Low temperature-aged garlic extract suppresses psychological stress by modulation of stress hormones and oxidative stress response in brain

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Abstract

Background: Garlic is a folk medicine known for its multiple physiological activities, but the neuro-modulatory effect of garlic against psychological stress has rarely been explored. The current study was conducted to determine the potential antipsychological stress effect of low temperature-aged garlic (LTAG).

Methods: After acute restraint stress exposure, mice were administered with raw garlic (RG, 500 mg/kg, p.o.) or LTAG (500 mg/kg, p.o.). We investigated corticosterone, cortisol, and monoamines levels, and the mRNA expression of genes relevant to oxidative stress.

Results: RG and LTAG treatment significantly decreased stress-related hormones such as corticotropin-releasing factor, adrenocorticotropic hormone, corticosterone, and cortisol. Moreover, RG and LTAG administration significantly restored acute restraint stress-induced changes in concentrations of brain neurotransmitters (serotonin, norepinephrine, dopamine, and epinephrine). In addition, RG and LTAG improved the antioxidant defense system by causing an increase in mRNA expression of superoxide dismutase, catalase, and glutathione peroxidase in the brain.

Conclusion: This study suggests an antipsychological stress and neuroprotective effect of RG and LTAG under stress conditions.

Keywords: Aged garlic; Corticosterone; Cortisol; Monoamines; Oxidative stress; Psychological stress

1. INTRODUCTION

Stress is a term for various physical and psychological factors that cause tension when applied to the body. Excessive stimulation may cause an imbalance of the body's homeostasis by breaking down adaptation mechanisms.[1] Stresses, such as failure to adapt to environmental change, fear of the future, anxiety, and environmental pollution caused by chemical substances, are seriously threatening to health, leading to insomnia and depression.[2]

The secretion of neurotransmitters and hormones by the hypothalamus is controlled in response to external stimuli and stress. The secretion of neurotransmitters such as dopamine (DA), norepinephrine (NE), and serotonin (5-hydroxytryptamine [5-HT]) from the hypothalamus through the central nervous system regulates physiological activities such as emotional state, heart rate, blood pressure, and increased blood flow in the skeletal muscle. Hormone secretion into the blood is carried out through the hypothalamic–pituitary–adrenal (HPA) axis system, ie, the hormone circulation system from the hypothalamus to the pituitary and adrenal glands. When subjected to any external stimulus or stress, the HPA axis releases corticotropin-releasing factor (CRF) from the hypothalamus. When the hormone binds to a receptor that specifically binds to the CRF of the pituitary gland, it secretes adrenocorticotropic hormone (ACTH). ACTH reaches the adrenal gland through the blood and lymph nodes, releasing corticosterone-like steroid hormones and catecholamine neurotransmitters into the blood, thereby regulating the heart rate, blood pressure, and energy metabolism. The negative feedback system regulates the secretion of these factors.[3,4]

Currently, in clinical practice, medication and long-term psychotherapy are combined to treat mental stress such as anxiety and depression. Benzodiazepine-type drugs, such as diazepam, lorazepam, clonazepam, and alprazolam, and azapirone-type drugs, such as buspirone, which selectively acts on 5-HT receptors, are used to relieve anxiety.[5] In recent years, studies on stress control substances derived from natural products that can complement the side effects of these drugs have been actively conducted, and DA and 5-HT have been studied as the main regulators.[6–9]

Garlic is a perennial plant of the *Allium* genus and the Liliaceae family. It is widely used as a raw material of functional foods and medicines because of its antibacterial, anticancer, antioxidant, blood pressure lowering, and immunity enhancing properties.[10–13] However, due to the strong aroma and pungent taste of raw garlic, it is unpleasant to consume. To overcome this, there have been many studies to alleviate the strong aroma and taste while maintaining the physiological activity function of garlic, such as heat treatment or changing the aging period.[14–16] Although there are several studies regarding the physiological activities of aged garlic extract,[17–19] there have been few studies on the antipsychological stress effect of low temperature-aged garlic (LTAG) extract. Therefore, the current study was

conducted to determine the potential antipsychological stress effect of LTAG.

2. METHODS

2.1. Sample preparation

Garlic was purchased from Uiseong-Gun, Gyeongsangbuk-do, Korea. The garlic was sealed in a container and aged for 60 days at 60°C. The aged garlic was peeled, lyophilized, and pulverized with a Philips mini blender (HR 2860, Ya Horng Electronic Co., China) for 10 minutes. LTAG powder (5 g) was added to 200 mL of 70% EtOH and ultrasonic extraction was performed for 30 minutes. After standing at room temperature for 1 hour, the supernatant was recovered. This was repeated three times and the recovered supernatant was filtered with No. 2 filter paper, evaporated under vacuum (EYELA N-1000, Tokyo Riakikai Co., Ltd., Japan), and then lyophilized with a Bondiro Lyophpride freeze dryer (Ilshine Lab Co. Ltd., Korea) at −70°C under reduced pressure (<20 Pa). The dry residue was stored at −70°C.

2.2. Animals and treatment

The 6-week-old male ICR mice were purchased from Orient Bio (Seong-Nam, Korea). All animal experiments were approved according to the guidelines of the Institutional Animal Care and Use Committee of the National Institute of Agricultural Sciences (NIAS201602), and all procedures were conducted in accordance with the Animal Experiments Guidelines of the National Institute of Agricultural Science. The mice were housed under controlled temperature (23°C ± 3°C) with a relative humidity of 40% to 60% and 12-hour light/dark cycles. Food and water were provided ad libitum. After one week of acclimatization, the mice were divided into groups (n = 5) and treated as shown in Table 1. Raw garlic (RG) and LTAG doses (500 mg/kg, p.o.) were selected based on pilot dose–response experiments. For the pilot study, normal (nonstressed) mice were administered with a single dose of 250, 500, or 1000 mg/kg (p.o.). At 250 mg/kg dosage, there was a tendency toward improved mental stress but this was not significant; however, there was a significant effect on the improvement of mental stress at 500 mg/kg and 1000 mg/kg doses, and there was no significant difference between the concentrations (data not shown). Therefore, we used 500 mg/kg dose in this study. RG and LTAG were prepared in 0.5% (w/v) carboxymethyl cellulose in phosphate buffered saline (PBS). All the mice in groups 2 to 4 were subjected to acute restraint stress.

2.3. Acute restraint stress procedure in mice

Mice were immobilized for 1 hour using an individual rodent restraint device, restraining all physical movement without causing pain.[17,18] LTAG (500 mg/kg) and RG (500 mg/kg) were administered p.o. 1 hour before acute restraint stress procedure.

2.4. Biochemical investigation

One hour after the acute restraint stress, mice were euthanized with CO_2. Blood samples were collected from the abdominal aorta with heparinized syringes, centrifuged at 3000 × g for 15 minutes, and stored at −70°C until further analysis. After blood collection, the whole brain was isolated, washed with PBS, and homogenized (1:10 w/v) in PBS. The homogenates were centrifuged at 15 000 × g, at 4°C for 15 minutes, and supernatants were used for neurochemical and biochemical analysis.

2.5. Plasma cortisol and corticosterone estimation

Plasma cortisol (Cusabio technology LLC. Houston, TX, USA) and corticosterone (Abcam, Cambridge, MA, USA) were estimated by using enzyme-linked immunosorbent assay (ELISA) as per the procedure described by the kit manufacturer. The results are expressed as pg/mL.

2.6. Monoamine estimation

The monoamines, 5-HT and NE, were determined in the brain homogenates using an ELISA method according to the manufacturer's instructions (Abcam). The results are expressed as ng/mg protein.

2.7. Real-time reverse transcription polymerase chain reaction analyses

Real-time reverse transcription polymerase chain reaction, using a real-time thermal cycler (Qiagen rotorgene Q, Qiagen, Valencia, CA, USA) was performed according to the manufacturer's instructions. Total RNA was extracted from brain tissue by using an RNeasy mini plus kit (Qiagen), according to the manufacturer's instructions, and cDNA was synthesized from the isolated total RNA. The real-time PCR reaction was performed using 2× SYBR Green mix (Qiagen). All results were normalized to glyceraldehyde 3-phosphate dehydrogenase expression.

2.8. Statistical analysis

Statistical analysis was performed using SPSS (version 21.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to calculate the mean and SEM. One-way analysis of variance was performed, and when significance ($p < 0.05$) was found, the differences of mean values were identified with Duncan's multiple range tests.

3. RESULTS

3.1. Effect of LTAG on stress hormone levels in acute restraint stress-induced mice

Corticosterone and cortisol levels were measured because changes in the level of plasma glucocorticoids are commonly used as a measure of stress in animals.[19] In the normal group, the plasma corticosterone level was 142.02 pg/mL. In the control group, the corticosterone level was significantly increased to 1388.02 pg/mL, 9.77-fold higher than that in the normal group. In the RG and LTAG groups, the corticosterone level was significantly decreased: it was 39.4% and 43.2%, respectively, of that in the control group, and was not significantly different between RG and LTAG groups (Fig. 1A). Similarly, the plasma cortisol level was 42.46 pg/mL in the normal group, and was significantly increased in the control group, while the RG and LTAG groups had significantly decreased cortisol levels compared to those in the control group (Fig. 1B).

Table 1

Experimental groups and treatments in mice

No.	Groups	Mice condition	Treatment and dose, mg/kg, p.o.
1	Normal	Nonstressed	0.5% CMC in PBS
2	Control	ARS	0.5% CMC in PBS
3	RG	ARS	Raw garlic (500)
4	LTAG	ARS	Low temperature-aged garlic (500)

ARS = acute restraint stress; CMC = carboxymethyl cellulose; LTAG = low temperature-aged garlic; PBS = phosphate buffered saline; RG = raw garlic.
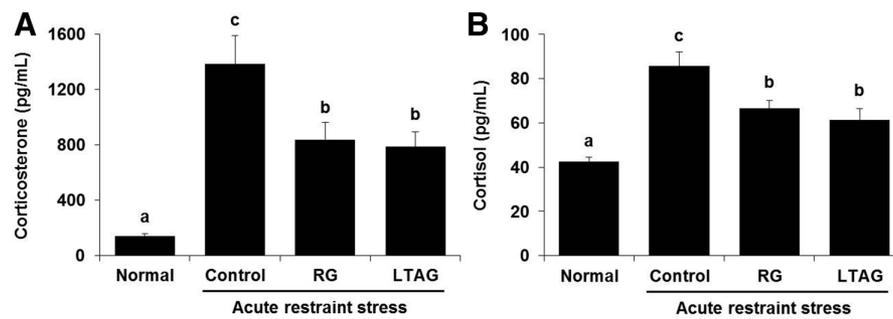
Fig. 1 Effect of LTAG on plasma corticosterone (A) and cortisol (B) levels in acute restraint stress mice. Mice were immobilized for a further 1 h after administration of RG or LTAG (500 mg/kg, p.o.). Data represent mean ± SEM. Values expressed by different letters are significantly different at $p < 0.05$. LTAG, low temperature-aged garlic; RG, raw garlic.

CRF and ACTH are known to release corticosterone-like steroid hormones and catecholamine neurotransmitters into the blood.[3,4] We measured the CRF and ACTH levels in the plasma. As shown in (Supplementary Fig. 1, available at http://links.lww.com/JCMA/A20), acute restraint stress significantly increased the CRF and ACTH levels compared with those in the normal group. Treatment with RG or LTAG resulted in a significant decrease in CRF and ACTH levels.

3.2. Effect of LTAG on monoamine levels in acute restraint stress-induced mice

The results of the monoamine (5-HT, NE, DA, and epinephrine) levels in the brain tissue from each group are shown in Fig. 2 and 3 and (Supplementary Fig. 2, available at http://links.lww.com/JCMA/A20). As a central neurotransmitter, 5-HT is involved in various functional changes in the brain.[20] As shown in Fig. 2, 5-HT levels decreased with restraint stress by 55.6% compared with that in the normal group but were increased by 2.09- to 2.11-fold after the administration of RG and LTAG, respectively. There was no significant difference between the RG group and the LTAG group.

NE levels in the normal, control, RG, and LTAG groups were 595.82, 382.53, 493.53, and 569.79 ng/mg protein, respectively; significantly lower in the control group than in the normal group, but significantly increased by RG and LTAG treatments when compared to control (Fig. 3).

After acute restraint stress, DA and epinephrine levels were reduced in the control group compared to the normal group. However, treatment with LTAG increased DA and epinephrine (Supplementary Fig. 2, available at http://links.lww.com/JCMA/A20).
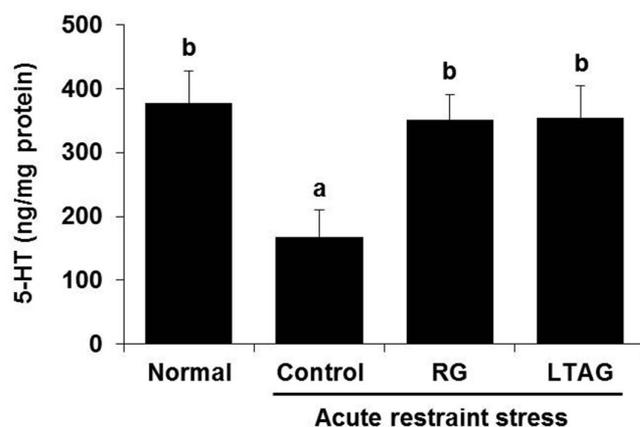
3.3. Effect of LTAG on ROS production and oxidative stress-related enzyme activities in acute restraint stress-induced mice

Restraint stress has been indicated to induce oxidative damage in tissues.[21] Thus, we examined whether LTAG exerts inhibitory effects on the production of reactive oxygen species (ROS) using DCF-DA in the brain tissue. When the mice were subjected to acute restraint stress, a 5-fold increase in the generation of ROS compared to that in the normal group was observed. Treatment with RG or LTAG significantly decreased the acute restraint stress-induced production of ROS in the brain (Supplementary Fig. 3, available at http://links.lww.com/JCMA/A20).

To investigate whether the LTAG effect is mediated by its ability to increase the activity of cellular antioxidant enzymes, we measured the activity of cellular antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in the brain tissue. In the control group, the activities of SOD, CAT, and GPx were significantly decreased compared to the normal group. However, treatment with RG or LTAG increased the activities of these enzymes (Supplementary Fig. 4, available at http://links.lww.com/JCMA/A20).

In addition, we studied the effects of LTAG on mRNA expression of the antioxidant enzymes in the brain. As shown in Fig. 4A–C, restraint stress caused a significant decline in the mRNA expression of SOD, CAT, and GPx compared to the normal group. Both RG and LTAG administration resulted in a significant increase in the mRNA expression of antioxidant enzymes compared to control. However, LTAG was more effective than RG in restoring the stress-induced loss in SOD, CAT, and GPx mRNA expression. In addition, we evaluated the
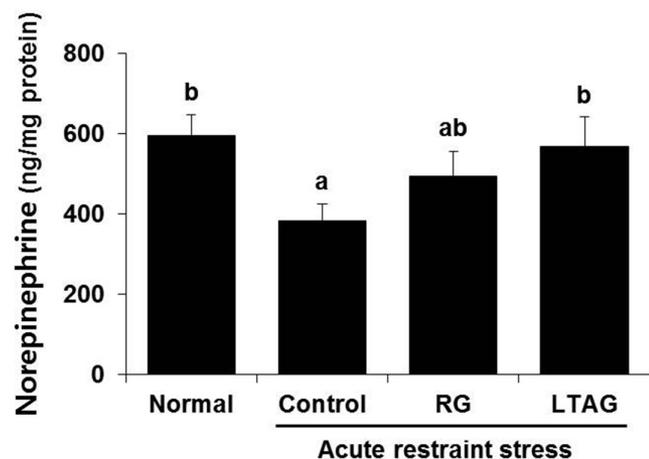

Fig. 2 Effect of LTAG on serotonin (5-HT) levels in brain tissue of acute restraint stress mice. Mice were immobilized for a further 1 h after administration of RG or LTAG (500 mg/kg, p.o.). Data represent mean ± SEM. Values expressed by different letters are significantly different at $p < 0.05$. LTAG, low temperature-aged garlic; RG, raw garlic.

Fig. 3 Effect of LTAG on norepinephrine (NE) levels in brain tissue of acute restraint stress mice. Mice were immobilized for a further 1 h after administration of RG or LTAG (500 mg/kg, p.o.). Data represent mean ± SEM. Values expressed by different letters are significantly different at $p < 0.05$. LTAG, low temperature-aged garlic; RG, raw garlic.
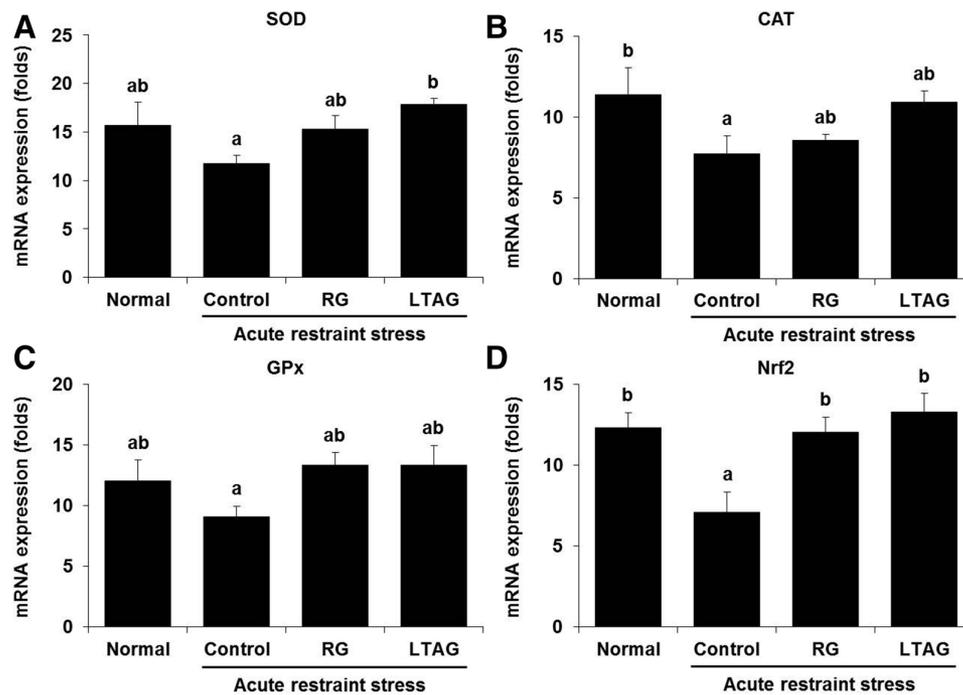
Fig. 4 Effect of LTAG on mRNA expression of antioxidant enzyme genes, SOD (A), CAT (B), GPx (C), and Nrf2 (D), in acute restraint stress mice. Mice were immobilized for a further 1 h after administration of RG or LTAG (500 mg/kg, p.o.). Data represent mean ± SEM. Values expressed by different letters are significantly different at $p < 0.05$. LTAG, low temperature-aged garlic; RG, raw garlic.

mRNA expression of Nrf2, which is a key transcription factor that regulates antioxidant enzymes.[22] RG and LTAG treatments significantly increased Nrf2 expression compared to control (Fig. 4D) with LTAG having the greatest effect.

Taken together, these data indicate that LTAG extract could increase the expression and activities of antioxidant enzymes, indicating that LTAG helps overcome psychological stress.

4. DISCUSSION

Exposure to stress activates the nervous and endocrine system in vivo, also affecting the immune response.[23] If stress is neglected, headache, insomnia, anxiety disorder, stressful hypertension, irritable bowel syndrome, cardiac arrhythmia, bronchial asthma, and/or chronic pain may occur.[24] Therefore, relieving stress is essential for improving mental and physical health, and the quality of life.

In vivo restraint stress is a mental stress model commonly used because it is similar to mental stress in humans and does not cause more direct pain than other stress-inducing methods.[25–27] In this study, mice were restraint-stressed and the effect of RG and LTAG extract treatment was observed. When exposed to stress, multiple reactions occur within the body through the HPA axis, which stimulates hypothalamic-derived CRF secretion by sensing external stimuli. The secreted CRF stimulates the secretion of ACTH from the pituitary gland, and ACTH stimulates the adrenal cortex to secrete cortisol. In addition, sympathetic nerves are stimulated to secrete epinephrine and NE from the adrenal gland and peripheral nerves, increasing blood pressure, heart rate, and blood sugar, and thus, protecting against stress.[3,4] In the stress situation, corticosterone and cortisol are secreted from the adrenal cortex. These hormones break down proteins and fats to raise blood sugar to supply energy to cope with the stress. Excessive corticosterone and cortisol secretion, due to persistent stress, causes problems such as excessive protein degradation, nitrogen balance inhibition, and immune function loss.[28] In mice, corticosterone release is dependent on CRF and ACTH.[29] CRF, ACTH, corticosterone, and cortisol content in the plasma was increased in the control group compared to the normal

group, and the CRF, ACTH, corticosterone, and cortisol levels in the RG and LTAG groups were significantly decreased compared to the control group (Fig. 1 and Supplementary Fig. 1). In addition, we observed a tendency of blood sugar to decrease in acute restraint stress mice (Supplementary Fig. 5, available at http://links.lww.com/JCMA/A20). The mechanism whereby RG and LTAG decrease corticosterone levels is not well established. It is known that S-allyl cysteine, a main compound in RG and LTAG, inhibits corticosterone production,[30] and therefore, it may have an effect on suppressing psychological stress. Moreover, RG and LTAG extracts regulate cholesterol synthesis,[31,32] and this can inhibit the corticosterone synthesis pathway. Thus, we suggest that these two mechanisms could be associated with the decreased plasma corticosterone and cortisol levels in RG and LTAG-dosed restraint-stressed mice.

The effective role of monoamines in the pathophysiology of depressive disorder has been extensively investigated and depletion of monoamine neurotransmitters leads to depressive disorder.[33,34] The monoamine hypothesis has demonstrated that 5-HT, DA, NE, and epinephrine are important neurotransmitters involved in the etiology of depression.[35] In the current study, neurotransmitters, including 5-HT, NE, DA, and epinephrine were evaluated in brain tissues of mice and it was found that these neurotransmitters were suppressed by restraint stress exposure. However, treatment with RG or LTAG increased these neurotransmitters. This study is in line with previous studies in which monoamine neurotransmitter levels were increased by the application of antidepressant drugs.[36]

The major mechanism against oxidative stress in the brain is the glutathione system.[37] Studies have shown that mental stress can impair the function of this system.[38] Furthermore, the restraint stress procedure decreased SOD and CAT activities in both cerebral cortex and hippocampus. In another study, a similar decrease in the SOD and CAT defense system was observed in the brain due to stress.[39] Moreover, Lucca et al.[40] demonstrated a decrease in CAT and SOD activities in the prefrontal cortex and hippocampus of stressed mice, suggesting that an alteration in the endogenous antioxidant defense system is responsible for the induction of depression-like behavior in mice. Hence, the present experimental findings, the decreased SOD, CAT, and GPx

mRNA expression and decreased enzyme activities in the brain, suggested mental stress. Treatment with RG or LTAG restored their expression and activities.

In conclusion, our results indicate that restraint stress impairs the antioxidant defense system in the brain, whereas RG or LTAG extract restore it, reestablishing the glutathione system in the brain of stressed mice. Furthermore, both RG and LTAG decreased the corticosterone, cortisol, and monoamine levels, and this could be linked to antioxidant system improvement in the brain, suggesting that both of these extracts may function as antistress agents.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://links.lww.com/JCMA/A20.

REFERENCES

1. Schneiderman N, Ironson G, Siegel SD. Stress and health: psychological, behavioral, and biological determinants. *Annu Rev Clin Psychol* 2005;**1**:607–28.
2. Choi GA, Cho SI, Kim KS, Choi CW, Wei TS, Yang SJ, et al. Effect of Banhahoobak-tang (Banxiahoupo-tang) Extract (BHTe) on psychological stress. *J Oriental Neuropsychiatry* 2015;**26**:117–30.
3. Charney DS. Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr Scand Suppl* 2003;**417**:38–50.
4. Millan MJ. The neurobiology and control of anxious states. *Prog Neurobiol* 2003;**70**:83–244.
5. Sugimoto Y, Furutani S, Itoh A, Tanahashi T, Nakajima H, Oshiro H, et al. Effects of extracts and neferine from the embryo of *Nelumbo nucifera* seeds on the central nervous system. *Phytomedicine* 2008;**15**:1117–24.
6. Aragão GF, Carneiro LM, Junior AP, Vieira LC, Bandeira PN, Lemos TL, et al. A possible mechanism for anxiolytic and antidepressant effects of alpha and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Pharmacol Biochem Behav* 2006;**85**:827–34.
7. Lee JS, Kim HG, Lee HW, Kim WY, Ahn YC, Son CG. Pine needle extract prevents hippocampal memory impairment in acute restraint stress mouse model. *J Ethnopharmacol* 2017;**207**:226–36.
8. Lin YE, Lin SH, Chen WC, Ho CT, Lai YS, Panyod S, et al. Antidepressant-like effects of water extract of *Gastrodia elata* Blume in rats exposed to unpredictable chronic mild stress via modulation of monoamine regulatory pathways. *J Ethnopharmacol* 2016;**187**:57–65.
9. Vazhayil BK, Rajagopal SS, Thangavelu T, Swaminathan G, Rajagounder E. Neuroprotective effect of *Clerodendrum serratum* Linn. leaves extract against acute restraint stress-induced depressive-like behavioral symptoms in adult mice. *Indian J Pharmacol* 2017;**49**:34–41.
10. Kang MJ, Yoon HS, Shin JH. Chemical properties and biological activity of garlic (*Allium sativum* L.) shoots. *J Agric Life Sci* 2012;**46**:129–39.
11. Chun HJ, Park JE. Effect of heat treatment of garlic added diet on the blood of spontaneously hypertension rat. *J Korean Soc Food Sci Nutr* 1997;**26**:103–8.
12. Katsuzi N, Park M, Ha SD, Kim GH. Effects of garlic extract for protecting the infection of influenza virus. *J Korean Soc Food Nutr* 2000;**29**:128–33.
13. Kim HK, Kwak HJ, Kim KH. Physiological activity and antioxidative effect of garlic (*Allium sativum* L.) extract. *Food Sci Biotechnol* 2002;**11**:500–6.
14. Jang EK, Seo JH, Lee SP. Physiological activity and antioxidative effects of aged black garlic (*Allium sativum* L.) extract. *Korean J Food Sci Technol* 2008;**40**:443–8.
15. Lawson LD, Wang ZJ. Allicin and allicin-derived garlic compounds increase breath acetone through allyl methyl sulfide: use in measuring allicin bioavailability. *J Agric Food Chem* 2005;**3**:1974–83.
16. Cho KJ, Cha JY, Yim JH, Kim JH. Effects of aging temperature and time on the conversion of garlic (*Allium sativum* L.) components. *J Korean Soc Food Sci Nutr* 2011;**40**:84–8.
17. Freitas AE, Bettio LEB, Neis VB, Santos DB, Ribeiro CM, Rosa RB. Agmatine abolishes restraint stress-induced depressive-like behavior and hippocampal antioxidant imbalance in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 2014;**50**:143–50.
18. Kumar A, Goyal R. Quercetin protects against acute immobilization stress induced behavior and biochemical alterations in mice. *J Med Food* 2008;**11**:469–73.
19. Malisch JL, Saltzman W, Gomes FR, Rezende EL, Jeske DR, Garland T Jr. Baseline and stress-induced plasma corticosterone concentrations of mice selectively bred for high voluntary wheel running. *Physiol Biochem Zool* 2007;**80**:146–56.
20. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* 1999;**38**:1083–152.
21. Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. *FASEB J* 1996;**10**:1532–8.
22. Li N, Alam J, Venkatesan MI, Eiguren-Fernandez A, Schmitz D, Di Stefano E, et al. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. *J Immunol* 2004;**173**:3467–81.
23. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull* 2004;**130**:601–30.
24. Cohen S, Janicki-Deverts D, Miller GE. Psychological stress and disease. *JAMA* 2007;**298**:1685–7.
25. Glavin GB, Paré WP, Sandbak T, Bakke HK, Murison R. Restraint stress in biomedical research: an update. *Neurosci Biobehav Rev* 1994;**18**:223–49.
26. Reyes TM, Walker JR, DeCino C, Hogenesch JB, Sawchenko PE. Categorically distinct acute stressors elicit dissimilar transcriptional profiles in the paraventricular nucleus of the hypothalamus. *J Neurosci* 2003;**23**:5607–16.
27. Sawchenko PE, Li HY, Ericsson A. Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms. *Prog Brain Res* 2000;**122**:61–78.
28. Yun SJ, Pack SH, Shin MH. The effect of the meridian massage on the change of the low back pain RPE (rating of perceived exertion) and the changes of serum cortisol as well as stress index of middle aged women. *J Kor Soc Cosm* 2013;**19**:751–6.
29. Spiga F, Waite EJ, Liu Y, Kershaw YM, Aguilera G, Lightman SL. ACTH-dependent ultradian rhythm of corticosterone secretion. *Endocrinology* 2011;**152**:1448–57.
30. Becerril-Chávez H, Colín-González AL, Villeda-Hernández J, Galván-Arzate S, Chavarría A, Eduarda de Lima M, et al. Protective effects of S-allyl cysteine on behavioral, morphological and biochemical alterations in rats subjected to chronic restraint stress: antioxidant and anxiolytic effects. *J Funct Foods* 2017;**35**:105–14.
31. Ried K. Garlic lowers blood pressure in hypertensive individuals, regulates serum cholesterol, and stimulates immunity: an updated meta-analysis and review. *J Nutr* 2016;**146**:389S–96S.
32. Ried K, Travica N, Sali A. The effect of aged garlic extract on blood pressure and other cardiovascular risk factors in uncontrolled hypertensives: the AGE at Heart trial. *Integr Blood Press Control* 2016;**9**:9–21.
33. Pinto Brod LM, Fronza MG, Vargas JP, Lüdtke DS, Luchese C, Wilhelm EA, et al. Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. *Prog Neuropsychopharmacol Biol Psychiatry* 2016;**65**:201–7.
34. Belmaker RH, Agam G. Major depressive disorder. *N Engl J Med* 2008;**358**:55–68.
35. Nutt DJ. Relationship of neurotransmitters to the symptoms of major depressive disorder. *J Clin Psychiatry* 2008;**69**:4–7.
36. Dang H, Sun L, Liu X, Peng B, Wang Q, Jia W, et al. Preventive action of Kai Xin San aqueous extract on depressive-like symptoms and cognition deficit induced by chronic mild stress. *Exp Biol Med* 2009;**234**:785–93.
37. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* 2000;**62**:649–71.
38. Sahin E, Gumuslu S. Alterations in brain antioxidant status, protein oxidationand lipid peroxidation in response to different stress models. *Behav Brain Res* 2004;**155**:241–8.
39. Jindal A, Mahesh R, Bhatt S. Etazolate a phosphodiesterase 4 inhibitor reverses chronic unpredictable mild stress-induced depression-like behavior and brain oxidative damage. *Pharmacol Biochem Behav* 2013;**105**:63–70.
40. Lucca G, Comim CM, Valvassori SS, Reus GZ, Vuolo F, Petronilho F. Effects of chronic mild stress on the oxidative parameters in the rat brain. *Neurochem Int* 2009;**54**:358–62.