Effects of *Codonopsis pilosula* water extract on MicroRNA expression profile in D-galactose-induced senile mice

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Abstract: This paper aims to observe and analyze effects of *Codonopsis pilosula* water extract on micro RNA (miRNA) expression profile in liver tissue of senile mice. The 110 Konminmice were randomly divided into five groups, including D-galactose-induced senile model group, normal control group, and low, middle and high dose intervention groups. Continuous modeling lasted 40 days. General symptoms and changes of body mass of the model mice were monitored and observed. The levels of serum glutamic pyruvic transaminase (ALT) and alkaline phosphatase (ALP) of mice were compared, and miRNA of differential expression during aging of D-galactose-induction and high-dose *Codonopsis pilosula* intervention was analyzed. The serum ALT and ALP levels in the aging model group were significantly higher than those in the normal control group ($P<0.05$). The serum ALT and ALP levels of *Codonopsis pilosula* intervention group were lower than those of aging model group, and decrease in ALP value of high dose intervention group was higher ($P<0.05$). The expression profile of miRNA in the aging model group was significantly different from that in normal control group and high-dose *Codonopsis pilosula* intervention group, and miRNA expression profile in high-dose *Codonopsis pilosula* intervention group was clustered with that in the normal control group. The differentially expressed miRNAs of D-galactose-induced senescence and *Codonopsis pilosula* anti-aging usually belong to 7 miRNA clusters. The target gene function of the differentially expressed miRNAs during senescence process was enriched in 29 signal pathways. There were 67 regulatory signal pathways in differentially expressed miRNA target genes during *Codonopsis pilosula* intervention. The effect of miRNA targeting may play an important role during D-galactose-induced senescence and *Codonopsis pilosula* anti-aging period.

Keywords: *Codonopsis pilosula*, D-galactose induced senile mice, Hepatic microRNA expression profile, effect, observation and analysis.

INTRODUCTION

The human body is based on the five internal organs as the center. In the aging period, the main manifestation is hypofunction of the five internal organs. Among them, the liver is mainly responsible for hematopoietic function, which has direct relation with aging. A large number of clinical studies show that the process of aging is quite complex and involves multiple biological processes. In recent years, with the improvement of people's ideological consciousness, people gradually lay more emphasis on longevity and health in Chinese medicine, providing effective intervention for aging.

MicroRNA (miRNA) is non-coding RNA about 20nt-25nt long. Through post-transcriptional inhibition of the target mRNA, it can play an important role in vital movement (development, tumor or aging, etc) after effective regulation (Li G, 2015). *Codonopsis pilosula* (as shown in fig. 1) is an authentic herb in Gansu. With sweet flavor and mild nature, it can strengthen the middle warmer and benefiting vital energy. Research shows that anti-aging effect of *Codonopsis pilosula* is widely recognized.

In this study, biochip and bioinformatics tools were used to analyze the effects of *Codonopsis pilosula* water extracts on miRNAs expression profile of liver tissues of D-galactose-induced senile mice. Moreover, target gene function of miRNA was observed and explored, and relevant anti-aging mechanisms of *Codonopsis pilosula* was figured out.

MATERIALS AND METHODS

General information

The 110 Kunming mice of SPF grade were selected as the subjects. These mice were aged at 2 months, including 55 female mice and 55 male mice with average body weight of (18.2±3.5) g. All mice were divided into normal control group, aging model group, and low, middle and high dose *Codonopsis pilosula* intervention groups. Each group contained 22 mice (11 male and 11 female) (Ofori-Kwakye, 2016).

Methods

Identified white *Codonopsis pilosula* was used in this experiment include. Take 100g of white *Codonopsis pilosula* as material and boil them twice, combine and filter the two extracting solutions, condense them into

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0.5g•mL-1 water decoction, then place them in the refrigerator (4°C) for standby application. Preparation of water decoction was conducted once every 5 days (Wang *et al*., 2016); prepare 12g • L-1 water decoction using D-galactose (shown in fig. 2, produced by Sigma, the United States) and normal saline. Moreover, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) test kit (Shanghai Mlbio Diagnostic Technology Co., Ltd.) were used (Wroblewska K, 2015).

Applied instrument and equipment included 7020 automatic biochemical analyzer by Hitachi, Japan, and Gene ChipR Scanner 3000 laser scanner by Affymetrix, the United States (Abdel M, 2016).

The experimental method: for aging model group, *Codonopsis pilosula* low dose intervention group, middle dose intervention group and high dose intervention group, the mice were injected with D-galactose solution (50g• L-1) subcutaneously in the neck and back, with the injection volume of 0.025mL•g-1. The normal control group mice were injected with volume dose of normal saline (Wang J *et al*., 2016); for aging model preparation, body weight loss and metabolic disorders are symbols of modeling success. Through intragastric administration, dose of 15g• kg-1 was administered every morning in mice of the *Codonopsis pilosula* intervention group. The volume saline infusion was applied to the normal control group and the aging model group. Continuous modeling was performed for 40 days (Huang Y *et al*., 2016).

Determination of biochemical parameters: Blood samples were taken from the caudal vein with serum separated at 3000r • min-1 centrifugation for 10 minutes. ALT and ALP were determined by biochemical analyzer (Geng *et al*., 2015).

miRNA microarray detection: Total RNA of mouse liver tissue was extracted with liquid nitrogen and Trizol, and 30424 probes of Affymetrix miRNA 4.0 microarray were detected and analyzed. The chip after hybridization was scanned by Gene ChipR Scanner 3000 laser scanner and the scanned chip images were analyzed by AGCC software. The fluorescence signal values above 1000 were selected through normalization and "dead dot" elimination measures. The differential expression miRNA was Cy5 / Cy3 ≥ 2.0 or ≤0.5 or more (Hazra, 2015).

Bioinformatics analysis: Cluter V3.0 database was used for clustering analysis of microarray data. Analysis of miRNA cluster was carried out using miRBase Release 21 database. Argonaute database was used to analyze target genes of differentially expressed miRNA. CapitalBio biomolecule function annotation system MAS V4.0 was adopted to reflect the significance of the involved signal pathways (Ibrahim, 2016).

DNA damage: implement single cell gel electrophoresis, wash mice liver tissue with PBS buffer solution at 37°, add PBS buffer and collect cell suspension into a 1.5mL Ephendorf tube. After that, adjust cell density into 104-105/mL with PBS, and measure cell viability with trypan blue; single-cell gel electrophoresis was conducted using Singh description method. After finishing above works, observe fluorescent microscope, take pictures, and select tail length, tail moment and Olive tail moment as DNA damage indexes.

Fig. 1: The picture of *Codonopsis pilosula.*

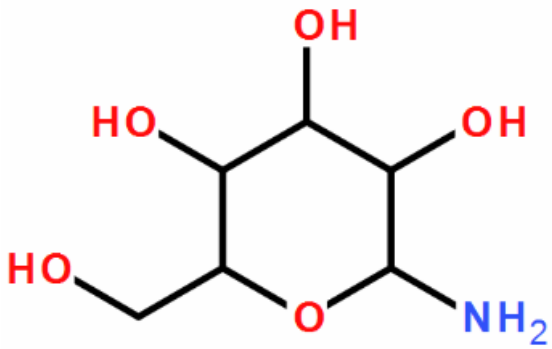

Fig. 2: D-galactose molecular structure.

STATISTICAL METHODS

The Excel software was used to analyze the data involved in the experiment. The measurement data were expressed as ($\overline{X} \pm s$). Single factor analysis of variance was used to compare the differences between groups. Statistical significance was found when the P value was less than 0.05.

RESULTS

Effects of *Codonopsis pilosula* on Morphology and Body Weight of D-galactose-induced Senile Mice: After 4 weeks of modeling, the model group had aging symptoms, there was no significant change in the reference group, *Codonopsis pilosula* intervention group was close to the normal group, and meanwhile the high dose *Codonopsis pilosula* intervention group was more active. As shown in table 1 below, the mean body mass of aging model mice decreased less with time (P <0.05), low dose *Codonopsis pilosula* intervention group had no

significant changes in body mass compared with the aging model mice, while body weight change of high dose *Codonopsis pilosula* intervention group was significantly larger than that of the aging model group (P <0.05).

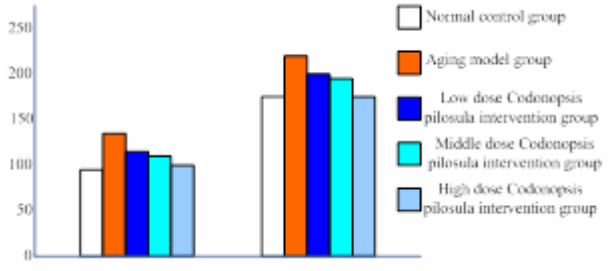

Fig. 3: Comparison of liver function indicators

Fig. 4: Clustering analysis of miRNA expression profiles in liver tissue of normal reference group and aging model group and high dose *Codonopsis pilosula* intervention group

Effect of *Codonopsis pilosula* on liver function of D-galactose induced senile mice: Compared with the normal control group, serum ALT and ALT values of the mice in aging model group were significantly higher (P <0.05).Compared to aging model group, indicators of *Codonopsis pilosula* intervention groups of different concentrations were significantly lower (P<0.05). The ALP reduction in high dose *Codonopsis pilosula* intervention group was close to the reference group, as shown in fig. 3.

Effect of *Codonopsis pilosula* on expression profile miRNA in liver tissues of D-galactose- induced senile mice. During D-galactose-induced senescence, miRNA expression encounters abnormal regulation, while *Codonopsis pilosula* intervention can reverse miRNA abnormal expression (Shown as fig. 4). The microarray results showed that most differentially expressed miRNAs were miRNA gene clusters i.e., gene batteries consisting of two or more genes on the chromosome. The details are shown in table 2 and table 3.

Comparison of DNA damage in hepatocytes. As shown in table 2, by comparing DNA damage of hepatocyte in all groups and the normal reference group, DNA tail length, tail moment and Olive tail moment of hepatocytes in the low, medium and high dose intervention groups were significantly higher (P <0.05).

Signaling Pathway Analysis of *Codonopsis pilosula* Intervening in miRNA Target Gene of Hepatic Tissue of D-galactose-induced Aging Mice. During D-galactose induced aging of mice, differentially expressed miRNAs had 197 confirmed target genes. Among the 70 involved in KEGG signaling pathway, 29 were significantly enriched (P <0.05). During high dose *Codonopsis pilosula* intervened aging, there were 122 differentially expressed miRNA target genes, including 90 signaling pathways involved, and 67 pathways were significantly enriched(P <0.05), as shown in table 3 below.

DISCUSSION

Aging process is a complex and irreversible phenomenon. However, aging can be delayed, which becomes the current medical focus of attention. Micro RNA (miRNA) is a type of non-coding RNA. Ofori-Kwakye K., *et al*. made analysis on the adjustment of the inhibition effect of post transcriptional target mRNA, which is of great significance to human life activities. In this study, with animal experimental model and liver function biochemical detection method, we verified investigate the role of *Codonopsis pilosula* water extracts in anti-aging. Moreover, we explored the relevant mechanisms and analyzed small molecules RNA (i.e. miRNA) which plays an important regulatory role in cells.

The results showed that miRNA expression profile of the aging model group was significantly different from that in the normal control group and high dose *Codonopsis pilosula* intervention group, and miRNA expression profile of the high dose *Codonopsis pilosula* intervention group was clustered with that of the normal control group; differential expression miRNA of D-galactose-induced senescence and anti-senescence of *Codonopsis pilosula* generally belonged to 7 gene clusters. After bioinformatics analysis, the results showed that target gene function of differentially expressed miRNAs in the senescence process was enriched in 29 signal pathways. There were 67 regulatory signal pathways in target genes of differentially expressed miRNA during the *Codonopsis pilosula* intervention. It is reported that expression and function of miRNA gene cluster have synergistic characteristics. The results of this study are consistent with the related reports, and can fully confirm the important significance of *Codonopsis pilosula* water extract in anti-aging process.

Table 1: D-galactose-induced senile mice changes in body weight growth and effect of *Codonopsis pilosula* intervention ($\overline{x}$ ±s)

Group	Normal control group (n=22)	Aging model group (n=22)	Low dose intervention group (n=22)	Middle dose intervention group (n=22)	High dose intervention group (n=22)
Week 1	21.6±1.5	21.8±2.6	22.0±3.1	22.2±2.4	21.9±1.1
Week 2	24.2±2.1	23.1±1.9	23.4±3.2	23.1±1.4	24.8±1.2
Week 3	25.6±2.8	24.5±1.9	24.8±3.1	24.6±1.5	26.3±1.3
Week 4	27.3±2.6	25.4±1.7	25.8±2.3	25.6±1.5	27.8±1.5
Week 5	28.7±2.2	26.3±1.4	26.0±2.9	26.8±2.1	28.6±2.7
Week 6	30.8±1.7	26.8±1.4	27.4±3.1	27.9±2.1	30.2±1.5
Week 7	31.5±2.1	27.6±1.3	28.2±2.9	29.2±2.3	31.1±2.0

Table 2: Comparison of DNA damage of hepatocytes ($\overline{x}$ ± s)

Group	Tail length	Tail moment	Olive tail moment
Normal reference group (n=22)	4.27±0.66	0.85±0.11	0.60±0.04
Aging model group (n=22)	20.56±1.64	7.16±1.51	6.17±0.39
Low dose intervention group (n=22)	16.38±0.94	5.96±0.47	5.94±0.34
Medium dose intervention group (n=22)	12.38±1.24	4.76±0.45	4.12±0.28
High dose intervention group (n=22)	9.94±1.35	2.26±0.34	1.85±0.11

Table 3: Signaling pathway analysis of *Codonopsis pilosula* Intervening in miRNA target gene of hepatic tissue of D-galactose-induced Aging Mice (the first 20)

Signaling Pathway	P Value
Melanoma	2.15287E-22
MAPK signaling pathway	6.48646E-18
Focal adhesion	1.30867E-15
Colorectal cancer	1.64856E-14
Endometrial cancer	2.97439E-14
Cell cycle	1.89766E-12
Prostate cancer	1.50736E-11
Glioma	3.15345E-11
Cytokine-cytokine receptor interaction	4.78430E-11
P53signaling pathway	4.88869E-11
Pancreatic cancer	5.77523E-11
Bladder cancer	3.57348E-10
Toll-like receptor signaling pathway	3.61756E-10
Regulation of actin cytoskeleton	6.69369E-10
Renal cell carcinoma	1.29728E-09
Non-small cell lung cancer	4.46949E-09
Adherens junction	6.79577E-09
Thyroid cancer	8.27420E-09
Fc epsilon RI signaling pathway	9.77237E-09
ErbB signaling pathway	1.42986E-08

CONCLUSION

In summary, differentially expressed miRNA forms associated with D-galactose-induced aging in mice and *Codonopsis pilosula* water extracts were screened by miRNA expression microarrays and bioinformatics tools were used to express signaling pathways that exerted a significant impact on function during the process, thus providing reference for aging and anti-aging treatment with *Codonopsis pilosula.*

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