Natural Product Sciences
19(4) : 337-341 (2013)

Effects of Gypenosides on Acute Stress in Mice

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Abstract – The effects of gypenosides (GPS) on electric footshock (EF)-induced acute stress in mice were investigated. Mice were treated orally with GPS (30 - 400 mg/kg) once a day for 5 days. After 2 days of GPS treatment, mice were exposed to EF stimuli (intensity, 2 mA; interval, 10 s; duration, 3 min) for acute stress for 3 days. Spontaneous locomotor activity was increased by acute EF stress, which was decreased by treatment with GPS (100 and 400 mg/kg). In addition, the increased levels of dopamine and serotonin by acute EF stress in the brain were reduced by treatment with GPS (100 and 400 mg/kg). The serum levels of corticosterone increased by acute EF stress were also reduced by GPS (100 and 400 mg/kg). These results suggest that GPS shows the ameliorating effects on acute EF stress by modulating the activity of dopaminergic and serotonergic neurons, and the serum levels of corticosterone. Clinical trials of GPS need to be conducted further so as to develop promising anti-stress agents.
Keywords – Gypenosides, Acute electric footshock stress, Spontaneous locomotor activity, Dopamine and serotonin, Corticosterone, Mice

Introduction

Various stresses can cause physical changes as well as mental performances. Acute stress can make human and animals exciting to protect against the nociceptive stimuli, which increase the heart rate and blood pressure (Kovacs *et al.,* 2005). In contrast, chronic stress has been associated with the many illnesses, including anxiety disorders and depression (Kendler *et al.,* 1999). Both acute and chronic stresses activate the hypothalamic-pituitary-adrenal (HPA) axis, which are characterized by a sudden rise in adrenocorticotrophic hormone followed by the release of glucocorticoids, such as corticosterone and cortisol (Keeney *et al*., 2006; Rivier and Plotsky, 1986). In addition, the brain levels of dopamine and serotonin are increased under the conditions of acute stress, whereas the repeated and chronic stress leads to decrease in dopamine and serotonin levels in the brain (Sheikh *et al*., 2007).

Many stress models, including electric footshock (EF) stimulus, forced swimming, noise stimulus, restraint and immobilization have been employed to examine the stressful responses in mice and rats (Xie *et al*., 2008). Spontaneous locomotor activity increases after being exposed to acute stress (Katz *et al*., 1981). In contrast, locomotor activity, grip strength, body weight and endurance decrease after exposure to chronic stress (Retana-Márquez *et al*., 2003).

Gynostemma pentaphyllum Makino (Cucurbitaceas, GP) is a traditional medicinal herb that has shown various effects on diabetes, fatigue, hyperlipidemia, immunity, oxidative stress and tumor (Razmovski-Namovski *et al*., 2005). Recently, it has been reported that ethanol extract from GP (GP-EX) had an anti-stress function by improving the loss of body weight and the reduction of grip strength which were induced by chronic EF stress, as well as an immunomodulatory effect in mice (Choi *et al*., 2008; Im *et al*., 2012). GP-EX also had an ameliorating effect on chronic stress-induced anxiety disorders, which were evaluated by the elevated plus-maze and marble burying tests (Choi *et al*., 2013). In addition, GP-EX had a protective effect against neurotoxicity in the 6-hydroxy-dopamine (6-OHDA)-lesioned rat model of Parkinson's disease (PD) (Choi *et al*., 2010). Gypenosides (GPS) are the dammarane-type gynosaponin-enriched components isolated from GP (Razmovski-Namovski *et al*., 2005). GPS shows a neuroprotective effect in the 1-methyl-4-

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phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of PD (Wang *et al*., 2010a).

In this study, the main GPS was obtained from GP (Shang *et al*., 2006; Wang *et al*., 2010b), and the pharmacological effects of GPS on acute EF stress-induced behaviors in mice were investigated in order to further define the anti-stress function of GP-EX. After being exposed to acute stress by electric EF stimuli, we examined the behavioral changes using the spontaneous locomotor test and the biochemical changes on the levels of dopamine, serotonin and corticosterone.

Experimental

Materials – GPS was purchased from Ankang Dongke Maidisen Nature Pharmaceutical Co. (Xi'an, China) (Shang *et al*., 2006; Wang *et al*., 2010b). Dopamine, serotonin, isoproterenol and 5-hydroxyindoleacetic acid (HIAA) were purchased from Sigma Co. (St. Louis, MO, USA). A corticosterone kit was purchased from USCN Life Sci. (E0504m, Wuhan, China). All other chemicals were of HPLC grade.

Animals – Mice (ICR, male, 20 - 25 g) were purchased from Samtako Co. (Animal Breeding Center, Osan, Korea). Animals were housed in a temperature (23 ± 2 ºC) and humidity (50 ± 2%) controlled environment with a 12 h light/dark cycle (lights on at 07:00), and with *ad libitum* access to standard mouse food and water. The present study was performed in accordance with the guidelines for the care and use of laboratory animals of Chungbuk National University Laboratory Animal Research Center (approval number: CBNU-481-12-01).

Experimental design and the exposure to acute EF stress – Mice were randomly divided into the groups containing 8 - 12 animals. The control groups received saline (0.9%). GPS was the groups which were treated orally with GPS (30 - 400 mg/kg) for 5 days once a day including a 2 day-adaptation period. Stress was the groups which, after 2 days of GPS treatment, were exposed to the acute EF stimuli (intensity, 2 mA; interval, 10 s; duration, 3 min) in an electrified shock chamber at 14:00 every day for 3 days using an electric shock generator (Seil Electric Co., Daejeon, Korea). During the periods of acute stress, mice were treated with GPS approximately 2 h before the exposure of EF stress. After the final treatment with GPS and behavioral tests, mice were anaesthetized and sacrificed to obtain brain tissues and serum for biochemical analyses.

The spontaneous locomotor activity test – Spontaneous locomotor activity was measured every day using a tilting-type ambulometer (Model AMB-10, O'Hara, Tokyo, Japan). Each mouse was placed in a round cage (diameter, 20 cm; depth, 18 cm) and the numbers of horizontal movements were detected automatically for 30 min.

Measurement of dopamine and serotonin levels – After the final behavioral tests, the whole brain tissues were homogenized in perchloric acid (1 M, 300 μl) and isoproterenol (100 pmol, internal standard) or HIAA (300 pmol, internal standard) and the homogenates were centrifuged at 12,000 × *g* at 4 ºC for 20 min. The supernatants were filtered using pore filters (Millex-GV, 0.45 μm, Waters, Milford, MA, USA) and the filtrate (100 μl) was injected into an HPLC system (Satoh *et al*., 2008; Yanagisa *et al*., 1982).

Measurement of corticosterone – After the final behavioral tests, blood was collected from the heart of sacrificed mice and centrifuged at 12,000 × *g* at 4 ºC for 15 min to obtain serum. The serum levels of corticosterone were assessed using an enzyme-linked immunosorbent assay kit.

Statistical analysis – Data were analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey's test for evaluating the dose-dependent effects of GPS. Two-way ANOVA followed by Tukey's test was also employed to evaluate the effects of GPS on acute EF stress. All data were expressed as means ± S.E.M. with p values < 0.05 being considered statistically significant.

Results

Effects of GPS on spontaneous locomotor activity – Treatment with GPS (30 - 400 mg/kg) did not alter the counts of spontaneous locomotor activity at day-3, compared with the control groups (Fig. 1). In contrast, the counts of spontaneous locomotor activity after being exposed to acute EF stress were increased by 16.8% ($p < 0.05$) at day-3, compared with the control groups (n = 12). However, the counts of spontaneous locomotor activity were reduced by 3.2%, 5.0%, 10.9% ($p < 0.05$), 12.3% ($p < 0.05$) and 13.1% ($p < 0.05$) by treatment with GPS (30, 50, 100, 200 and 400 mg/kg) respectively for 5 days, compared with the acute EF-stressed groups (n = 12) (Fig. 1). In addition, at day-2, the counts of spontaneous locomotor activity were increased by 7.9% by acute EF stress, and they were reduced by 0.7 - 6.1% by treatment with GPS (30 - 400 mg/kg), but it was not significant (data not shown).

Effects of GPS on the levels of dopamine and serotonin in the brain – Treatment with GPS (30 - 400 mg/kg) for 5 days did not alter the levels of dopamine and serotonin in the brain, compared with the control groups

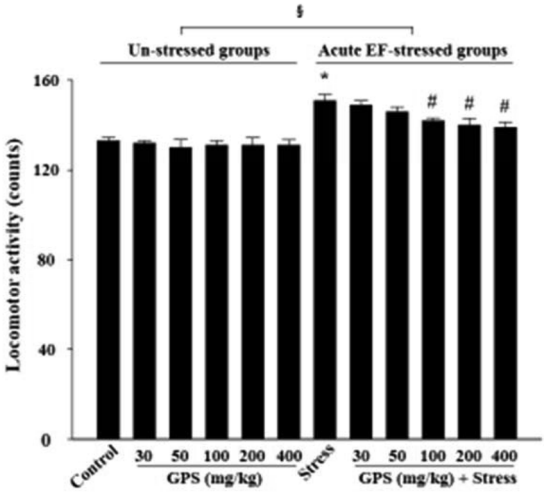

Fig. 1. Effects of GPS on spontaneous locomotor activity after exposure to acute EF stress in mice. Mice (ICR, male, 25 - 30 g) were treated orally with GPS (30 - 400 mg/kg) or saline (0.9%) once a day for 5 days. After 2 days of GPS treatment, mice were also exposed to EF stimuli (2 mA, with an interval and duration of 10 s for 3 min) for 3 days (Stress groups). Spontaneous locomotor activity was performed as described in the Experimental section. The results are expressed as means ± S.E.M. (n = 12). $^{*}p < 0.05$ compared with the control groups; $^{\#}p < 0.05$ compared with the acute EF-stressed groups (one-way ANOVA followed by Tukey's test). $^{\S}p < 0.05$ compared with the un-stressed groups (two-way ANOVA followed by Tukey's test).

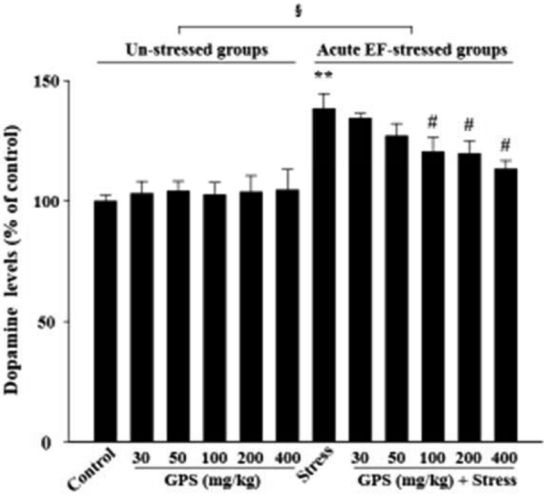

Fig. 2. Effects of GPS on the levels of dopamine in the brain**.** Mice (ICR, male, 25 - 30 g) were treated orally with GPS (30 - 400 mg/kg) or saline (0.9%) once a day for 5 days. Mice were also exposed to EF stimuli (2 mA, with an interval and duration of 10 s for 3 min) for 3 days (Stress groups). The brains were removed and the levels of dopamine were determined by an HPLC method as described in the Experimental section. The levels of dopamine in the control groups were 7.2 ± 1.1 ng/mg tissue in the brain. The results are expressed as means ± S.E.M. (n = 8). $^{*}p < 0.01$ compared with the control groups; $^{\#}p < 0.05$ compared with the chronic EF-stressed groups (one-way ANOVA followed by Tukey's test). $^{\S}p < 0.05$ compared with the un-stressed groups (two-way ANOVA followed by Tukey's test).

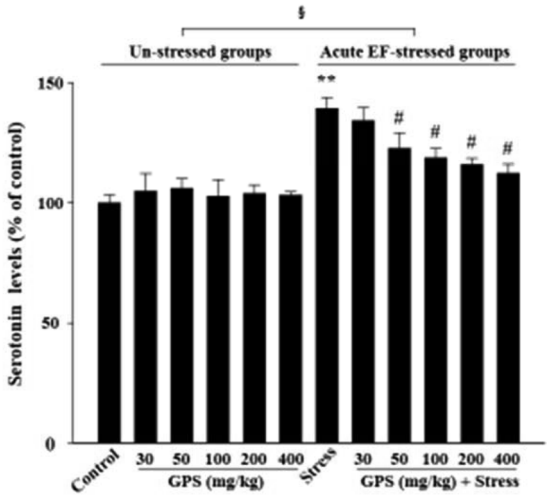

Fig. 3. Effects of GPS on the levels of serotonin in the brain. The brains were removed and the levels of serotonin were determined by an HPLC method as described in the Experimental section. The levels of serotonin in the control groups were 4.1 ± 0.5 ng/mg tissue in the brain. For further comments, see Fig. 2.

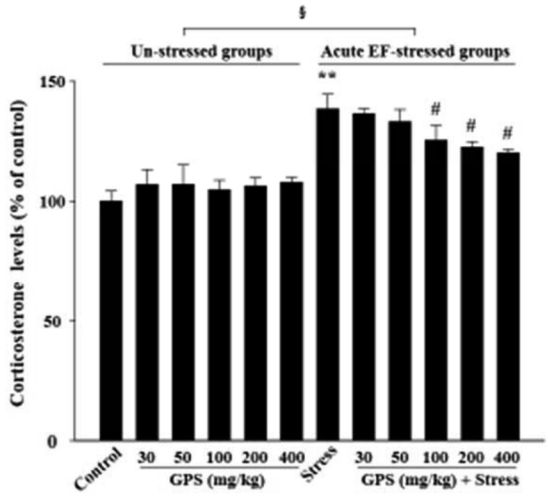

Fig. 4. Effects of GPS on the levels of corticosterone in the serum. The blood samples were collected and the serum levels of corticosterone were determined by an enzyme-linked immunosorbent assay kit as described in the Experimental section. The levels of corticosterone in the control groups were 156.3 ± 10.2 ng/ml in the serum. The results are expressed as means ± S.E.M. (n = 8). For further comments, see Fig. 2.

(Fig. 2). The levels of dopamine significantly were increased by 38.1% ($p < 0.01$) after exposure to acute EF stress for 3 days, compared with the control groups (n = 8), and they were recovered by 4.3%, 10.6%, 17.6% ($p < 0.05$), 18.5% ($p < 0.05$) and 23.7% ($p < 0.05$), respectively by treatment with GPS (30, 50, 100, 200 and 400 mg/kg), compared with the acute EF-stressed groups (n = 8) (Fig. 2).

In addition, the levels of serotonin were increased by

38.9% ($p < 0.01$) after exposure to acute EF stress, compared with the control groups (n = 8) (Fig. 3). However, the increased levels of serotonin by acute EF stress were reduced by 4.2%, 15.5% ($p < 0.05$), 18.9% ($p < 0.05$), 20.2% ($p < 0.05$) and 21.9% ($p < 0.05$), respectively by treatment with GPS (30, 50, 100, 200 and 400 mg/kg), compared with the acute EF-stressed groups (n = 8) (Fig. 3).

Effects of GPS on the levels of corticosterone in the serum – The serum levels of corticosterone were not altered by treatment with GPS (30 - 400 mg/kg) for 5 days, compared with the control groups (Fig. 4). The levels of corticosterone in the serum were increased by 37.2% ($p < 0.01$) by exposure to acute EF stress, compared with the control groups (n = 8) (Fig. 4). However, the increased levels of corticosterone were decreased by 3.2%, 6.7%, 15.4% ($p < 0.05$), 17.0% ($p < 0.05$) and 19.1% ($p < 0.05$), respectively by treatment with GPS (30, 50, 100, 200 and 400 mg/kg), compared with the acute EF-stressed groups (n = 8) (Fig. 4).

Discussion

Recently, GP-EX has been found to have an ameliorating effect on chronic EF stress-induced anxiety disorders in mice (Choi *et al*., 2013). GP-EX has been reported to have approximately 90 kinds of GPS (Razmovski-Naumovski *et al*., 2005). In present study, the ameliorating effects of GPS on acute EF stress-induced behaviors were investigated in order to define the main functional components of GP-EX.

In the initial excited state of acute stress, the locomotor behaviors could be increased due to an increase in muscle tone and blood pressure (Kovacs *et al*., 2005). The acute stress also increases grip strength due to the excited states (Benaroya-Milshtein *et al*., 2004). In this study, the spontaneous locomotor activity were increased after being exposed to acute EF stress for 3 days, and they were ameliorated by treatment with GPS (100 - 400 mg/kg) in a dose-dependent manner (Fig. 1). The changes of grip strength increased by acute EF stress were also reduced by treatment with GPS (200 - 400 mg/kg), but it was not significant (data not shown).

It has been suggested that spontaneous locomotor activity is associated with dopaminergic neurons of the brain as the dopamine receptor blocking agents can reduce this activity (Andén *et al*., 1970). The acute stress-induced changes of grip strength are also associated with dopamine levels and dopaminergic neurons in the brain (Dunnett *et al*., 1998; Kirby *et al*., 1995). Acute stress increases the brain levels of dopamine and serotonin, while chronic stress reduces dopamine and serotonin levels in the brain (Fujino *et al*., 2002; Sheikh *et al*., 2007; Sorg *et al*., 1991). In addition, both acute and chronic stresses lead to the increase in tyrosine hydroxylase activity and the release of dopamine (Kvetnansky *et al*., 1970). The serum levels of corticosterone are increased by both acute and chronic stresses, which keep the sympathetic nervous systems exciting (Murakami *et al*., 2005).

In this study, treatment with GPS reduced the levels of dopamine and serotonin that were increased by acute EF stress in a dose-dependent manner (Figs. 2 and 3). The serum levels of corticosterone, which were increased by acute EF stress, were also decreased by treatment with GPS (100 - 400 mg/kg) in a dose-dependent manner (Fig. 4). Together, these results suggest that GPS has the capability to reduce the excitability via inhibiting the activation of dopaminergic and serotonergic neurons, and the serum levels of corticosterone by HPA axis in mice.

It is suggested that the stressful stimuli induce the production of reactive oxygen species and increase the release of catecholamines and glucocorticoids (Kandel, 2000). GPS has been found to protect aortic endothelial cells against oxidative damage (Tanner *et al*., 1999). GPS also exhibits a protective effect on glutamate-induced oxidative neurotoxicity in PC12 cells (Shang *et al*., 2006) and on the MPTP-induced mouse model of PD (Wang *et al*., 2010a). In addition, GP-EX has a protective effect against neurotoxicity in the 6-OHDA-lesioned rat model of PD (Choi *et al*., 2010). It is therefore possible to explain that the responses induced by acute EF stress might be relieved by treatment with GPS through the anti-oxidative activity in rodents.

Furthermore, GPS and GP-EX recover the brain levels of dopamine and serotonin which are reduced by chronic EF stress in mice (Choi *et al*, 2008; 2013). In contrast, the increased dopamine and serotonin levels by acute EF stress were reduced by treatment with GPS (Figs. 2-4). These results suggest that GPS and GP-EX have an adaptogenic effect on the modulation of monoamine-related neuronal functions, including anxiety disorders and stress.

In this study, treatment with GPS (50 - 400 mg/kg) did not exhibit the adverse effects, such as weight loss, diarrhea, vomiting and death. The values of LD_{50} of total GPS are 755-838 mg/kg (injected into the abdominal cavity) and 402 (± 18.2 mg/kg, i.p.) in mice (Guo and Wang, 1993). The water extract (750 mg/kg) of GP has also been shown not to produce any significant toxicity in rats during a 6-month period of treatment (Attawish *et al*., 2004).

In conclusion, GPS (100 - 400 mg/kg) had the ameliorating effects on acute EF stress in mice, which was evaluated by measuring the spontaneous locomotor activity and the levels of dopamine, serotonin and corticosterone. Clinical trials of GPS need to be conducted further so as to develop promising anti-stress agents.

Acknowledgements

This work was supported by the research grant of Chungbuk National University in 2011.

References

Andén, N.E., Butcher, S.G., Corrodi, H., Fuxe, K., and Ungerstedt, U., Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmacol.*. **11**, 303-314 (1970).

Attawish, A., Chivapat, S., Phadungpat, S., Bansiddhi, J., Techadamrongsin, Y., Mitrijit, O., Chaorai, B., and Chavalittumrong, P., Chronic toxicity of *Gynostemma pentaphyllum*. *Fitoterapia* **75**, 539-551 (2004).

Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., Yaniv,A I., and Pick, C.G., Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *Eur. J. Neurosci.* **20**, 1341-1347 (2004).

Choi, H.S., Lim, S.A., Park, M.S., Hwang, B.Y., Lee, C.K., Kim, S.H., Lim, S.C., and Lee, M.K., Ameliorating effects of the ethanol extracts from *Gynostemma pentaphyllum* on electric footshock stress. *Kor. J. Pharmacogn*. **39**, 341-346 (2008).

Choi, H.S., Park, M.S., Kim, S.H., Hwang, B.Y., Lee, C.K., and Lee, M.K., Neuroprotective effects of herbal ethanol extracts from *Gynostemma pentaphyllum* in the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Molecules* **15**, 2814-2824 (2010).

Choi, H.S., Zhao, T.T., Shin, K.S., Kim, S.H., Hwang, B.Y., Lee, C.K., and Lee, M.K., Anxiolytic effects of herbal ethanol extract from *Gynostemma pentaphyllum* after exposure to chronic stress in mice. *Molecules* **18**, 4342-4356 (2013).

Dunnett, S.B., Torres, E.M., and Annett, L.E., A lateralised grip strength test to evaluate unilateral nigrostriatal lesions in rats. *Neurosci. Lett.* **246**, 1-4 (1998).

Fujino, K., Yoshitake, T., Inoue, O., Ibii, N., Kehr, J., Ishida, J., Nohta, H., and Yamaguchi, M., Increased serotonin release in mice frontal cortex and hippocampus induced by acute physiological stressors. *Neurosci. Lett.* **320**, 91-95 (2002).

Guo, W.Y. and Wang, W.X., Cultivation and utilisation of *Gynostemma pentaphyllum.* Publishing House of Electronics, Science and Technology University pp. 1-261 (1993).

Im, S.A., Choi, H.S., Choi, S.O., Kim, K.H., Lee, S., Hwang, B.Y., Lee, M.K., and Lee, C.K., Restoration of electric footshock-induced immunosuppression in mice by *Gynostemma pentaphyllum* components. *Molecules* **17**, 7695-7708 (2012).

Kandel, E.R., Schwartz, J.H., Jessell, T.M., Siegelbaum, S.A., and Hudspeth, A.J., Principles of Neural Science, 5th ed.; McGraw-Hill Medical: New York, NY, USA, (2000).

Katz, R.J., Roth, K.A., and Carroll, B.J., Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neurosci. Biobehav. Rev.* **5**, 247-251 (1981).

Keeney, A., Jessop, D.S., Harbuz, M.S., Marsden, C.A., Hogg, S., and Blackburn-Munro, R.E., Differential effects of acute and chronic social defeat stress on hypothalamic-pituitary-adrenal axis function and hippocampal serotonin release in mice. *J. Neuroendocrinol.* **18**, 330-338 (2006).

Kendler, K.S., Karkowski, L.M., and Prescott, C.A., Causal relationship between stressful life events and the onset of major depression. *Am. J. Psychiatry.* **156**, 837-841 (1999).

Kirby, L.G., Allen, A.R., and Lucki, I., Regional differences in the effects of forced swimming on extracellular levels of 5-hydroxytryptamina and 5-hydroxyindoleacetic acid. *Brain Res.* **682**, 189-96 (1995).

Kovacs, K.J., Mikios, I.H., and Bali, B. (ed) Psychological and physical stressors. Handbook of stress and the brain. Part I, 775-792 (2005).

Kvetnansky, R., Weise, V.K., and Kopin, I.J., Elevation of adrenal tyrosine hydroxylase and phenylethanolamine-N-methyltransferase by repeated immobilization of rats. *Endocrinology* **87**, 744-749 (1970).

Murakami, S., Imbe, H., Morikawa, Y., Kubo, C., and Senba, E., Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci. Res.* **53**, 129-139 (2005).

Razmovski-Naumovski, V., Huang, T.H-W., Tran, V.H., Li, G.Q., Duke, C.C., and Roufogalis, B.D., Chemistry and pharmacology of *Gynostemma pentaphyllum*. *Phytochem. Rev.* **14**, 197-219 (2005).

Retana-Márquez, S., Bonilla-Jaime, H., Vázquez-Palacios, G., Domínguez-Salazar, E., Martínez-García, R., and Velázquez-Moctezuma, J., Body weight gain and diurnal differences of corticosterone changes in response to acute and chronic stress in rats. *Psychoneuroendocrinol*. **28**, 207-227 (2003).

Rivier, C.L. and Plotsky, P.M., Mediation by corticotropin-releasing factor (CRF) of adenohypophysial hormone secretion. *Ann. Rev. Physiol*. **48**, 475-494 (1986).

Satoh, K., Nonaka, R., Ohashi, N., Shimizu, M., Oshio, S., and Takeda, K., The effects of in utero exposure to a migrant, 4,4'-butylidenebis (6-t-butyl-m-cresol), from nitrile-butadiene rubber gloves on monoamine neurotransmitter in rat. *Biol. Pharm. Bull.* **31**, 2211-2215 (2008).

Shang, L.S., Liu, J.C., Zhu, Q.J., Zhao, L., Feng, Y.X., Wang, X.P., and Cao, W.P., Gypenosides protect primary cultures of rat cortical cells against oxidative neurotoxicity. *Brain Res*. **1120**, 163-174 (2006).

Sheikh, N., Ahm, A., Siripurapu, K.B., Kuchibhotla, V.K., Singh, S., and Palit, G., Effect of *Bacopa monniera* on stress induced changes in plasma corticosterone and brain monoamines in rats. *J. Ethnopharmacol*. **111**, 671-676 (2007).

Sorg, B.A. and Kalivas, P.W., Effects of cocaine and footshock stress on extracellular dopamine levels in the ventral striatum. *Brain Res.* **559**, 29-36 (1991).

Tanner, M.A., Bu, X., Steimle, J.A., and Myers, P.R., The direct release of nitric oxide by gypenosides derived from the herb *Gynostemma pentaphyllum*. *Nitric Oxide* **3**, 359-365 (1999).

Xie, X.Z., Chi, X.L., Zhou, W.X., Ma, Y., and Zhang, Y.X., Progress in research of animal stress models. *Chin. J. New Drugs* **17**, 1375-1380 (2008).

Wang, P., Niu, L., Gao, L., Li, W.X., Jia, D., Wang, X.L., and Gao, G.D., Neuroprotective effect of gypenosides against oxidative injury in the substantia nigra of a mouse model of Parkinson's disease. *J. Int. Med. Res*. **38**, 1084-1092 (2010a).

Wang, P., Niu, L., Guo, X.D., Gao, L., Li, W.X., Jia, D., Wang, X.L., Ma, L.T., and Gao, G.D., Gypenosides protects dopaminergic neurons in primary culture against MPP^+-induced oxidative injury. *Brain Res. Bull*. **83**, 266-271 (2010b).

Yanagisa, M., Hasegawa, H., and Ichiyama, A., Assay of tryptophan hydroxylase and aromatic L-amino acid decarboxylase based on rapid separation of the reaction product by high-performance liquid chromatography. *J. Biochem*. **92**, 449-456 (1982).

Received August 23, 2013
Revised October 9, 2013
Accepted November 9, 2013