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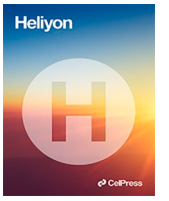

Research article

Abscisic acid ameliorates D-galactose -induced aging in mice by modulating AMPK-SIRT1-p53 pathway and intestinal flora

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ABSTRACT

Abscisic acid (ABA) is a plant hormone with various biological activities. Aging is a natural process accompanied by cognitive and physiological decline, and aging and its associated diseases pose a serious threat to public health, but its mechanisms remain insufficient. Therefore, the purpose of this study was to investigate the ameliorative effects of ABA on D-galactose (D-Gal)-induced aging in mice and to delve into its molecular mechanisms. Aging model was es-tablished by theintraperitoneal injection of D-Gal. We evaluated the oxidative stress by measuring superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT) levels in serum. Proteins content in brain were determined by Western blot. D-Gal-induced brain damage was monitored by measuring the levels of acetylcholinesterase (AChE) content and hematoxylin-eosin staining (H&E). To evaluate the effects of ABA on aging, we measured the gut microbiota. The results demonstrated that ABA increased SOD, CAT and AChE, decreased MDA level. H&E staining showed that ABA could improve D-Gal-induced damage. In addition, ABA regulated the B-cell-lymphoma-2 (BCL-2) family and Phosphatidylinositol 3-kinase/Protein kinase B (PI3K/AKT) signaling pathway, while further regulating the acetylation of p53 protein by modulating the AMPK pathway and activating SIRT1 protein, thereby inhibiting the apoptosis of brain neurons and thus regulating the aging process. Interestingly, ABA improved the ratio of intestinal bacteria involved in regulating multiple metabolic pathways in the aging process, such as Bacteroides, Firmicutes, Lactobacillus and Ak-kermansia. In conclusion, the present study suggests that ABA may be responsible for improving and delaying the aging process by enhancing antioxidant activity, anti-apoptosis and regulating intestinal flora.

1. Introduction

With the global increase in life expectancy, individuals aspire to enhance their quality of life. However, aging is an inherent aspect

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of the life cycle, leading to degenerative changes in the functions of various tissues and organs [1]. In addition, extensive research has demonstrated a strong correlation between aging and various diseases such as cardiovascular diseases, diabetes, and neurodegenerative disorders [2]. Therefore, there is a growing interest in investigating methods to delay aging and unravel the underlying mechanisms, making it a prominent topic in current research.

Several doctrinal theories have been developed to explain the mechanisms of aging [3]. Among these theories, the oxygen free radical theory is widely accepted. According to this theory, aging is caused by cell and tissue damage resulting from oxidative stress and the accumulation of reactive oxygen species (ROS). In order to prevent free-radical-mediated diseases such as aging, it is important to inhibit ROS. Additionally, as individuals age, their immune function declines and there are changes in the physiological function of the intestines, including alterations in the type and quantity of intestinal flora. These changes have a direct impact on the overall physiological function of the body [4,5]. Intestinal microorganisms, known as 'microbial organs,' play a crucial role in various physiological functions such as digestion, immunity, nutrition, metabolism, and cognition [6]. This highlights the strong relationship between gut microbes and aging. Numerous studies have identified the manipulation of gut microbiota as a potential therapeutic strategy for improving aging and related diseases [7].

Recently, there has been a growing interest in exploring natural ingredients for their potential anti-aging properties [8–10]. Researchers have been particularly focused on screening drugs to develop new anti-aging treatments. Natural products have gained attention due to their safety and beneficial biological activities. Consequently, there is a rising trend in seeking natural products with antioxidant, anti-apoptotic, and anti-inflammatory properties for the prevention and treatment of aging. Abscisic acid, a natural plant hormone found in various plants and animal tissues, has been found to possess significant pharmacological activities, including anti-inflammatory effects [11–17]. However, the protective effect of ABA on subacute aging induced by D-galactose (D-gal) has not been reported in the current study.

The purpose of this study is to investigate the anti-aging activity and molecular mechanism of ABA by detecting the oxidative stress index, histopathological state and apoptosis signal pathway in brain tissue of aging mice induced by D-gal, and further probing the correlation between aging body and intestinal flora, which provides a groundbreaking pharmacological basis for the development of ABA anti-aging drugs.

2. Materials and methods

2.1. Chemicals and reagents

ABA and D-Gal were purchased from Sigma-Aldrich (Louis, USA). Assay kits for the measurements of Superoxide Dismutase (SOD), Malondialdehyde (MDA), Catalase (CAT) and acetylcholinesterase (AchE) were obtained from Jiancheng Biotech (Nanjing, China). The primary antibodies such as Bax, Bcl-2, caspase 3, p-PI3K, PI3K, *p*-AKT, AKT, AMPK, p53, SIRT1 and CHOP, and all horseradish peroxidase (HRP)-conjugated secondary antibodies for western blotting, were obtained from Cell Signaling Technology (Boston, USA). β-actin was purchased from Proteintech Group (Chicago, USA).

2.2. Animals

Male ICR mice (6 weeks), weighing20 to 24 g (License number: SCXX 2023-0008), were obtained from Institute of Laboratory Animals Science, CAMS & PUMC (Beijing, China), housed in Laboratory Animal Center of Jilin Agricultural Science and Technology University. All animals were maintained under of the regulated temperature (22–24 °C) and humidity (45–65%) on the 12 h light-dark cycle with access to standard commercial food and water. The present protocol in the experiment were in accordance with Animal Ethics Committee of Jilin Agricultural Science and Technology University and Laboratory Animal Center of Jilin Agricultural Science and Technology University (20220828002), and every effort were made to reduce animal suffering.

2.3. Experimental design

After 3-week acclimatization of feeding, all mice were randomly divided into 4 groups (n = 10): normal group (saline), D-Gal group (D-Gal treated, 100 mg/kg (1%)), D-Gal + ABA low-dose group (20 mg/kg), and D-Gal + ABA high dose group (40 mg/kg), subacute aging mice was induced by consecutive subcutaneous injection of D-Gal once a day at the different dose at different weeks for 8 weeks. Normal group received equal amounts of saline rather than D-Gal in same way. In addition to normal and D-Gal received saline, ABA low and high dose group orallyadministeredtwo doses of ABA treatment daily at fifth week for 4 weeks.

At the end of the experiment, the mice were fasted overnight and weighed after the last drug treatment. Before the mice were sacrificed, the tails of the mice in each group were stimulated to defecate, and they were immediately collected in the 2 mL sterile cryopreservation tube and stored at −80 °C for later sample analysis. Then, all mice were anesthetized by intraperitoneal injection of 70 mg/kg sodium pentobarbital (Shanghai Beizhuo Biochemical Technology Co., Ltd.) (Shanghai, China), and the eyeballs were immediately removed for blood collection, and serum was collected by centrifugation at 3500×*g* at 4 °C for 10 min. The anesthetized mice were then sacrificed by dislocation, and the brains were dissected out, washed with cold sterile saline and weighed, and then immediately stored at −80 °C for subsequent experimental analysis.

2.4. Histomorphometric analysis

Fresh brains were fixed in 4% paraformaldehyde, then paraffin-embedded and cut into 5 μm-thick sections, which were then deparaffinized in xylene and hydrated with various concentrations of ethanol. The sections were then routinely stained using hematoxylin-eosin staining (H&E) and analyzed histomorphometrically under the light microscopy [18].

2.5. Biochemical assays

According to the manufacturer's instructions, the activities of superoxide dismutase (SOD, Xanthine oxidase method), catalase (CAT, Ammonium molybdate method) and the level of malonaldehyde (MDA, Thiobarbituric acid method) in serum were measured using commercial kits. Furthermore, the brain was carried out and centrifuged with ice-cold saline (3000 r/min, 4 °C, 15 min), and the supernatant was used for assay [19]. The activity of AChE was determined by producing TNB with choline and chrominant. Protein concentration was measured using the method of bicinchoninic acid (BCA) method with bovine serum albumin as the standard.

2.6. Western blot analysis

The hippocampal of the mice was lysed in cold RIPA (Beyotime, China) with protease and phosphatase inhibitors. After centrifugation at 10,000×*g* and 4 °C for 15 min, supernatants were decanted by BCA protein assay kits (Beyotime, China), mixed with SDS loading buffer, and then boiled for 10 min. The proteins were separated by 6–10% SDS-polyacrylamide gel and transferred to PVDF membranes (Millipore, USA). The membrane was blocked with 5% BSA (w/v) for 2 h, and then incubated overnight at 4 °C with the primary antibody. After washing three times with TBST, the membrane was incubated with HRP-conjugated secondary antibodies for 60 min at room temperature. Protein bands were visualized using the ECL system and quantified by LAS 4000 mini gel-imaging system (General Electric, Boston, USA). After that, the target protein levels were normalized with β-actin control [20].

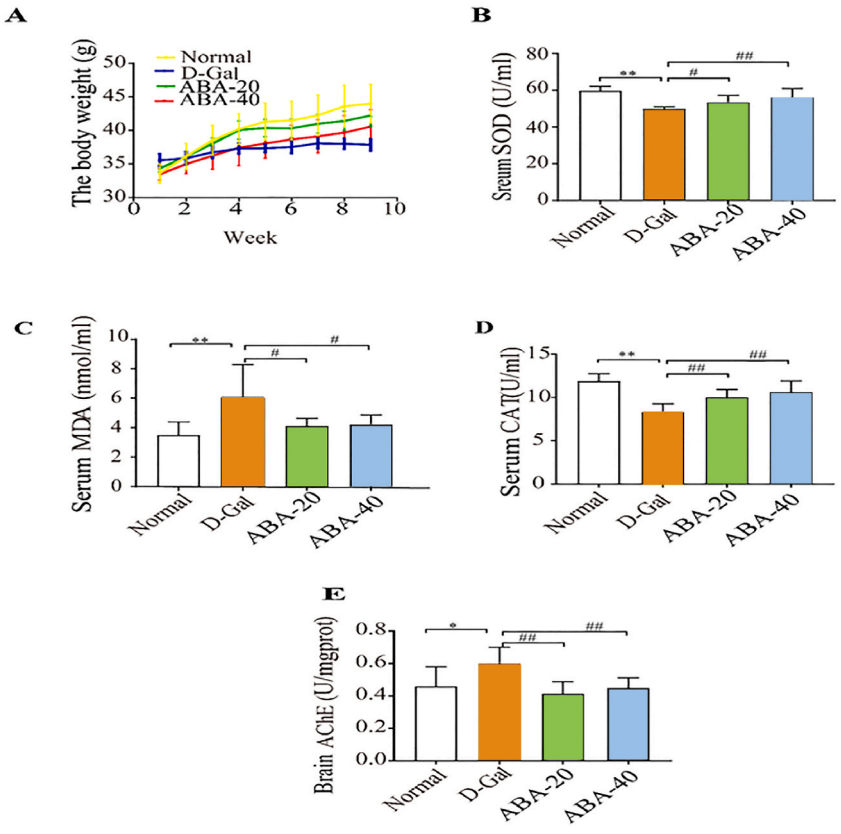

Fig. 1. ABA ameliorates D-Gal-induced oxidative stress injury and cholinergic function (serum). (A) Body weight of mice in each group; (B) Antioxidant enzyme SOD activity; (C) level of Lipid peroxidation MDA; (D) Catalase CAT activity; (E) level of Acetylcholinesterase AChE. Data expressed as mean ± S.D. (n = 10). *$p < 0.05$, **$p < 0.01$, as compared with Normal group; #$p < 0.05$, ##$p < 0.01$, as compared with D-Gal group.

2.7. 16S rDNA gene sequencing

Total DNA from animal fecal samples was extracted using a DNA Extraction Kit (Omega Bio-Tek, USA). PCR amplification of the V3–V4 variable region was performed using 16S rDNA gene-specific primers with the following primer sequences: 341F(5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′- GGACTACHVGGGTATCTAAT-3′). Illumina Miseq sequencing was performed by Shanghai Reabio Technology Co., Ltd.

2.8. Statistical analysis

All experimental data were analyzed using SPSS 24.0 (SPSS Inc., Chicago, IL). Measurement data are presented as the mean ± standard deviation (means ± S.D.). The significance of the difference was determined by one-way ANOVA and post hoc Dunnett's test. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. The general condition and body weights of mice

During eight-week administration of D-Gal, the daily behaviors and body weight of mice were observed and measured weekly. It has been shown that mice in the normal group did not experience symptoms such as weight loss, reduced activity, slow movement, and hair loss. Mice lost weight after being injected with D-Gal. After ABA intervention, weight was gradually restored (Fig. 1A). Five weeks after the injection of D-Gal, the mice aging model group, including the obvious symptoms of slow in response, food intake, depression reduce gradually, and wither and lackluster fur, aging model was successfully established by subcutaneous injection trigger D-Gal. Compared with D-Gal group, mice in normal and ABA-treated group alleviated these symptom of aging.

3.2. ABA ameliorates D-gal-induced oxidative stress injury and cholinergic

The effect of ABA on brain function in mice of D-Gal-treated was evaluated by measuring the serum and levels of SOD, MDA, CAT and AChE in brain. As shown in Fig. 1B–E and Fig. 2A–E, after 8 weeks of D-Gal induction, the levels of MDA and AChE in the D-Gal group were significantly increased compared with normal group ($p < 0.05$), while the activities of SOD and CAT were significantly inhibited ($p < 0.05$). However, ABA treatment significantly reversed MDA and AChE levels, and SOD and CAT activities were significantly restored, especially in the ABA-40 dose group.

3.3. ABA improves hippocampal morphological changes in aging mice

In order to investigate the damage of dentate gyrus region (DG), histopathological changes were examined by HE staining with a light microscope in Fig. 3. The results of HE staining showed that there were no significant pathological changes in DG neurons in the normal group, but the apoptotic like cells in DG area of the brain of aging mice induced by D-gal increased significantly, unclear

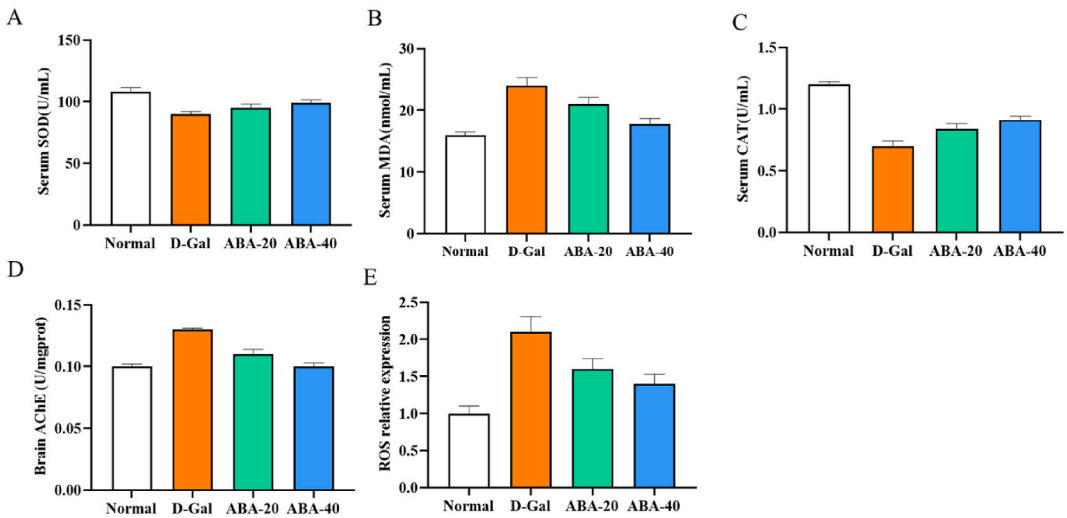

Fig. 2. ABA ameliorates D-Gal-induced oxidative stress injury and cholinergic function (brain). (A) Antioxidant enzyme SOD activity; (B) Level of Lipid peroxidation MDA; (C) Catalase CAT activity; (D) Level of Acetylcholinesterase AChE; (E) ROS relative expression. Data expressed as mean ± S.D. (n = 10). *$p < 0.05$, **$p < 0.01$, as compared with Normal group; #$p < 0.05$, ##$p < 0.01$, as compared with D-Gal group.

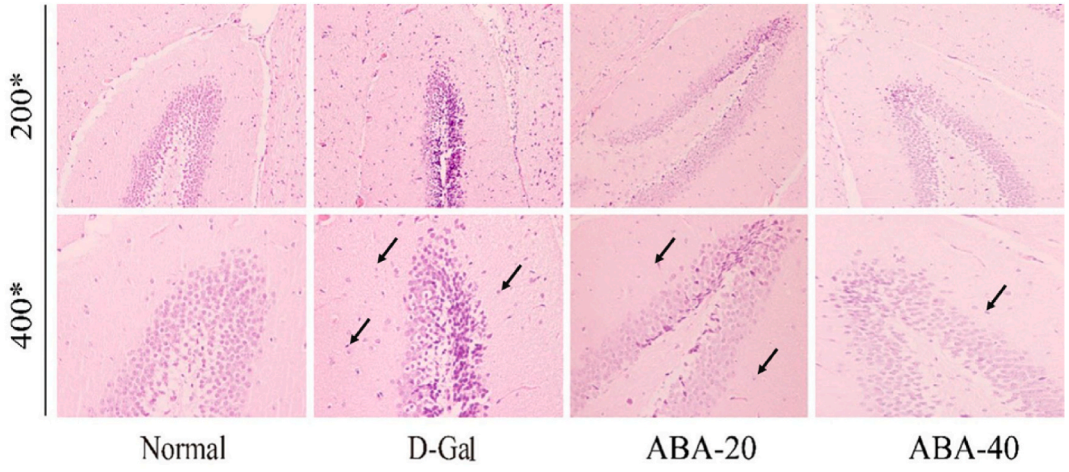

Fig. 3. Improvement of histopathology of the dentate gyrus in the hippocampal region of mice by ABA. H&E staining of hippocampal region of brain tissue, amplification multiple: × 200 and × 400.

irregularly shaped cells, nuclear shrinkage cells and sparse cell arrangement. However, the mice in ABA treatment group showed the significant reduction in DG neuron loss, indicating that ABA effectively reduced the injury of D-gal to the brain tissue of aging mice.

3.4. ABA alleviates D-gal-induced apoptosis in aging mice brain cells

In order to investigate the regulatory role of ABA in apoptosis, we detected the expression of markers in apoptosis protein, such as Bax, Bcl-2 and Caspase-3 through Western blot in D-Gal induced mice. As shown in Fig. 4A, the protein factors related to the apoptosis signaling pathway were detected by gel electrophoresis individually in this study, and each factor was separately subjected to a loading control analysis. Our results showed that ABA treated group decreased the protein levels of Bax and Caspase-3 while increased the level of Bcl-2 compared with D-Gal group (Fig. 4B–D), suggesting that ABA had a potent inhibitory effect on apoptosis. In addition, to further confirm whether ABA's anti-aging is related to regulating the expression of PI3K/AKT pathway, we detected the expression of PI3K, p-PI3K, AKT and *p*-AKT by Western blot. As shown in Fig. 4E, F, D-Gal induction inhibited the expression of p-PI3K, *p*-AKT proteins. However, ABA treatment significantly restored the p-PI3K, *p*-AKT proteins ratio compared with D-Gal group, with the most significant improvement in the ABA-40 dose group, which suggesting that ABA exerted anti-aging effects by regulating the PI3K/AKT signaling pathway.

3.5. ABA ameliorates aging process by modulating AMPK-SIRT1-p53 pathway

To further explore the potential mechanism of ABA anti-aging, the expression level of AMPK-SIRT1-p53 pathway in the brain was analyzed by Western blot. As shown in Fig. 5A–D, compared with the normal group, SIRT1 and AMPK proteins were significantly decreased in the D-Gal group, while p53 expression was increased. However, ABA administration significantly increased the levels of AMPK and SIRT1, while the expression of p53 decreased. Considering all the above findings, we hypothesized that ABA may regulate the acetylation of p53 protein by adjusting AMPK pathway and activating SIRT1 protein, thereby inhibiting the apoptosis of brain neurons and thus regulating the aging process.

3.6. Ameliorating effect of ABA on the changes of intestinal flora in D-Gal-induced aging mice

It has been demonstrated that the composition of intestinal flora can affect the rate of aging [21]. Therefore, we conducted 16S rDNA sequencing technology to analyze the fecal samples of aging mice to investigate the changes in the intestinal flora of aging mice, so as to further explain the related changes of aging phenotype and explore the possible mechanism of ABA anti-aging function. In this research, the analysis mainly focuses on the phylum and genus level, and to depict the profile histogram. Sequencing results generated 1205617 reads, which had passed all quality filters with a 97% identity threshold to obtain a total of 1965 species-classification OTUs (Fig. 6A). Chao1, shannon and simpson was nearly saturated, indicating that the majority of the microbial has been sufficiently captured (Fig. 6B–E).

At the phylum level, Bacteroides, Firmicutes and Verrucomicrobia accounted for over 90% (Fig. 7A). D-Gal group, Normal groups showed adecrease in the proportion of Bacteroides, but it was increased in the D-Gal + ABA-40 group. Firmicutes class had a high proportion in D-Gal, Normal and D-Gal + ABA-20, but the proportion was low in D-Gal + ABA-20 group. Moreover, the level of Proteobacteria was close to Normal, which showed that ABA may reduce the content of Proteobacteria in the intestines of the elderly mice, and thus promote their intestinal health. Importantly, at the genus level, Lactobacillus, Akkermansia, Bacteroides, alistipes, Barnesiella, Clostridium and Desulfovibrio occupy over 80% of the total area (Fig. 7B). ABA-20 group had the highest Lactobacillus

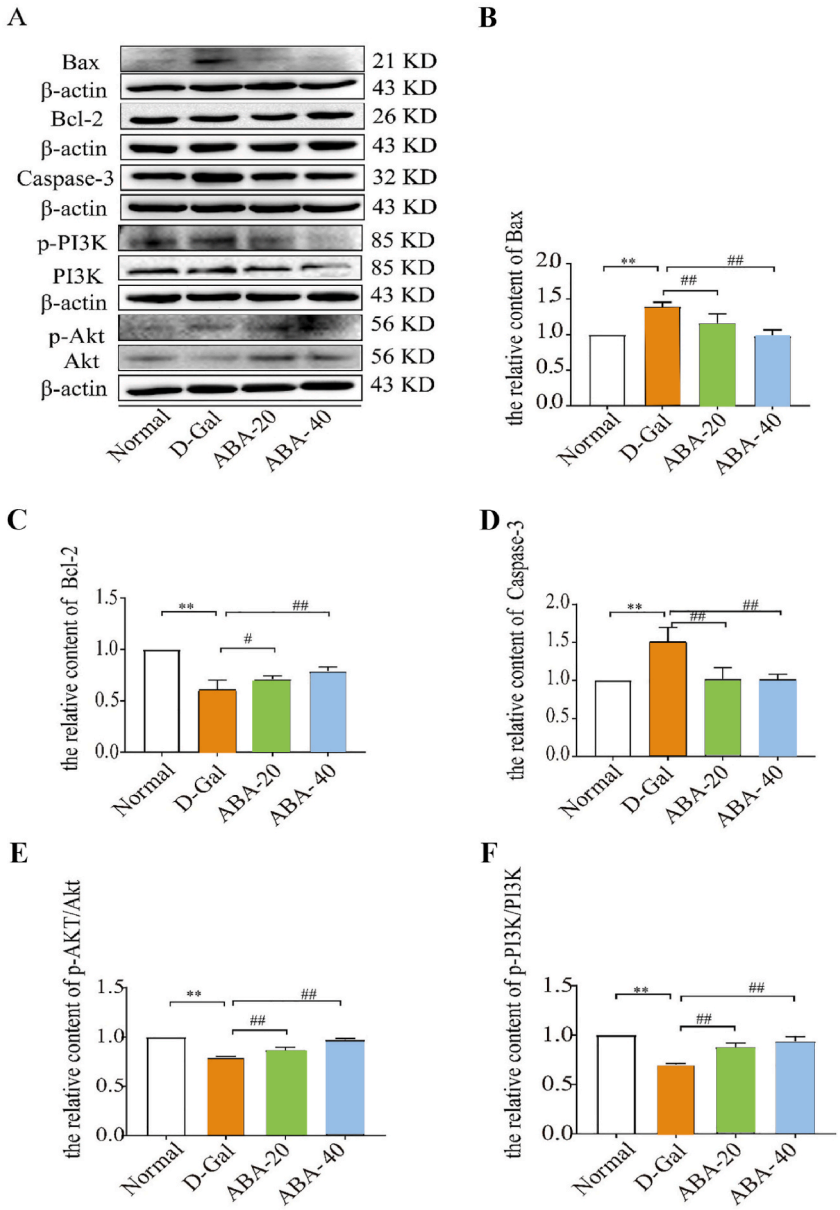

Fig. 4. ABA alleviates D-Gal-induced apoptosis in aging mice brain cells. (A) The effects of ABA on PI3K/Akt, Caspase 3 and Bcl-2/Bax in the brain tissue of aging mice were detected by Western blot. (B–F) Analysis of Bax, Bcl-2, Caspase 3, p-PI3K/PI3K, *p*-AKT/AKT proteins expression. Data expressed as mean ± S.D. (n = 10). $*p < 0.05$, $**p < 0.01$, as compared with Normal group; $\#p < 0.05$, $\#\#p < 0.01$, as compared with D-Gal group.

level. Lactobacillus was gradually increasing in Normal, D-Gal, ABA-20 and ABA-40 groups, and Bacteroides and Barnesiella cover a greater percentage in ABA-40 group. The correlation was calculated using a Spearmans correlation test (Fig. 7C). Some bacteria biomass was found to have a strong significant correlation with bacterial (cor>0.6 or cor < −0.6), including Aeromonas, Enterorchabdus, Bifidibacterium, Fusobacterium, Erysipeotrichace, while these microbiotas could influence multiple metabolic pathways in the aging process.

4. Discussion

The organ index and body weight are very important for assessing D-gal-induced aging in mice. The evidence shows that aging mice exhibit symptom changes such as hair thinning, inhibited weight gain, reduced activity, body weight and organ index decreased. Our results showed that the rate of weight gain declined in D-Gal model group, which was consistent with previous results in the literature

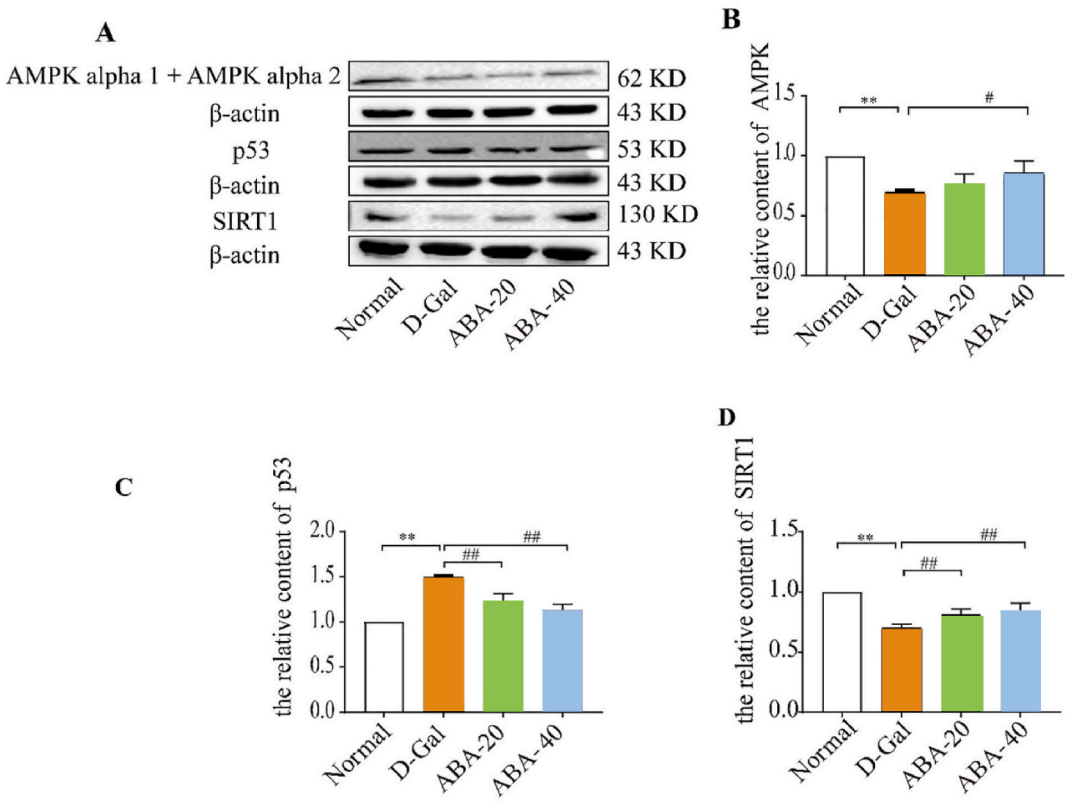

Fig. 5. ABA ameliorates aging process by modulating AMPK-SIRT1-p53 pathway. (A) The effects of ABA on AMPK-SIRT1-p53 in the brain tissue of aging mice were detected by Western blot. (B,C,D) Analysis of Bax, Bcl-2, Caspase 3, p-PI3K/PI3K, *p*-AKT/AKT proteins expression. Data expressed as mean ± S.D. (n = 10). $*p < 0.05$, $**p < 0.01$, as compared with Normal group; $\#p < 0.05$, $\#\#p < 0.01$, as compared with D-Gal group.

[22,23]. In addition, we observed that the ABA treatment significantly increased rate of weight gain compared with those treated with D-Gal group, and these results indicated that ABA can mitigate weight loss due to D-Gal.

The balance of redox in the body is very important to regulate aging. Previous research has shown that D-Galactose induced oxidative stress in the serum/tissue via changing oxidative indicator levels such as MDA, SOD, and CAT [24–26]. MDA, which is the end product of free radical-mediated lipid peroxidation. The level of MDA always reflects on oxidation in vivo. Indirectly, it reflects the degree of cell damage [27,28]. SOD and CAT are important defense of antioxidant defense system. SOD is a strong free radical scavenging enzyme, natural presence in the body. It can dismutation of harmful superoxide (O_2) into hydrogen peroxide (H_2O_2). Although hydrogen peroxide is still a reactive oxygen species that is harmful to the body, CAT in the body immediately breaks it down into completely harmless water [22,23]. In present research, the antioxidant SOD and CAT are important antioxidant enzymes against superoxide radicals in endogenous antioxidant defense systems, eliminates ROS and prevent its induced organ injury. MDA is the end -product of polyunsaturated fatty acid peroxidation in bio membranes, which reflected the level of cell damage. In order to make sure whether ABA regulate D-Gal-induced aging in mice, we detected several ROS- clearance antioxidant enzymes in the brain, including SOD, MDA and CAT. In this research, D-Gal has been used to induce brain injury. We intraperitoneal injection of D-Gal caused a significant increase in the MDA, as well as a decrease in CAT, SOD level. However, the activities of oxidative indicators were improved after ABA supplementation, which indicating that ABA could be a potential pharmaceutical candidate for the prevention of aging.

The brain is vulnerable to oxidative stress injury because it is highest oxygen of oxygen consumption and its relative deficiency of antioxidant enzyme compared with other organs. we have speculated that aging associated with decline in brain functions accompanying progressive memory loss. Dentate gyrus (DG) plays an important role in study, memory and responsible for memory functions in hippocampus. In the progress of aging, many behavioral changes such as decline, memory, learning and cognitive capacity because the structure and function of brain shows gradual degeneration [29]. After D-Gal treatment, D-Gal exist excessive apoptosis. In the current study, our results showed that after ABA intervention, apoptotic cells of dentate gyrus were significant reduction.

It has been proved that the aging process induced by D-Gal is related to cell apoptosis in mice. Apoptosis is the multi-step process of programmed cell death that plays an important role in the tissue development and cell turnover [30]. It is well known that implementation of apoptosis needs to the Bcl-2 family of proteins and caspase signaling cascades. Bcl-2 is a proto-oncogene that inhibits of apoptosis. It keeps away from apoptosis through inhibit pro-apoptotic BAX. Bcl-2 shows anti-apoptotic effect and BAX shows effect as a pro-apoptotic protein. Caspase-3 is considered to be another important protein of apoptosis, which participates in regulating apoptosis through multiple channels [31]. In the present work, ABA regulated Bcl-2 family proteins, and pro-apoptotic protein Bax belongs to

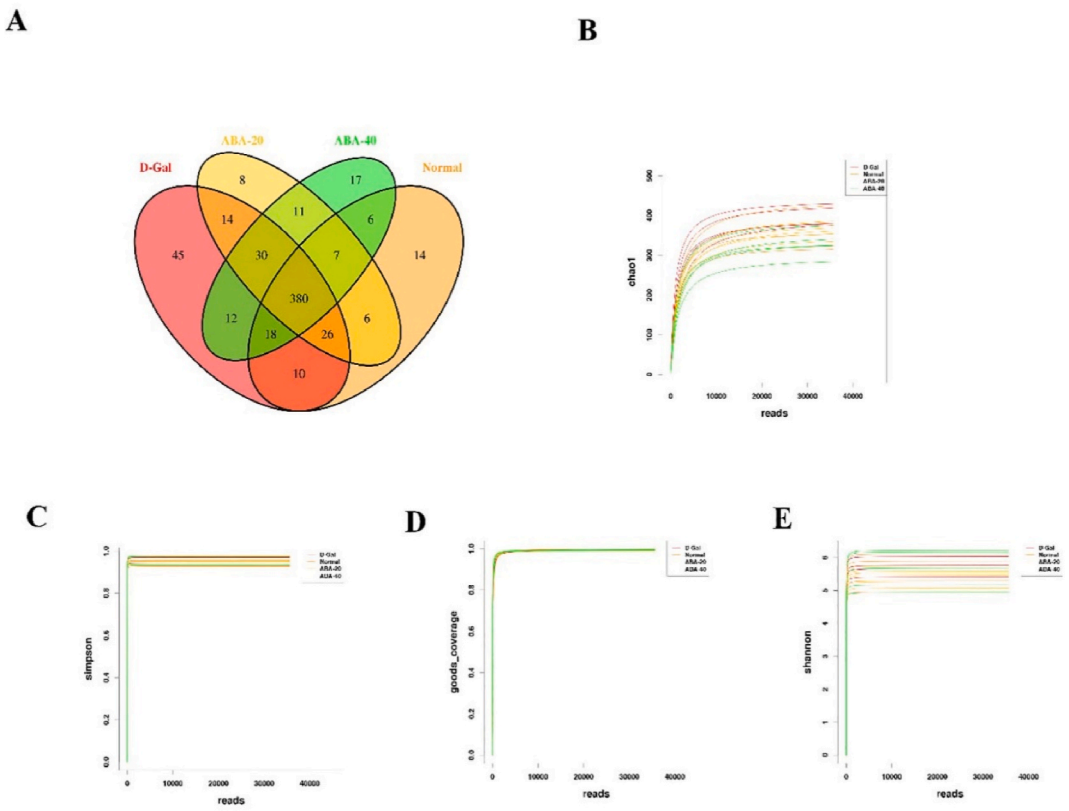

Fig. 6. Analysis of α-diversity index of intestinal flora in D-Gal-induced aging mice by ABA. (A) Venn Diagrams: Species Tax-onomy OTUs; (B) Chao1 index analysis; (C) Shannon index analysis; (D) simpson index analysis.

Bcl-2 family proteins, which regulated downstream caspase-3 to inhibit cell apoptosis.

Many literatures have confirmed that PI3K/Akt signaling pathway is relevant to cell proliferation, agingand survival [32–34]. PI3K, a kind of phosphatidylinositol kinase in cells, can be activated by a series of growth factor receptors. Alteration of AKt protein structure by activated PI3K produces next levels of signaling. In our study, exposure to D-Galreduced the relative expression degree of proteins such as PI3K and AKt their phosphorylation levels, that is the relative expressions of p-PI3K and *p*-AKT. These results are similar to inhibition of PI3K/AKT pathway. Thus, conjecture that ABA may inhibitsgrowth factor receptors or signaling transduction complexes to activate PI3K, which may reduce the downstream AKT kinase activity, and then inhibit a series of phosphorylated cascade activation reactions triggered by AKT.

SIRT1, a member of nicotinamide adenine dinucleotide (NAD+)- dependent histone deacetylases, have a vital role in biological functions, including cell survival, aging, apoptosis, metabolism, and oxidative stress [32,35–37]. Increasing evidence show that increased SIRT1 activity may have beneficial effects on aging and senescence related diseases in mammals [38,39]. SIRT1 regulates several important transcription factors, including p53. p53 is a tumor suppressor, which can be activated by numerous stressors to induce cell apoptosis, cell cycle arrest and plays a vital role in aging [40]. In this study, we elucidated that oxidative stress triggered by activation of the SIRT1 and p53 in aging mice. D-Gal-induced oxidative stress in aging mice is mediated by activation of SIRT1 and p53. D-Gal can enhance p53 and down-regulate the protein expression level of SIRT1. ABA and D-Gal can inhibit p53 and increase SIRT1 levels. Overall, oxidative stress is the key pathway of D-Gal-induced aging. ABA inhibits senescence through Sirt1 and p53, which may reduce oxidative stress.

As reported, the gut microbiome changes were related to the age [41]. Gut microbiota architecture has altered in elderly. Maintaining a “young” intestinal flora composition may delay the aging [21,42,43]. The intestinal flora is not only a participant in the metabolic process, but also a regulator of its metabolic process. The metabolism of the host is regulated by its own genome and symbiotic bacteria. Some brain functions are regulated by intestinal flora including emotional behaviors, stress-related responsiveness, pain and food intake. ABA affects the intestinal flora of mice, and all groups had different bacterial abundances. By analyze Chao 1 and species richness, Bacteroides and Firmicutes are the two most abundant phyla in the intestinal flora of mice, which is consistent with the previously reported literature. Besides, Lactobacillus and Akkermansia are the dominant genus. At phylum, Bacteroides and Firmicutes play a key role in host gut metabolism. Bacteroides metabolizes pyruvate into acetic acid and propionic acidby some metabolic pathways [44], thereby inhibiting intestinal inflammation. For genus, Lactobacillus and Akkermansia considered to be beneficial bacteria. Lactobacillus stimulating intestinal peristalsis for maintaining health [45]. A low level of Akkermansia may cause the intestinal mucosa to become thinner, thereby weakening the intestinal barrier function and making it easier for enteric pathogens

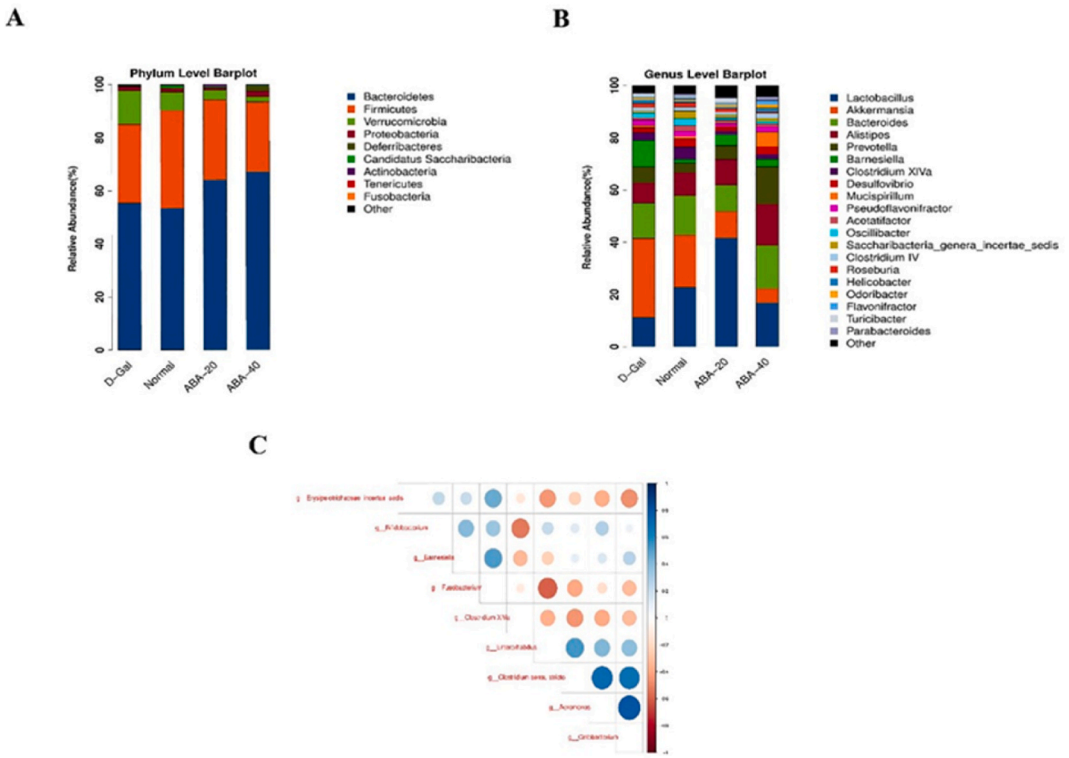

Fig. 7. Effects of ABA on the phylum and genus levels of the intestinal flora. (A) Phylum level barplot; (B) Genus level barplot; (C) Spearmans correlation analysis.

to invade the body [46]. This evidence showed that ABA delaying aging of mice may be related to inhibiting the growth of harmful bacteria and increasing the number of beneficial bacteria (Fig. 8).

5. Conclusions

In conclusion, the present study revealed that ABA inhibited D-Gal-induced brain cell apoptosis in aging mice by regulating the expression levels of PI3K/AKT and its downstream apoptotic protein family, while further regulating the AMPK-SIRT1 pathway and inhibiting the acetylation of p53 protein, thereby inhibiting the apoptosis of brain neurons and regulating the aging process. Importantly, ABA regulated the proportion of intestinal bacteria in aging mice, such as Bacteroides, Firmicutes, Lactobacillus and Akkermansia, which evidences lay the research foundation for the development of clinical anti-aging drugs.

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Institutional review board statement

The animal study protocol was approved by the Animal Ethics Committee of Jilin Agricultural Science and Technology University (20220828002).

Data availability statement

Data will be provided upon request.

CRediT authorship contribution statement

Yongchun Zheng: Writing – original draft, Funding acquisition. **Xueyan Chen:** Resources, Data curation. **Chuanbo Ding:** Supervision, Methodology. **Xinglong Liu:** Resources, Project administration, Investigation. **Lihua Chi:** Writing – review & editing, Methodology, Data curation. **Shuai Zhang:** Writing – review & editing, Supervision, Project administration.

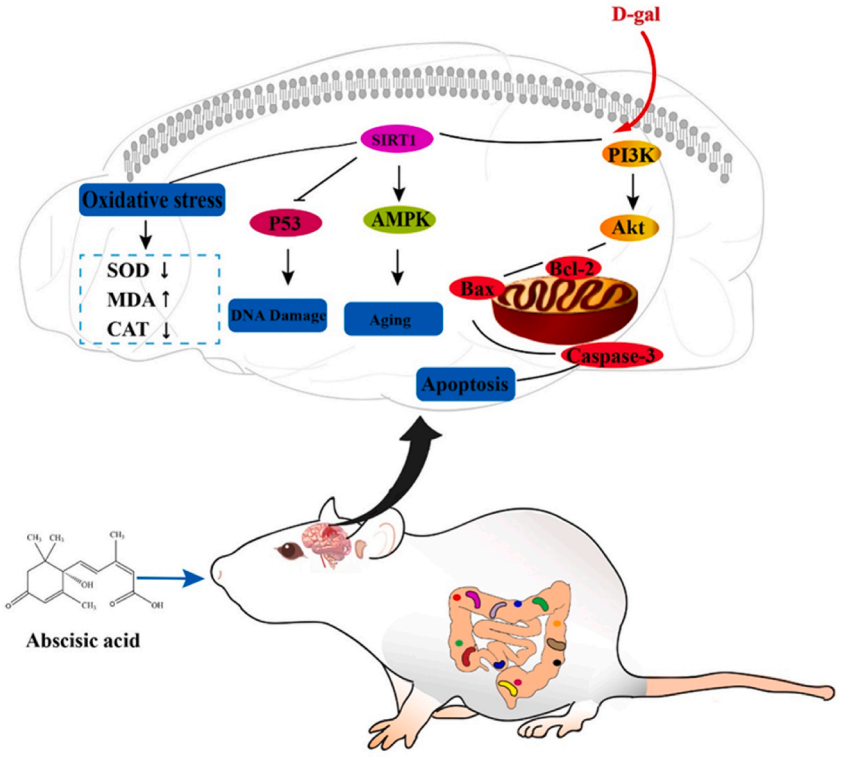

Fig. 8. ABA induced by D-Gal mouse the possible mechanisms of aging.

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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Appendix A. Supplementary data

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References

[1] J.C. Clemente, L.K. Ursell, L.W. Parfrey, R. Knight, The impact of the gut microbiota on human health: an integrative view, Cell (2012), https://doi.org/10.1016/j.cell.2012.01.035.
[2] I. Katzir, M. Adler, O. Karin, N. Mendelsohn-Cohen, A. Mayo, U. Alon, Senescent cells and the incidence of age-related diseases, Aging Cell 20 (2021) e13314, https://doi.org/10.1111/ACEL.13314.
[3] D. H, Aging: a theory based on free radical and radiation chemistry, J. Gerontol. 11 (1956) 298–300, https://doi.org/10.1093/GERONJ/11.3.298.
[4] P. K, L. Hu, I. G.-P, E.X.Y. T, H. K, L.E. F, K.A. M, R. P, D.I. D.-M, S. G, J.K. N, S. S, E. H, S. P, Neurogenesis and prolongevity signaling in young germ-free mice transplanted with the gut microbiota of old mice, Sci. Transl. Med. 11 (2019), https://doi.org/10.1126/SCITRANSLMED.AAU4760.
[5] M.J. C, S. C, O. O, R. G.-D, H. de W, E. F, J.R. M, D. F, T. D, G. F, C. S, D. van S, M. O, N. H, K. O, C. H, D. O, A.P. F, F. S, C. T, C. H, R.P. R, P.W. O, Composition, variability, and temporal stability of the intestinal microbiota of the elderly, Proc. Natl. Acad. Sci. U.S.A. 108 (Suppl 1) (2011) 4586–4591, https://doi.org/10.1073/PNAS.1000097107.
[6] M. L, Z. W, Microbiota and aging, Adv. Exp. Med. Biol. 1086 (2018) 141–156, https://doi.org/10.1007/978-981-13-1117-8_9.
[7] X.L. L, Y.C. Z, H.Y. Z, M. W, Y.N. Z, M. Y, Z.Q. C, C.B. D, W.C. L, Taxifolin retards the D-galactose-induced aging process through inhibiting Nrf2-mediated oxidative stress and regulating the gut microbiota in mice, Food Funct. 12 (2021) 12142–12158, https://doi.org/10.1039/D1FO01349A.
[8] X. S, D.W. S, J.W. H, W.Y. S, E.J. S, M.K. K, K.H. H, Y.K. K, S. K, T.J. K, T.B. K, K.H. L, Anti-inflammatory effect of Impatiens textori Miq. extract via inhibition of NLRP3 inflammasome activation in in vitro and in vivo experimental models, J. Ethnopharmacol. 170 (2015) 81–87, https://doi.org/10.1016/J.JEP.2015.05.001.

[9] M. R.-K, A. B, M. R, Plants antioxidants: from laboratory to clinic, J. Nephropathol. 2 (2013) 152–153, https://doi.org/10.12860/JNP.2013.26.
[10] N. A, G. M, S. G, E. G, Natural compounds and plant extracts as therapeutics against chronic inflammation in Alzheimer's disease–a translational perspective, CNS Neurol. Disord.: Drug Targets 13 (2014) 1175–1191, https://doi.org/10.2174/1871527313666140917110635.
[11] M. S, M. A, R. K, S. E.-M, Central microinjection of phytohormone abscisic acid changes feeding behavior, decreases body weight, and reduces brain oxidative stress in rats, Nutr. Neurosci. 22 (2019) 678–687, https://doi.org/10.1080/1028415X.2018.1431093.
[12] X. C, C. D, W. L, X. L, Y. Z, Y. Z, L. D, S. K, M. H, X. P, Y. Z, H. C, Abscisic acid ameliorates oxidative stress, inflammation, and apoptosis in thioacetamide-induced hepatic fibrosis by regulating the NF-κB signaling pathway in mice, Eur. J. Pharmacol. 891 (2021), https://doi.org/10.1016/J.EJPHAR.2020.173652.
[13] M. M, G. L, T. V, L. E, L. S, E. Z, G. M, Chronic intake of Micrograms of abscisic acid improves Glycemia and lipidemia in a human study and in high-Glucose fed mice, Nutrients 10 (2018), https://doi.org/10.3390/NU10101495.
[14] P. D, G. A, G.C. T, A. S, Abscisic acid identification in Okra, Abelmoschus esculentus L. (Moench): perspective nutraceutical use for the treatment of diabetes, Nat. Prod. Res. 34 (2020) 3–9, https://doi.org/10.1080/14786419.2019.1637874.
[15] S. B, M. M, E. M, G. S, L. S, C. F, V. B, L. E, A. D.F, E. Z, Abscisic acid Stimulates Glucagon-like peptide-1 secretion from L-cells and its oral administration increases plasma glucagon-like peptide-1 levels in rats, PLoS One 10 (2015), https://doi.org/10.1371/JOURNAL.PONE.0140588.
[16] R. N, S. E.-M, M. A, Phosphatidylinositol-3-kinase and protein kinase C are involved in the pro-cognitive and anti-anxiety effects of phytohormone abscisic acid in rats, Biomed. Pharmacother. 96 (2017) 112–119, https://doi.org/10.1016/J.BIOPHA.2017.09.089.
[17] E.K.K. G, D. M, B.K. T, I. S, L. H, F. A, B. G, L.G. A, S. L, Elevated plasma abscisic acid is associated with asymptomatic falciparum malaria and with IgG-/caspase-1-dependent immunity in Plasmodium yoelii-infected mice, Sci. Rep. 8 (2018), https://doi.org/10.1038/S41598-018-27073-1.
[18] M. Fan, H. Shi, H. Yao, W. Wang, Y. Zhang, C. Jiang, R. Lin, Bmscs promote differentiation of enteric neural precursor cells to maintain neuronal homeostasis in mice with enteric nerve injury, Cell. Mol. Gastroenterol. Hepatol. 15 (2023) 511–531, https://doi.org/10.1016/j.jcmgh.2022.10.018.
[19] D. Luo, Z. Huang, G. Jia, H. Zhao, G. Liu, X. Chen, Naringin mitigates lps-induced intestinal barrier injury in mice, Food Funct. 14 (2023) 1617–1626, https://doi.org/10.1039/d2fo03586c.
[20] T. Lan, T. Tang, Y. Li, Y. Duan, Q. Yuan, W. Liu, Y. Ren, N. Li, X. Liu, Y. Zhang, et al., Ftz polysaccharides ameliorate kidney injury in diabetic mice by regulating gut-kidney axis, Phytomedicine 118 (2023) 154935, https://doi.org/10.1016/j.phymed.2023.154935.
[21] M. C, E. B, P. B, P.W. O, W.M. D.V, Maintenance of a healthy trajectory of the intestinal microbiome during aging: a dietary approach, Mech. Ageing Dev. 136–137 (2014) 70–75, https://doi.org/10.1016/J.MAD.2013.12.004.
[22] H. J, Y. L, H.S. K, Dried plum and chokeberry ameliorate d-galactose-induced aging in mice by regulation of Pl3k/Akt-mediated Nrf2 and Nf-kB pathways, Exp. Gerontol. 95 (2017) 16–25, https://doi.org/10.1016/J.EXGER.2017.05.004.
[23] J. Jiao, Y. Wei, J. Chen, X. Chen, Y. Zhang, Anti-aging and redox state regulation effects of A-type proanthocyanidins-rich cranberry concentrate and its comparison with grape seed extract in mice, J. Funct.Foods 30 (2017) 63–73, https://doi.org/10.1016/J.JFF.2016.12.039.
[24] J. Z, J. Z, L. Z, X. S, L.A. W, Y. W, Antioxidant and anti-aging activities of ethyl acetate extract of the coral tooth mushroom, hericium coralloides (agaricomycetes), Int. J. Med. Mushrooms 21 (2019) 561–570, https://doi.org/10.1615/INTJMEDMUSHROOMS.2019030840.
[25] G. L, J. Y, L. Z, Y. W, C. W, Q. C, Onjisaponin B prevents cognitive impairment in a rat model of D-galactose-induced aging, Biomed. Pharmacother. 99 (2018) 113–120, https://doi.org/10.1016/J.BIOPHA.2018.01.006.
[26] D.J. E, M. S, Y. S, Role of galactose in cellular senescence, Exp. Gerontol. 73 (2016) 1–4, https://doi.org/10.1016/J.EXGER.2015.11.003.
[27] E. K, M. R, A. K, A. S, M. A, N. M, I. F, Protective effect of atorvastatin on d-galactose-induced aging model in mice, Behav. Brain Res. 334 (2017) 55–60, https://doi.org/10.1016/J.BBR.2017.07.029.
[28] Z. D, M. X, J. H, L. C, J. X, X. C, R. J, L. W, Y. W, The protective effect of Ginsenoside Rg1 on aging mouse pancreas damage induced by D-galactose, Exp. Ther. Med. 14 (2017) 616–622, https://doi.org/10.3892/ETM.2017.4514.
[29] K.B. W, S.K. K, I.K. A, H. B, A. B, P. D.-T, H. G, D.J. H, A.K. H, W.S. K, L. F, L. N, M.S. P, D.A. R, J. S, A.B. S, A.E. S, C.K. T, W.K. T, C. R, A.M. D, A.M. F, Neurodevelopmental origins of lifespan changes in brain and cognition, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 9357–9362, https://doi.org/10.1073/PNAS.1524259113.
[30] S. A, A. G.-C, M. C, M.F. M, A. A, Advantages and disadvantages of apoptosis in the aging process, Ann. N. Y. Acad. Sci. 1443 (2019) 20–33, https://doi.org/10.1111/NYAS.14020.
[31] J.H. Zhang, Y. Zhang, B. Herman, Caspases, apoptosis and aging, Ageing Res. Rev. 2 (2003) 357–366, https://doi.org/10.1016/S1568-1637(03)00026-6.
[32] H. W, F. H, Z. Z, P. W, Y. L, H. L, N. L, J. W, J. Z, Y. W, S. L, Feedback activation of SGK3 and AKT contributes to rapamycin resistance by reactivating mTORC1/4EBP1 Axis via TSC2 in breast cancer, Int. J. Biol. Sci. 15 (2019) 929–941, https://doi.org/10.7150/IJBS.32489.
[33] S. L, S. L, X. W, J. Z, Y. C, F. W, E. D, The PI3K-Akt pathway inhibits senescence and promotes self-renewal of human skin-derived precursors in vitro, Aging Cell 10 (2011) 661–674, https://doi.org/10.1111/J.1474-9726.2011.00704.X.
[34] F. C, J.T. L, P.M. N, L.S. S, J.G. S, W.L. B, R.A. F, J.A. M, Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy, Leukemia 17 (2003) 590–603, https://doi.org/10.1038/SJ.LEU.2402824.
[35] M.T. D, H.G. K, J.H. C, H.G. J, Metformin induces microRNA-34a to downregulate the Sirt1/Pgc-1α/Nrf2 pathway, leading to increased susceptibility of wild-type p53 cancer cells to oxidative stress and therapeutic agents, Free Radic. Biol. Med. 74 (2014) 21–34, https://doi.org/10.1016/J.FREERADBIOMED.2014.06.010.
[36] B. S, M. M, P. P, A. G.-B, J. R, M. B, The sirtuin family members SIRT1, SIRT3 and SIRT6: their role in vascular biology and atherogenesis, Atherosclerosis 265 (2017) 275–282, https://doi.org/10.1016/J.ATHEROSCLEROSIS.2017.08.027.
[37] F. N, L. W, B.L. T, SIRT1 in the brain-connections with aging-associated disorders and lifespan, Front. Cell. Neurosci. 9 (2015), https://doi.org/10.3389/FNCEL.2015.00064.
[38] A.Z. H, L. G, SIRT1 in neurodevelopment and brain senescence, Neuron 81 (2014) 471–483, https://doi.org/10.1016/J.NEURON.2014.01.028.
[39] D. H, M. S, Impact of Sirt1 on mammalian aging, Aging (Albany. NY) 2 (2010) 315–316, https://doi.org/10.18632/AGING.100156.
[40] F. R, J. C, D. B, Two faces of p53: aging and tumor suppression, Nucleic Acids Res. 35 (2007) 7475–7484, https://doi.org/10.1093/NAR/GKM744.
[41] J.M. R.C, [Intestinal microbiota and ageing: a new intervention route?], Rev. Esp. Geriatr. Gerontol. 51 (2016) 290–295, https://doi.org/10.1016/J.REGG.2015.12.012.
[42] H.J. Z, V.J. Q, The microbiota and microbiome in aging: potential implications in health and age-related diseases, J. Am. Geriatr. Soc. 63 (2015) 776–781, https://doi.org/10.1111/JGS.13310.
[43] S. Khanna, Y. Vazquez-Baeza, A. González, S. Weiss, B. Schmidt, D.A. Muñiz-Pedrogo, J.F. Rainey, P. Kammer, H. Nelson, M. Sadowsky, A. Khoruts, S. L. Farrugia, R. Knight, D.S. Pardi, P.C. Kashyap, Changes in microbial ecology after fecal microbiota transplantation for recurrent C. difficile infection affected by underlying inflammatory bowel disease, Microbiome 5 (2017), https://doi.org/10.1186/S40168-017-0269-3.
[44] J.C. S, M.A. Z, R.J. de S, N.C. C, M. F, M. S, M.R. S, A.B. B, P.J. M, P. S, S.E. T, M. G, J. B, M.G. S, S.S. A, Ethnic and diet-related differences in the healthy infant microbiome, Genome Med. 9 (2017), https://doi.org/10.1186/S13073-017-0421-5.
[45] W.P. H, P.S. T, The potential of lactic acid bacteria for the production of safe and wholesome food, Z. Lebensm. Unters. Forsch. 198 (1994) 193–201, https://doi.org/10.1007/BF01192595.
[46] J.C. C, L.K. U, L.W. P, R. K, The impact of the gut microbiota on human health: an integrative view, Cell 148 (2012) 1258–1270, https://doi.org/10.1016/J.CELL.2012.01.035.