo, Japan). A spin adduct was measured on an ESR spectrometer exactly 2 min later. Experimental conditions were as follows: central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5; and temperature, 298 K.

Alkyl radical scavenging activity

Alkyl radicals were generated by AAPH. The phosphate-buffered saline (pH 7.4) reaction mixtures containing 10 mM AAPH, 10 mM 4-POBN, and the indicated concentrations of tested samples were incubated at 37°C in a water bath for 30 min and then transferred to a 100 µL quartz capillary tube. The spin adduct was recorded on an ESR spectrometer. Measurement conditions were as follows: central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3×10^5; and temperature, 298 K.

Animals and experimental design

Subjects were male Sprague-Dawley rats (*n*=31) (Dae Han Bio Link CO. LTD., Korea) weighing approximately 100 g at the start of the experiments. The rats fed *ad libitum* access to water and to pelleted commercial diet (Samyang Co., Korea); temperature and relative humidity were kept at 24±1 °C and 60±10%, respectively. Rats were maintained in a reversed light cycle of 12 h with lights off at 08:00 h. Acclimatization to local conditions lasted at least one week. The experimental room was maintained under the same conditions as the animal room. Treatments were randomly assigned to animals in order to obtain six groups: no shock with saline treatment (control group, *n*=5), shocked rats with saline treatment (stress group, *n*=6), shocked rats with extract of lemongrass treatment (ST + lemongrass group, *n*=5), shocked rats with extract of lavender treatment (ST + lavender group, *n*=5), shocked rats with extract of peppermint treatment (ST + peppermint group, *n*=5) and shocked rats with extract of betaine treatment (ST + betaine group, *n*=5) as a positive control. The extract of medicinal herbal plants were dissolved or dispersed in saline, respectively. Aqueous extract from medicinal herbal plants (100 mg/kg, in saline, p.o.) and saline were given a daily dose of 15 mL/kg between 10:00 and 11:00 for 2 weeks. Rats were transferred to a separate quiet room, where they received footshocks via the grid floor of a Plexiglas cage (30×30×30 cm) between 13:00 and 14:00 h. Light intensity in cage was approximately 400 lx. Two shocks trains were administered per minute for 5 min, i.e. each rat received 10 shocks. Each shock train was 1 s-long and consisted of 0.01 s shocks separated by 0.02 s-long breaks. Current potential and intensity were 100 V and 3 mA, respectively. Control rats were placed into a similar box for 5 min, but shocks were not delivered. The box was cleaned after each shocking session with ethanol. Weekly measurement of body weight was obtained. All animal-based procedures were approved by the Institutional Animal Care and Use Committee at Konkuk University (KU10064).

Sampling procedures

One hour after the last stressor, blood was drawn from the heart puncture into heparin tubes and centrifuged at 2,000 rpm for 5 min and 4°C. The plasma samples were stored at -70°C until assayed. Following blood collection, whole brain, liver, heart and kidneys were rapidly washed in saline buffer, collected into cryovials, weighed and immediately stored in liquid nitrogen for biochemical analysis.

Determination of antioxidant enzyme activities in liver

Glutathione

Liver samples were pulverised in a cooled ceramic percussion mortar with 6% metaphosphoric acid, and the mixture was centrifuged (55,000g for 30 min) at 4 °C. Total glutathione (GSH + oxidized glutathione (GSSG), which are useful indicators of oxidative stress in cells and tissues) concentration was determined enzymatically according to the procedure of Floreani *et al*. [19] with slight modification. The supernatant (0.05 mL) was mixed with 100 mM phosphate buffer (pH 7.4, 0.39 mL) containing 5 mM EDTA, 10 mM 5,5-dithiobis-(2-nitrobenzoic acid) (0.025 mL), and 5 mM nicotinamide adenine dinucleotide phosphate (NADPH, 0.08 mL). After 3 min of equilibration at 25 °C, the reaction was started by adding 2 units of glutathione reductase (type III from bakers yeast). The formation of 5,5-dithio-2-nitrobenzoic acid was continuously recorded at 412 nm with a UV/visible spectrophotometer. The total amount of GSH in the samples was determined from a standard curve obtained by plotting known amounts of GSH versus the rate of change of absorbance at 412 nm.

Superoxide dismutase assay

Liver samples were homogenised in nine volumes of 20 mM phosphate buffer containing 0.1 M KCl, 1 mM EDTA, and 0.5% Triton X-100 (pH 7.4). The homogenate was centrifuged (55,000g at 4 °C) for 30 min, and the supernatant was used for the enzyme assays. The supernatant was mixed with 1 M GSSG and 5 mM NADPH in 0.1 M phosphate/0.5 mM EDTA buffer (pH 7.0), and the formation of $NADP^+$ was monitored with a spectrophotometer at 340 nm [20]. For the SOD assay, the homogenate was mixed with 1 mM xanthine, 0.2 mM cytochrome, and 0.05 M potassium cyanide in 0.05 M potassium phosphate/0.1 mM EDTA buffer, and then xanthine oxidase was added to the reaction mixture. SOD activity was measured from the inhibition of the reduction rate of cytochrome by superoxide radical as observed spectrophotometrically at 550 nm [21]. The activities of SOD were expressed as international units/mg of liver sample.

Glutathione-S-transferase

The activity of GST towards 1-chloro-2,4-dinitrobenzene (CDNB) was determined by the method of Habig *et al* [20]. The method was based on the reaction of CDNB with the –SH group of GSH, which is catalysed by GST contained in the liver samples. The reaction proceeded in the presence of 1 mM GSH in phosphate buffer (pH 6.5) at 37 °C. GST activity was expressed as nM GSH/min.mg protein. The GST assay kit utilised CDNB, which is suitable for the broadest range of GST isozymes. Following conjugation of the thiol group of the GSH to the CDNB substrate, there was an increase in the absorbance at 340 nm.

Glutathione peroxidase activity assay

The activity of GPx was determined following the oxidation of NADPH with t-butyl hydroperoxide as a substrate [22]. This reaction was followed by the action of GPx contained in the liver samples examined on t-butyl hydroperoxide (3 mM) in 0.5 M phosphate buffer, pH 7.0, at 37 °C. The activity of GPx was expressed as nM NADPH/min/mg protein.

Catalase

CAT activity was evaluated by the rate of hydrogen peroxide (H_2O_2) decomposition [23]. The method was based on H_2O_2 degradation by the CAT contained in the examined samples. In this procedure 50 mM phosphate buffer (pH 7.0) was used with 30 mM H_2O_2 as substrate. CAT activity was expressed as µM H_2O_2/min/mg protein.

Protein determination, electrophoresis, and immunoblotting

The plasma was mixed with Laemmli buffer and boiled in a boiling water bath for 10 min. The proteins were separated electrophoretically and transferred to PVDF membranes. The following antibodies were used for immunodetection: goat anti-rat IL-6 (1 mg/ml; R&D systems), goat anti-rat TNF-α (1 mg/ml; R&D systems, Minneapolis, MN, USA). Goat anti-rat and goat anti-mouse secondary antibodies conjugated to horseradish

peroxidase (1:1000; Promega; Madison, WI, USA) followed by enhanced chemiluminescence reagents (Amersham Biosciences) were used to detect the target proteins. Immunoreactive bands were imaged using a ChemiDoc XRS system (Bio-Rad).

Biochemical analyses

Plasma cortisol concentrations were determined by a double-antibody radioimmunoassay method using a commercially available kit (Biochem Immunosystem). Plasma dehydroepiandrosterone-sulfate (DHEA-S) levels were also measured by using a commercially available radioimmunoassay kit (Radim S.p.A.).

Monoamine neurotransmitter levels in brain

Rat brain monoamine neurotransmitters, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured by HPLC coupled with electrochemical detection. Briefly, the brain tissues were homogenized in an ice-cold solution of 0.4 M perchloric acid (6.6 µL/mg) containing 5 mM sodium bisulfate and 0.04 mM EDTA for avoiding oxidation, using a Polytron homogenizer, and then centrifuged at 14,000 rpm for 30 min at 4°C. HPLC was used to assay 5-HT and 5-HIAA. The HPLC procedure was performed according to the method previously described Byers *et al*. [24] with some modifications. Briefly, an Agilent HC-C18 analytical column (250 mm × 4.6 mm, 5 µm; Agilent, USA) was used. The mobile phase consisted of 20% methanol and 80% aqueous solution, which contained 30 mM citric acid, 40 mM sodium acetate, 0.2 mM ethylenediamine-tetraacetic acid (EDTA) disodium salt and 0.5 mM octanesulfonic acid sodium salt, at a flow rate of 1.0 mL/min and at pH value of 3.8. The level of 5-HT and 5-HIAA were detected using a Waters 474 scanning fluorescence detector (Waters, USA) with the excitation and emission wavelengths set at 280 nm and 330 nm, respectively. The HPLC system was connected to a computer to quantify all compounds by comparing the area under the peaks with the area of reference standards with specific HPLC software (Chromatography Station for Windows).

Statistical analysis

All data were presented as the means ± standard deviation, and statistical analyses were performed using Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, U.S.A). The differences between means were assessed by the Duncan's multiple range tests, and statistical significance was defined at $P < 0.05$.

RESULTS

Free radical scavenging activities of medicinal herbal plant extracts

In the present study, DPPH and alkyl radical scavenging activities of medicinal herbal plant extracts were measured using an ESR spectrometer. DPPH is a stable radical that is used to screen the free radical-scavenging ability of compounds or antioxidant activity of plant extracts. The effects of medicinal herbal plant extracts including lemongrass, lavender and peppermint on DPPH radical scavenging activity are shown in Fig. 1A. A lower value of IC_{50} indicates greater antioxidant activity. It was observed that DPPH radical scavenging activities, at the concentration inhibiting 50% of free radical generation (IC_{50}) of distilled water extract from lemongrass (0.0079 ± 0.0018 mg/mL) and peppermint (0.0123 ± 0.0014 mg/mL) had significantly higher activity than that of extract from lavender (0.0462 ± 0.011 mg/mL). Additionally, a decrease of ESR signals was observed with the dose increment of lemongrass extract (Fig. 1B). On the other hand, the effects of medicinal herbal plant extracts on alkyl radical scavenging activity are shown in Fig. 1C. The peppermint extract (0.0076 ± 0.001 mg/mL) were exhibited stronger alkyl radical scavenging activity compared to that of lavender (0.0123 ± 0.0023 mg/mL). In addition, the decrease of ESR signal was observed with the dose increment of the extract from peppermint (Fig. 1D). These results indicate that medicinal herbal plants effectively scavenged various reactive radicals such as DPPH and alkyl radicals.

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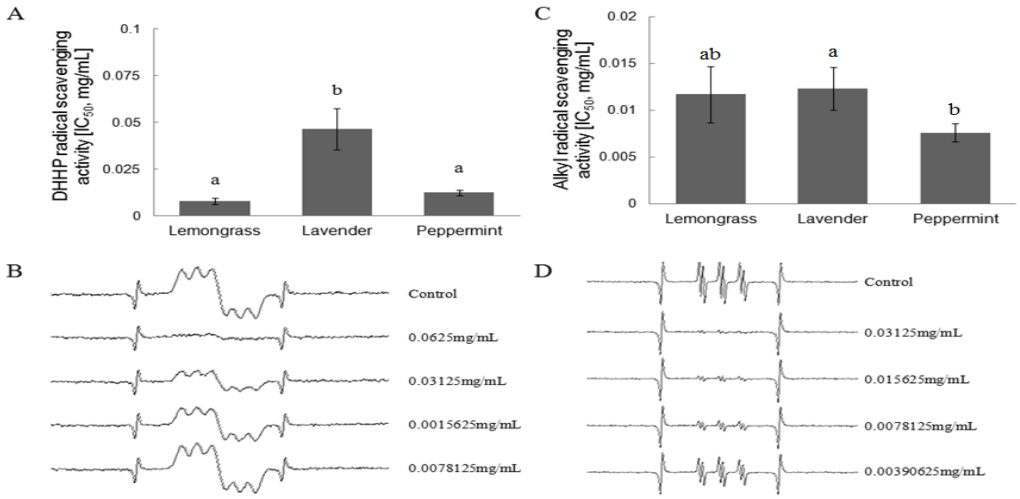

Figure 1: Free radical scavenging activities of the medicinal herbal plant extracts. IC_{50} values (expressed in mg dry weight of each extracted from medicinal herbal plants per mL organic solvent) of the samples that exhibited (A) DPPH radical scavenging activity, (B) ESR spectra on DPPH radical scavenging activity of lemongrass extract, (C) alkyl radical scavenging activity and (D) ESR spectra on alkyl radical scavenging activity of peppermint extract.

Body weight and organ weights in medicinal herbal plants supplemented rats

The effects of medicinal herbal plant extracts on the body weight gain and organ weight of rats are shown in Table 1. The body weight gain for 2 weeks was significantly higher in the stress group than the peppermint-treated stress group without the change of food intakes. The kidney weight in the stress group was significantly higher compared to that in the ST + peppermint group. On the other hand, the liver, heart and brain weight were not affected by medicinal herbal plant extracts treatment, which is indicating that medicinal herbal plant extracts has no side effects.

Table 1: Body weight and organ weights in medicinal herbal plants supplemented rats

	Initial body weight (g)	Body weight gain (g/14 d)	Liver (g)	Kidney (g)	Heart (g)	Brain (g)
Control	99.9 ± 11.0^{NS}	50.9 ± 4.4^{ab}	4.71 ± 0.6^{NS}	1.27 ± 0.06^{ab}	0.62 ± 0.07^{NS}	1.51 ± 0.26^{NS}
Stress	100.2 ± 11.1	56.9 ± 5.1^{a}	5.15 ± 1.10	1.32 ± 0.10^{a}	0.65 ± 0.04	1.52 ± 0.25
ST+Lemongrass	97.7 ± 9.6	50.4 ± 6.9^{ab}	4.78 ± 0.44	1.22 ± 0.11^{ab}	0.65 ± 0.06	1.63 ± 0.15
ST+Lavender	99.4 ± 6.6	49.5 ± 3.5^{ab}	4.68 ± 0.33	1.26 ± 0.07^{ab}	0.66 ± 0.08	1.59 ± 0.31
ST+Peppermint	99.6 ± 6.7	46.9 ± 2.0^{b}	4.76 ± 0.48	1.26 ± 0.06^{b}	0.67 ± 0.03	1.54 ± 0.10
ST+Betaine	99.6 ± 7.5	50.4 ± 0.3^{ab}	4.56 ± 0.18	1.26 ± 0.10^{ab}	0.61 ± 0.04	1.64 ± 0.14

Data are shown as the mean ± SEM values (n = 5~6 per group). ^{NS} Not significantly different among the groups. $^{a, b}$ Mean values with different superscript letters in the same column are significantly different ($P < 0.05$).

GSH level and antioxidant enzyme activities in liver

In the present study, the effects of medicinal herbal plant extracts on the antioxidant enzyme activities in rat liver sample are shown in Table 2. The GSH level was markedly increased in the medicinal herbal plant extract treatment group compared to the stress group. Especially, the GSH level was 1.81-fold and 1.67-fold increased in the ST + lemongrass group and ST + betaine group compared to the stress group. In our study, GST activity, which is known to play an important role in the intracellular defense against oxygen radical damage, was markedly increased by 2.04-fold in the ST + lemongrass group compared to the stress group. The activity of GPx was significantly higher in the ST + lemongrass or betaine groups compared to those of the stress group. Also, the activity of CAT was markedly increased in the ST + lemongrass, ST + peppermint and ST + betaine groups compared to those of the stress group. These results suggest that medicinal herbal plants such as lemongrass, lavender and peppermint may result in reduced oxidative stress in rats, with improved

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antioxidant capacity evidenced by the increased concentrations of antioxidants and substantiated antioxidant enzyme activities.

Table 2: Antioxidant enzyme activities in medicinal herbal plants supplemented rats

Biomarkers	GSH (nmol GSH/min/mg protein)	SOD (%)	GST (µmol GST/min/mg protein)	GPx (nmol NADPH/min/mg protein)	CAT (µmol H_2O_2/min/mg protein)
Control	17.25 ± 1.52[b]	91.31 ± 1.08[NS]	22.49 ± 1.48[b]	0.98 ± 0.01[b]	0.097 ± 0.013[b]
Stress	12.96 ± 0.82[c]	90.44 ± 2.11	13.08 ± 0.55[c]	0.58 ± 0.06[d]	0.037 ± 0.006[c]
ST+Lemongrass	23.45 ± 1.49[a]	92.61 ± 1.79	26.62 ± 1.18[a]	1.13 ± 0.11[a]	0.159 ± 0.014[a]
ST+Lavender	18.37 ± 2.71[b]	91.90 ± 1.01	7.16 ± 0.98[d]	0.49 ± 0.07[d]	0.049 ± 0.009[c]
ST+Peppermint	17.32 ± 1.52[b]	89.46 ± 5.66	5.39 ± 0.56[d]	0.54 ± 0.03[d]	0.105 ± 0.018[b]
ST+Betaine	21.58 ± 1.75[a]	95.11 ± 5.39	21.25 ± 1.96[b]	0.69 ± 0.05[c]	0.141 ± 0.092[a]

Data are mean ± SEM values (n = 5~6 per group). GSH, total glutathione; SOD, superoxide dismutase; GST, glutathione-S-transferase; GPx, glutathione peroxidase; CAT, catalase.
[a, b, c, d] Mean values with different superscript letters in the same column are significantly different ($P < 0.05$). [NS] Not significantly different among the groups.

Expression of plasma IL-6 and TNF-α in medicinal herbal plants supplemented rats

Fig. 2 shows the immunoblot analysis of IL-6 and TNF-α protein in plasma of control and experimental rats. Induction of IL-6 and TNF-α protein in plasma, observed in lemongrass or betaine-treated groups were markedly reduced compared to the stress group.

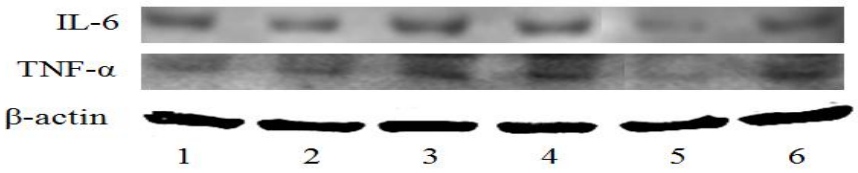

Figure 2: Expressions of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in plasma. Immunoblot analysis of IL-6, TNF-α and β-actin, respectively. Lane 1 : Control, lane 2 : ST + lemongrass, lane 3 : ST + lavender, lane 4 : ST + peppermint, lane 5 : ST + betaine, lane 6 : Stress group.

Plasma stress-related hormone levels

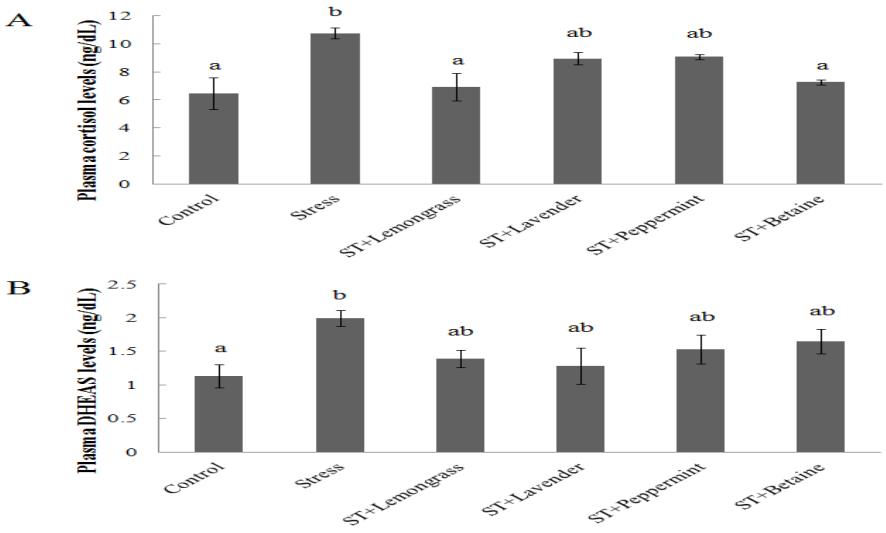

Figure 3: Plasma stress-related hormone concentrations (A) cortisol (B) dehydroepiandrosterone-sulfate (DHEA-S) in medicinal herbal plants supplemented rats. Data are presented as means ± SEM bar. [a, b] Means with different superscript among the groups are different (p<0.05).

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The effects of medicinal herbal plant extracts on the plasma stress-related hormone levels are shown in Fig. 3. The plasma cortisol level was significantly increased in the stress group compared to the control group (Fig. 3A). On the other hand, the plasma cortisol levels were markedly reduced by 1.55-fold in the lemongrass-treated group and 1.48-fold in the betaine-treated group compared to the stress group. The plasma dehydroepiandrosterone-sulfate (DHEA-S) level was significantly higher in the stress group than the control group. On the other hand, treatment of medicinal herbal plans extracts tended to decrease the plasma DHEA-S level (Fig. 3B).

Effects of the extracts of medicinal herbal plants on brain monoamine neurotransmitter levels

The effects of medicinal herbal plant extracts on the monoamine neurotransmitter levels in rat brain are shown in Fig. 4. The 5-HT level was significantly decreased in the stress group compared to the control group (Fig. 4A). However, betaine tended to suppress the decreasing in the 5-HT level in rat brain. Especially, the 5-HT level were markedly increased in the lemongrass and lavender-treated group compared to the stress group. Similar with the 5-HT level in rat brain, the 5-HIAA level was significantly reduced in the stress group compared to the control group (Fig. 4B). On the other hand, the 5-HIAA level was markedly increased in the lavender-treated group compared to the stress group.

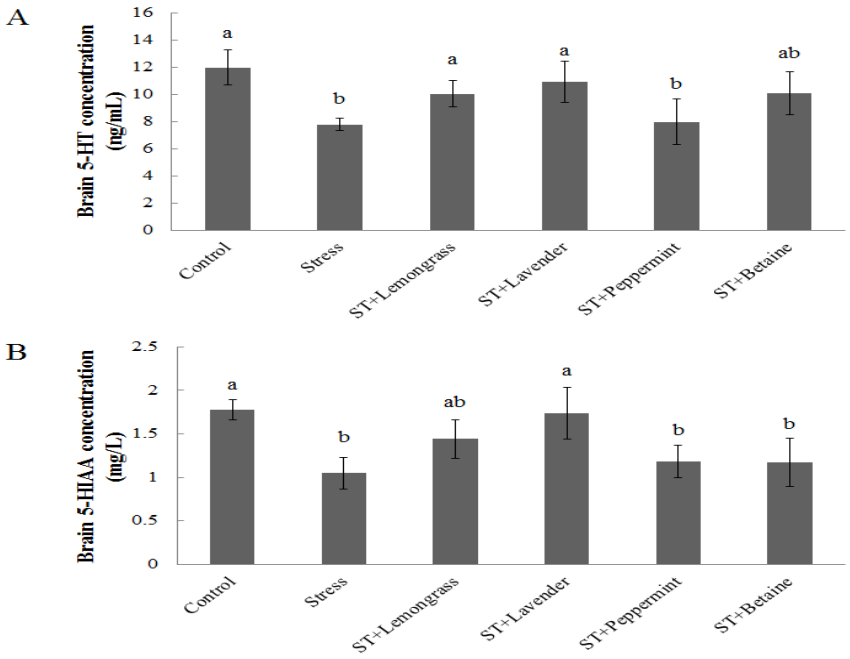

Figure 4: Monoamine neurotransmitter levels (A) 5-hydroxytryptamine (5-HT), (B) 5-hydroxyindoleacetic acid (5-HIAA) of the brain in medicinal herbal plants supplemented rats. Data are presented as means ± SEM bar. [a, b] Means with different superscript among the groups are different ($p<0.05$).

DISCUSSION

Plants have played an important role in maintaining human health and improving the quality of human life for thousands of years. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants relied on traditional medicine for their primary health care needs, and most of this therapy involved the use of plant extracts or their active components [25]. In the present study, we evaluated *in vitro* antioxidant activities and *in vivo* anti-inflammatory and anti-stress activities of aqueous extract from medicinal herbal plants including lemongrass, lavender and peppermint. Several previous studies have demonstrated that medicinal plant extracts such as lemongrass, potent activities for diabetes, digestive disorder as well as antioxidant, anti-inflammatory that is related to its polyphenolic content 26-30]. However, little is known about their anti-stress effect *in vivo*. In our *in vitro* antioxidant activity, it was observed that DPPH radical scavenging activities of lemongrass and peppermint extracts were significantly higher than lavender extract. Moreover, we observed that medicinal herbal plant extract, especially lemongrass extract markedly improved the antioxidant enzyme activities such as GPx as well as GSH content and GST level in rat liver, compared to

the stress group. These results indicate that medicinal herbal plants provide antioxidant effects by acting as an efficient scavenger against several free radicals *in vitro* and *in vivo*.

It has been known that oxygen radical generation is one of part of a complex network of events resulting in acute and/or chronic inflammation *in vivo* [31]. Especially, it has been reported that initial injury in liver by production of ROS, leading to increased activity of myeloperoxidase (MPO) and the release of pro-inflammatory cytokines such as IL-6 and TNF-α [32]. Our present study revealed that the expression of IL-6 and TNF-α protein in plasma were markedly reduced in the lemongrass or betaine-treated groups compared to the stress group *in vivo*. Similar to our results, it was reported that galangin, which is the active constituent of the rhizome of *Alpinia galanga* treatment down-regulated the plasma TNF-α and IL-6 levels and mRNA expression of these cytokines, but enhanced the antioxidant status in fructose-fed rat liver [33].

The stress-induced effects are outcomes of altered activity of several systems such as central neurotransmitters, neurohormonal factors particularly those linked with the pituitary-adrenal axis, and free radical generation [34]. Cortisol is among the major stress hormones and is secreted by activated hypothalamic-pituitary-adrenal (HPA) axis under physical and/or mental stress [35, 36]. In our *in vivo* study, we confirmed that the plasma cortisol level was markedly reduced in the lemongrass-treated group and the betaine-treated group, compared to the stress group. Similarly, Yin *et al*. [37] reported that biocompounds from *Paecilomyces tenuipes* significantly decreased the increased serum cortisol level induced by chronic unpredictable stress in rat. In our study, we have confirmed that stress induced by footshocks markedly reduced the 5-HT and 5-HIAA levels in whole rat brain. On the other hand, brain 5-HT level was significantly increased in the lemongrass or lavender-treated group compared to the stress group. Similar to our results, it was reported that the stress induced by forced swimming test produced the increment of serum cortisol and reductions in brain 5-HT and 5-HIAA levels in male ICR mice. However, the ethanolic extracts of *Curcuma longa* elevated the decreased brain monoamine neurotransmitter levels including 5-HT and 5-HIAA induced by swim stress [38]. Some investigators showed the anti-stress potential of *Ocimum sanctum* compounds in relation to their modulatory effects on the central monoaminergic and antioxidant systems [39]. These differential alterations in the antioxidant systems *in vitro* and *in vivo* as well as variable effect of medicinal herbal plants in rat brain may arise from the antioxidant capacities by acting as an efficient scavenger against several free radicals. Therefore, it is greatly expected that medicinal herbal plants such as lemongrass, lavender and peppermint would be natural source which can be present substitutes for the food and pharmaceutical industries because of its potent antioxidant and anti-stress capacities.

CONCLUSIONS

In conclusion, our results showed that aqueous extract from medicinal herbal plants exhibited strong antioxidant and anti-stress activities *in vivo* as well as *in vitro*. Further pre-clinical and clinical studies seem warranted to assess in more detail possible anti-stress actions of the aqueous extract of medicinal herbal plants, and its therapeutic role.

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