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Research Article

The effects of Korean Red Ginseng on stress-related neurotransmitters and gene expression: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Background: Korean Red Ginseng (KRG) is an effective anti-stress treatment. In this study, we investigated the therapeutic potential effects of KRG on relieving stress in a general population using transcriptome analysis.

Methods: We conducted an 8-week clinical pilot study on 90 healthy men who reported stress. The study was completed by 43 participants in the KRG group and 44 participants in the placebo group. Participants were randomized 1:1 to the KRG and placebo groups. We evaluated the stress by stress response inventory (SRI) at baseline and 8 weeks. The main outcomes were changes in the levels of neurotransmitters (NTs) and NT-related gene expression. NTs were analyzed using automated (GC) content, and levels of gene expression were measured by reads per kilobase of transcript per million mapped reads (RPKM).

Results: The KRG group showed significantly preserved epinephrine decrease compared with placebo group at 8 weeks (changes in epinephrine, KRG vs. placebo; -1623.2 ± 46101.5 vs. -35116.3 ± 86288.2, $p = 0012$). Among subjects who higher SRI score, meaning stress increased compared to baseline, the KRG group showed a smaller decrease in serotonin than the placebo group (changes in serotonin, KRG vs. placebo; -2627.5 ± 5859.1 vs, -8087.4 ± 7162.4, $p = 0.005$) and a smaller increase in cortisol than the placebo group (changes in cortisol, KRG vs. placebo; 1912.7 ± 10097.75 vs. 8046.2 ± 8050.6 , $p = 0.019$) in subgroup analysis. Transcriptome findings indicated that KRG intake affects gene expression related with metabolism of choline, adrenalin, and monoamine.

Conclusion: These findings suggest that KRG has beneficial effects on the amelioration of stress response in NTs, and this effect is more prominent in stressful situations. Further clinical studies are required to confirm the anti-stress effect of KRG.

1. Introduction

Modern people experience various stressful situations such as psychosocial stress and work stress in their daily lives. In the human body, specific adaptive responses are activated in various organs to respond to stress. Adaptive responses to various stressors are mainly governed by the central nervous system and the sympathetic nervous system, which control catecholamine secretion and activation of the hypothalamic-pituitary-adrenal axis, resulting in increased cortisol level. A rapid increase in catecholamine and cortisol concentrations affects the whole body and triggers a cascade of responses known as the stress response [1,2]. Depending on the type of stressor and the magnitude of stress response, the degree of involvement of stress-related neurotransmitters/hormones varies [3]. Thus, measuring the levels of stress-related neurotransmitters/hormones provide information to quantify the

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stress response. According to accumulating studies, stress increases the risk of medical problems such as cardiovascular disease and endocrine disorders and even can lead to death [4–6]. Although appropriate interventions and treatments are needed to cope with stress, there is no definite therapy because of the individual differences in response and adaptation to stress due to personal genetics [7], experiences [8], and socio-economic status [9].

Korean Red Ginseng (KRG) has been used as a natural remedy for both physical and mental health. According to accumulating studies, KRG showed potential anti-stress effects that can help restore homeostasis and abnormal physiological changes caused by the stresses of daily life [10–12]. In addition, Baek et al [13]. reported through double-blind, randomized, placebo-controlled trials that KRG stimulated the sympathetic nerve in people with high stress levels and reduced the epinephrine level that mediates the stress response. However, in evaluating the anti-stress effect of KRG, no clinical study has confirmed their effectiveness through stress-related neurotransmitters and the expression of related genes. Advancements in technology have enabled efficient and affordable analysis of biological molecules, resulting in a range of techniques capable of scrutinizing transcripts, proteins, metabolites, and genomes [14]. In particular, transcriptome analysis has identified the functions of genes and identification of mechanisms responding to environmental stresses [15]. For example, transcriptome analysis has revealed that signaling pathways control various downstream elements that allow rapid changes in a cell's transcriptional environment within minutes of exposure to stress [16]. Therefore, this study investigated the potential anti-stress effects of KRG in adults by performing transcriptome analysis from whole blood samples.

2. Materials and method

2.1. Study population

The study was an 8-week randomized, double-blind, placebo-controlled clinical trial conducted at a single center to assess the impact of KRG on stress in men (Clinical Research Information Service (CRIS), KCT0004714). This study was conducted in compliance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board (IRB) of Yongin Severance Hospital in Yongin, South Korea. Prior to participating in the study, all patients provided written informed consent. The study recruited participants from Yongin Severance Hospital between April 2019 and March 2021. All participants were men between 35 and 60 years old. Following the initial screening, participants were assigned randomly in a 1:1 ratio to either of two groups: one group was given four tablets of KRG (2 g) daily, while the other was administered four placebo tablets (2 g) daily. The study was completed by 43 participants in the KRG group and 44 participants in the placebo group. The KRG dose used in this study was 2 g of KRG tablet/day, containing ginsenosides Rb1 (8.03 mg/g), Rb2 (2.80 mg/g), Rg3 (2.50 mg/g), Rg1 (1.18 mg/g), Rc (3.29 mg/g), Rf (1.47 mg/g), Re (1.29 mg/g), and Rd (1.0 mg/g). The KRG tablets were prepared by dehydrating extracts of KRG (3 g per 2 g tablet). The placebo tablets were comprised of corn starch and cellulose and were indistinguishable from the KRG tablets in color, shape, and taste.

2.2. Stress response analysis

At each visit, stress response was evaluated using the Stress Response Inventory (SRI), a self-administered questionnaire widely employed to assess the extent of response to stress. The SRI had a total of 39 questions and seven subscales (tension, aggression, somatization, anger, depression, fatigue, and frustration) that provided a valid measure of psychological, physical, cognitive, and behavioral stress responses. Each question was measured on a 5-point Likert scale [17]. The cutoff score defining a high stress individual was 81 or higher. During the study, we analyzed the changes in SRI between baseline and week 8. Increasing SRI change was defined as an increase in SRI evaluated at week 8 compared to that at baseline.

2.3. Gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) analysis

To prepare for derivatization, 100 μl of the supernatant was transferred to a new e-tube and thoroughly dried. Detailed information on the study methods was published previously [18]. First, the dried extract was subjected to oximation by addition of 50 μl of methoxyamine hydrochloride (20 mg/ml in pyridine), followed by incubation at 30∘C for 90 minutes. Second, the silylation step was carried out by introducing 50 μl of MSTFA into the reaction mixture, which was then incubated at 37∘C for 30 minutes. The concentration of the derivatized samples was set to 20,000 ppm, and an internal standard (IS) of daidzein (0.25 mg/ml) was included. Before instrument analyses, all samples were passed through Millex-GP 0.22-μm filters (Merck Millipore, Billerica, MA, USA) to remove any impurities. An Agilent 7890A GC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent 7693 autosampler (Agilent Technologies) and a Pegasus HT TOF-MS (Leco Corporation, St. Joseph, MI, USA) was utilized to perform GC-TOF-MS analysis. Chromatographic separation was carried out using an Rtx-5MS column (30m length * 0.25mm inner diameter; J&W Scientific, USA), and helium was used as the carrier gas at a constant flow rate of 1.5 ml/min. The program used for analysis of samples was based on a methodology from a prior study [18]. To minimize bias and systematic errors, the analyses were carried out in a random order, utilizing three biological replicates for each type.

2.4. Blood RNA sample extraction

Blood was collected from each participant into a PAXgen Blood RNA tube (BD Bioscience, Cat. 762165). Total RNAs were purified from blood samples using a MagMAX kit (Thermofisher, Cat. 4451894) as follows. Blood was lysed with protease, the RNA was bound to binding beads and washed with wash solution and then was eluted from the beads for analysis. The integrity of the isolated RNA was assessed using the Agilent 2100 BioAnalyzer and TECAN Infinite F200. RNA samples with a concentration greater than 65ng/μL, a quantity greater than 1μg, and an RNA Integrity Number (RIN) greater than 6 were considered suitable for further analysis. For 150bp paired-end sequencing, libraries were created using the TruSeq stranded mRNA Sample Preparation Kit (Illumina, CA, USA). Purification and fragmentation of mRNA molecules were carried out using oligo (dT) magnetic beads and 1μg of total RNA. Single-stranded cDNA was synthesized from fragmented mRNA using random hexamer primers. Double-stranded cDNA was obtained using this single-stranded DNA as a template. The cDNA libraries were subjected to a series of end repair, A-tailing, and adapter ligation and were amplified through Polymerase Chain Reaction (PCR). The quality of the cDNA libraries was assessed using the Agilent 2100 BioAnalyzer (Agilent, CA, USA). Quantification of the libraries was conducted according to the manufacturer's protocol using the KAPA library quantification kit (Kapa Biosystems, MA, USA). After cluster amplification of denatured templates, paired-end (2 × 150bp) sequencing was performed using the Illumina NovaSeq6000 (Illumina, CA, USA).

2.5. Transcriptome data analysis

To filter out low-quality reads, the following criteria were applied: reads containing more than 10% skipped bases (marked as 'N'), reads containing more than 40% bases with quality scores less than 20, and reads with an average quality score less than 20. The entire filtering process was conducted using in-house scripts. The aligner TopHat was used to map the filtered reads to the reference genome associated with the species [19]. Cufflinks v2.1.1 [20] was utilized to determine the gene expression level by referencing the gene annotation database specific to the species. To enhance the precision of the measurement, the options of multi-read-correction and frag-bias-correct were utilized. The default values were retained for all other options. The differential expression analysis was carried out using Cuffdiff [21]. The options of multi-read-correction and frag-bias-correct were utilized to improve the accuracy of the analysis. The default values were retained for all other options. DEGs were detected by applying a q value threshold less than 0.05, which corrects for errors arising from multiple testing [22].

2.6. Statistical analysis

All continuous variables are presented as mean ± SD, and the categorical variables are summarized as percentages for the KRG and placebo groups. The KRG group was compared to the placebo group based on the primary outcome, which consisted of neurotransmitters and stress response inventory, as well as the secondary outcome of metabolic profile. To determine the significance of the differences from baseline in each group, Student's t-test was applied. The Wilcoxon signed rank test was employed to calculate the significance of subgroup analysis. The comparison between changes from baseline in the two treatment groups was used to analyze the effects of treatment in relation to control levels. This was accomplished using the Wilcoxon rank sum test in SAS version 9.1 (SAS Inc., Cary, NC, USA, which was used for all analyses. Significance was determined using a two-sided statistical test, with a p-value less than 0.05 being considered statistically significant.

3. Results

Ninety adults were involved in this study and were randomly assigned to either the KRG group (n = 45) or the placebo group (n = 45) at a 1:1 ratio. Forty-three participants in the KRG group completed the study, with two missing measurements. In the placebo group, one participant withdrew consent, resulting in a total of 44 participants completing the study (see Fig. 1). Table 1 shows that there were no significant differences in baseline characteristics between the two groups of participants. Changes in the stress response inventory (SRI) between baseline and 8 weeks for both groups are presented in Table 2. A statistically significant decrease in SRI was confirmed in the KRG group. Also, the placebo group showed a significant decrease in SRI; no statistically significant difference in change was confirmed by group. Table 3 shows the changes in stress-related neurotransmitters between baseline and 8 weeks in the KRG and placebo groups. In the KRG group, statistically significant decrease in acetylcholine, dopamine, norepinephrine, serotonin, and dehydroepiandrosterone was confirmed at 8 weeks. In particular, epinephrine showed significantly less change in the KRG group compared to the placebo group at 8 weeks (changes in epinephrine, KRG vs. placebo; −1623.2 ± 46101.5 vs. −35116.3 ± 86288.2, p = 0012). Fig. 2 is about the flow of the entire paper. Therefore, mentioning in result may overlap with other articles.

Since this study did not control the degree of individual stress exposure or environment, subgroup analysis was performed on changes in stress-related neurotransmitters (NT) of all participants whose SRI increased at 8 weeks from baseline (Table 4). In participants with increased stress, a significant decrease in serotonin was observed in both the KRG and placebo groups. However, in the KRG group, the degree of reduction was smaller than that of the placebo group (changes in serotonin, KRG vs. placebo; −2627.5 ± 5859.1 vs. −8087.4 ± 7162.4, p = 0.005). In addition, although cortisol increased in both groups under increased stress, the degree of increase was not significant in the KRG group but was significant in the placebo group (changes in cortisol, KRG vs. placebo; 1912.7 ± 10097.75 vs. 8046.2 ± 8050.6, p = 0.019). In other words, cortisol increased in both KRG and placebo groups under increased stress, but the change in the KRG group was small. Transcriptome analysis was performed for 31 neurotransmitter-related genes to determine the change patterns to confirm the effect of KRG on expression levels (Table 5). The RNA sequencing results were obtained from whole blood samples (baseline and after 8 weeks) of the KRG and placebo groups. The RPKM value was used to measure gene expression levels, and any gene with a difference in RPKM value between the 1st and 8th rounds was considered a significant

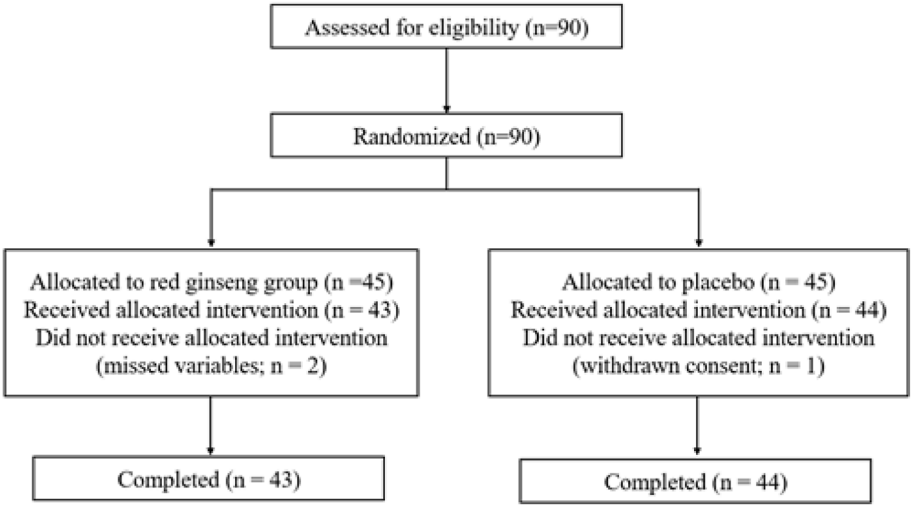

Fig. 1. Flow chart for selection of study participants.

Table 1
Baseline Characteristics of the Study Population According to Lipid Accumulation Product Quartiles

	KRG	Placebo	P-value
N	43	44	
Age, years	49.3 ± 8.5	48.2 ± 8.6	0.542
Physical measurement			
Body mass index (kg/m^2)	25.6 ± 3.3	25.3 ± 3.1	0.726
Waist circumference (cm)	90.4 ± 9.5	90.1 ± 8.3	0.902
SBP (mmHg)	132.5 ± 16.6	130.1 ± 13.6	0.468
DBP (mmHg)	83.9 ± 14.4	83.2 ± 10.8	0.797
Comorbid condition, n (%)			
Hypertension	12 (27.9)	9 (20.6)	0.417
Diabetes	12 (27.9)	7 (15.9)	0.176
Dyslipidemia	10 (23.3)	11 (25.0)	0.849
Smoking, n (%)	19 (44.2)	12 (27.3)	0.100
Serum marker			
Total-cholesterol (mg/dL)	182.6 ± 30.8	187.0 ± 39.8	0.565
AST (IU/L)	24.7 ± 9.0	24.0 ± 6.1	0.684
ALT (IU/L)	32.4 ± 24.2	28.4 ± 14.4	0.347
Fasting plasma glucose (mg/ dL)	112.8 ± 18.5	105.6 ± 13.7	0.041
Insulin (μIU/mL)	10.9 ± 6.9	10.9 ± 6.2	0.972
Blood Urea Nitrogen (mg/dL)	14.0 ± 2.9	13.8 ± 3.5	0.763
Creatinine (mg/dL)	0.9 ± 0.1	0.9 ± 0.1	0.819
eGFR(mL/min/1.73m^2)	95.5 ± 12.6	95.8 ± 13.8	0.933
White blood cells (× 10^{3}L)	6.0 ± 1.5	6.0 ± 1.6	0.994

Data are expressed as the mean ± SD or percentage. *P-values were calculated using ANOVA or the chi-squared test.

DEG (Differential Expression Gene) if both the p-value and q-value were less than 0.05. A total of 5 genes in the KRG group showed significant change in expression level. The ADRB2 gene, which is an adrenaline-related gene, showed a tendency to significantly decrease in expression in the KRG group. Among choline-related genes, CHKB, CEPT1, and CHRNB1 showed changes in the KRG group. CHKB and CHRNB1 showed a decreasing trend, while the CEPT1 gene showed a tendency to increase. Lastly, expression of the COMTD1 gene, which encodes a monoamine degrading enzyme, tended to decrease in the KRG group.

4. Discussion

In this study, we tried to objectively confirm the stress-relieving effects of KRG, which were posited in previous studies [13,23,24], through stress-related NTs. We also aimed to measure gene expression by transcriptome analysis and to clarify the mechanism of the stress response. The protective effects of ginsenosides of KRG against stress are well studied [25–29]. Although stress can be easily assessed through questionnaires and lifestyle assessments, it is difficult to quantify because stress responses vary from person to person. By measuring stress-related neurotransmitters/hormones levels to quantify the level of stress, the effect of KRG on stress can be quantified. Ninety adult males aged 30 years or older were randomly selected to participate in a double-blind, placebo-controlled study. Since this study was conducted with general participants and the stress level of each participant was not controlled, the analysis of the effect of KRG compared to placebo was performed by subgroup analysis for participants whose SRI increased from baseline. Statistically significant changes in serotonin and cortisol were confirmed in comparative analysis of stress-related NTs in participants in KRG and placebo groups with SRI increased from baseline. For serotonin, both the KRG and the placebo groups decreased between baseline and 8 weeks, while cortisol increased in both groups. However, the change of decrease in serotonin was significantly smaller in the KRG group than in the placebo group at 8 weeks. In addition, the increase in cortisol was significantly smaller in the KRG group than in the placebo group. Activation of the hypothalamic-pituitary-adrenal axis, which involves catecholamines such as epinephrine, is known to increase cortisol level in response to stress. Also, Tafet et al [30] reported that serotonin uptake was increased through an increase in serotonin transporter gene expression when normal blood samples were cultured with cortisol in vitro.

Most total body serotonin is secreted by enterochromaffin (EC) cells within the gastrointestinal mucosa [31], and Kim et al [32] showed that an extract of KRG can act on the intestinal EC cells to increase serotonin level in plasma. It was also reported that saponin could improve depression-like behavior in a rodent model by increasing the level of monoamines including serotonin [33]. It is known that KRG inhibits the stress-induced increase in plasma corticosterone level by inhibiting the action of adrenocorticotropic hormone in the adrenal gland in a chronic restraint stress-induced depression animal model [24]. This study, which was the first to confirm the effect of KRG on stress-related NTs in the general population, is consistent with previous results from in vitro and animal studies, as well as studies on specific occupational groups of humans, which showed some NT effects. Thus, this study will be the basis of understanding of the potential anti-stress effect of KRG. When comparing baseline levels to those at 8 weeks for all participants, the KRG group showed a statistically significant decrease in the change of epinephrine level compared to the placebo group.

Table 2
Changes in Stress Response Inventory (SRI) of KRG and Placebo Baseline and at 8week

SRI	KRG			Placebo			
	Baseline	At 8weeks	p-value	Baseline	At 8weeks	p-value	Changed p-value
	23.1 ± 19.6	17.4 ± 17.4	0.005	29.1 ± 27.4	19.3 ± 19.5	0.001	
Change	−5.644 ± 12.911			−9.814 ± 18.870			0.228
Tension	3.4 ± 3.5	2.9 ± 3.7	0.160	4.5 ± 4.6	2.9 ± 3.6	0.021	
Change	−0.556 ± 2.707			−1.581 ± 4.338			0.180
Aggression	1.0 ± 1.6	0.6 ± 1.2	0.077	1.8 ± 3.3	0.9 ± 2.0	0.010	
Change	−0.356 ± 1.317			−0.930 ± 2.272			0.148
Somatization	1.4 ± 1.5	0.9 ± 1.4	0.005	1.5 ± 1.9	1.1 ± 1.5	0.101	
Change	−0.511 ± 1.160			−0.419 ± 1.636			0.760
Anger	4.40 ± 3.8	3.2 ± 3.2	0.004	4.9 ± 4.9	3.1 ± 3.4	0.013	
Change	−1.156 ± 2.567			−1.721 ± 4.350			0.457
Depression	3.9 ± 4.7	2.9 ± 4.1	0.026	5.3 ± 6.2	3.6 ± 4.4	0.007	
Change	−1.000 ± 2.915			−1.674 ± 3.871			0.357
Fatigue	4.3 ± 3.5	3.3 ± 2.8	0.008	5.1 ± 3.8	3.7 ± 2.8	0.001	
Change	−1.000 ± 2.393			−1.419 ± 2.648			0.438
Frustration	4.7 ± 4.3	3.7 ± 3.9	0.043	6.0 ± 5.7	3.7 ± 4.4	0.004	
Change	−1.067 ± 3.440			−2.326 ± 4.999			0.170

Table 3
Changes in Stressed Related Neurotransmitters of KRG and Placebo Baseline and at 8week

	KRG			Placebo			
	Baseline	At 8weeks	p-value	Baseline	At 8weeks	p-value	Changed p-value
Acetylcholine	273380.5 ± 95774.7	174377.3 ± 111316.6	0.000	275880.8 ± 94365.2	159626.4 ± 81090.4	0.000	
Change	−99003.3 ± 104127.2			−116254.4 ± 89135.1			0.345
Dopamine	230371.2 ± 76400.3	86891.7 ± 72061.2	0.000	220483.1 ± 54324.5	87386.1 ± 68676.2	0.000	
Change	−143479.4 ± 85883.2			−133097.0 ± 60875.8			0.283
Norepinephrine	101757.5 ± 79855.3	49906.8 ± 38730.8	0.000	78509.5 ± 41199.9	45217.8 ± 38916.9	0.000	
Change	−51850.7 ± 75639.9			−33291.7 ± 46886.2			0.197
Epinephrine	30307.5 ± 31858.4	28684.3 ± 36479.7	0.810	60555.3 ± 84719.7	25439.0 ± 26185.7	0.010	
Change	−1623.2 ± 46101.5			−35116.3 ± 86288.2			0.012
DHEA	24406.1 ± 11305.3	14140.4 ± 10855.7	0.000	29631.5 ± 25968.3	15068.7 ± 9420.1	0.001	
Change	−10265.7 ± 14352.6			−14562.8 ± 27566.5			0.323
Serotonin	32366.1 ± 6028.2	28562.9 ± 4119.8	0.000	34106.6 ± 6447.4	27978.4 ± 4603.4	0.000	
Change	−3803.2 ± 6610.5			−6128.2 ± 7547.8			0.135
Cortisol	20725.6 ± 8385.5	21494.5 ± 5956.4	0.631	18038.8 ± 8930.1	20768.4 ± 6294.3	0.106	
Change	768.9 ± 10791.5			2729.6 ± 10953.5			0.354

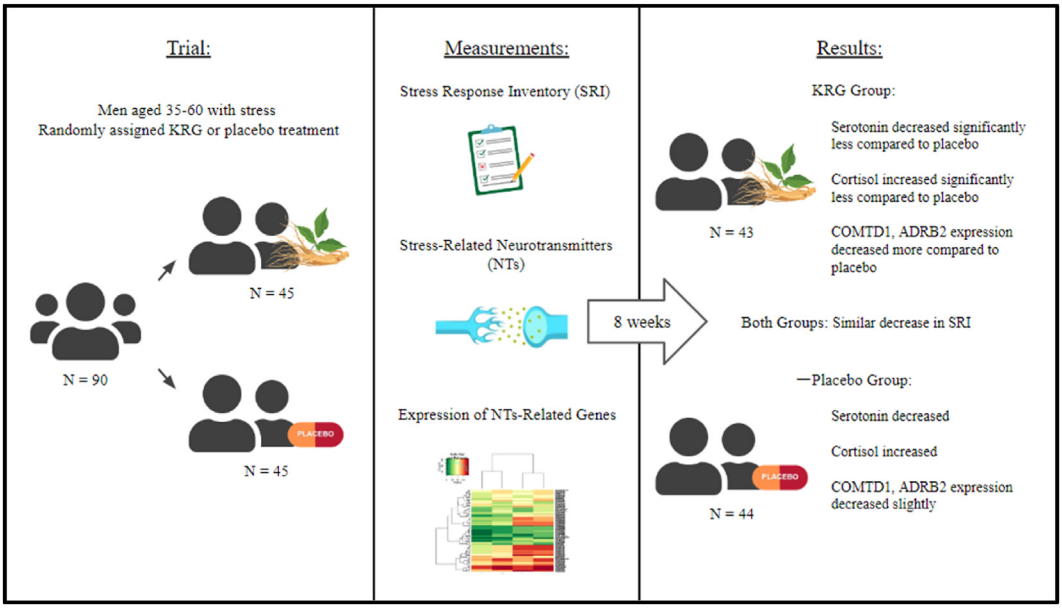

Fig. 2. This study was completed by 43 participants in the KRG group and 44 participants in the placebo group. Our objective was to investigate the impact of ginseng on stress by analyzing changes in neurotransmitter levels and gene expression between the two groups. Under conditions of increased stress, the KRG group exhibited a smaller reduction in serotonin level and a lesser increase in cortisol level compared to the placebo group. Furthermore, we observed a significant decrease in the expression of the ADRB2 gene, which is associated with adrenaline, in the KRG group, which also showed significant changes in the expression of COMTD1, a gene related to monoamine degrading enzyme production.

Table 4
Subgroup Analysis of Increasing SRI Changes in Stress Related Neurotransmitters of KRG and Placebo Baseline and at 8 weeks

	KRG (n = 17)			Placebo (n = 14)			
	Baseline	At 8weeks	p-value	Baseline	At 8 weeks	p-value	Changed p-value
Acetylcholine	273659.6 ± 78043.5	184540.5 ± 117316.1	0.011	266696.5 ± 94078.2	171401.8 ± 70471.3	0.014	
Change	−89119.1 ± 107630.3			−95294.6 ± 106506.4			0.889
Dopamine	224095.8 ± 67519.2	75492.0 ± 49584.2	0.000	227582.3 ± 53301.6	98771.5 ± 81226.0	0.000	
Change	−148603.8 ± 41538.4			−128810.8 ± 61863.1			0.361
Norepinephrine	121247.5 ± 97825.3	43713.4 ± 30469.8	0.017	78990.8 ± 27676.7	38816.7 ± 29741.5	0.002	
Change	−77534.1 ± 101391.1			−40174.1 ± 32837.6			0.255
Epinephrine	20985.8 ± 14289.7	20120.2 ± 15565.8	0.891	93384.9 ± 123412.9	40005.0 ± 38104.1	0.183	
Change	−865.6 ± 22220.0			−56379.9 ± 130533.2			0.144
DHEA	24134.5 ± 10388.1	13067.7 ± 7645.5	0.008	27594.9 ± 22457.1	15580.8 ± 8681.7	0.170	
Change	−11066.8 ± 12664.9			−12014.1 ± 26920.9			0.911
Serotonin	30924.9 ± 5628.1	28297.4 ± 3390.9	0.048	34921.0 ± 4674.2	26833.6 ± 6111.5	0.004	
Change	−2627.5 ± 5859.1			−8087.4 ± 7162.4			0.005
Cortisol	21629.9 ± 8380.5	23542.6 ± 5386.9	0.508	15572.5 ± 6673.1	23618.6 ± 5505.0	0.008	
Change	1912.7 ± 10097.75			8046.2 ± 8050.6			0.019

Table 5
RNA Expression Changes in Stress Related Neurotransmitters of KRG and Placebo Baseline and at 8 weeks

Symbol	RPKM average	KRG				Placebo			
Serotonin		DEG	p-value	Qvalue	Direction	DEG	p-value	Qvalue	Direction
HTR6	0.03	0.00	9.9E-1	1.0E+0	UP	0.00	9.6E-1	1.0E+0	UP
HTR7	0.22	−0.05	8.5E-1	9.9E-1	DOWN	−0.04	7.2E-1	1.0E+0	DOWN
HTR7P1	0.53	−0.14	3.8E-1	8.9E-1	DOWN	−0.02	9.6E-1	1.0E+0	DOWN
SLC6A4	0.13	0.15	5.9E-1	9.9E-1	UP	0.10	6.3E-1	1.0E+0	UP
TPH1	0.06	0.34	3.7E-2	1.9E-1	UP	0.21	2.1E-1	1.0E+0	UP
TPH2	0.00	0.01	7.2E-1	9.9E-1	UP	0.84	9.4E-1	1.0E+0	UP
Dopamin									
DRD3	0.12	0.09	9.6E-1	1.0E+0	UP	0.45	5.1E-1	1.0E+0	UP
DRD4	0.10	−0.51	6.1E-2	2.8E-1	DOWN	0.04	9.2E-1	1.0E+0	UP
Adrenaline									
ADRA2A	0.05	0.55	1.3E-1	4.6E-1	UP	−0.13	4.2E-1	1.0E+0	DOWN
ADRB1	0.17	−0.25	4.5E-1	9.7E-1	DOWN	−0.30	2.5E-1	1.0E+0	DOWN
ADRB2	5.48	−0.30	5.3E-3	4.8E-2	DOWN	−0.20	1.0E-1	8.6E-1	DOWN
ADM	18.70	−0.11	5.7E-1	9.9E-1	DOWN	0.12	5.5E-1	1.0E+0	UP
ADM2	0.29	−0.42	1.1E-2	8.0E-2	DOWN	0.10	3.5E-1	1.0E+0	UP
ADM5	0.71	−0.25	9.8E-2	3.8E-1	DOWN	0.01	6.5E-1	1.0E+0	UP
Choline									
CHDH	0.11	−0.39	1.6E-1	5.4E-1	DOWN	−0.11	5.6E-1	1.0E+0	DOWN
CHKA	4.46	0.11	2.0E-1	6.1E-1	UP	0.17	9.2E-2	8.3E-1	UP
CHKB	78.84	−0.49	4.9E-5	2.3E-3	DOWN	−0.26	5.9E-2	7.5E-1	DOWN
CHPT1	15.80	−0.07	6.9E-1	9.9E-1	DOWN	−0.23	1.5E-1	9.7E-1	DOWN
CEPT1	12.63	0.28	3.9E-3	3.8E-2	UP	0.19	3.0E-2	6.2E-1	UP
CHRNA10	1.66	−0.23	1.9E-2	1.2E-1	DOWN	−0.01	9.5E-1	1.0E+0	DOWN
CHRNB1	4.46	−0.41	1.2E-3	1.7E-2	DOWN	−0.36	2.9E-3	3.5E-1	DOWN
CHRNE	0.91	−0.24	6.4E-2	2.9E-1	DOWN	−0.06	6.8E-1	1.0E+0	DOWN
Monoamine metabolism									
COMT	34.46	−0.15	1.0E-2	7.7E-2	DOWN	−0.03	6.9E-1	1.0E+0	DOWN
COMTD1	4.78	−0.61	3.3E-5	1.9E-3	DOWN	−0.42	2.9E-2	6.2E-1	DOWN
Estrogen									
ESR1	0.12	0.00	8.4E-1	9.9E-1	DOWN	0.13	5.0E-1	1.0E+0	UP
EBAG9	5.63	−0.01	9.4E-1	9.9E-1	DOWN	0.05	5.2E-1	1.0E+0	UP
ESRRA	19.95	−0.15	1.4E-1	4.8E-1	DOWN	−0.06	6.1E-1	1.0E+0	DOWN
Androgen									
ADTRP	2.24	−0.66	1.4E-2	9.5E-2	DOWN	−0.16	5.9E-1	1.0E+0	DOWN
AIG1	4.80	−0.14	1.8E-1	5.8E-1	DOWN	−0.06	4.8E-1	1.0E+0	DOWN
AR	0.04	−0.27	6.4E-1	9.9E-1	DOWN	0.39	4.5E-1	1.0E+0	UP
ARNILA	0.36	−0.03	9.5E-1	1.0E+0	DOWN	−0.17	5.2E-1	1.0E+0	DOWN

The stress response is mediated by the sympathetic nervous system, which is stimulated by epinephrine. This is consistent with a previous study [13] showing that KRG can stabilize the sympathetic nervous system by reducing epinephrine for individuals with high stress levels, and that KRG inhibits catecholamine secretion in animal experiments [23]. In order to understand the specific biological processes and fundamental mechanisms of stress-induced changes in NTs confirmed in this study, it is necessary to evaluate their-related gene expression levels. Therefore, in this study, to confirm the anti-stress effects of KRG of stress-related gene expression levels, transcriptome analysis was performed for all participants. The expression of the adrenergic receptor (ADRB2) decreased by 30% in the KRG group, showing a significant statistically significant change compared to the placebo group, which decreased by 20%. This is consistent with the trend of epinephrine reduction confirmed in the KRG group in the results of stress-related NTs changes of all participants described above. Activation of both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) occurs in response to stress [34,35]. Peripheral release of norepinephrine (NE) and corticosteroids occurs in response to strong stressors [35,36]; NE release is increased, which results in NE activation of the adrenergic receptor ADRB2 [37]. Therefore, the results of this study show that stress can be linked to NE-ADRB2-related mechanisms and confirmed that KRG can have a protective effect against stress. In addition, a significant difference was confirmed in catechol-O-methyltransferase domain containing 1 (COMTD1) among monoamine metabolism-related genes. A 61% decrease was seen in the KRG group, whereas only a 42% decrease was seen in the placebo group. Further studies are needed to investigate the differences in genes such as Choline Kinase Beta (CHKB), which affect choline metabolism. COMTD1 is a catechol-O-methyltransferase (COMT) domain-containing protein, while COMT is an intracellular enzyme localized in the post-synaptic membrane of neurons and is responsible for the degradation of dopamine, adrenaline, and noradrenaline [38]. A previous animal study demonstrated that fluoxetine (SSRI) downregulated the expression of the COMT gene at the mRNA level [39]. Therefore, KRG can reduce serotonin degradation by down-regulating COMT gene expression when stress increases SSRI, confirming the protective effect against stress at the gene level.

Our study has several limitations. First, there was no significant difference observed in SRI between the KRG and placebo groups. This may be attributed to the strong placebo effects that are often observed in clinical trials of nutraceuticals [40]. Second, since the study was conducted in a single center and comprised only 90 adults, there may be limitations in generalizing the results. Individuals may have different responses to stress, and differences in stress-related gene expression may occur depending on the characteristics, duration, and intensity of individual stress [41]. For example, ADRB2 expression on the surface of target cells caused by chronic stress is known to affect downstream signal transduction pathways by reducing the number of receptors present on the cell surface due to desensitization when exposed to noradrenaline (NE) for a long time [42]. In addition, it the ADRB2 SNP rs1042714 may

affect the response to stress by directly influencing the number of ADRB2 proteins expressed on the cell surface [43]. Therefore, future studies that consider individual genotype differences are needed. Despite these limitations, this study is the first to confirm the effects of KRG on stress in humans, including its metabolites and related transcripts. This study provides evidence and biological mechanisms that may be involved in the stress-relieving effects of KRG.

In a randomized double-blind RCT, it was confirmed that KRG reduced epinephrine level to stabilize the autonomic nervous system and reduce the serotonin reduction, helping to stabilize the HPA axis by reducing the increase in cortisol. In addition, KRG also affects the levels of stress-related genes by reducing the expression of those related to monoamine metabolism and adrenergic receptors, confirming the molecular biological mechanism of the stress response. To gain a more comprehensive understanding of how KRG exerts its anti-stress effect, it is necessary to conduct further research, such as analyzing total transcriptomes.

Declaration of competing interest

The authors report no conflicts of interest in this work.

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