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Research Article

# Effects of Korean red ginseng on auditory, cognitive, and liver functions in a naturally aged mouse model

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ABSTRACT

*Background:* Korean Red Ginseng and ginsenosides have been studied for their efficacy against various diseases, including those related to aging. However, most aging studies use D-galactose to induce aging, which often does not accurately represent natural aging. This study aimed to verify improvements in auditory, cognitive, and liver function through administering red ginseng to an 18-month-old naturally aging mouse model.
*Methods:* Auditory function was assessed using Auditory Brainstem Response (ABR) and Auditory Middle Latency Response (AMLR). Cognitive function was evaluated electrophysiologically with P300 and mismatch negativity (MMN), and behaviorally using the Y-maze. Additionally, biochemical tests and histological analysis were conducted to assess liver function. The effects of red ginseng on gene expression regulation were also examined in the cochlea, auditory cortex, and liver, focusing on age-related disease processes.
*Results:* Red ginseng significantly decreased hearing thresholds and improved central auditory function. It also enhanced cognitive behavior and function in response to external stimulation. Furthermore, red ginseng regulated alkaline phosphatase (ALP), albumin (Alb), and total protein (TP) levels, notably decreasing aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Hematoxylin and eosin (H&E) staining of liver tissue showed significant improvement in fat droplets. These effects appear to be mediated by the regulation of aging-related genes *Dec*, *c-Jun*, *Stat5b*, and *Lims2*.
*Conclusion:* These results suggest that red ginseng improves auditory, cognitive, and liver functions in a naturally aged mouse model.

## 1. Introduction

Aging is characterized by gradual biological changes that occur over time, ultimately leading to the decline of physical and cognitive functions. With increasing average lifespan due to advances in medical care and improved living conditions, addressing age-related health issues has become crucial [1]. Aging makes the body more susceptible to various diseases and increases the incidence of chronic conditions and functional impairments [2]. Moreover, hearing loss, cognitive decline, and liver function impairment are particularly prevalent in the elderly population [3–6]. These issues often lead to additional problems, such as social isolation and depression, which adversely affect overall health [7, 8]. Therefore, the study of aging should not only focus on prolonging lifespan but also prioritize enhancing the quality of life for the elderly population.

The accelerated aging model using D-galactose (D-gal) is commonly used to mimic the aging process in experimental research [9]. While this model provides a useful method for studying aging mechanisms and evaluating anti-aging therapies, it has several limitations and potential drawbacks. Specifically, its focus on oxidative stress, acute nature, and variability in experimental protocols may limit the generalizability of findings to human aging [9]. Generally, the average lifespan of

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laboratory mice is approximately 24 months [10], with mice aged 18–24 months considered 'old,' corresponding to humans aged 56–69 years [11]. Although natural aging models require long-term study, they accurately reflect the aging process in a natural context. Therefore, in this study, a naturally aged mouse model using 18-month-old mice was employed to represent elderly individuals with relevant aging characteristics.

Korean Red Ginseng, derived from *Panax ginseng* Meyer, has been traditionally utilized for its health-enhancing properties, including boosting the immune system and alleviating fatigue [12]. In recent years, it has garnered significant attention in aging-related research. Studies have demonstrated that red ginseng can delay the aging process by mitigating oxidative damage to cells and inhibiting inflammatory responses through its antioxidant effects [13–16]. Moreover, red ginseng possesses neuroprotective properties that help prevent cognitive decline, and its immune-boosting effects enhance resistance to age-related diseases [17,18].

In this study, we investigated the effects of red ginseng on auditory, cognitive, and liver functions in a naturally aging model using 18-month-old mice. To provide a comprehensive assessment, we employed electrophysiological, behavioral, biochemical, histological, and molecular biological methodologies. Specifically, we focused on the expression of age-related genes, such as *Dec*, *Onecut*, *c-Jun*, *Stat5b*, and *Lims2*, in the ear, brain, and liver of naturally aging mice. The results indicate that red ginseng significantly enhances auditory, cognitive, and liver functions in naturally aged mice, suggesting its potential as a therapeutic agent for mitigating age-related functional decline.

## 2. Materials and methods

### 2.1. Animals

C57BL/6J mice were obtained from Orient Bio, Inc. (Seongnam, Korea). The animals were housed under a 12-h light-dark cycle, with ad libitum access to food and water, maintained at a temperature of 23.0 ± 1.0 °C and humidity of 50.0 ± 5.0 %. The mice were kept in this environment until the age of 18 months, which is recognized as aging in mice.

### 2.2. Ethical statement

All experimental procedures involving mice were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and approved by the Animal Care and Use Committee of Kyung Hee University (KHUASP(SE)-15-17), Republic of Korea.

### 2.3. Sample preparation

Red Ginseng (RG) extracts were provided by KT&G Corporation Central Research Institute. Korean red ginseng extract (crude saponin 70 mg/g, solid component 60 %, or more) contained Rb1 (0.46 %), Rb2 (0.23 %), Rc (0.28 %), Rd (0.09 %), Re (0.12 %), Rf (0.10 %), Rg1 (0.07 %), Rg2 (0.14 %), Rg3 (0.12 %), Rh1 (0.10 %), and other minor ginsenosides.

### 2.4. Auditory function evaluation

Auditory function was assessed using channel recording (Smart EP, Intelligent Hearing Systems, MA, USA). Mice were anesthetized with a mixture of xylazine (Bayer, Germany), ketamine (Yuhan Corporation, Korea), and saline solution (JW Pharmaceutical, Korea) (1.1:4:4.9, respectively) administered intramuscularly before testing. Mice were placed in an electrically and acoustically shielded sound attenuation booth (TCA-500D, Sontek, Korea). Stimulation was delivered through earphones (Etymotic ER-EA). Mice were divided into three groups (n = 7/group) and chronically treated with RG in their drinking water for 6 months until the age of 24 months.

### 2.5. Evaluation of auditory brainstem response (ABR)

Hearing thresholds were measured using Auditory Brainstem Response (ABR) at 3 and 6 months after RG treatment. For the ABR recordings, alternating clicks and 8, 16, 24, and 32 kHz tone bursts (TBs) (rise-plateau-fall; 2-1-2 cycles) were delivered through earphones (Etymotic ER-3A) at a rate of 20.1 stimuli/s and high-frequency transducers. Physiological filters were set to pass electrical activity between 100 and 3000 Hz. Each mouse's monaural response was recorded and averaged in a 10.24 ms time window, collecting 1000 sweeps. The ABR test threshold was determined by decreasing the click, 8, 16, 24, and 32 kHz TB in 5 dB steps near the hearing threshold. ABR parameters were assessed based on hearing thresholds.

### 2.6. Evaluation of auditory middle latency response (AMLR)

Central auditory function and neurotransmission speed of the auditory nerve were evaluated using Auditory Middle Latency Response (AMLR) at 3 and 6 months after RG treatment. To measure AMLR, rarefaction clicks (0.1-ms duration) were delivered via earphones at a rate of 9.1 stimuli/s. Filters were set to pass activity between 10 and 250 Hz. The average of 250 sweeps was determined in a 70-ms time window. AMLR parameters were assessed based on amplitude and latency at 80 dB pSPL.

### 2.7. Evaluation of P300 and mismatch negativity (MMN)

Neural-based evaluations of cognitive function and central auditory function in the auditory cortex were determined using P300 at 3 and 6 months after RG treatment. An oddball paradigm was employed with a total of 80 cycles of stimuli (6 or 9 kHz) to elicit the mouse P300. Deviation stimuli randomly occurred 5 % of the time over all 80 cycles. P300 amplitude was defined as the highest voltage recorded within the delay time range of 275–550 ms after stimulation began. The initial stage of information processing and the brain's automatic alerting mechanism were assessed using Mismatch Negativity (MMN) at 3 and 6 months after RG treatment. An oddball paradigm was used with 80 cycles of stimuli (6 or 9 kHz), with deviation stimuli occurring randomly 5 % of the time. MMN amplitude was defined as the difference between the baseline and the maximum negative deflection of the subtracted curve (deviation ERP minus standard ERP) within 100–250 ms.

### 2.8. Behavioral test

The Y-maze test was conducted using equipment consisting of three plastic arms forming a "Y" shape. The walls of the arms were 15.5 cm high, following the published protocol [19]. Mice were placed in the start arm and allowed to explore freely for 8 min. The total number of arm entries and the sequence of entries were recorded to calculate the percentage of alternation.

### 2.9. Histology analysis

Upon completion of the experiment, livers were removed and fixed in 4 % formaldehyde solution. Livers were embedded in O.C.T. tissue tek, cryo-sectioned at 25 μm, and stained with hematoxylin and eosin using standard methods [20].

### 2.10. Biochemical test

After 6 months of drug treatment, mouse blood was collected and centrifuged at 2500 rpm for 10 min to obtain serum, which was immediately frozen. All biochemical serum evaluations were conducted

spectrophotometrically using commercial diagnostic kits (Hoffmann-La Roche Ltd., Basel, Switzerland). The parameters evaluated included: lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total proteins (TP), and albumin (Alb).

### 2.11. Real time qPCR

Quantitative Polymerase Chain Reaction (qPCR) was performed to assess the expression of aging-related genes in the cochlea, auditory cortex, and liver. Total RNA was extracted using Trizol reagent (Thermo Fisher Scientific, Seoul, Korea). Relative mRNA expression levels were measured by qPCR, with *β-actin* used for normalization. Total RNA (1 μg) was reverse-transcribed using the Reverse Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). qPCR reactions (10 μL) included 5 μL of SYBR Select Master Mix (Applied Biosystems, Thermo Fisher Scientific), 1 μL of cDNA template, 1 μL of forward primer (10 pmol), 1 μL of reverse primer (10 pmol), and 2 μL of RNase-free water. The primer sequences are shown in Table 1. The qPCR parameters were: initial denaturation at 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 20 s, with a final extension at 73 °C for 5 min. Gene expression was analyzed using the $2-\Delta\Delta Ct$ method.

### 2.12. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using GraphPad Prism (version 5). Statistical comparisons were made using repeated one-way analysis of variance (ANOVA) with post-hoc tests. Statistical significance was set at $p < 0.05$.

## 3. Results

### 3.1. Efficacy of RG on auditory function in aging mice

RG was examined for its effects on auditory function using the ABR test (Fig. 1A–E). In 18-month-old mice, ABR tests showed a hearing threshold over 60 dB. After RG administration, a significant reduction in hearing thresholds was observed for click, 8, 16, 24, and 32 kHz frequencies. Mice treated with 300 mg/kg RG showed slightly lower thresholds at 16, 24, and 32 kHz compared to those treated with 100 mg/kg. The reduction was more pronounced with six months of RG treatment compared to three months, with the most significant decrease at 24 kHz in both 100 mg/kg and 300 mg/kg groups ($p < 0.001$). These findings suggest that RG mitigates age-related auditory decline. RG's impact on central auditory function was assessed using the AMLR test. RG increased Na and Nb amplitudes, with the 300 mg/kg group showing significant Nb amplitude increases ($p < 0.01$) compared to controls (Fig. 1F and G). Nb latency significantly decreased in the 100 and 300 mg/kg groups ($p < 0.05$ and $p < 0.01$), with a trend towards reduced Pa latency (Fig. 1H and I). Thus, RG enhances central auditory function, potentially ameliorating age-related auditory decline.

**Table 1**
Primers for Real Time qPCR.

| Gene | Forward Sequence | Reverse Sequence |
|---|---|---|
| β-actin | GAA GAG CTA TGA GCT GCC TGA | TGA TCC ACA TCT GCT GGA AGG |
| Dec1 | ATC AGC CTC CTT TTT GCC TTC | AGC ATT TCT CCA GCA TAG GCA G |
| Dec2 | ATT GCT TTA CAG AAT GGG GAG CG | AAA GCG CGC GAG GTA TTG CAA GAC |
| Onecut1 | TCG GCG CTC CGC TTA G | CCT TCC CGT GTT CTT GCT CTT |
| Onecut2 | ATG CCG GTC TCA GGG GAC TCT C | GGC GAA GAG TGT TCG GCG TTG GAG |
| c-Jun | ACC TTC AAC ACC CCA GCC ATG | GGC CAT CTC TTG CTC GAA GTC |
| Stat5b | CAT TTT CCC ATT GAG GTG CG | GGG TGG CCT TAA TGT TCT CC |
| Lims2 | TGC AGT CAT GTG ATC GAG GGT | TTC ATA GCA CCT CTT ACA CAC GG |

### 3.2. Efficacy of RG on electrophysiology cognitive function in aging mice

P300 and MMN assessments were used to evaluate cognitive performance. RG administration increased P300 amplitude, with significant enhancement noted at 6 months after 300 mg/kg RG treatment ($p < 0.05$). P300 latency showed a significant reduction after 3 months of 100 and 300 mg/kg RG treatment ($p < 0.01$ and $p < 0.001$, respectively) (Fig. 2A and B). MMN amplitude exhibited an increasing trend post RG administration, with significant elevation after 6 months of treatment ($p < 0.05$). A reduction trend was also observed in MMN latency (Fig. 2C and D). These findings suggest that RG exerts a beneficial effect on age-related cognitive decline.

### 3.3. Y-maze

The Y-maze test was used to investigate the exploratory behavior of naturally aged mice treated with RG (Fig. 2E). Correct responses were entries into new arms, while returns to previously visited arms were errors. The total number of arm entries and their sequences were recorded to compute the alternation percentage. RG administration increased the alternation rate, with a significant rise in the group administered 300 mg/kg for 6 months ($p < 0.01$), indicating an enhancement in cognitive function impaired by aging.

### 3.4. Biochemical analysis

Biochemical parameters were assessed to evaluate RG's effects on liver function in naturally aged mice (Fig. 3A–F). AST and ALT levels were significantly decreased in the RG-treated group compared to naturally aged mice ($p < 0.05$), indicating a potential hepatoprotective effect. Although ALP, Alb, and TP levels did not show significant differences between groups, there was a trend towards increased levels in the treated group. LDH levels remained consistent across all groups. These results suggest that RG may ameliorate liver function markers in aging mice, with some parameters indicating a tendency towards improvement.

### 3.5. Histology analysis

Histological analysis using H&E staining examined liver samples (Fig. 3G–I). Naturally aged mice exhibited prominent fat droplets and noticeable inflammation, which were reduced in the RG-treated group. RG-administered mice showed significantly diminished fat droplets in size and number compared to the control group. These histological findings support the biochemical data, suggesting RG's protective effect on liver tissue in naturally aged mice, reducing lipid accumulation and inflammatory responses.

### 3.6. Age-related gene expression in aging mice cochlea, auditory cortex, liver

To confirm the expression of *Dec1*, *Dec2*, *Onecut1*, *Onecut2*, *c-Jun*, *Stat5b*, and *Lims2* in naturally aged mice, RT-qPCR was performed using the cochlea, auditory cortex, and liver (Fig. 4). In all tissues of 18-month-old mice, *Dec1*, *Dec2*, *Onecut1*, *Onecut2*, *c-Jun* ($p < 0.001$), and *Stat5b* ($p < 0.01$) expression was significantly increased, while *Lims2* was significantly decreased ($p < 0.001$). In the cochlea (Fig. 4A–G), RG treatment significantly reduced *Dec1*, *Dec2*, *c-Jun*, and *Stat5b* expression compared to the control group ($p < 0.05$, $p < 0.01$, $p < 0.001$), with no significant differences in *Onecut1*, *Onecut2*, and *Lims2* expression. In the auditory cortex (Fig. 4H–N), RG treatment significantly reduced *Dec1*, *Dec2*, *c-Jun*, and *Stat5b* expression ($p < 0.05$, $p < 0.01$, $p < 0.001$) and increased *Lims2* expression ($p < 0.01$). In the liver (Fig. 4O–U), RG treatment significantly reduced *Dec1*, *Dec2*, *c-Jun*, and *Stat5b* expression

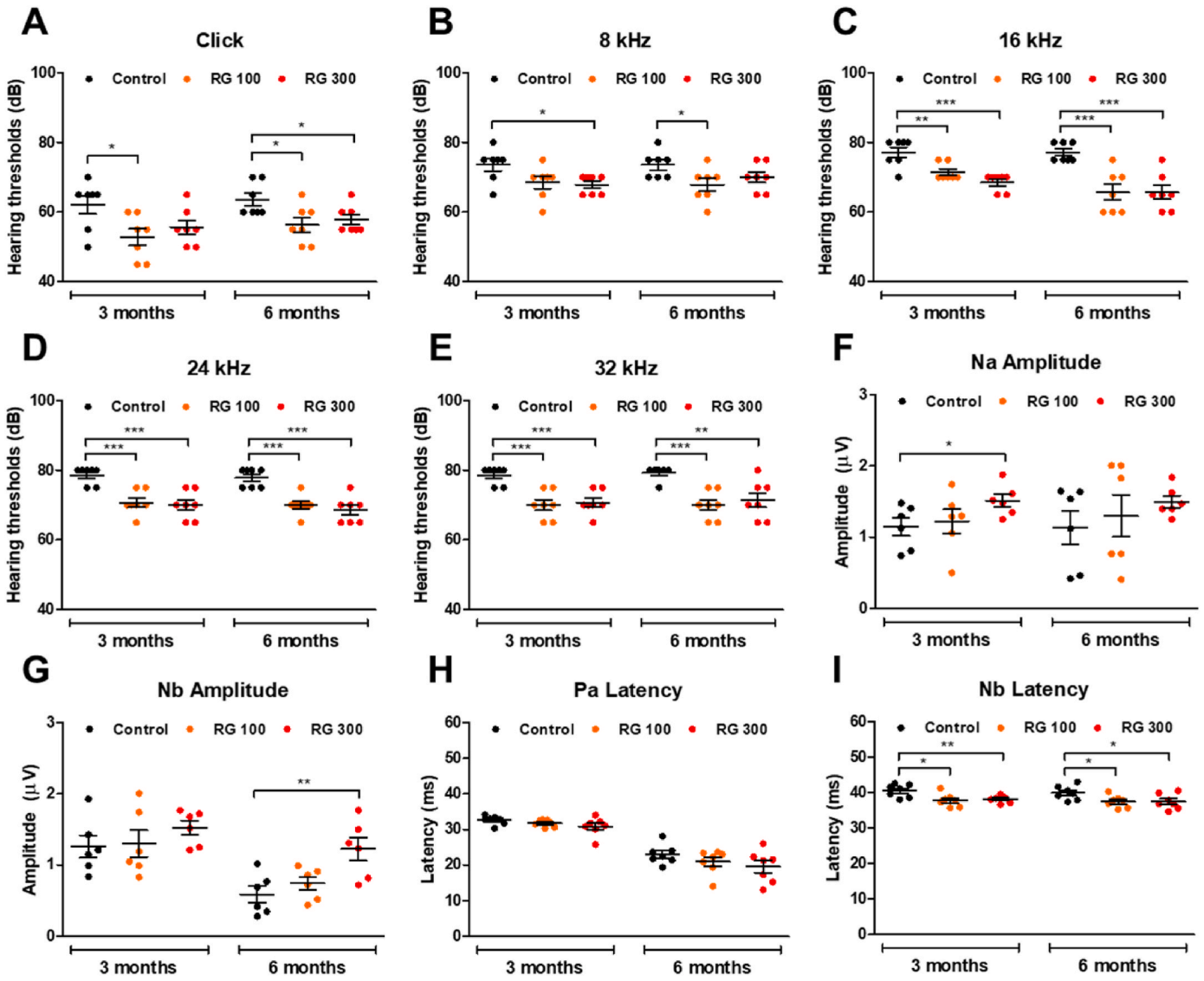


**Fig. 1.** Effects of red ginseng (RG) on auditory functions at 3 and 6 months after 100 or 300 mg/kg treatment in 18-month-old aging mice. (A–E) Hearing thresholds (dB) in auditory brainstem response (ABR). (F–G) Amplitude (μV) of Na and Nb in auditory middle latency response (AMLR). (H–I) Latency (ms) of Pa and Na in AMLR. CON represents the control group, consisting of aging mice that were not treated with RG. Data are presented as means ± SEMs. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (CON vs. RG 100 mg/kg and RG 300 mg/kg).

($p < 0.01$, $p < 0.001$) and increased *Lims2* expression at 100 mg/kg ($p < 0.01$) and 300 mg/kg ($p < 0.001$). These findings suggest that RG modulates *Dec1*, *Dec2*, *c-Jun*, *Stat5b*, and *Lims2* gene expression in the cochlea, auditory cortex, and liver, impacting age-related auditory, cognitive, and liver function decline.

## 4. Discussion

Hearing disorders are increasingly prevalent among the elderly, especially those aged 65 and older [21]. Primary causes include degeneration of inner ear structures, genetic predispositions, prolonged exposure to loud noises, and comorbidities such as diabetes and cardiovascular disease [22]. Hearing loss has profound consequences, contributing to cognitive decline, social isolation, depression, and increased risk of falls and injuries [23]. ABR assesses the integrity of auditory pathways from the ear to the brainstem by measuring the latency and amplitude of electrical activity in response to auditory stimuli [24]. AMLR evaluates neural activity in the auditory cortex and thalamocortical pathway, focusing on latency and amplitude of waves occurring 10–50 ms post-stimulus [25]. Together, ABR and AMLR provide a comprehensive assessment of auditory function from the peripheral auditory nerve to the auditory cortex, aiding in diagnosing and monitoring auditory and neurological disorders. In this study, 18-month-old mice were administered red ginseng (RG) at doses of 100 or 300 mg/kg. ABR and AMLR tests were conducted after 3 and 6 months of treatment. During the ABR test, hearing thresholds were assessed using click stimuli and pure tones at 8, 16, 24, and 32 kHz, starting at 80 dB and decreasing in 5 dB increments. The hearing threshold in decibels (dB) represents the softest sound intensity that elicits a detectable neural response from the ear to the brainstem. Mice treated with RG showed a significant reduction in hearing thresholds across all sound stimuli, with notable improvements at 16, 24, and 32 kHz compared to the control (CON) group. Aging mostly causes the increase of hearing thresholds at high frequencies [26]. These results suggest that RG effectively ameliorates age-related hearing loss. The Na and Nb amplitudes in AMLR indicate the strength of neural response in the auditory cortex, while the Pa and Nb latencies reflect the speed of neural conduction and the integrity of central auditory pathways. Mice treated with RG at 300 mg/kg showed a dose-dependent increase in all amplitudes and a decrease in latencies compared to the CON group. In

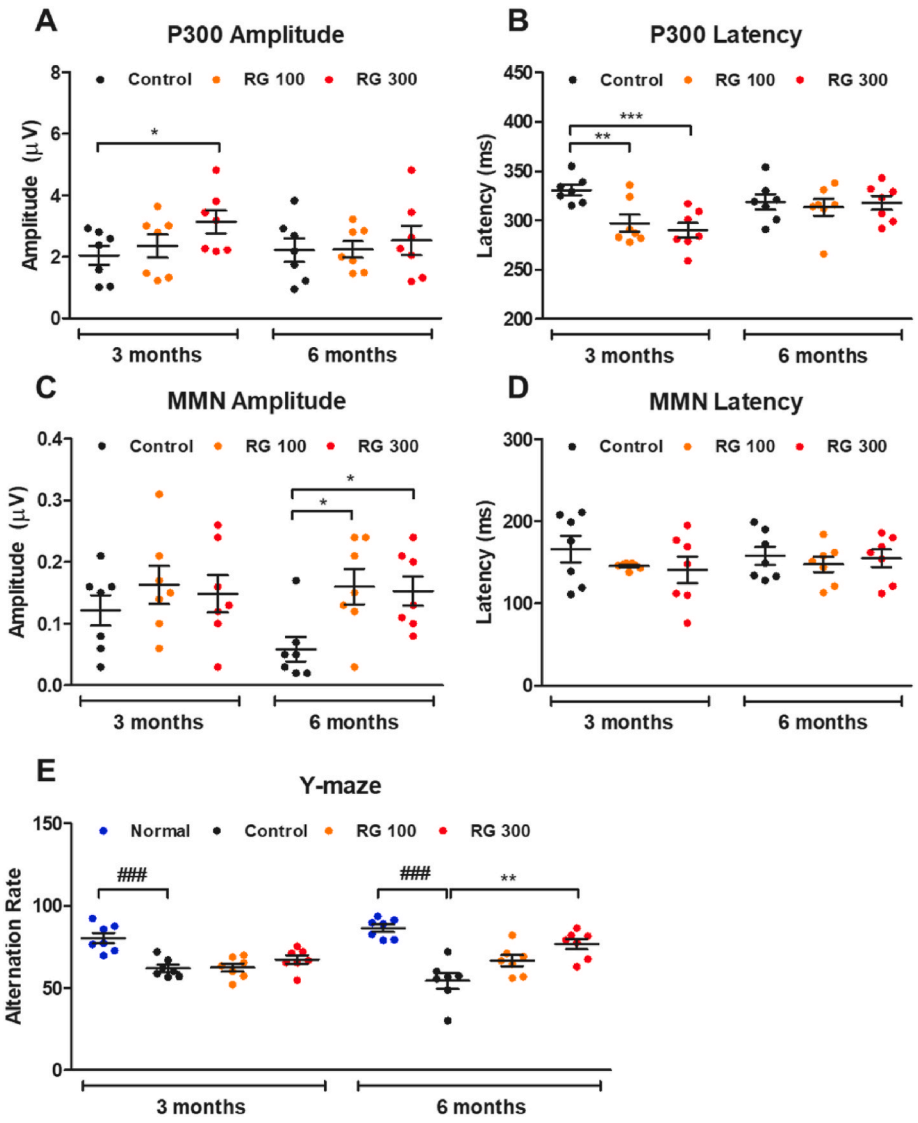


**Fig. 2.** Effects of red ginseng (RG) on cognitive functions at 3 and 6 months after 100 or 300 mg/kg treatment in 18-month-old aging mice. (A–B) Amplitude (μV) and latency (ms) of P300. (C–D) Amplitude (μV) and latency (ms) of mismatch negativity (MMN). (E) Alternation rate in Y-maze behavior test. NOR represents the normal group, consisting of 6-week-old mice, while CON represents the control group, consisting of aging mice that were not treated with RG. Data are presented as means ± SEMs. $^{###}p < 0.001$ (NOR vs. CON). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (CON vs. RG 100 mg/kg and RG 300 mg/kg).

aging individuals, AMLR typically shows increased latency and reduced amplitude compared to younger adults, indicating declines in neural conduction speed and auditory cortical responsiveness [27,28]. Therefore, RG not only improves hearing thresholds across various frequencies but also enhances both amplitude and latency responses to sound stimuli, suggesting its efficacy in ameliorating auditory deficits associated with aging.

Cognitive decline is a prominent symptom of aging, manifesting as diminished memory, attention, and executive function [29]. This decline is primarily due to neurodegenerative changes, such as neuronal loss and synaptic dysfunction [30]. Electrophysiological responses, like P300 and MMN, provide insights into cognitive function during aging. In older adults, the reduced amplitude and increased latency of P300 responses reflect declines in cognitive efficiency and attentional processing [31,32]. Similarly, MMN amplitude decreases with age, indicating impairments in auditory discrimination and sensory memory [33]. These markers link changes in neural processing to cognitive decline. In this study, P300 and MMN tests showed that RG treatment ameliorated age-related decreases in amplitude and increases in latency, suggesting improved cognitive functions. Notably, the P300 results align with finding from a 2-week clinical trial in healty young males, where a daily dose of 4500 mg RG led to decreased P300 latency [34]. The Y-maze is a representative behavioral test used to evaluate cognitive function. It assesses spatial memory and spontaneous alternation, measuring working memory and cognitive flexibility [35]. Age-related declines in Y-maze performance indicate deteriorating hippocampal function. RG administration at 300 mg/kg improved cognitive flexibility and working memory in aging mice by significantly increasing alternation rates after 6 months. Compared to another study using a scopolamine-induced hypomnesic mouse model, a similar trend in alternation rates in the Y-maze was observed after oral administration of hydrolyzed RG extract [36]. Integrating electrophysiological measures with behavioral tests provides a comprehensive understanding of RG's ameliorative effects on cognitive function in aging mice, highlighting both neural and behavioral improvements.

Liver function management is crucial in aging due to the central role the liver plays in maintaining overall health. Aging-related decline in liver function is characterized by reduced hepatocyte regenerative capacity, decreased hepatic blood flow, diminished metabolic efficiency, and increased hepatic steatosis and fibrosis [37]. These changes impact

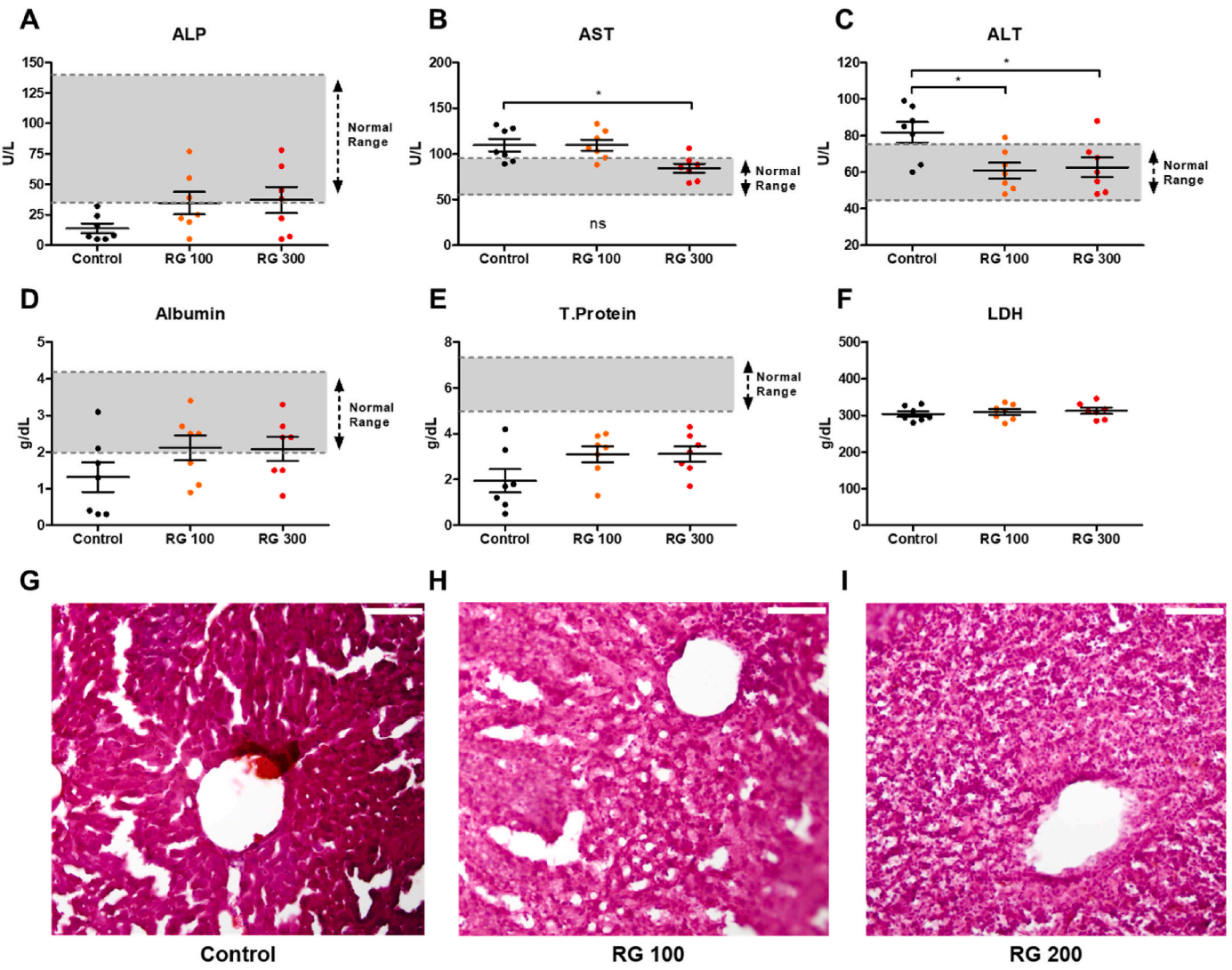


**Fig. 3.** Effects of red ginseng (RG) on liver functions at 3 and 6 months after 100 or 300 mg/kg treatment in 18-month-old aging mice. (A–F) Biochemical levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein (T.Protein), lactate dehydrogenase (LDH). (G–I) Histological images of liver tissue by Hematoxylin and eosin (H&E) staining. CON represents the control group, consisting of aging mice that were not treated with RG. Data are presented as means ± SEMs. *$p < 0.05$ (CON vs. RG 100 mg/kg and RG 300 mg/kg). Scale bar represented 100 μm.

drug metabolism, detoxification, and metabolic homeostasis. Biomarkers such as ALP, AST, ALT, Alb, TP, and LDH are commonly used to monitor liver health [38]. Elevated levels of ALP, AST, ALT, and LDH indicate liver injury or metabolic dysfunction, whereas albumin and total protein levels reflect the liver's protein synthesis capacity, which tends to decrease in chronic liver disease. Albumin helps maintain oncotic pressure and transports various substances, while total protein indicates overall protein status, including immune function. In this study, 18-month-old mice remained within or close to biochemical reference ranges [39,40]. After 6 months of RG treatment at 100 and 300 mg/kg, increases in ALP, Alb, and TP levels, alongside significant decreases in AST and ALT levels, suggest that RG has a regulatory effect on liver function. Age-related decreases in ALP [41], Alb [42], and TP [43] suggest RG modulates these biomarkers. Notably, the statistically significant decreases in AST and ALT levels observed in this study align with findings from a clinical trial involving patients with nonalcoholic statohepatitis, where a daily dose of 2000 mg RG led to decreased AST and ALT levels [44]. Hepatic steatosis can lead to non-alcoholic fatty liver disease (NAFLD) and cirrhosis [45]. Hematoxylin and Eosin (H&E) staining showed a reduction in hepatic fat droplets in mice treated with 300 mg/kg RG compared to untreated aging mice. Normally, the liver contains few fat droplets, however, aging livers show increased fat accumulation, fibrosis, and lipofuscin buildup [46]. The observe reduction in fat droplets with RG treatment suggests a protective effect against hepatic steatosis, which may help maintain liver function during aging. This efficacy of RG in reducing hepatic fat droplets was also observed in another study using high-fat diet-induced rats [47]. Thus, RG demonstrates potential in preserving both liver function and structure during aging, possibly by modulating liver biomarkers and reducing hepatic steatosis.

Aging is associated with complex genetic changes and study for this is important for understanding the molecular mechanisms underlying aging and age-related diseases. Recent studies have highlighted the roles of genes such as *Dec*, *Onecut*, *Stat5b*, *c-Jun*, and *Lims2* in age-related diseases. Differentiated Embryo Chondrocyte (DEC) genes, including *Dec1* and *Dec2*, are crucial for regulating circadian rhythms, which decline with age, leading to physiological changes. DEC genes encode bHLH transcription factors essential for circadian rhythm regulation, similar to PER genes [48]. The Onecut transcription factors are vital for liver, pancreas, and nervous system health, with dysregulation linked to cancer and metabolic diseases [49]. Age-related changes in *Onecut1* and *Onecut2* affect liver regeneration, metabolism, and inflammation,

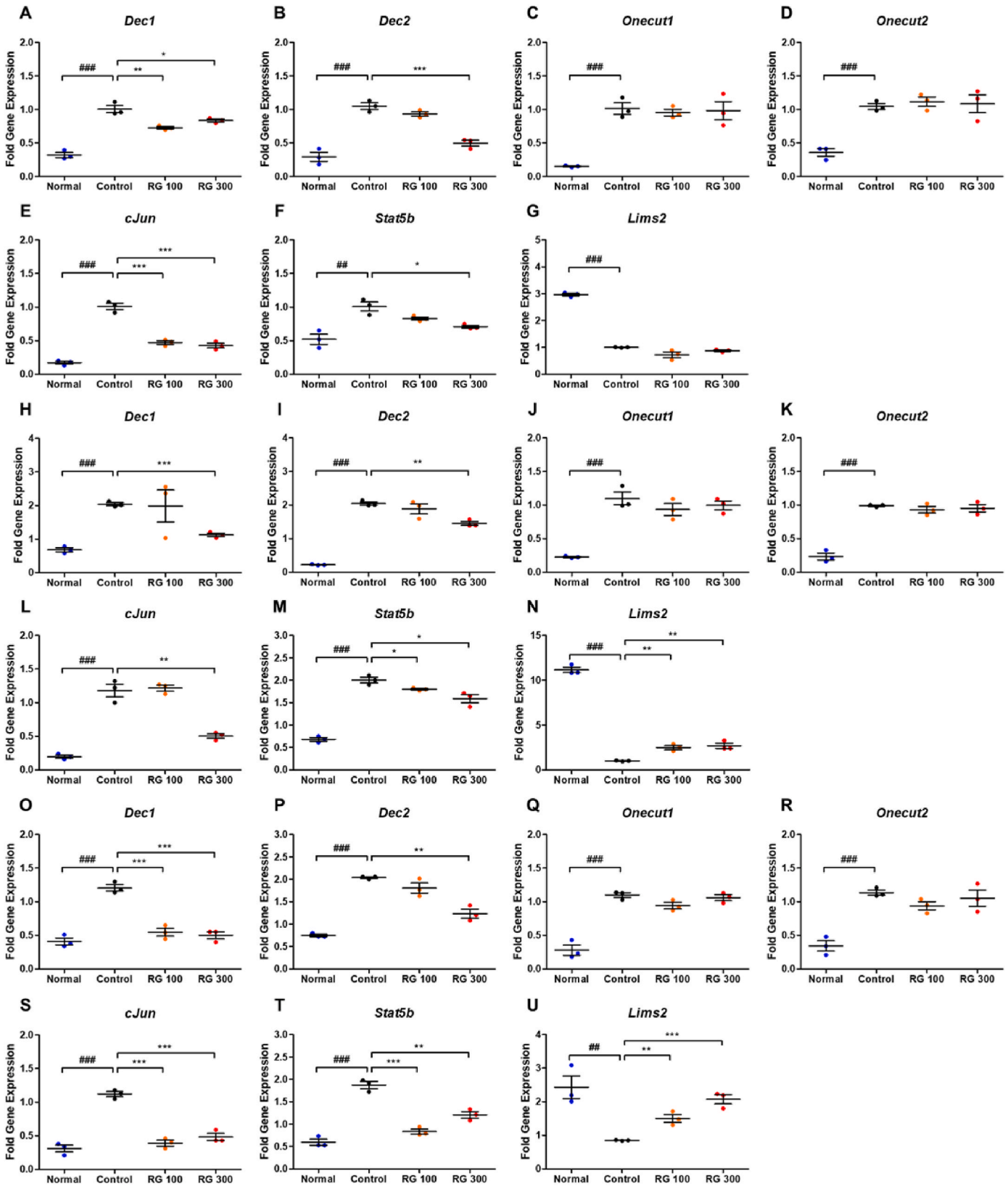


**Fig. 4.** Effects of red ginseng (RG) on gene expression of *Dec1*, *Dec2*, *Onecut1*, *Onecut2*, *c-Jun*, *Stat5b*, and *Lims2* at 6 months after 100 or 300 mg/kg treatment in cochlea (A–G), auditory cortex (H–N), and liver (O–U) of 18-month-old aging mice. NOR represents the normal group, consisting of 6-week-old mice, while CON represents the control group, consisting of aging mice that were not treated with RG. Data are presented as means ± SEMs. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ (NOR vs. CON). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (CON vs. RG 100 mg/kg and RG 300 mg/kg).

contributing to liver dysfunction [50]. *c-Jun*, part of the AP-1 transcription factor complex, regulates cell proliferation, differentiation, apoptosis, and stress response, and is involved in hearing loss, diabetes, chronic inflammation, and cancer [51]. *Stat5b*, part of the STAT family, mediates responses to cytokines and growth factors, influencing cell growth, differentiation, apoptosis, and immunity, with its activation linked to aging-related cancers [52,53]. *Lims2* (*Pinch2*) plays a role in cell adhesion, migration, and survival, with its downregulation observed in cancer [54,55]. In this study, gene expression in the cochlea, auditory cortex, and liver of 18-month-old mice treated with 100 or 300 mg/kg of RG for 6 months was assessed. Compared to 6-week-old mice, aging mice showed downregulation of *Lims2* and upregulation of other genes. The expression levels of the gene in the ear, brain, and liver were almost identical. RG administration generally reversed these aging-related gene expression changes, highlighting the influence of these genes on aging pathways and their importance in aging research.

Aging is associated with complex genetic changes, and studying these changes is essential for understanding the molecular mechanisms underlying aging and age-related diseases. Recent studies have highlighted the roles of genes such as *Dec*, *Onecut*, *Stat5b*, *c-Jun*, and *Lims2* in these processes. Differentiated Embryo Chondrocyte (DEC) genes, including *Dec1* and *Dec2*, encode bHLH transcription factors that are essential for circadian rhythm regulation, which decline with age, leading to various physiological changes, similar to PER genes [48]. The Onecut transcription factors are vital for the health of the liver, pancreas, and nervous system, with dysregulation linked to cancer and metabolic diseases [49]. Age-related changes in *Onecut1* and *Onecut2* affect liver regeneration, metabolism, and inflammation, contributing to liver dysfunction [50]. *c-Jun*, a component of the AP-1 transcription factor complex, regulates cell proliferation, differentiation, apoptosis, and the stress response, and it is involved in conditions such as hearing loss, diabetes, chronic inflammation, and cancer [51]. *Stat5b*, a member of the STAT family, mediates responses to cytokines and growth factors, influencing cell growth, differentiation, apoptosis, and immunity, with its activation linked to aging-related cancers [52,53]. *Lims2* (*Pinch2*) plays a role in cell adhesion, migration, and survival, and its downregulation has been observed in cancer [54,55]. In this study, we assessed the expression of these genes (*Dec*, *Onecut*, *c-Jun*, *Stat5b*, and *Lims2*) in the cochlea, auditory cortex, and liver of 18-month-old mice treated with 100 or 300 mg/kg of RG for 6 months. Compared to 6-week-old mice, the aging group exhibited a significant downregulation of *Lims2* and upregulation of *Dec*, *Onecut*, *Stat5b*, and *c-Jun* in all three tissues, suggesting these genes are key players in the aging process. Interestingly, RG treatment generally reversed these aging-related changes. *Lims2* expression, which was downregulated in aging mice, showed partial restoration upon RG administration, while the upregulation of *Dec*, *Stat5b*, and *c-Jun* was mitigated, indicating RG's potential to modulate gene expression in aging. These alterations in gene expression have important implications for aging and related diseases. For example, the restoration of *Lims2* could enhance cell adhesion and survival, potentially reducing cancer risk. Similarly, the normalization of *Dec*, *Stat5b*, and *c-Jun* expression may help mitigate circadian rhythm disruptions, immune dysfunction, and chronic inflammation, all of which are hallmarks of aging. Our findings are consistent with other research that suggests RG has a regulatory effect on aging-related pathways. For instance, previous studies have demonstrated RG's ability to modulate stress response genes and immune function [56,57], which aligns with the observed changes in Stat5b and c-Jun expression in our study. Additionally, the effect of RG on circadian rhythm genes, such as *Dec*, is supported by literature [58] linking RG to improved sleep patterns and metabolic health in aging populations.

In conclusion, the findings from this study highlight the potential of Korean Red Ginseng as a therapeutic agent for mitigating age-related declines in auditory, cognitive, and liver functions. Administering red ginseng to 18-month-old mice resulted in significant improvements in these critical aging indicators. These results suggest that red ginseng may counteract the degeneration of auditory pathways, enhance cognitive functions, and preserve liver health.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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