



Research article

Korean Red Ginseng attenuates anxiety-like behavior during ethanol withdrawal in rats



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ARTICLE INFO

Article history:

Received 18 February 2014

Received in Revised form

23 May 2014

Accepted 30 May 2014

Available online 7 June 2014

Keywords:

amygdala

anxiety

dopamine

ethanol withdrawal

Panax ginseng

ABSTRACT

Background: Korean Red Ginseng (KRG) is known to have antianxiety properties. This study was conducted to investigate the anxiolytic effects of KRG extract (KRGE) during ethanol withdrawal (EW) and the involvement of the mesoamygdaloid dopamine (DA) system in it.

Methods: Rats were treated with 3 g/kg/d of ethanol for 28 d, and subjected to 3 d of withdrawal. During EW, KRGE (20 mg/kg/d or 60 mg/kg/d, p.o.) was given to rats once/d for 3 d. Thirty min after the final dose of KRGE, anxiety-like behavior was evaluated in an elevated plus maze (EPM), and plasma corticosterone (CORT) levels were determined by a radioimmunoassay (RIA). In addition, concentrations of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) in the central nucleus of the amygdala (CeA) were also measured by high performance liquid chromatography (HPLC).

Results: The EPM test and RIA revealed KRGE inhibited anxiety-like behavior and the over secretion of plasma CORT during EW. Furthermore, the behavioral effect was blocked by a selective DA D2 receptor (D2R) antagonist (eticlopride) but not by a selective DA D1 receptor (D1R) antagonist (SCH23390). HPLC analyses showed KRGE reversed EW-induced decreases of DA and DOPAC in a dose-dependent way. Additionally, Western blotting and real-time polymerase chain reaction (PCR) assays showed that KRGE prevented the EW-induced reductions in tyrosine hydroxylase (TH) protein expression in the CeA and TH mRNA expression in the ventral tegmental area (VTA).

Conclusion: These results suggest that KRGE has anxiolytic effects during EW by improving the meso-amygdaloid DA system.

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1. Introduction

Alcoholism is a chronic relapsing disorder that is primarily driven by negative reinforcement via the reduction of withdrawal symptoms including anxiety, depression, hyperirritability, and insomnia. Of these symptoms, anxiety appears to be the most critical [1]. Abstinent alcoholics are more likely to return to drinking to ease psychological feelings of anxiety or depression, rather than to alleviate physical withdrawal symptoms. Similarly,

ethanol-dependent rats exhibit elevated anxiety-like behaviors during ethanol withdrawal (EW) and excessive ethanol self-administration following a period of EW [2], and a number of pharmacological antianxiety agents reduce ethanol self-administration and the cue-induced reinstatement of alcohol seeking [3].

The central nucleus of the amygdala (CeA) is important for the integration of stress with the rewarding effects of ethanol and plays a crucial role in the development of anxiety and ethanol

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dependence [4]. The CeA receives dense dopamine (DA) projections from the ventral tegmental area (VTA) and withdrawal from chronic ethanol consumption results in the general decline of the mesoamygdaloid DA system, which involves a reduction in firing rates of VTA DA neurons and decreased DA levels in the CeA [5–7]. These dopaminergic changes are closely related to EW-induced anxiety and ethanol intake. The pharmacological reversal of reduced DA levels in the CeA ameliorates EW-induced anxiety in rats [6,7], DA D2 receptors (D2R) exhibit low sensitivity in the CeA of type 1 alcoholics [8], and chronic mild stress increases ethanol intake in genetically modified low D2R mice [9]. Based on such evidence, the rectification of dysregulation in the mesoamygdaloid DA system during EW appears to be a promising target for the treatment of EW-induced anxiety and alcoholism.

Korean Red Ginseng (KRG) is a steamed form of *Panax ginseng* Meyer with enhanced pharmacological activities that have beneficial effects for those with physical and mental exhaustion, including fatigue and anxiety [10,11]. KRG is also frequently prescribed to treat alcoholism, but the underlying pharmacological mechanisms have yet to be fully elucidated [12]. Experimental evidence suggests that improved neurotransmission in the brain is an important neuropharmacological mechanism supporting the effects of KRG. For example, *Panax ginseng* attenuates repeated cocaine-induced behavioral sensitization via the inhibition of elevated DA release in the nucleus accumbens [13] and ameliorates morphine withdrawal-induced anxiety and depression through the restoration of the balance between corticotrophin releasing factor and neuropeptide Y in the brain [14]. Considering the critical role that mesolimbic DA plays in ethanol dependence and the similarities between ethanol and opiate addictions, the present study evaluated the possible anxiolytic effects of KRG during EW and the involvement of the mesoamygdaloid DA system in this process.

2. Materials and methods

2.1. Animals and experimental design

Adult male Sprague-Dawley rats (250–270 g) were obtained from Hyochang Science (Daegu, Korea) and acclimatized for 1 wk prior to the experimental manipulations. All rats were provided with ad libitum access to food and water and maintained at a temperature of 21–23°C, a relative humidity of 50%, and with a 12 h light/dark cycle throughout the course of the study. All procedures were conducted in accordance with the National Institutes of Health guidelines concerning the care and use of laboratory animals and were approved by the Animal Care and Use Committee of Daegu Haany University, Daegu, South Korea. This study used standardized KRG extract (KRGE) that was manufactured from the roots of 6-yr-old fresh ginseng (*P. ginseng* Meyer) provided by the Central Research Institute, Korea Ginseng Corporation (Daejeon, Korea). A high performance liquid chromatography (HPLC) fingerprint of the KRGE was developed (Fig. 1A), and the KRGE contains 2.9 mg/g Rb1, 1.3 mg/g Rg1, 1.1 mg/g Rg3, and other ginsenosides.

EW was induced in the experimental group via intraperitoneal (i.p.) injection of ethanol (3 g/kg/d; 20%, w/v, dissolved in saline) for 28 d followed by 3 d of withdrawal, whereas the control rats received i.p. injections of saline. During the withdrawal period, the rats were orally administered KRGE (20 mg/kg/d or 60 mg/kg/d) dissolved in distilled water (DW) or only DW once/d for 3 d (Fig. 1B). Thirty min after the third dose of KRGE, the rats were tested for anxiety-like behavior in an elevated plus maze (EPM) to evaluate the possible anxiolytic effects of KRGE during EW. Immediately after the EPM test, each rat was decapitated and the entire brain was removed and stored at –80°C. Tissue samples from the CeA and VTA were punched out for neurochemical analyses; coordinates for the CeA [anterior-posterior (AP) = –2.0 mm, medial-

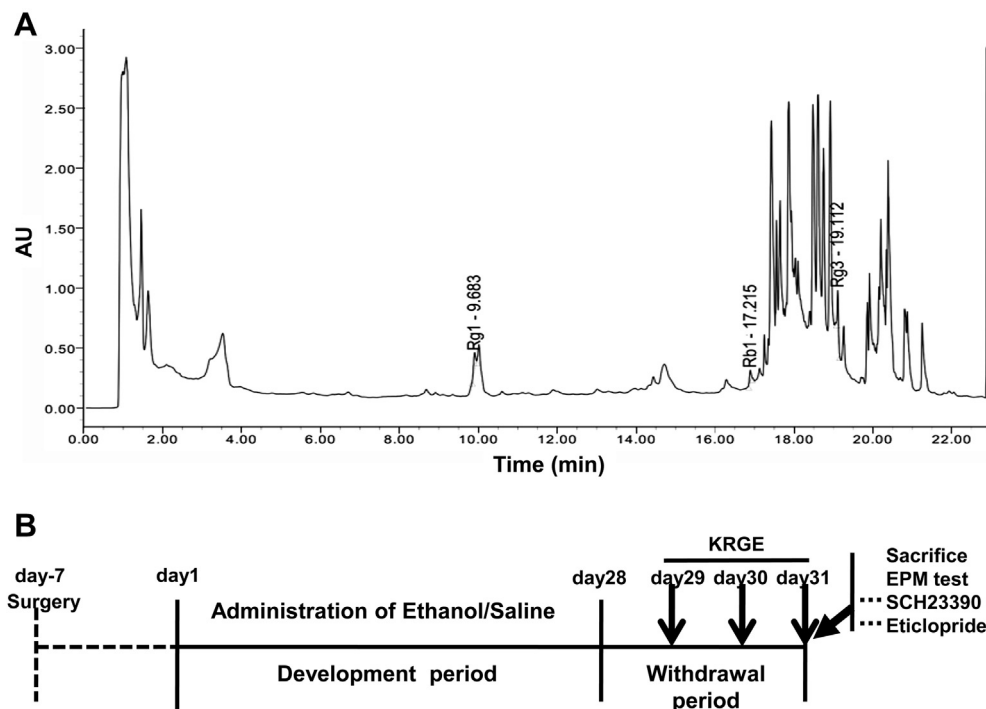


Fig. 1. (A) High performance liquid chromatography profile of Korean Red Ginseng extract (KRGE); flow rate: 1.0 mL/min, column: Waters XTerra RP18 (150 mm × 4.6 mm, 5 μm). (B) Time schedule for study of anxiolytic effects of KRGE on ethanol withdrawal (EW)-induced anxiety.

lateral (ML) = -4.2 mm, dorsal-ventral (DV) = -7.8 mm) and VTA (AP = -6.0 mm, ML = -0.7 mm, DV = -7.8 mm) were based on the Paxinos and Watson rat brain atlas [7,15]. At the same time, blood samples were collected for a radioimmunoassay (RIA) of corticosterone (CORT) levels.

2.2. EPM test

The EPM (Shanghai Yishu Co., Shanghai, China) consisted of a plus-shaped maze that was elevated 50 cm above the ground and equipped with a video tracking system. Each of the four arms was 40 cm long × 10 cm wide; two of the opposing arms were enclosed by 30 cm high black wooden walls (closed arms) whereas the other two opposing arms were devoid of walls (open arms). The EPM test is thought to induce anxiety due to the natural fear of open and elevated spaces that exists in rodents. The number of entries into open arms and the time spent in open arms are negatively correlated with the anxiety level of the rat.

Thirty min after the third dose of KRGE, all rats were individually subjected to the EPM test as described previously [7]. Briefly, without any pretest handling, each rat was placed in the center of the maze, after which the cumulative time spent in each arm and the numbers of entries into the open or closed arms were recorded during a 5 min test session. The percentage of time (T) spent in open arms was calculated as follows:

Percentage of $T_{\text{spent in open arms}}$

$$= \frac{T_{\text{spent in open arms}}}{(T_{\text{spent in closed arms}} + T_{\text{spent in open arms}})}$$

2.3. Plasma CORT assay

Approximately 1.5 mL of blood collected from each rat was mixed with EDTA (20 mg/mL, 20 μ L) and centrifuged (1,000 × g) at 4°C for 10 min. The plasma was separated out and CORT was measured using an ImmChem double antibody 125 I RIA kit (MP Biomedicals, Orangeburg, NY, USA) with the values expressed as ng/mL [7].

2.4. Local infusions of D1R and D2R antagonists

To determine the involvement of amygdaloid DA receptors in the expected anxiolytic effects of KRGE during EW, another set of experiments was conducted using the same EW schedule described above, in which the rats were given an intra-CeA infusion of either a D1R antagonist (SCH23390) or a D2R antagonist (eticlopride) 5 min prior to the third dose of KRGE (60 mg/kg). These rats were also tested in the EPM.

All rats were placed under anesthesia (sodium pentobarbital, 50 mg/kg, i.p.) and stainless steel guide cannulae (15 mm; 23 gauge) were bilaterally implanted into the amygdala using a stereotaxic instrument with the cannula tips 2 mm above the CeA using the same coordinates described above [15]. The guide cannulae were secured in place using two small stainless steel screws anchored to the skull with dental acrylic cement [16]. Animals were allowed 7 d of recovery following the surgery. The D1R antagonist SCH23390 (0.6 μ g/200 nL/side; Tocris Bioscience, Ellisville, MO, USA) and the D2R antagonist eticlopride (0.7 μ g/200 nL/side; Tocris Bioscience) were separately dissolved in modified Ringer's solution (MRS; 150mM NaCl, 3.0mM KCl, 1.4mM CaCl₂, and

0.8mM MgCl₂ in 10mM phosphate buffer with a pH of 7.1) and individually delivered over a period of 60 s using motorized syringe pumps (Sage Instruments, Boston, MA, USA) [17]. Immediately following the EPM test, the rats were decapitated and their brains were removed to verify the guide cannula placements.

2.5. HPLC analysis

The CeA tissue samples were sonicated in 1 mL 0.1 M perchloric acid (HClO₄) and centrifuged (26,000 × g) at 4°C for 15 min. Then, a 20 μ L aliquot of supernatant was injected directly into an HPLC machine with a coulometric detector (Coulchem II; ESA, Bedford, MA, USA). The HPLC system was composed of a C18 reverse-phase column (5 U ODS; Altex, Ann Arbor, MI, USA) and an electrochemical transducer with a glassy carbon electrode set at 350 mV. The mobile phase contained 0.16 M citric acid (pH 3.0), 0.02mM EDTA with 0.69mM sodium octanesulfonic acid as an ion-pairing reagent, 4% (v/v) acetonitrile, and 1.7% (v/v) tetrahydrofuran. The peaks and values of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) were identified and calculated based on a comparison of their retention times and peak heights with those of standards. The protein concentrations in the brain homogenate samples were determined using a Bicinchoninic acid (BCA) protein assay with the HPLC results expressed as ng/g of protein.

2.6. Western blot analysis

The frozen CeA tissues were homogenized in lysis buffer [20mM Tris, 5mM EDTA, 1% Nonidet P-40 (vol/vol), and protease inhibitors], incubated on ice for 20 min, and centrifuged (19,000 × g) at 4°C for 20 min. Then the supernatants were resolved via electrophoresis on a 12% sodium dodecyl sulfate-polyacrylamide gel and the proteins were transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). The membrane was incubated with either an anti-mouse tyrosine hydroxylase (TH) antibody or an anti-goat β -actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), washed with tris buffered saline with Tween-20 (TBST; 10mM Tris-Cl pH 7.5, 150mM NaCl, and 0.05% Tween-20), and incubated for 1 h with the appropriate peroxidase conjugated secondary antibodies. Bands corresponding to TH and β -actin were visualized using enhanced chemiluminescence Western blot detection reagents (Amersham Biosciences, Piscataway, NJ, USA).

2.7. Real-time polymerase chain reaction analysis

Total RNA was extracted from the VTA tissue samples using Trizol (Invitrogen, Carlsbad, CA, USA) and the cDNA was synthesized by reverse transcription using an oligo (dT) primer. Then, a real-time polymerase chain reaction (PCR) procedure was performed using a Light Cycler 1.5 (Roche, Mannheim, Germany) and a Light Cycler DNA Master SYBR green-I kit according to the manufacturer's instructions. The primers (synthesized by Bioneer Corporation, Daejeon, Republic of Korea) were as follows: 5'-ATGCCACCCCCAGCGCCCC-3' (sense) and 5'-GACACTTTTCTGGGAACCA-3' (antisense) for TH and 5'-GTCGTAC-CACTGGCATTGTG-3' (sense) and 5'-GCCATCTCTGCTCGAAGTC-3' (antisense) for β -actin. The housekeeping gene β -actin was used as an endogenous reference and the relative expression levels of TH mRNA were calculated using the following formulas: Δ CT = CT (TH) - CT (β -actin) and $\Delta\Delta$ CT = Δ CT (treated) - Δ CT (saline), expressed as $2^{-\Delta\Delta$ CT}.

2.8. Statistical analyses

All data were expressed as mean \pm standard deviation (SD) and analyzed statistically by one-way analysis of variance (ANOVA)

followed by Newman-Keuls multiple comparison tests using the commercially available GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). A p value of < 0.05 was considered statistically significant.

3. Results

3.1. Effects of KRGE on anxiety-like behavior and the involvement of DA receptors

In a preliminary experiment, 20 mg/kg KRGE and 60 mg/kg produced no significant behavioral changes in rats either in locomotor activity or in anxiety-like behavior [locomotor activity: $F(2, 15) = 0.3, n = 6, p > 0.05$; anxiety-like behavior: $F(2, 15) = 1.1, n = 6, p > 0.05$] (Fig. 2), but when KRGE doses were > 60 mg/kg, there was a small increase in locomotion, grooming, and nodding (data not shown). Therefore, in the present study, the doses of 20 mg/kg and 60 mg/kg were evaluated.

The presence of anxiety-like behavior was evident in rats undergoing EW during the EPM tests, as this group spent less time in the open arms than the saline-treated controls [$F(3, 18) = 19.9, p < 0.001$; saline-treated control group ($31.2 \pm 6.3\%$, $n = 6$) vs. ethanol-treated control group ($10.2 \pm 2.2\%$, $n = 6$), $p < 0.001$]. Both doses of KRGE administered (20 mg/kg/d and 60 mg/kg/d) significantly attenuated anxiety-like behavior [ethanol-treated control group vs. ethanol + KRGE20 group ($23.8 \pm 5.4\%$, $n = 5$), $p < 0.01$; ethanol-treated control group vs. ethanol + KRGE60 group ($29.8 \pm 6.1\%$, $n = 5$), $p < 0.001$] with more increased percentages observed in the 60 mg/kg group than in the 20 mg/kg group, however, the *post hoc* test failed to show a significant difference between the two groups (ethanol + KRGE20 group vs. ethanol + KRGE60 group, $p > 0.05$) (Fig. 3A).

To evaluate the role played by DA receptors in the anxiolytic effects of KRGE during the EPM test, D1R (SCH23390) and D2R (eticlopride) antagonists were individually administered to the rats.

Given prior to the administration of KRGE (60 mg/kg), the intra-CeA infusion of eticlopride, but not SCH23390, almost completely blocked the anxiolytic effects of KRGE [$F(4, 16) = 13.8, p < 0.001$; saline + MRS + DW group ($26.6 \pm 5.3\%$, $n = 4$) vs. ethanol + MRS + DW group ($10.5 \pm 2.4\%$, $n = 4$), $p < 0.001$; ethanol + MRS + DW group vs. ethanol + MRS + KRGE60 group ($23.4 \pm 5.4\%$, $n = 4$), $p < 0.01$; ethanol + MRS + KRGE60 group vs. ethanol + eticlopride + KRGE60 group ($10.2 \pm 2.5\%$, $n = 4$), $p < 0.01$; ethanol + MRS + KRGE60 group vs. ethanol + SCH23390 + KRGE60 group ($27.4 \pm 6.1\%$, $n = 4$), $p > 0.05$] (Fig. 3B). Taken together, these results suggest that the anxiolytic effects of KRGE during EW were mediated by D2R in the CeA.

3.2. Effects of KRGE on plasma CORT levels during EW

Plasma CORT levels, a hormonal marker of anxiety in rats, were measured with an RIA to confirm the anxiolytic effects of KRGE. Plasma CORT levels were significantly higher in ethanol-treated control rats ($858.4 \pm 181.3, n = 4$) than in saline-treated controls [$F(3, 13) = 18.2, p < 0.001$; ethanol-treated control group ($858.4 \pm 181.3, n = 4$) vs. saline-treated control group ($318.6 \pm 57.3, n = 5$), $p < 0.001$]. Also in agreement with the behavioral data, the administration of both doses of KRGE significantly inhibited EW-related increases in plasma CORT levels [ethanol-treated control group vs. ethanol + KRGE 20 mg/kg group ($473.2 \pm 131.6, n = 4$), $p < 0.001$; ethanol-treated control group vs. ethanol + KRGE 60 mg/kg group ($350.0 \pm 80.7, n = 4$), $p < 0.001$] (Fig. 4).

3.3. Effects of KRGE on DA and DOPAC levels in the CeA

The HPLC analyses revealed significant decreases in the levels of DA and DOPAC in the CeA during EW. Treatment with KRGE dose-dependently reversed these deficiencies (Table 1) demonstrating that the anxiolytic effects of KRGE are mediated by the amygdaloid dopaminergic system.

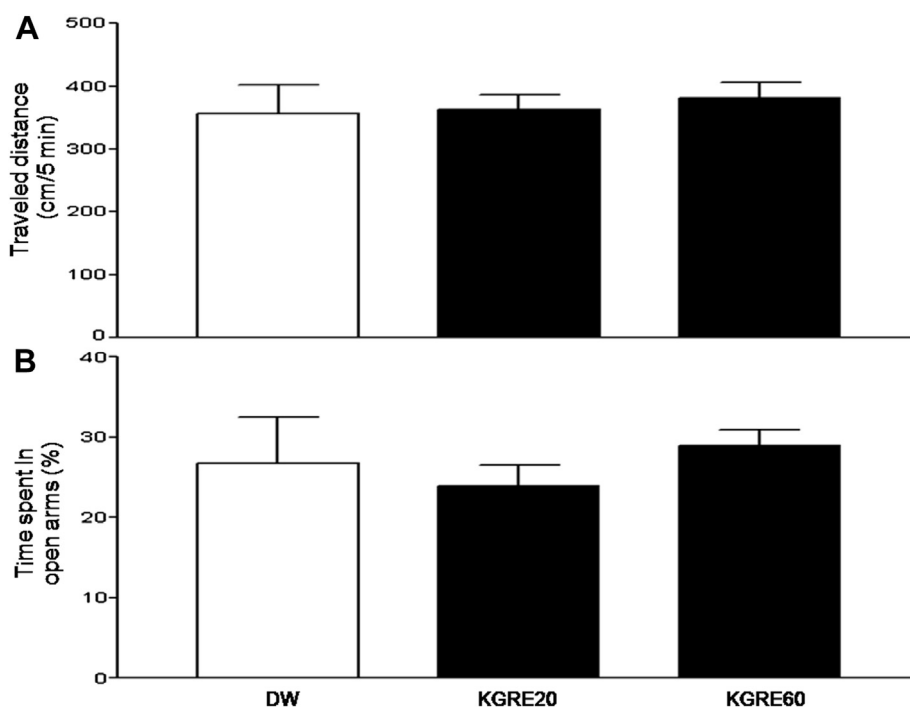


Fig. 2. Effects of Korean Red Ginseng extract (KRGE) on naive rat locomotor activity and anxiety-like behavior. Locomotor activity was measured by the total distance traveled for 5 min, anxiety-like behavior was determined by the percentage of time spent in the open arms of the EPM. Statistical analyses showed no significant differences. Data are expressed as mean \pm SD ($n = 6$). DW, distilled water; KRGE20, 20 mg/kg/d of KRGE for 3 d; KRGE60, 60 mg/kg/d of KRGE for 3 d.

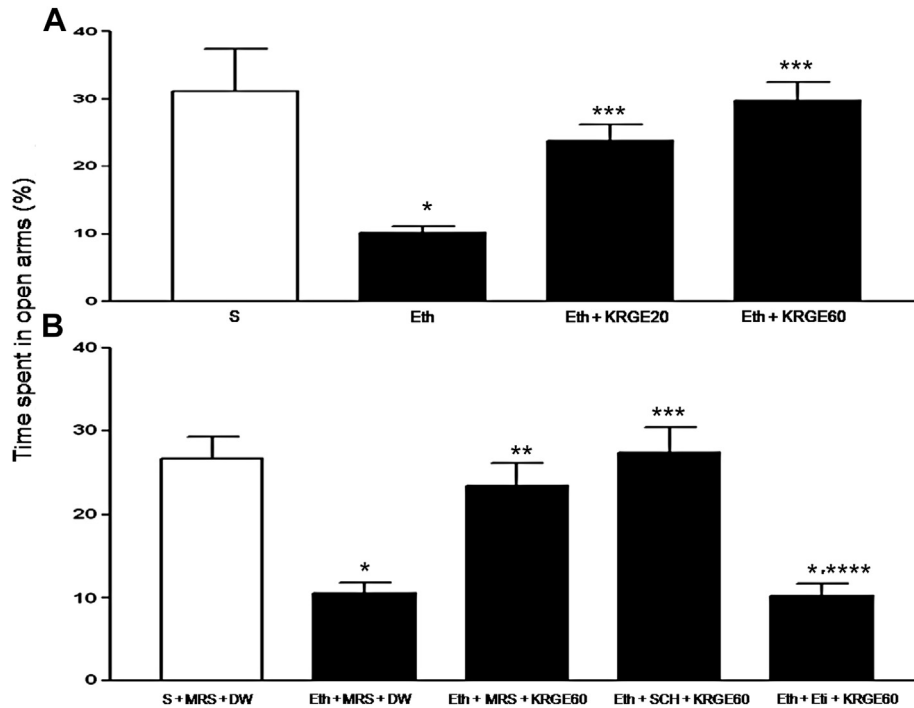


Fig. 3. Effects of Korean Red Ginseng extract (KRGE) on anxiety-like behavior during ethanol withdrawal (EW). The anxiety-like behavior was measured in the elevated plus maze (EPM) test. Data are expressed as mean \pm SD ($n = 4-6$) of the percentages of time spent in the open arms of the EPM for a 5-min test period. * $p < 0.001$, compared with S group (or S + MRS + DW group); ** $p < 0.01$, *** $p < 0.01$, compared with Eth group (or Eth + MRS + DW group); **** $p < 0.01$, compared with Eth + MRS + KRGE60 (analysis of variance, followed by the *post hoc* Newman-Keuls multiple comparison test). Eti, eticlopride; Eth, ethanol; KRGE20, 20 mg/kg/d of KRGE for 3 d; KRGE60, 60 mg/kg/d of KRGE for 3 d; MRS, modified Ringer's solution; S, saline; SCH, SCH23390.

3.4. Effects of KRGE on TH protein expression in the CeA and TH mRNA expression in the VTA

Western blot analyses revealed a reduction in the expression of TH proteins in the CeA of ethanol-treated controls compared to saline-treated controls [$F(2, 9) = 24.6$, $p < 0.001$; saline-treated control group (100%, $n = 4$) vs. ethanol-treated control group ($36.2 \pm 8.3\%$, $n = 4$), $p < 0.001$]. However, the

administration of KRGE (60 mg/kg) prevented these reductions [ethanol-treated control group vs. ethanol + KRGE60 ($95.2 \pm 23.4\%$, $n = 4$), $p < 0.001$] (Fig. 5). The real-time PCR analyses revealed that EW significantly decreased the expression of TH mRNA in the VTA [$F(2, 9) = 8.6$, $p < 0.01$; saline-treated control group (100%, $n = 4$) vs. ethanol-treated control group ($60.6 \pm 10.0\%$, $n = 4$), $p < 0.01$]. However, the expression of TH mRNA in the CeA was spared (data not shown). Similar to protein

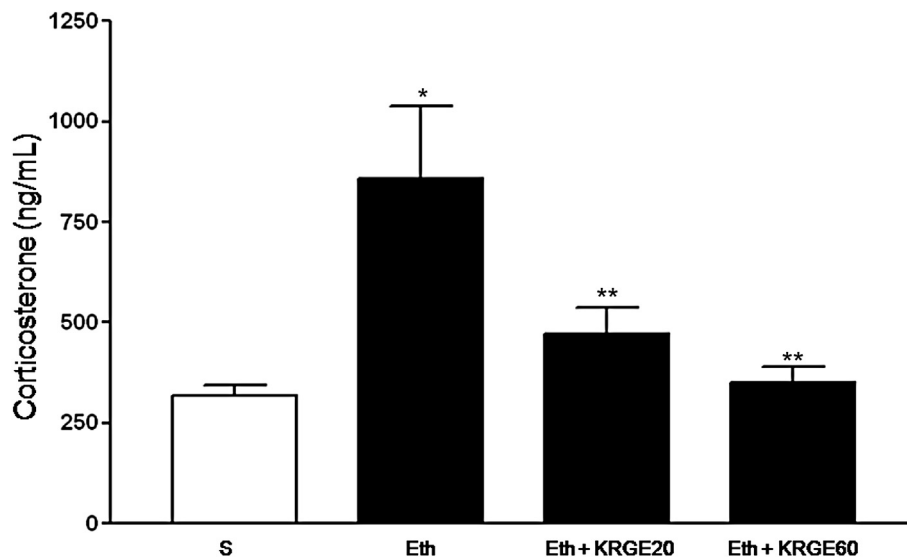


Fig. 4. Effect of Korean Red Ginseng extract (KRGE) on plasma corticosterone (CORT) levels during ethanol withdrawal (EW). The plasma CORT levels were examined by radioimmunoassay (RIA). The plasma CORT levels were analyzed with RIA. Data are expressed as mean \pm SEM ($n = 4, 5$) of the concentrations of plasma CORT. Eth, ethanol; KRGE20, 20 mg/kg/d of KRGE for 3 d; KRGE60, 60 mg/kg/d of KRGE for 3 d; S, saline. * $p < 0.001$, compared with S group; ** $p < 0.001$, compared with Eth group (analysis of variance, followed by the *post hoc* Newman-Keuls multiple comparison test).

Table 1

Effect of Korean Red Ginseng extract (KRGE) on dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the central nucleus of the amygdala (CeA) during ethanol withdrawal (EW)

Groups	DA ¹⁾	DOPAC ¹⁾
Saline (5)	1819.4 ± 314.7	304.2 ± 53.6
Ethanol (4)	527.6 ± 110.6***	116.5 ± 20.1***
Ethanol + KRGE20 (4)	1208.4 ± 231.4**, ****	218.4 ± 32.5*, ****
Ethanol + KRGE60 (4)	1741.3 ± 301.7*****, ****	283.8 ± 58.4*****, ****
F (3, 13) (p)	22.5 (p < 0.001)	15.1 (p < 0.001)

Data are expressed as mean ± SD (ng/g protein). The numbers in parentheses indicate the number of rats in each group

p* < 0.05, *p* < 0.01, and ****p* < 0.001, compared with S group, *****p* < 0.05, ******p* < 0.01, and *****, *p* < 0.001, compared with ethanol group, *****, *p* < 0.05, compared with ethanol + KRGE20 group (analysis of variance, followed by the *post hoc* Newman-Keuls multiple comparison test)

DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; KRGE20, 20 mg/kg/d of KRGE for 3 d; KRGE60, 60 mg/kg/d of KRGE for 3 d

¹⁾ The levels of DA and DOPAC in the CeA were determined by high performance liquid chromatography analyses

expression in the CeA, KRGE (60 mg/kg) prevented the reduction of TH mRNA expression in the VTA during EW [ethanol-treated control group vs. ethanol + KRGE 60 mg/kg group (90.3 ± 22.2%, *n* = 4), *p* < 0.05] (Fig. 6).

4. Discussion

Consistent with previous findings, the present study demonstrated that rats undergoing EW exhibit anxiety-like behavior as they spent less time in the open arms of the EPM [7,18]. The behavioral testing also revealed that both the 20 mg/kg and 60 mg/kg doses of KRGE significantly increased the time spent in the open arms, which reflects the anxiolytic effects of KRGE. The anxiety-reducing behavioral effects of KRGE were supported by biochemical evidence showing that KRGE inhibited plasma CORT secretion.

Taken together, these findings provide strong support for the anxiolytic effects of KRGE during EW in rats.

Imbalances between inhibitory and excitatory systems in the brain during EW, including hypoactivation of the GABAergic system but hyperactivation of the glutamatergic system, a deficiency of DA but excessive release of norepinephrine, and the downregulation of neuropeptide Y but upregulation of corticotrophin releasing factor, are the main causative factors underlying EW-induced anxiety [7,19]. Of these factors, DA deficiency in the CeA appears to be the most critical, because the mesoamygdaloid DA system is a convergent site wherein the effects of the positive and negative reinforcement of ethanol are processed [20,21]. Therefore, in the present study, the mesoamygdaloid DA system was selected as a principal site in which to investigate the underlying mechanisms of the anxiolytic effects of KRGE. HPLC analyses revealed a marked reduction in amygdaloid DA and DOPAC levels during EW, which is consistent with the results of a previous study from our lab and a study by Rubio et al [6,7]. However, the HPLC analyses showed that pretreatment with KRGE (20 or 60 mg/kg) significantly inhibited the decreases of DA and DOPAC in a dose-dependent manner. In traditional Oriental medicine, KRGE is a *Qi* tonic herb that is used to treat deficiency syndromes, because it can invigorate reduced physiological functions. Hence, the HPLC findings suggest that the anxiolytic effects of KRGE are mediated by a replenishment of the EW-induced DA deficiency in the CeA.

TH is the rate-limiting enzyme of DA synthesis and the expressions of TH protein and mRNA in the mesolimbic region are affected by chronic ethanol consumption. For example, there is a mean 20% decrease in TH protein levels in the dorsal and ventral striata of alcohol-fed rats compared to controls [22] and lower accumbal TH-positive densities are found in selectively bred Sardinian alcohol-preferring rats compared to unselected Wistar rats [23]. In the present study, Western blot analyses demonstrated a significant decrease in TH protein expression in the CeA during EW. To further characterize the relationship between the protein

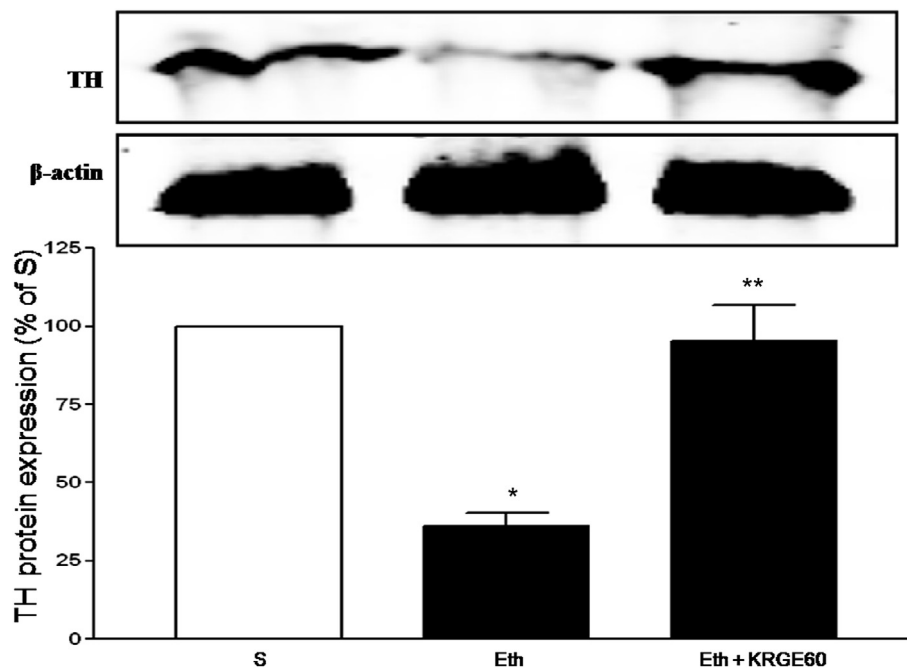


Fig. 5. Effect of Korean Red Ginseng extract (KRGE) on the tyrosine hydroxylase (TH) protein expression in central nucleus of the amygdala (CeA) during ethanol withdrawal (EW). The TH protein expressions were measured by Western blotting assays. Data are expressed as mean ± SEM (*n* = 4) of the percentages of S group. **p* < 0.001, compared with S group; ***p* < 0.001, compared with Eth group (analysis of variance, followed by the *post hoc* Newman-Keuls multiple comparison test). Eth, ethanol; KRGE60, 60 mg/kg/d of KRGE for 3 d; S, saline.

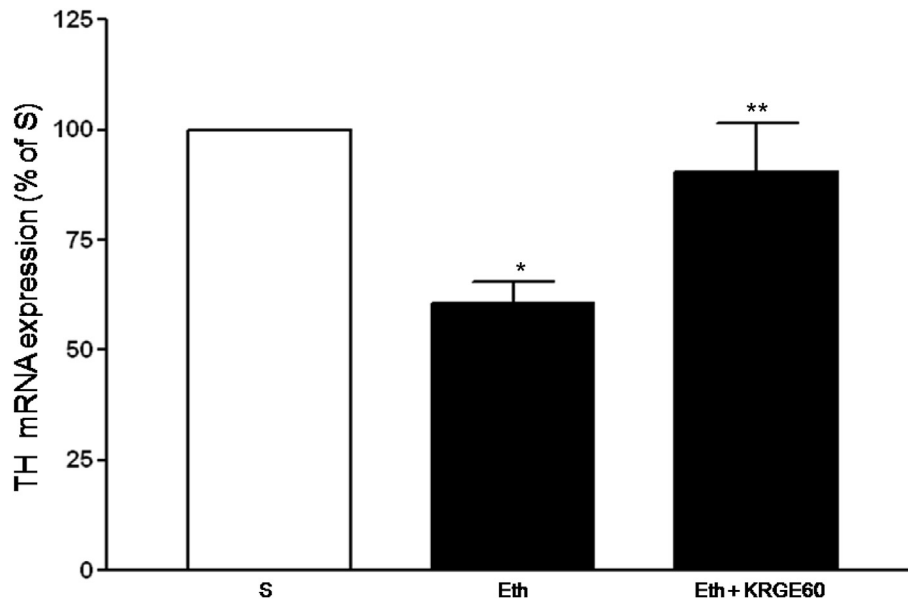


Fig. 6. Effect of Korean Red Ginseng extract (KRGE) on the tyrosine hydroxylase (TH) mRNA expression in ventral tegmental area (VTA) during ethanol withdrawal (EW). The TH mRNA levels were determined by real-time polymerase chain reaction (PCR) assays. Data are expressed as mean \pm SEM ($n = 4$) of the percentages of S group. * $p < 0.01$, compared with S group; ** $p < 0.05$, compared with Eth group (analysis of variance, followed by the *post hoc* Newman-Keuls multiple comparison test). Eth, ethanol; KRGE60, 60 mg/kg/d of KRGE for 3 d; S, saline.

levels and gene transcription of TH, real-time PCR assays were conducted. There were no significant differences in amygdaloid TH mRNA levels between ethanol-treated control rats and saline-treated control rats (data not shown), but there was a significantly lower expression of TH mRNA in the VTA of EW rats compared to saline-treated control rats.

The dopaminergic fibers in the CeA arise from DA neurons in the VTA. This suggests that the reduction in TH protein expression in the CeA during EW may stem from decreased TH gene transcription in the VTA, which would be the cause of the diminished amygdaloid DA production. Moreover, these findings indicate that TH gene transcription in the VTA may be more vulnerable to EW than gene transcription in the CeA. Meanwhile, the same Western blot and PCR assays showed that KRGE (60 mg/kg) almost completely prevented the reduction in TH protein levels as well as rescued the decrease in TH mRNA expression. Taken together, the biochemical and behavioral findings of the present study suggest that KRGE produces anxiolytic effects via improvements in EW-induced mesoamygdaloid DA system dysfunction.

DA receptors are members of the seven transmembrane domain G protein-coupled receptor family and are generally categorized into two different DA receptor subfamilies; the D1R (D1R and D5R) and D2R (D2R, D3R, and D4R) families [24]. DA afferents from the VTA innervate the CeA and activate both D1R and D2R; however, autoradiographic and local infusion studies have shown that D1R and D2R have a differentiated distribution [25,26] and modulate anxiety differently. Behaviorally, the activation of D1R in the CeA has anxiogenic consequences, while the activation of D2R can produce either anxiogenic or anxiolytic effects depending on the nature of the stress experienced [27–29]. In the present study, the anxiolytic effects of KRGE (60 mg/kg) on EW-induced anxiety-like behavior were blocked by the prior intra-CeA infusion of eticlopride (a selective D2R antagonist) but not SCH23390 (a selective D1R antagonist), indicating that the anxiolytic effects of KRGE are mediated via D2R in the CeA.

In summary, rats treated with KRGE (20 mg/kg/d or 60 mg/kg/d, three times) during EW exhibited an attenuation of EW-induced anxiety-like behavior, an inhibition of enhanced plasma CORT

secretion, and a reversal of decreased levels of amygdaloid DA and DOPAC. In addition, KRGE (60 mg/kg/d, three times) restored the EW-induced decrease in TH protein levels in the CeA and TH mRNA levels in the VTA. Together, these findings suggest that KRGE exerts its anxiolytic effects during EW via improvements in the mesoamygdaloid DA system.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by the National Research Foundation of Korea (NRF) funded by Korea government (MSIP; No. 2011-0030124) and the Natural Science Foundation of Heilongjiang Province for Returned Scholars, China (LC201028).

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