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# Effect Of Lotus (*Nelumbo Nucifera*) Root Hot Water Extract On Electric Foot Shock Stress In Rats.

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**ABSTRACT**

The aim of this study was to evaluate the antioxidant and anti-stress activities of the hot water extract from lotus (*Nelumbo nucifera*) root. The hot water extract from lotus root exhibited potent 1,1-diphenyl-2-picrylhydrazyl and alkyl radical-scavenging activity *in vitro*. Moreover, glutathione content in the liver was significantly higher in rats treated with the lotus root extract than that in electric foot-shock stressed rats. The activities of antioxidant enzymes such as glutathione-S-transferase, glutathione peroxidase, and catalase markedly improved in the lotus root extract-treated group. On the other hand, plasma cortisol level significantly decreased in the lotus root extract-treated group than that in the stressed group. In contrast, brain 5-hydroxytryptamine level significantly increased in the lotus root extract- and/or betaine-treated groups than that in the stressed group. These results suggest that lotus root hot water extract has antioxidant and anti-stress activities by acting as an efficient scavenger of several free radicals and regulating the stress-related hormones.

**Keywords**: Antioxidant, Anti-stress, Hot water extract, Lotus (*Nelumbo nucifera*) root

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## INTRODUCTION

Chronic stress has been related to several psychological and immune disorders, including depression, anxiety, cardiovascular diseases, chronic fatigue, and neuronal degeneration [1, 2]. In the chronic stress conditions, the serum levels of glucocorticoids such as cortisol and corticosterone are increased, whereas the levels of dopamine and serotonin in the brain are decreased, and this is mediated by the hypothalamic-pituitary-adrenal axis [3]. Moreover, chronic stress increase the energy requirement within the body, leading to the generation of reactive oxygen species (ROS) resulting in the oxidative stress [4]. Therefore, there is a need for an effective natural anti-stress agent to prevent stress-induced disorders.

Approximately 80% of the world's population relies on traditional medicines for their primary health needs, and most of these therapies involve the use of aqueous plant extracts [5]. Lotus (*Nelumbo nucifera*), a member of the Nymphaeaceae family, is a well-known medicinal plant in Asian countries, including Korea, Japan, and China. Among the various parts of lotus, the root contains abundant starch and minerals, including calcium, copper, iron, magnesium, and zinc as well as important functional components such as flavonoids and alkaloids [6]. The lotus root has many therapeutic benefits, including anti-diarrheal properties, anti-hypertension and cholesterol-lowering effects, and antimicrobial, anti-hyperglycemic, and antioxidant activities [7-9]. Many researchers have studied the antioxidant effects of lotus root in several cellular systems. However, *in vivo* studies and the mechanisms involved in its pharmacological properties, including antioxidant and anti-stress activities, have not been elucidated. A number of chronic stress animal models, including electric foot-shock stress, immobilization, forced swimming, and noise stimuli have been used to induce anxiety disorders [10]. Therefore, the present study was designed to investigate the anti-stress and antioxidant potential of the hot water extract from lotus root in an *in vitro* and a foot-shock-stressed animal model.

## MATERIALS AND METHODS

### *Reagents*

1,1-diphenyl-2-picrylhydrazyl (DPPH), (4-pyridyl-1-oxide)-N-tert-butylnitrone, glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Superoxide dismutase (SOD) kits were obtained from Biovision (Milpitas, CA, USA). All other reagents were of the highest grade available commercially.

### *Preparation of the hot water extract from lotus root*

Lotus root was obtained from the local markets (Chungju, Korea). Fresh edible tissues of lotus root were washed with water. Samples were prepared by boiling in water for 5 h with a 1:20 (w/v) ratio of lotus root to distilled water. After the extract was filtered under suction through Whatman No. 1 filter paper, then the crude extract was concentrated to 40 mL by using a rotary evaporator under reduced pressure at a low temperature. The extract was freeze-dried and stored at -20 °C for the analysis of antioxidant and anti-stress activities.

### *Determination of total phenolic and total flavonoid contents*

The total phenolic content was analyzed by using the Folin-Ciocalteu assay [11]. The samples (5 mg/mL, 0.1 mL) were mixed with distilled water (2 mL) and Folin-Ciocalteu's phenol reagent (1 mL). After 5 min, 20% aqueous sodium carbonate solution (5 mL) was added and the mixture was incubated in the dark for 60 min. Absorbance was measured at 735 nm by using a UV−vis spectrophotometer, with distilled water as the blank. Total phenolic content was determined by using gallic acid (0−0.8 mg/mL) as a calibration standard, and the results are expressed as milligrams of gallic acid equivalents (GAE) per gram of sample. Total flavonoid content was determined by using a modified aluminum chloride coloration method [11]. Each sample (10 mg/mL, 0.6 mL) was mixed with distilled water (3.75 mL) and 5% aqueous sodium nitrite solution (0.225 mL). After 6 min, 10% aqueous aluminum chloride solution (0.45 mL) was added and the mixture was incubated for 5 min. Then, a sodium hydroxide solution (1 mol/mL, 1.5 mL) and distilled water were added for a total volume of 7.5 mL. Absorbance was measured at 510 nm by using a UV−vis spectrophotometer, with distilled water as the blank. Total flavonoid content was determined by using catechin (0−0.8 mg/mL) as a calibration standard. The results are expressed as milligrams of catechin equivalents (CE) per gram of sample.

***Determination of free radical-scavenging activity by using an electron spin resonance (ESR) spectrometer***

***DPPH radical-scavenging activity*** Lotus root hot water extract (60 µL) at various concentrations was added to 60 µL of DPPH (60 µM) in a methanol solution. After the solution was mixed vigorously for 10 s, it was transferred to a 100-µL Teflon capillary tube. DPPH radical scavenging activity was determined by using an ESR spectrometer (JEOL Ltd., Tokyo, Japan); a spin adduct was measured 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, $6.3 \times 10^5$; and temperature, 298 K.

***Alkyl radical-scavenging activity*** Alkyl radicals were generated by AAPH. Phosphate-buffered saline (pH 7.4) reaction mixtures containing 10 mM AAPH, 10 mM 4-POBN, and the indicated concentrations of test 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, $6.3 \times 10^5$; and temperature, 298 K.

***Animals and experimental design***

For these experiments, we used male Sprague-Dawley rats (*n* = 24) (Dae Han Bio Link CO. LTD., Korea) weighing approximately 100 g at the beginning of the study. The rats had *ad libitum* access to water and 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 1 week. The experimental room was maintained under the same conditions as the animal room. The animals were divided into four groups based on randomly assigned treatments: (1) no electric foot-shock with saline treatment (control group), (2) electric foot-shock with saline treatment (stress group), (3) electric foot-shock with hot water extract of lotus root treatment (ST-LR group), and (4) electric foot-shock with betaine treatment (ST-B group) as a positive control. Lotus root extract (100 mg/kg, in saline, p.o.) and saline were given daily at a dose of 15 mL/kg between 10:00 and 11:00 h for 2 weeks. Rats were transferred to a separate quiet room where they received electric foot shocks through the grid floor of a Plexiglas® cage (30×30×30 cm) between 13:00 and 14:00 h. Light intensity in the cage was approximately 400 lx. Two shock 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 in a similar box for 5 min, but shocks were not delivered. The box was cleaned with ethanol after each shock session. Body weight of the rats was measured weekly. All animals were treated in accordance with the Guidelines for Care and Use of Laboratory Animals of Chonnam National University, Yeosu, Republic of Korea.

***Sampling procedures***

One hour after the last stressor, blood was drawn from a 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, liver, heart, kidneys, and whole brain were rapidly washed in saline buffer, collected into cryovials, weighed, and immediately stored in liquid nitrogen for biochemical analysis.

***Determination of hepatic glutathione content and antioxidant enzyme activities***

Liver samples were pulverized in a cooled ceramic percussion mortar with 6% metaphosphoric acid and then centrifuged (55,000 × g for 30 min) at 4 °C. Total glutathione (GSH) concentrations were determined enzymatically according to the procedure described by Floreani et al. [12] with a slight modification. Briefly, 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 baker's 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. To obtain a 1:9 (w/v) whole homogenate, the liver tissue was homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at

25,000 rpm for 20 min at 4°C to remove any cell debris, and then the supernatant was used to determine the antioxidant enzyme activities. Superoxide dismutase (SOD) activity was estimated using commercial kits supplied by Biovision (Milpitas, CA, USA) according to the manufacturer's instructions. SOD activities were measured at 450 nm and then expressed as SOD activity (inhibition rate %). The activity of glutathione-S-transferase (GST) toward 1-chloro-2,4-dinitrobenzene (CDNB) was determined by using the method described by Habig et al. [13] and is based on the reaction of CDNB with the –SH group of GSH, which is then catalyzed by the 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 is expressed as nM GSH/min/mg protein. The GST assay kit utilized CDNB, which is suitable for the broadest range of GST isozymes. Following the conjugation of the GSH thiol group to the CDNB substrate, there was an increase in the absorbance at 340 nm. The activity of Glutathione peroxidase (GPx) in the liver samples was determined by measuring the oxidation of NADPH with *t*-butyl hydroperoxide (3 mM) as a substrate in 0.5 M phosphate buffer (pH 7.0 and 37 °C). The activity of GPx is expressed as nM NADPH/min/mg protein. Catalase (CAT) activity was determined by evaluating the rate of hydrogen peroxide ($H_2O_2$) decomposition. The method is based on $H_2O_2$ degradation by CAT contained in the samples. For this procedure, 50 mM phosphate buffer (pH 7.0) was used with 30 mM $H_2O_2$ as a substrate. CAT activity is expressed as µM $H_2O_2$/min/mg protein.

***Biochemical analyses in plasma***

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

***Monoamine neurotransmitter levels in rat brain***

The monoamine neurotransmitters, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), were measured in the rat brain by using HPLC coupled with a scanning fluorescence detector. 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 ethylenediamine-tetraacetic acid (EDTA) using a Polytron homogenizer and then centrifuged at 14,000 rpm for 30 min at 4 °C. The HPLC procedure was performed according to the method previously described by Byers et al. [14] 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 a 20% methanol and 80% aqueous solution containing 30 mM citric acid, 40 mM sodium acetate, 0.2 mM 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 levels of 5-HT and 5-HIAA were detected using a Waters 474 scanning fluorescent 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 using specific HPLC software (Chromatography Station for Windows).

***Statistical analysis***

All data are presented as means ± SEM, and statistical analyses were performed using Statistical Analysis System version 8.0 (SAS Institute, Cary, NC, USA). The differences between means were assessed by using the Duncan's multiple range test, and statistical significance was defined at $p < 0.05$.

## RESULTS

**Total phenolic and flavonoid contents and free radical-scavenging activity of the lotus root hot water extract**

In the present study, the total phenolic and flavonoid contents of lotus root hot water extract were 1.27 ± 0.06 mg GAE/g and 0.62 ± 0.07 mg CE/g, respectively (Table 1). The DPPH and alkyl radical-scavenging activities of the lotus root extract were measured by using an ESR spectrometer. DPPH is a stable radical that is used to screen the free radical-scavenging activity of compounds or plant extracts. The effect of lotus root extract on DPPH radicals is shown in Fig. 1A; a lower concentration inhibiting 50% of free radical generation ($IC_{50}$) indicates greater antioxidant activity. The hot water extract from lotus root exhibited potent DPPH radical scavenging activity at an $IC_{50}$ of 0.052 mg/mL. As shown in Fig. 1B, the hot water extract from lotus root

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also exhibited scavenging activity ($IC_{50}$=0.107 mg/mL) against alkyl radical. These results indicate that the lotus root hot water extract effectively scavenges various reactive radicals *in vitro*.

**Table 1: Total phenolic and flavonoid content of the hot water extract from lotus root**

| | Total phenolic content (mg of GAE/g of sample) | Total flavonoid content (mg of CE/g of sample) |
|---|---|---|
| Hot water extract from lotus root | 1.27 ± 0.06 | 0.62 ± 0.07 |

Data shown are expressed as mean ± SD. Each data point was derived from three independent experiments.

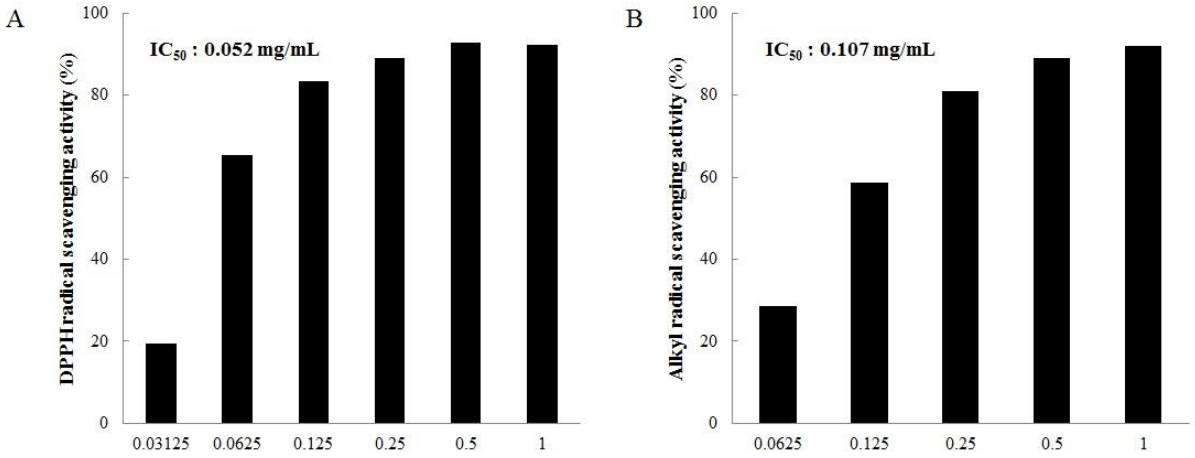


**Figure 1: Free radical-scavenging activities of the hot water extract from lotus root. $IC_{50}$ values (expressed in mg dry weight of extract from lotus root per mL organic solvent) from samples that exhibited (A) DPPH radical-scavenging activity and (B) alkyl radical-scavenging activity.**

**Body and organ weights in rats supplemented with the hot water extract from lotus root**

The effects of lotus root hot water extract on the body weight gain and organ weights of rats are shown in Table 2. The body weight gain over 2 weeks tended to decrease in the stressed group compared with the control group, however, the differences were not significant. The organ weights including liver, kidney, heart, and brain weights were not affected by lotus root hot water extract treatment, indicating that the extract has no side effects.

**Table 2: Body weight gain and organ weights in rats supplemented with the hot water extract from lotus root**

| | Initial body weight (g) | Body weight gain (g/14 d) | Liver (g) | Kidney (g) | Heart (g) | Brain (g) |
|---|---|---|---|---|---|---|
| Control | 103.1 ± 3.9 $^{NS}$ | 53.0 ± 11.1$^{NS}$ | 5.17 ± 0.19$^{NS}$ | 1.30 ± 0.03$^{NS}$ | 0.64 ± 0.05 $^{NS}$ | 1.38 ± 0.28 $^{NS}$ |
| Stress | 100.1 ± 3.9 | 47.0 ± 7.5 | 5.86 ± 1.21 | 1.28 ± 0.21 | 0.68 ± 0.03 | 1.64 ± 0.05 |
| ST-LR[1)] | 98.9 ± 5.2 | 51.0 ± 12.2 | 4.35 ± 0.57 | 1.17 ± 0.07 | 0.61 ± 0.04 | 1.59 ± 0.09 |
| ST-B[2)] | 98.9 ± 5.7 | 50.4 ± 0.8 | 4.52 ± 0.18 | 1.22 ± 0.11 | 0.60 ± 0.03 | 1.72 ± 0.08 |

Data are expressed as mean ± SEM ($n$ = 6 per group).

[1)] ST-LR: foot-shocked rats treated with lotus root hot water extract.

[2)] ST-B: foot-shocked rats treated with betaine.

[NS] Not significantly different among the groups.

[a,b] Mean values with different superscript letters in the same column are significantly different ($p < 0.05$).

**Hepatic GSH content and antioxidant enzyme activities**

Table 3 shows that the GSH content and the activities of antioxidant enzymes in the liver of rats fed a lotus root hot water extract. GSH content was significantly increased in rats receiving the lotus root hot water extract and/or betaine supplementations when compared with the stress group. GST activity, which is known to play an important role in the intracellular defense against oxygen radical damage, was 1.57-fold higher in the ST-LR group and 1.64-fold higher in the ST-B group than that in the stress group. In addition, CAT activity was significantly higher in the ST-LR and ST-B groups than that in the stressed group. Whereas, GPx activity was significantly decreased in in the ST-LR and ST-B groups than that in the stress group. These results suggest that lotus root reduces oxidative stress in rats, with improved antioxidant capacity, as evidenced by the increased concentration of GSH and substantiated antioxidant enzyme activities.

**Table 3: Hepatic antioxidant enzyme activity in rats supplemented with lotus root hot water extract**

| 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 | $20.47 \pm 0.75^{b}$ | $90.29 \pm 6.76^{NS}$ | $22.47 \pm 0.62^{a}$ | $0.76 \pm 0.02^{b}$ | $0.138 \pm 0.010^{ab}$ |
| Stress | $17.71 \pm 0.62^{c}$ | $90.48 \pm 1.62$ | $14.10 \pm 0.39^{b}$ | $0.85 \pm 0.02^{a}$ | $0.115 \pm 0.009^{b}$ |
| ST-LR[1)] | $21.57 \pm 0.78^{b}$ | $98.75 \pm 1.20$ | $22.12 \pm 1.16^{a}$ | $0.71 \pm 0.02^{c}$ | $0.162 \pm 0.012^{a}$ |
| ST-B[2)] | $24.66 \pm 0.71^{a}$ | $96.30 \pm 1.22$ | $23.06 \pm 2.21^{a}$ | $0.79 \pm 0.01^{b}$ | $0.143 \pm 0.011^{a}$ |

Data are expressed as mean ± SEM values ($n$ = 6 per group).
[1)] ST-LR: foot-shocked rats treated with lotus root hot water extract.
[2)] ST-B: foot-shocked rats treated with betaine.
Abbreviations: GSH, total glutathione; SOD, superoxide dismutase; GST, glutathione-S-transferase; GPx, glutathione peroxidase; CAT, catalase.
[NS] Not significantly different among the groups.
[a,b,c] Mean values with different superscript letters in the same column are significantly different ($p < 0.05$).

**Effects of the lotus root hot water extract on stress-related hormone levels in plasma**

The effects of lotus root hot water extract on stress-related hormone levels are shown in Fig. 3. Plasma cortisol level was markedly increased in the stress group than those of the control group (Fig. 3A). In contrast, plasma cortisol level was 0.73-fold and 0.87-fold lower in the ST-LR and SR-B groups, respectively, when compared with the stress group. On the other hand, plasma dehydroepiandrosterone-sulfate (DHEA-S) levels were not affected by lotus root hot water extract or betaine treatment in the electric foot-shock-stressed rats (Fig. 3B). The cortisol/DHEAS ratio was significantly increased in the stress group than those of the control group. However, the cortisol/DHEAS ratio was markedly decreased in the ST-LR group compared to that of the stress group (Fig. 3C).

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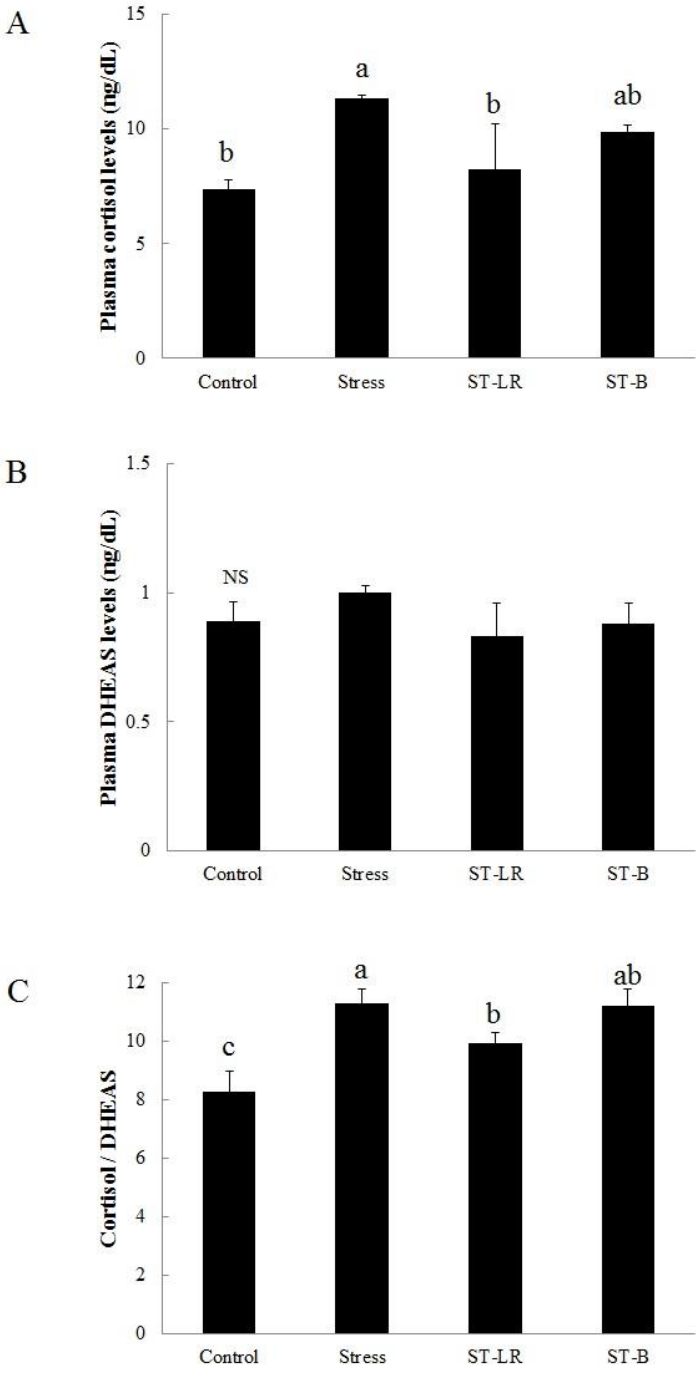


**Figure 2: Plasma stress-related hormone concentrations of (A) cortisol, (B) dehydroepiandrosterone-sulfate (DHEA-S), and (C) the cortisol/DHEAS ratio in rats supplemented with the hot water extract of lotus root. Data are presented as means ± SEM. [a,b] Means with different superscripts among the groups are significantly different ($p < 0.05$). [NS] Not significantly different among the groups. ST-LR: rats treated with hot water extract of lotus root, ST-B: foot-shocked rats treated with betaine.**

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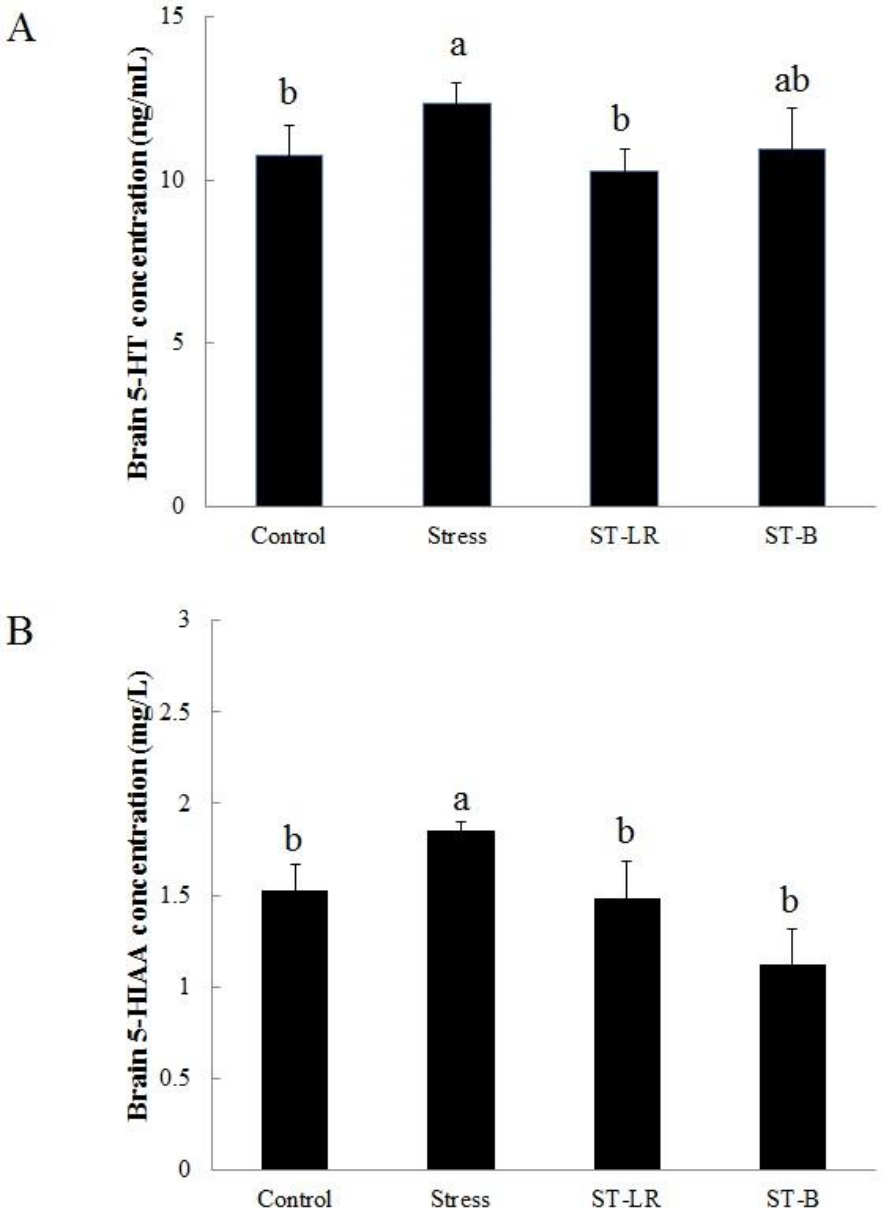


**Figure 3: Monoamine neurotransmitter levels of (A) 5-hydroxytryptamine (5-HT) and (B) 5-hydroxyindoleacetic acid (5-HIAA) in the brain of rats supplemented with hot water extract from lotus root. Data are presented as means ± SEM. [a,b] Means with different superscripts among the groups are significantly different ($p < 0.05$). ST-LR: rats treated with the hot water extract of lotus root, ST-B: foot-shocked rats treated with betaine.**

**Effects of the lotus root hot water extract on monoamine neurotransmitter levels in the brain**

The effects of lotus root hot water extract on monoamine neurotransmitter levels in the rat brain are shown in Fig. 4. 5-HT concentrations were significantly higher in the stress group than that in the control group (Fig. 4A). However, treatment with lotus root hot water extract effectively improved the electric foot shock stress-induced increase in 5-HT levels in the rat brain. Similarly, 5-HIAA concentrations were also significantly increased in the stress group, when compared with the control group (Fig. 4B). In contrast, 5-HIAA concentrations were markedly decreased in the ST-LR group compared to that of the stress group.

## DISCUSSION

Lotus root has been consumed as a vegetable in many countries such as Korea, Japan, and China. Previous studies have demonstrated that medicinal plant extracts exhibit protective effects against diabetes, digestive disorders, inflammation, and oxidative stress; these effects are related to their polyphenolic content [9, 15]. The antioxidant activity [9], cholesterol-lowering effects [7], and anti-obesity effects of lotus leaf or seed [16] have been recently reported; however, the stress-lowering effects of lotus root remain unknown. Reports by Ali et al. [17] demonstrated that pretreatment with betaine significantly ameliorates the endocrine effects induced by a stressful stimulus in desert sheep and goats. Therefore, in the present study, we investigated the anti-stress and antioxidant potential of hot water extract from lotus root and betaine as a standard drug by using an *in vitro* model as well as a foot-shock-stressed animal model.

In this study, the hot water extract from lotus root exhibited strong DPPH and alkyl radical-scavenging activity. Moreover, we observed that the lotus root extract markedly improved GSH levels as well as the activity of several antioxidant enzymes, including GST, GPx, and CAT in the rat liver. Similar results have been reported by many other researchers. Restraint stress caused depletion of GSH, SOD, CAT, and protein content in the mouse brain. However, a *Rostellularia diffusa* extract significantly increased levels of reduced GSH, SOD, and CAT [18]. You et al. [16] also reported that lotus root has abundant phenolic compounds and exhibits good anti-oxidant activities *in vitro*. Moreover, it has been reported that extracts from the lotus plant are effective in inhibiting meat oxidation [19]. These results indicate that the hot water extract from lotus root containing flavonoids and phenolic compounds has antioxidant properties by acting as an efficient scavenger of several free radicals *in vitro* as well as *in vivo*.

The adrenocortex stress profiles, including cortisol as a main stress hormone and DHEA, are secreted from the adrenal gland by the activated hypothalamus-pituitary-adrenal (HPA) axis under stress conditions [20]. Cruess et al. [21] demonstrated that an increased serum cortisol/DHEA ratio may be reflect the poor stress protection. In our *in vivo* study, we confirmed that the hot water extract from lotus root markedly reduced the cortisol/DHEAS ratio as well as the concentrations of plasma stress-related hormones, such as cortisol and DHEA-S. On the other hand, it has been reported that serotonin (5-HT) is a major neurotransmitter involved in the control of several functions including memory and stress [22]. In this study, we confirmed that stress induced by electric foot shocks markedly increases monoamine neurotransmitters, including 5-HT and 5-HIAA, in whole rat brain. However, both 5-HT and 5-HIAA levels were significantly decreased by treatment with the hot water extract from lotus root. Similar to our results, it has been reported that restraint-induced increases of brain 5-HT and 5-HIAA were reduced by treatment of rice bran oil as a good source of antioxidants [23]. Moreover, Ahmad et al. [24] reported that *Ocimum sanctum*, an Indian medicinal plant, normalized the depleted 5-HT and 5-HIAA levels in the frontal cortex, while it restored the decreased 5-HT level in the hippocampus. In our study, the alterations in the anti-oxidative enzyme systems and the anti-stress effects by treatment with the lotus root extract may be attributed to its antioxidant properties, which allows it to act as an efficient free radical scavenger, regulate stress-related hormones, and brain monoamine neurotransmitters. Therefore, hot water extract from lotus root exhibit potent antioxidant and anti-stress effects on electric foot shock stress in rats.

## CONCLUSIONS

On the basis of these findings, we conclude that the hot water extract from lotus root may exert both *in vitro* antioxidant and *in vivo* anti-stress effects by reducing the secretion of plasma cortisol and enhancing the monoamine neurotransmitter levels in the brain. However, further pre-clinical and clinical studies of lotus root extract need to be conducted to evaluate its benefits in stress management strategies.

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## REFERENCES

[1] Finlay JM, Zigmond MJ, Abercrombie ED. Neuroscience 1995; 64: 619–628.

[2] Steptoe A, Kivimäki M. Nat Rev Cardiol 2012; 9: 360–370.
[3] Sheikh N, Ahm A, Siripurapu KB, Kuchibhotla VK, Singh S, Pali G. J Ethnopharmacol 2007; 111: 671–676.
[4] Joshi T, Sah SP, Singh A. Indian J Exp Biol 2012; 50: 419–424.
[5] Zhang X. 2002. WHO Traditional Medicine Strategy 2002-2005. World Health Organization: Geneva, Switzerland.
[6] Xu SY, Shoemaker CF. J Food Sci 1986; 51: 445–449.
[7] Lee JJ, Park SY, Lee MY. Korean J Food Preserv 2006; 13: 634–642.
[8] Mukherjee PK, Pal SK, Saha K, Saha B. Phytother Res 2006; 9: 522–524.
[9] Jiang Y, Ng T, Wang C, Li N, Wen T, Qiao W, Zhang D, Cheng ZH, Liu F. Int J Food Sci Nutr 2010; 61: 346–356.
[10] Choi HS, Zhao TT, Shin KS, Kim SH, Hwang BY, Lee CK. Molecules 2013; 18: 4342–4356.
[11] Lin HY, Kuo YH, Lin YL, Chiang W. J Agric Food Chem 2009; 57: 6623−6629.
[12] Floreani M, Skaper SD, Facci L, Lipartiti M, Giusti P. FASEB J 1997; 14: 1309–1315.
[13] Habig WH, Pubst MJ, Jakoby WB. J Biol Chem 1974; 249: 7130–7139.
[14] Byers JP, Masters K, Sarver JG, Hassoun EA. Toxicology 2006; 228: 291–298.
[15] Lee HJ, Jeong HS, Kim DJ, Noh YH, Yuk DY, Hong JT. Arch Pharm Res 2008; 31: 342–349.
[16] You JS, Lee YJ, Kim KS, Kim SH, Chang KJ. J Sci Food Agric 2014; 94: 568–575.
[17] Ali BH, Al-Qarawi AA, Mousa HM. Res Vit Sci 2006; 80: 343–348.
[18] Nagasirisha M, Mohamed Saleem TS. Pharmacogn Mag 2014; 10: S614-621.
[19] Huang B, He J, Ban X, Zeng H, Yao X, Wang Y. Meat Sci 2011; 87: 46–53.
[20] Boudarene M, Legros JJ, Timsit-Berthier M. Encephale 2002; 28: 139–146.
[21] Cruess DG, Antoni MH, Kumar M, Ironson G, McCabe P, Fernandez JB, Fletcher M, Schneiderman N. Psychoneuroendocrinology 1999; 24: 537–549.
[22] Keeney A, Jeesop DS, HarbuzMS, Marsden CA, Hogg S, Blackburn-Munro RE. J Neuroendocrinol 2006; 18: 330–338.
[23] Mehdi BJ, Tabassum S, Haider S, Perveen T, Nawaz A, Haleem DJ. J Food Sci Technol 2015; 52: 4544−4550.
[24] Ahmad A, Rasheed N, Gupta P, Singh S, Siripurapu KB, Ashraf GM, Kumar R, Chand K, Maurya R, Banu N, Al-Sheeha M, Palit G. Phytomedicine 2012; 19: 639–647.