WHIOCE PUBLISHING PTE. LTD.
PROVIDING FIRST-CLASS SCIENTIFIC INFORMATION FOR TOP SCHOLARS

2026 Volume 11, Issue 1
Journal of Medicines Development Sciences
ISSN: 2382-6371(Online) ISSN: 2382-6363(Print)

Anti-Aging Effects of the Herbal Decoction Si Jun Zi Tang on Spleen Metabolites in Aging Mice: A Metabolomics Study

Er-xin Liang, Qi-wei Li, Ze-guang Li, Hua Liang*
Heilongjiang University of Chinese Medicine, Harbin 150006, Heilongjiang, China

Abstract: *Objective:* In this study, metabolomics analysis was conducted to assess changes in spleen metabolite profiles and pathways, aiming to identify key molecules and biochemical mechanisms that may contribute to its anti-aging effects. *Methods:* Thirty 3-month-old mice were divided into a young control group (n=10). The remaining 20 mice were aged to 6 months and then randomly assigned to a natural aging model group (n=10) or a Si Jun Zi Tang treatment group (n=10). Spleen tissues were analyzed using ultra-high-performance liquid chromatography coupled with high-definition quadrupole time-of-flight mass spectrometry. Data were analyzed with multivariate statistical analysis. *Results:* The spleen metabolic profiles differed significantly among the three groups. Multivariate analysis revealed 20 signature metabolites that were significantly altered in naturally aged mice compared to young controls. Of these, 12 were altered following Si Jun Zi Tang treatment. These signature metabolites were enriched in four key metabolic pathways. The treatment demonstrated a beneficial impact on these age-associated metabolites and pathways. *Conclusion:* In conclusion, Si Jun Zi Tang may mitigate age-related metabolic alterations in the spleen, thereby shedding light on the mechanisms underlying its anti-aging effects.

Keywords: Aging; Si Jun Zi Tang; Traditional Chinese Medicine; metabolomics; metabolite; spleen.

Online publication: March 16, 2026

1. Introduction

The global population is rapidly aging, presenting considerable social, familial, and personal challenges largely due to age-related disabilities and diseases. Extensive evidence has established that aging is not only a natural biological process characterized by a progressive decline in physiological functions; it is also an independent risk factor for a wide range of diseases[1-3]. These age-related diseases include Alzheimer's disease, type 2 diabetes and other metabolic disorders, sarcopenia, cardiovascular diseases, and Parkinson's disease[3-5]. Notably, the incidence rates of age-related diseases (i.e., dementia, stroke, coronary artery disease, and heart failure) and physical disabilities increase exponentially with age[4]. With the acceleration of global population aging and the growing burden of age-related diseases, effective strategies for prevention and management have become increasingly urgent. Therefore, there is a pressing need to identify interventions and therapies that can slow the aging process, prevent age-related diseases, and mitigate their onset, ultimately increasing disability-free survival and slowing disease progression and functional decline in individuals.

Traditional Chinese Medicine (TCM), with its profound theoretical framework and thousands of years of practical experience, emphasizes the importance of aligning the body and spirit to achieve a natural lifespan that can potentially

extend to one hundred years [6, 7]. TCM offers a unique perspective on aging, approaching it holistically and dynamically. TCM conceptualizes aging as a "deficiency" that is further complicated by blood stasis and Qi stagnation. Interventions based on TCM are tailored to individual differences in age, season, and constitution, utilizing a variety of formulas and treatment methods. Clinically, tonifying formulas are frequently employed to slow the aging process and alleviate aging-related symptoms, thereby contributing to the goal of prolonging life. Thus, TCM presents a wealth of therapeutic options for addressing the challenges of aging and aging-related diseases. Si Jun Zi Tang, also known as the Four Gentlemen Decoction or the Decoction of Four Noble Drugs, was initially documented in the *Taiping Huimin Hejiju Fang* of the Song Dynasty, approximately 1110 A.D. It is a well-known herbal formula composed of four key ingredients: ginseng, licorice, white atractylodes (Baizhu), and hoelen (Fuling). This herbal formula has long been utilized in TCM as the first among the Qi-tonic prescriptions to replenish Qi and is recognized for its beneficial effects on the spleen, stomach, immune system, and related disorders as well as its anti-aging effects [8-12]. However, the exact underlying mechanisms through which Si Jun Zi Tang exerts its health benefits, particularly regarding anti-aging and aging-related diseases, remain incompletely understood. This is partly owing to the formulation's complex composition, which involves multiple components, targets, and metabolic pathways.

In this study, metabolomics analysis was conducted to assess changes in spleen metabolite profiles and pathways, aiming to identify key molecules and biochemical mechanisms underlying the anti-aging effects of Si Jun Zi Tang. The results of this study will contribute to a better understanding of its potential therapeutic applications in age-related health interventions.

2. Materials and Methods

2.1. Si Jun Zi Tang

The source materials and amounts for Si Jun Zi Tang were as follows: Ginseng (Renshen), 9 g; White atractylodes (Baizhu), 9 g; Hoelen (Fuling), 9 g; Licorice (Gancao), 6 g. (The total weight of the herbal mixture was 33 g). These four herbs were procured from Guo Yi Tang at Heilongjiang University Of Chinese Medicine (Harbin, Heilongjiang, China). The herbal mixture was thoroughly soaked in water. Subsequently, an eight-fold volume of water was added, and the decoction was simmered for 30 min over low heat to extract the liquid. The herbal dregs were then boiled again with another eight-fold volume of water for an additional 30 min to facilitate a second extraction. The liquid extracts from both decoction cycles were combined. The solvent was recovered, and the mixture was concentrated under reduced pressure (80°C, 0.09 MPa) to a final volume of 100 mL. This resulted in a final Si Jun Zi Tang decoction with a crude herb concentration of 0.33 g/mL. A new batch of the decoction was prepared weekly and stored at 4°C for subsequent use.

2.2. Experimental animals and treatments

A total of 30 specific pathogen-free female mice (Institute of Cancer Research) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Shenyang, Liaoning, China) [License No: SCXK(Liao)2010-0001]. The mice were housed under standard laboratory conditions, with a natural light cycle, adequate ventilation, a temperature of 22±2°C, a humidity of 55%±20%, and *ad libitum* access to food and water. At three months of age, the mice were initially divided into two cohorts, with the first cohort used as the young control group (n=10). The remaining 20 mice were housed individually and fed a normal diet until they reached 6 months of age to represent a model of natural aging. They were then randomly and equally divided into two groups: the Si Jun Zi Tang group (n=10), which received treatment with Si Jun Zi Tang at a dosage of 9.75 g/kg body weight per day via oral gavage, and the normal saline (NS) group (n=10), which served as the naturally aged control and was treated with an equivalent volume of NS via the same gavage route. The treatment duration with either Si Jun Zi Tang or NS lasted for 30 days. Upon completion, the mice were sacrificed, and spleen tissues were immediately collected from experimental animals, snap-frozen, and stored at -80°C for subsequent metabolomics analysis of biochemical alterations.

This study was reviewed and approved by the Animal Protection and Utilization Committee of Heilongjiang University Of Chinese Medicine (Harbin, Heilongjiang, China; Approval No. HUCM-2014-08206)). All procedures involving the handling of animals were conducted in strict compliance with ethical guidelines and regulations governing

animal research.

2.3. Ultra-high-performance liquid chromatography coupled with high-definition *quadrupole time-of-flight mass* spectrometry (UPLC-Q-TOF-MS)

Ultra-performance liquid chromatography (UPLC) analysis was conducted using an ACQUITY™ UPLC system (Waters Corporation, Milford, MA, USA) to separate the metabolite compositions in spleen tissues from the three groups of mice (young control group, NS group, and the Si Jun Zi Tang group). The chromatographic separation was optimized in terms of the column type, mobile phase composition, gradient program, and temperature. The details are provided in **Suppl. Table 1**.

Supplementary Table 1. Chromatographic parameters for ultra-performance liquid chromatography (UPLC) analysis

Parameters	
Column	Acquity UPLC™ BEH C18 (2.1 mm × 50 mm, 1.7 μm)
Flow Rate	0.4 ml/min
Column Temperature	40°C
Sample Chamber Temperature	4°C
Mobile Phase A	Acetonitrile with 0.1% (v/v) Formic Acid
Mobile Phase B	Ultrapure Water with 0.1% (v/v) Formic Acid
UPLC mobile phase gradient elution	
Time (min)	0
Flow Rate (ml/min)	0.4
A%	2
B%	98

The eluent from the UPLC system was subsequently directed to a high-definition *quadrupole* time-of-flight mass spectrometer (Q-TOF-MS) for accurate mass detection. High-definition mass spectrometry (HDMS) analysis was performed using an LCT-Premier XE instrument (Waters Corporation, Milford, MA, USA) operating in either positive- or negative-electrospray ionization mode to capture a comprehensive profile of ionic metabolites. The HDMS parameters, such as capillary voltage, cone voltage, and desolvation temperature, are summarized in **Suppl. Table 2**.

Supplementary Table 2. Parameters for high-definition mass spectrometry

Parameters	**Positive Ion Mode (ESI+)**	**Negative Ion Mode (ESI-)**
Ionization Mode	Electrospray Ionization (ESI) Positive	Electrospray Ionization (ESI) Negative
Capillary Voltage (kV)	1.3	1.5
Sample Cone Voltage (V)	60	70
Desolvation Temperature (°C)	350	350
Source Temperature (°C)	110	110
Desolvation Gas Flow (L/h)	750	750
Collision Energy (eV)	60	60
Lock Mass (Leucine-Enkephalin)	$[M+H]^+$ = 556.2771	$[M-H]^-$ = 554.2615

2.4. Data acquisition, bioinformatics, and statistical analysis

Raw mass spectrometric data were acquired using MassLynx V4.1 (Waters Corporation, Milford, MA, USA). Subsequent data processing was conducted with Markerlynx and Progenesis QI 3.0.3 software (Waters Corporation), which transformed the raw instrumental data to form a data matrix through a bioinformatic workflow consisting of three key steps: (1) Peak picking: Identifying and quantifying individual molecular features from complex chromatographic data; (2) Deconvolution: Separating overlapping peaks to ensure the integrity of each signal; and (3) Peak alignment: Correcting for minor retention time shifts across multiple samples.

To mitigate technical variances and enable robust comparative analysis, the resulting data matrix was subjected to normalization and standardization via the EZInfo module within Progenesis QI 3.0.3. Further principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) multivariate statistical analyses were then performed using SIMCA®14.1. software (Sartorius Stedim Data Analytics)**.** PCA was used to provide an overview of the data structure, identify natural clusters, and detect potential outliers, while OPLS-DA was utilized to maximize the separation between predefined groups and identify potential biomarkers for aging or treatment with Si Jun Zi Tang.

The aging-related metabolites identified from the OPLS-DA were cross-referenced with the literature and freely accessible web-based public metabolomic databases, including the Human Metabolome Database (HMDB) and a metabolite mass spectral database (METLIN) to confirm their identities and biological roles. Metabolic pathway enrichment and functional analysis were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetaboAnalyst 5.0 to identify the most relevant pathways enriched with the spleen metabolites.

3. Results

3.1. Quality control and data reliability

Quality control (QC) was performed by analyzing a QC sample after every ten test samples from the spleen tissues. The PCA score plots of the QC samples demonstrated tight clustering in both positive- and negative-ionization modes, indicating high instrument stability throughout the data acquisition process. In contrast, the PCA score plots of the test samples labeled as “Other” from the spleen exhibited a more dispersed distribution in both the positive- (**Figure 1**) and negative-ionization (**Figure 2**) modes, reflecting the expected biological variability between the young control group (aged 3 months) and the naturally aged group (aged 6 months). The clear separation between the tightly clustered QC samples and the dispersed test samples confirmed that analytical variability was minimized, thereby ensuring the reliability of the metabolic profiles acquired in this study.

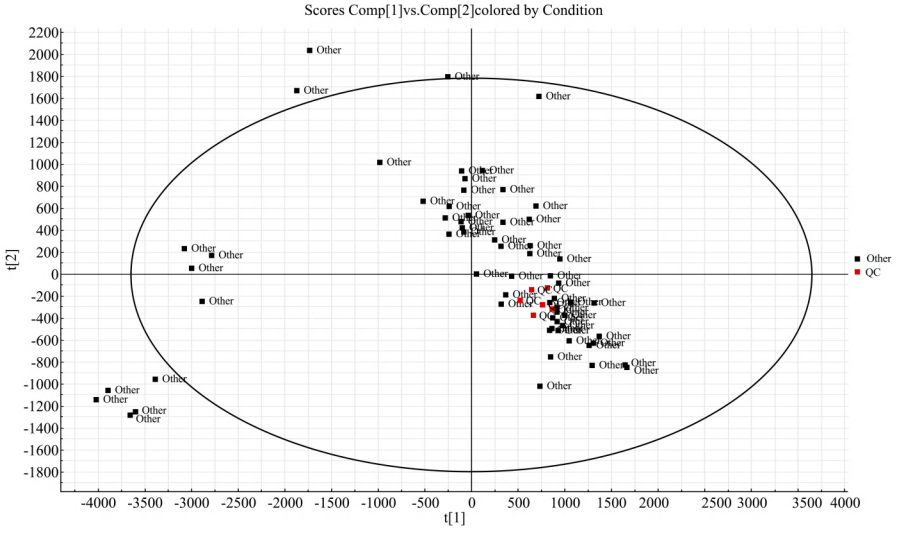

Figure 1. Principal component analysis (PCA) score plot of spleen metabolic data acquired in positive-ion mode. The PCA score plot was generated to compare the young control group (aged 3 months) with the naturally aged group (aged 6 months).

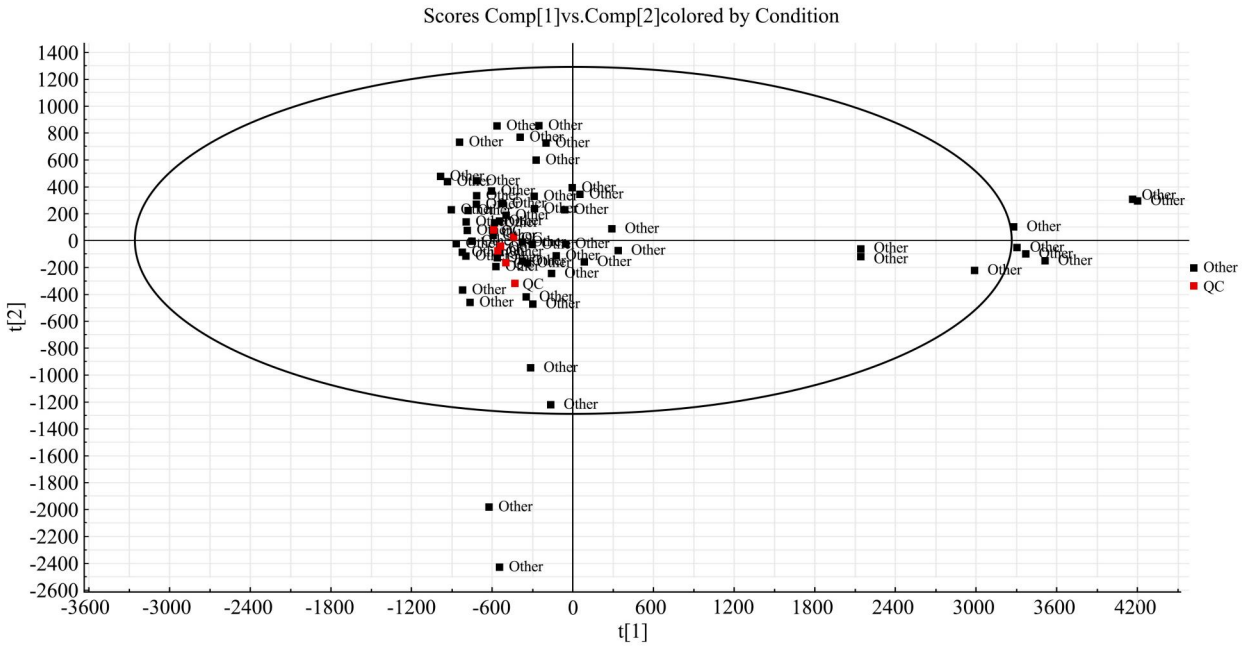

Figure 2. PCA score plot of spleen metabolic data acquired in negative-ion mode. The PCA score plot was created to compare the young control group (aged 3 months) and the naturally aged group (aged 6 months).

3.2. Base peak intensity chromatographic profiles revealed global age-associated changes in the spleen metabolome

The global metabolic profiles of spleen tissues from the young control group (aged 3 months) and the naturally aged group (aged 6 months) were analyzed using UPLC-HDMS in both positive- and negative-ion modes. Analysis of the resulting base peak intensity chromatograms revealed apparent differences between the two groups. Specifically, the chromatograms obtained from the aged mice **(Figures 3&4)** exhibited distinct alterations in the peak patterns and intensities compared to those of the young controls, indicating a comprehensive reorganization of the spleen metabolome associated with natural aging. These findings suggest global changes in the composition and relative abundance of specific metabolites between the young and aged mice.

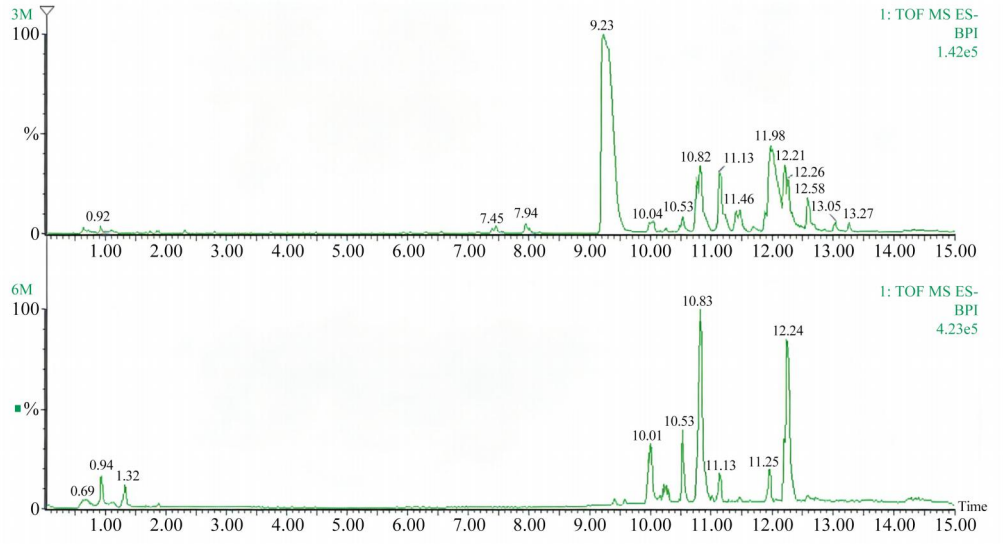

Figure 3. Chromatogram of spleen tissue samples in positive-ion mode. The UPLC-HDMS base peak intensity chromatogram was presented in positive-ion mode (upper panel: young control group, aged 3 months; lower panel, naturally aged group, aged 6 months). UPLC, ultra-high-performance liquid chromatography; HDMS, high-definition mass spectrometry.

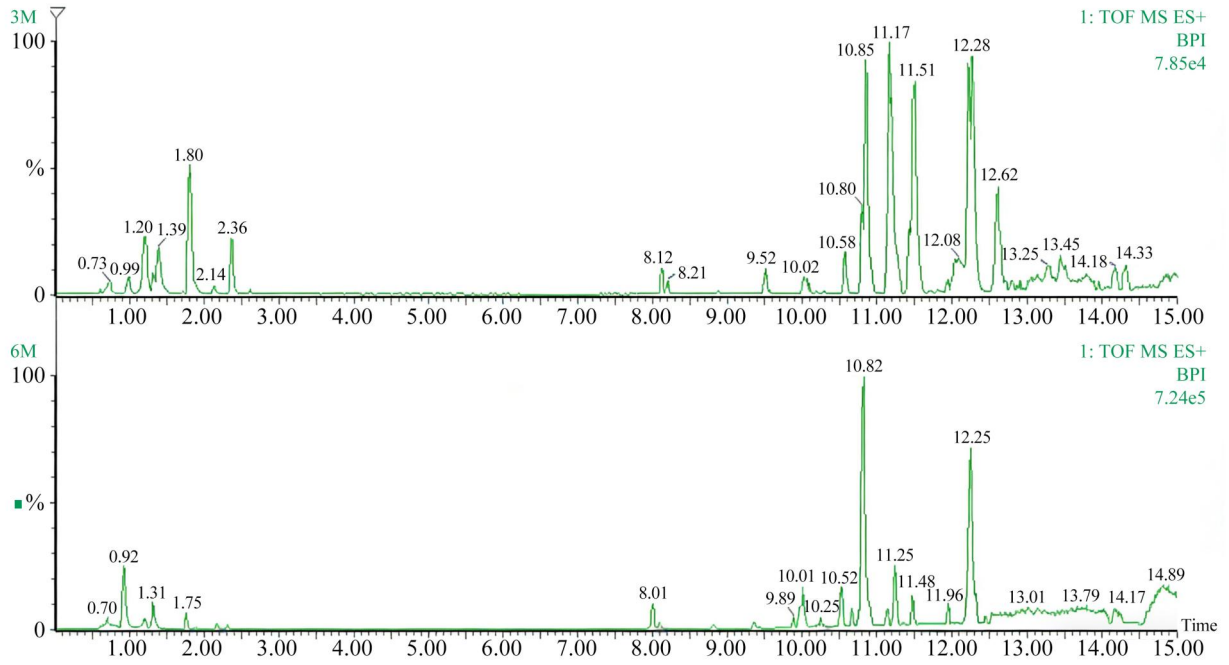

Figure 4. Chromatogram of spleen tissue samples in negative-ion mode. The UPLC-HDMS base peak intensity chromatogram was illustrated in negative-ion mode, with the upper panel representing the young control group (aged 3 months) and the lower panel representing naturally aged group (aged 6 months). UPLC, ultra-high-performance liquid chromatography; HDMS, high-definition mass spectrometry.

3.3. Prioritization and selection of spleen metabolites contributing to aging-related disparities using OPLS-DA S-plots and importance in projection (VIP) plots

To filter and prioritize the spleen metabolites contributing to aging-associated metabolic disparities, we conducted a comprehensive analysis of data acquired by UPLC-HDMS from the young control group (aged 3 months) and the naturally aged group (aged 6 months) using the robust multivariate statistical method, OPLS-DA, which is widely used to enhance the separation of groups and to minimize the influence of noise in the data. The OPLS-DA model generated both an S-plot and a VIP plot. Two criteria, a *P*-value <0.05 and a VIP score >1.0 were applied, and metabolites fulfilling these criteria were considered as potential biomarkers of aging. The results provide insight into the metabolic alterations associated with the aging process. Notably, in the OPLS-DA S-plots (**Figures 5&6**), metabolites were distributed in a characteristic "S" shape. Points located farther from the origin toward the ends of the "S," including the upper right corner (potential upregulated markers) and the bottom-left corner (potential downregulated markers). This spatial distribution represented metabolites with a higher combined measure of covariance and correlation, thereby indicating a greater contribution and high reliability for discriminating between the young control group (aged 3 months) and the aged group (aged 6 months). In parallel, the OPLS-DA VIP plots (**Figures 7&8**) presented a "V"-like distribution of metabolites, where those positioned higher along the arms of the "V" exhibited higher VIP scores, denoting their greater significance in the OPLS-DA model for distinguishing between the young control group and the naturally aged group.

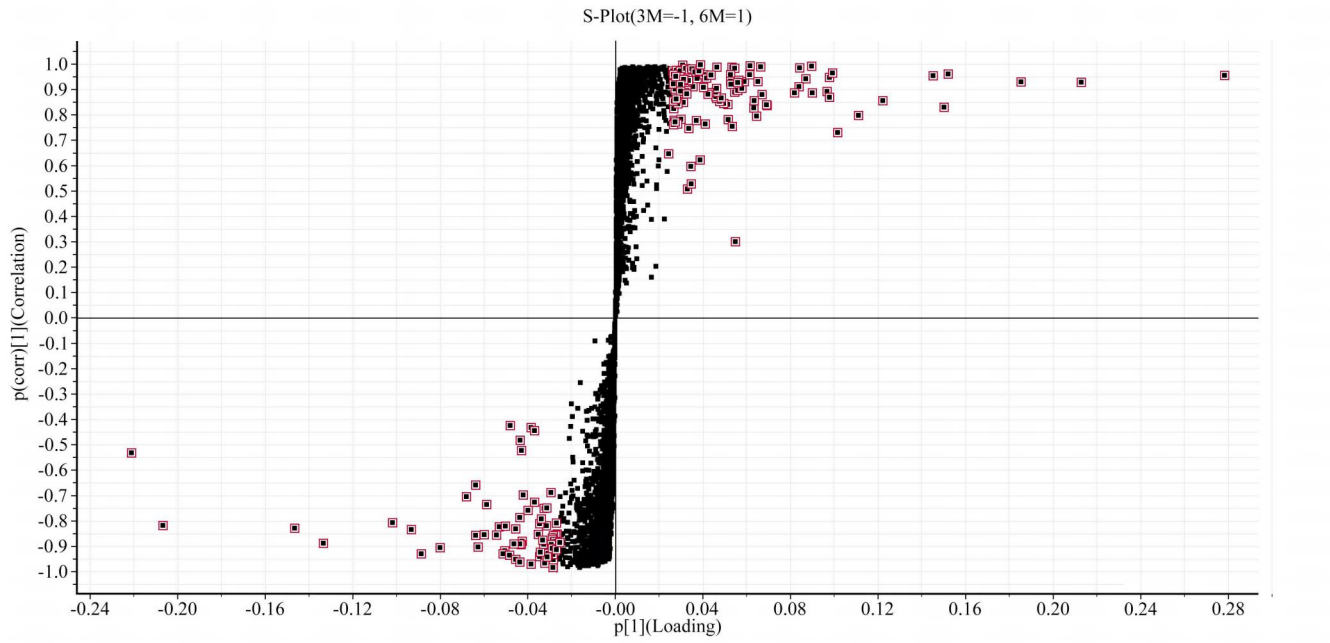

Figure 5. Orthogonal partial least squares discriminant analysis (OPLS-DA) S-plot of spleen metabolomic profiles in positive-ion mode. Spleen metabolomic profiles were analyzed and compared between the young control group (aged 3 months) and the naturally aged group (aged 6 months) using OPLS-DA. The S-plot, created for positive-ion mode, illustrated data points farther from the origin at the ends of the "S" curve, representing metabolites with a higher combined measure of covariance and correlation.

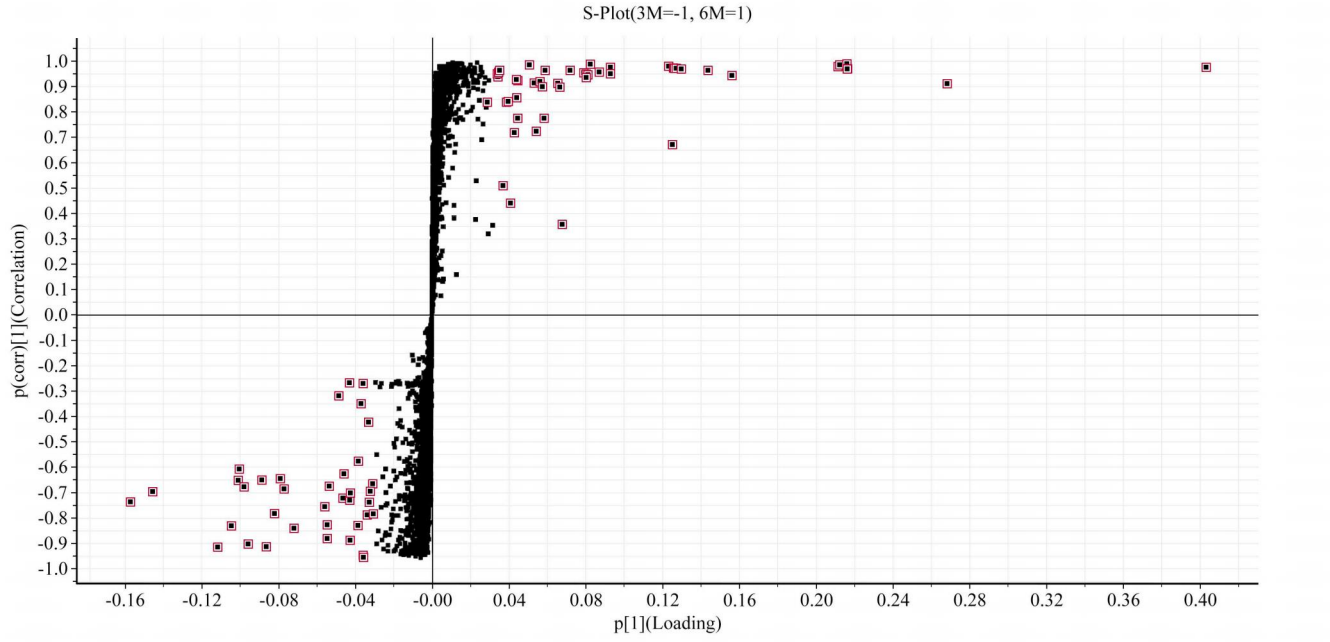

Figure 6. OPLS-DA S-plot of spleen metabolomic profiles in negative-ion mode. The S-plot was generated for negative-ion mode. Data points farther from the origin at the ends of the "S" curve represented metabolites with a higher combined measure of covariance and correlation, indicating a stronger contribution to the discrimination between the young control group (aged 3 months) and the aged group (aged 6 months). OPLS-DA, orthogonal partial least squares discriminant analysis.

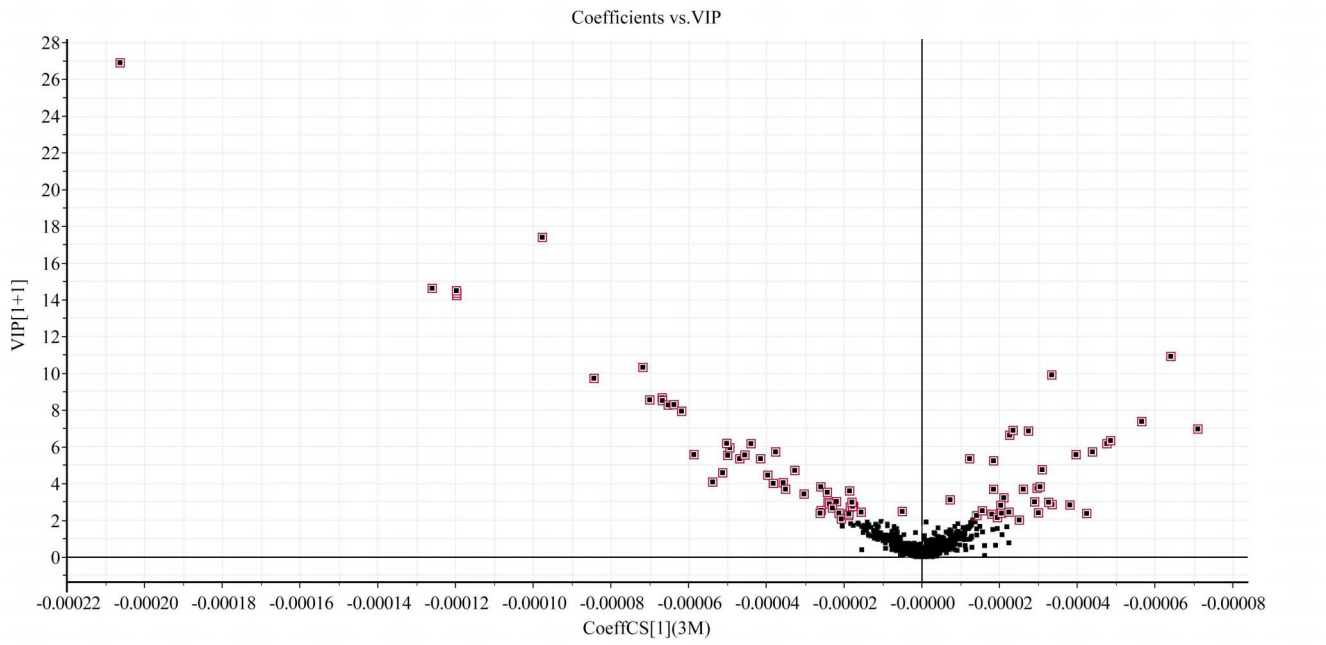

Figure 7. OPLS-DA VIP plot of spleen metabolomic profiles in positive-ion mode. Spleen metabolomic profiles in positive-ion mode from the young control group (aged 3 months) and the naturally aged group (aged 6 months) were analyzed using OPLS-DA, and the corresponding VIP plot was generated. Metabolites were arranged in a "V"-shaped distribution, where those higher on the arms had greater VIP scores, indicating stronger contributions to discrimination in the OPLS-DA model for distinguishing between the young control and naturally aged groups. OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection.

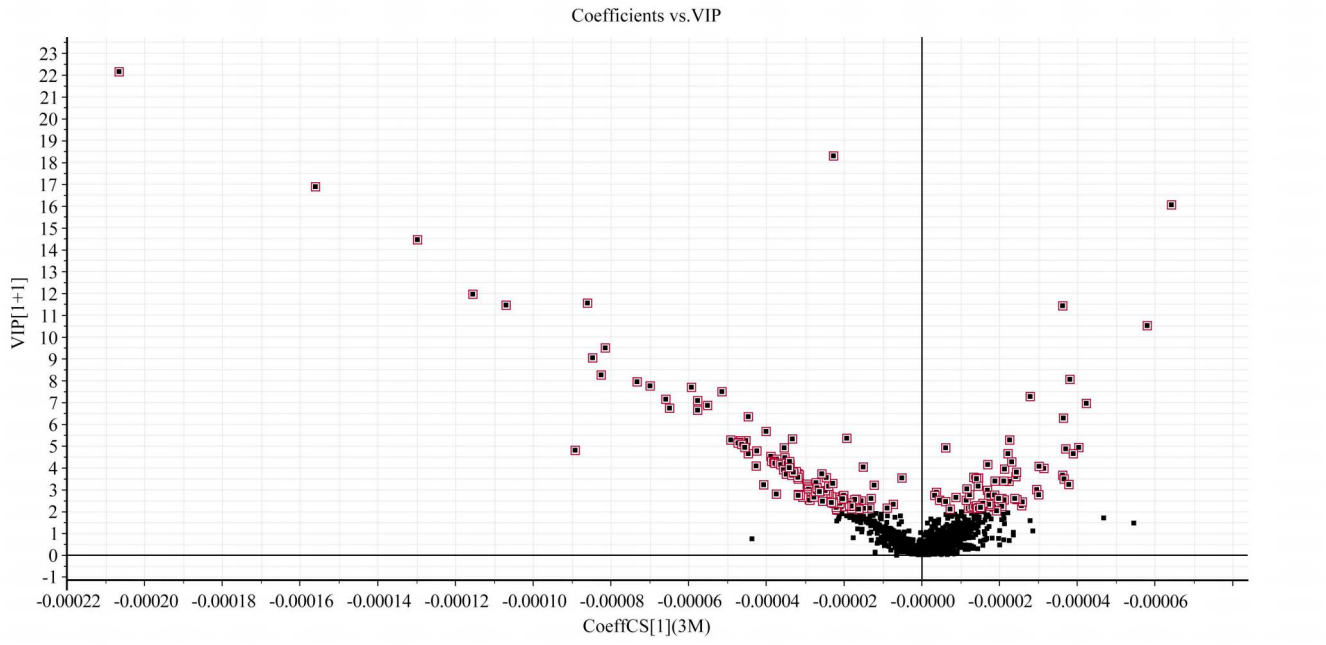

Figure 8. OPLS-DA VIP plot of spleen metabolomic profiles in negative-ion mode. Spleen metabolomic profiles in negative-ion mode from the young control group (aged 3 months) and the naturally aged group (aged 6 months) were analyzed using OPLS-DA, and the VIP plot was created. Metabolites were arranged in a "V"-shaped distribution, with those higher on the arms exhibiting greater VIP scores, indicating stronger contributions to discrimination in the OPLS-DA model for distinguishing between the young control and naturally aged groups. OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection.

3.4. Identification of signature spleen metabolites associated with natural aging in mice and their modulation by Si Jun Zi Tang

Following the application of the OPLS-DA model, which effectively distinguished the spleen metabolomic profiles of young and aged female mice, potential aging-related signature metabolites were selected. In the OPLS-DA VIP plot, metabolites with a VIP score > 2 were chosen and ranked according to their overall contribution to the separation between the young and aged groups, while the S-plot identified metabolites with a high confidence score and a significant magnitude of change. Metabolites identified from both the OPLS-DA S-plot and the VIP plot were integrated and cross-referenced with literature reports and metabolomic databases, including HMDB and METLIN, to confirm the identities and biological roles of these metabolites. This integration workflow resulted in the identification of 20 signature metabolites that were significantly altered in the spleen tissue of naturally aged female mice compared to that of young control mice (**Table 1**). These metabolites included key compounds involved in antioxidant defense (e.g., glutathione), fatty acid metabolism (e.g., L-palmitoylcarnitine, elaidic carnitine), and amino acid pathways (e.g., L-phenylalanine, L-tyrosine), constituting a core set of potential biomarkers for natural aging on the mouse spleen.

Among the 20 signature metabolites associated with natural aging in mice, 12 were found to be altered by Si Jun Zi Tang (**Table 1**). Specifically, metabolites such as glutathione, L-palmitoylcarnitine, and ubiquinol 8, which increased with aging, were regulated by Si Jun Zi Tang. This finding suggests that this TCM herbal decoction may enhance the antioxidant defense system and improve energy metabolism in aging mice. Additionally, metabolites like L-phenylalanine, L-tyrosine, and glycocholic acid showed decreased levels with aging, but Si Jun Zi Tang positively regulated these metabolites as well. This result indicates a potential rejuvenating effect of this TCM on these metabolic pathways. Collectively, Si Jun Zi Tang demonstrated a beneficial impact on several key metabolites associated with aging, suggesting its potential role in mitigating age-related metabolic changes in the spleens of mice.

Table 1. Signature metabolites associated with natural aging and their regulation by *Si Jun Zi Tang* in mice

NO.	HMBD ID	Biomarker	Formula	Rtime	VIP	Ion mode	Alterations in aging	Regulation by Si Jun Zi Tang
1	HMDB00125	Glutathione	C10H17N3O6S	0.9178	8.2467	ESI+	↑	√
2	HMDB00222	L-Palmitoylcarnitine	C23H45NO4	11.2473	6.14961	ESI+	↑	√
3	HMDB03040	Arabinosylhypoxanthine	C10H12N4O5	1.3119	5.5533	ESI+	↑	√
4	HMDB00201	L-Acetylcarnitine	C9H17NO4	0.9178	5.53221	ESI+	↑	-
5	HMDB06464	Elaidic carnitine	C25H47NO4	11.4817	5.33484	ESI-	↑	√
6	HMDB00848	Stearoyl carnitine	C25H49NO4	12.3356	4.5576	ESI-	↑	√
7	HMDB01060	Ubiquinol 8	C49H78O4	13.9547	3.6846	ESI-	↑	√
8	HMDB11617	Adenosine 2'-phosphate	C10H14N5O7P	0.6816	2.95845	ESI-	↑	-
9	HMDB00157	Hypoxanthine	C5H4N4O	0.9032	2.37686	ESI-	↑	√
10	HMDB31403	Cyclohexaneacetic acid	C8H14O2	0.6816	2.02449	ESI-	↑	√
11	HMDB00159	L-Phenylalanine	C9H11NO2	1.7403	7.3553	ESI-	↓	√
12	HMDB00158	L-Tyrosine	C9H11NO3	1.1536	6.31796	ESI-	↓	√
13	HMDB00138	Glycocholic acid	C26H43NO6	12.5706	5.33877	ESI+	↓	√
14	HMDB30396	Tryptophan	C11H12N2O2	2.3168	4.73355	ESI+	↓	√
15	HMDB15122	Cytarabine	C9H13N3O5	0.6816	3.6364	ESI+	↓	√
16	HMDB00944	Behenic acid	C22H44O2	12.058	2.9591	ESI+	↓	√
17	HMDB04256	7-Hydroxy-6-methyl-8-ribityl lumazine	C12H16N4O7	8.6863	2.40654	ESI-	↓	√
18	HMDB03411	D-Proline	C5H9NO2	0.6816	2.37234	ESI-	↓	√
19	HMDB01999	Eicosapentaenoic acid	C20H30O2	10.853	2.30617	ESI-	↓	√
20	HMDB11653	17-alpha,20-alpha-Dihydroxypregn-4-en-3-one	C21H32O3	8.0265	2.19773	ESI-	↓	-

ESI, electrospray ionization.

3.5. Pathway enrichment analysis of age-related signature metabolites and their regulation by Si Jun Zi Tang in mice

Metabolic pathway enrichment and functional analysis, performed using KEGG and MetaboAnalyst 5.0, identified the most biologically relevant pathways enriched with differential metabolites. Pathways with an impact value >0.05 were considered biologically significant. As shown in **Figure 9**, the 20 signature metabolites from mouse spleen tissue were enriched in four key pathways: 1) biosynthesis of phenylalanine, tyrosine, and tryptophan; 2) phenylalanine metabolism; 3) glutathione metabolism; and 4) tyrosine metabolism. These results suggest that Si Jun Zi Tang may mitigate age-related metabolic changes in mice by potentially targeting these pathways.

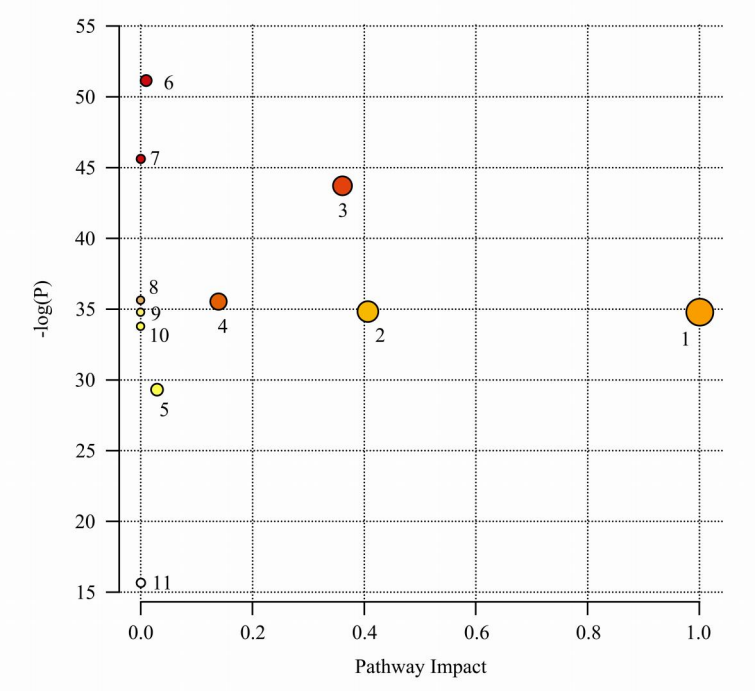

Figure 9. Scatter plot of metabolic pathways in mouse spleen tissue. Metabolic pathway analysis was performed using MetaboAnalyst 5.0, and a scatter plot was created to illustrate the most relevant pathways enriched with differential metabolites. Pathways exceeding an impact value threshold of 0.05 were considered biologically relevant.

4. Discussion

This study utilized metabolomics technology and multivariate statistical analyses to analyze changes in metabolites of spleen tissues across three groups of mice: a young (3-month-old) control group, a naturally aging (6-month-old) model group, and a Si Jun Zi Tang treatment group. A total of 20 age-related metabolic markers were identified, with 12 markers that specifically responded to treatment with Si Jun Zi Tang, of which 10 metabolites showed statistical significance. Further analysis of metabolic pathway enrichment revealed that these metabolic markers were primarily involved in four key metabolic pathways in the spleen: the biosynthesis of phenylalanine, tyrosine, and tryptophan; phenylalanine metabolism; glutathione metabolism; and tyrosine metabolism. Through systematic metabolomics and multivariate statistical analyses, these findings suggest that Si Jun Zi Tang may exert its anti-aging effects in mice by targeting these metabolic pathways, thereby providing scientific evidence for the relationship between syndromes and their corresponding prescriptions in TCM.

While often overlooked in research on aging, the spleen plays a pivotal role in aging and age-related diseases. As a key component of the lymphatic system, the spleen produces and regulates lymphocytes, which are essential cells for mounting adaptive immune responses against various diseases, including those prevalent in older individuals [13-15]. With

age, the immune system typically experiences a functional decline, known as immunosenescence, leading to increased susceptibility to infections, chronic inflammation (or "inflammaging"), and chronic diseases [16]. The ability of the spleen to maintain the pool of and regulate immune cells becomes vital in countering this decline. By promoting effective immune surveillance and response, the spleen can help mitigate chronic, low-grade inflammation, an underlying factor in numerous age-related conditions and diseases, such as cardiovascular disease, type 2 diabetes, and neurodegenerative disorders like Alzheimer's disease. Emerging biomedical research suggests that improving and supporting spleen function through lifestyle interventions, such as an anti-inflammatory diet and regular exercise, may bolster immune resilience and potentially slow the aging process [17]. This perspective aligns with the theory of TCM, which views the spleen as the "foundation of postnatal life," playing a pivotal role in generating Qi (defensive energy) and blood, as well as in digestion and nutrient absorption [18]. A well-functioning spleen is believed to enhance Qi, defensive energy, and immunity, thereby improving the body's intrinsic resistance to diseases, including those related to aging, and promoting longevity. Furthermore, the proper function of the spleen is intricately linked to the prevention of aging and age-related disorders, as it governs the transformation of food into energy that nourishes and supports other organ systems [19, 20]. Additionally, modern medical science has revealed that the spleen acts as a reservoir for inflammatory myeloid cells [21]. During acute stress, these cells can be mobilized into circulation, exacerbating systemic inflammation and potentially contributing to remote organ damage, including cognitive deficits [21]. Thus, investigating how TCM herbs and formulations modulate the metabolic pathways in the spleen is important for elucidating the mechanistic basis for their anti-aging effects. This research may potentially bridge ancient wisdom with contemporary biomedicine to develop novel strategies for healthier aging, especially in the era of rapid global aging.

It is worth noting that our metabolomics study of spleen metabolites in naturally aging mice, treated with or without the TCM decoction Si Jun Zi Tang, identified four key metabolic pathways in response to the Si Jun Zi Tang intervention. Among these, the glutathione metabolism pathway is noteworthy as it is a critical mechanism for cellular protection. This pathway plays a pivotal role in eliminating reactive oxygen species and enhancing the activity of antioxidant enzymes, thus maintaining redox homeostasis [22, 23]. Extensive research has shown that glutathione inhibits the pathogenesis of major age-related neurodegenerative disorders, including Parkinson's and Alzheimer's diseases [24-27]. The regulation of glutathione under oxidative stress involves a complex cycle of depletion and synthesis. In response to cellular damage, glutathione is degraded by gamma-glutamyl transferase as a protective measure [23]. To counteract this loss and maintain homeostasis, the body synthesizes glutathione *de novo* [23]. However, under sustained oxidative stress, this synthesis may be insufficient, leading to a decline in intracellular glutathione levels. Consequently, a critical dependency on exogenous glutathione or its precursors arises to mitigate ongoing cellular damage. A primary route for replenishing glutathione is through the precursor L-cystine, which is converted into cysteine and is subsequently incorporated into newly synthesized glutathione [23]. As glutathione is consumed, the levels of feedback inhibitors that regulate its synthesis decrease, facilitating upregulation of the biosynthetic pathway and enhancing the conversion of L-cystine into glutathione. Based on these findings, along with the present study, we propose that Si Jun Zi Tang may modulate the glutathione metabolism pathway, potentially increasing the bioavailability of precursors or the activity of synthesizing enzymes, thereby boosting cellular glutathione content and strengthening the antioxidant defense system against degenerative processes.

Nevertheless, this study has several limitations that should be acknowledged. As a metabolomics study, we identified 20 potential age-related markers, of which 10 were significantly influenced by treatment with Si Jun Zi decoction in mice. In addition, we predicted the enriched metabolic pathways through bioinformatics analysis. However, it is important to note that these findings have yet to undergo validation. Given the intriguing results of this pilot study, our research team is planning further studies aimed at verification and elucidation of the underlying mechanisms. Additionally, the current data were generated in a naturally aging mouse model; thus, validation in additional animal studies and in *in-vitro* cell culture models is necessary to determine whether this herbal formulation can prevent age-related diseases and promote healthy aging in humans.

5. Conclusion

In conclusion, the metabolomics and bioinformatics analyses of spleen metabolites and the enriched pathways in naturally aging mice revealed that the TCM formulation Si Jun Zi Tang significantly affects key age-related spleen metabolites and pathways. These findings indicate that Si Jun Zi Tang may exert a beneficial influence on splenic metabolism, potentially counteracting the metabolic dysregulation associated with aging. This study provides a mechanistic basis for the TCM formula's anti-aging effects, which may help further elucidate the mechanisms behind these effects and potentially bridge TCM wisdom with contemporary biomedicine in developing novel strategies for healthier aging, especially in the context of rapid global population aging.

List of Abbreviations

normal saline (NS)
Human Metabolome Database (HMDB)
Kyoto Encyclopedia of Genes and Genomes (KEGG)
orthogonal partial least squares discriminant analysis (OPLS-DA)
principal component analysis (PCA)
quadrupole time-of-flight mass spectrometer (Q-TOF-MS)
Quality control (QC)
Traditional Chinese Medicine (TCM)
Ultra-performance liquid chromatography (UPLC)

Disclosure statement

The author declares no conflict of interest.

References

[1] López-Otín C, Blasco MA, Partridge L, et al., 2013, The hallmarks of aging. Cell. 153: 1194-217.

[2] Dey AB, 2017, World report on aging and health. Indian J Med Res. 145: 150–1.

[3] Li Q, Xiao N, Zhang H, et al., 2025, Systemic aging and aging-related diseases. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 39: e70430.

[4] Kritchevsky SB, Cummings SR, 2025, Geroscience: A Translational Review. Jama. 334: 1094-102.

[5] Guo J, Huang X, Dou L, et al., 2022, Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. Signal transduction and targeted therapy. 7: 391.

[6] Greten HJ, Understanding TCM, 2013, The Fundamentals of Chinese Medicine. Volume Part I Heidelberg School of Chinese Medicine; Heidelberg, Germany.

[7] Matos LC, Machado JP, Monteiro FJ, et al., 2021, Understanding Traditional Chinese Medicine Therapeutics: An Overview of the Basics and Clinical Applications. Healthcare (Basel, Switzerland). 9(3): 257.

[8] Yang S, Li J (transl), 1993, Li Dongyuan's Treatise on the Spleen and Stomach. Boulder: Blue Poppy Press.

[9] Jin H, Cai C, Li B, et al., 2019, Modified Si-Jun-Zi-Tang Attenuates Airway Inflammation in a Murine Model of Chronic Asthma by Inhibiting Teff Cells via the mTORC1 Pathway. Front Pharmacol. 10: 161.

[10] Gao X, Zhang X, Ge XZ, et al., 2020, Jiawei Sijunzi decoction delays aging of mouse spleen tissue through mTOR signaling pathway. Acad J Sec Mil Med Univ. 41(12):1410-1413.

[11] Liang H, Liang E, Li Q, et al., 2021, Qi Y, Yan W and Zeguang L. Analysis of the effect of Sijunzi decoction on naturally grown 16-month-old mice based on proteomics. http://kns.cnki.net/kcms/detail/21.1546.r.20211009 .2317.137.html *Chinese Journal of Traditional Chinese Medicine*. 2021: 1–14.

[12] Yuan Y, Zhang Y, Zheng R, et al., 2022, Elucidating the anti-aging mechanism of Si Jun Zi Tang by integrating network pharmacology and experimental validation in vivo. Aging (Albany NY). 14(9): 3941-3955.

[13] Alexandre YO, Mueller SN, 2023, Splenic stromal niches in homeostasis and immunity. *Nature reviews Immunology*. 23: 705-19.

[14] Groenen AG, Lipscomb M, Bossardi Ramos R, et al., 2024, Resolvin D1 suppresses macrophage senescence and splenic fibrosis in aged mice. *Prostaglandins, leukotrienes, and essential fatty acids*. 202: 102634.

[15] Long B, Koyfman A, Gottlieb M, 2021, Complications in the adult asplenic patient: A review for the emergency clinician. *The American journal of emergency medicine*. 2021; 44: 452-7.

[16] Liu Z, Liang Q, Ren Y, et al., 2023, Immunosenescence: molecular mechanisms and diseases. *Signal transduction and targeted therapy*. 8: 200.

[17] Zhou H, Han X, Huang C, et al., 2024, Exercise-induced adaptive response of different immune organs during ageing. *Ageing research reviews*. 102: 102573.

[18] Tang HJ, Jin HJ, Zhao CY, et al., 2024, Spleen and Aging: Potential Mechanisms and Applications of Traditional Chinese Medicine Interventions. *Frontiers in Medical Science Research*. 6: 44-50.

[19] Yu YH, Yu YS, Cheng XH, 2023, Clinical effects of a traditional Chinese medicine nursing programme to intervene in gastric pain of the spleen and stomach with Qi deficiency. *World journal of clinical cases*. 11: 5056-62.

[20] Wang H, Wang Y, 2024, What Makes the Gut-Lung Axis Working? From the Perspective of Microbiota and Traditional Chinese Medicine. *The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale*. 2024: 8640014.

[21] Xu D, Zhang Y, Xie B, et al., 2020, The spleen mediates chronic sleep restriction-mediated enhancement of LPS-induced neuroinflammation, cognitive deficits, and anxiety-like behavior. *Aging*. 12: 15446-61.

[22] Averill-Bates DA, 2023, The antioxidant glutathione. *Vitamins and hormones*. 121: 109-41.

[23] Gasmi A, Nasreen A, Lenchyk L, et al., 2024, An Update on Glutathione's Biosynthesis, Metabolism, Functions, and Medicinal Purposes. *Current medicinal chemistry*. 31: 4579-601.

[24] Conrad M, Schick J, Angeli JP, 2013, Glutathione and thioredoxin dependent systems in neurodegenerative disease: what can be learned from reverse genetics in mice. *Neurochemistry international*. 62: 738-49.

[25] Meldrum BS, 2000, Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *The Journal of nutrition*. 130: 1007s-15s.

[26] Singh A, Kukreti R, Saso L, et al., 2019, Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. *Molecules (Basel, Switzerland),* 24.

[27] Iskusnykh IY, Zakharova AA, Pathak D, 2022, Glutathione in Brain Disorders and Aging. *Molecules (Basel, Switzerland)*, 27.