Pharmacokinetic differences of grape seed procyanidin by gavage administration between Alzheimer's Disease and normal in rats

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ABSTRACT: Background: Grape Seed Procyanidins(GSP) is a kind of natural polyphenols with strong antioxidant capacity. Studies have shown that GSP have a significant effect in the alleviation of Alzheimer's Disease (AD).

Objective: In order to investigate whether there is a pharmacokinetics difference in GSP between normal and AD rats, a rapid UPLC-MS/MS method for detecting the content of it in plasma samples was established, and we analyzed the plasma concentrations of procyanidin B_2, procyanidin B_3, catechin and epicatechin in normal and AD rats over time to determine the plasma concentration of GSP.

Methods: We used 400μL of methanol for the protein precipitation solvent in the plasma treatment. The chromatographic separation was performed on a C_{18} column at 20℃. The mobile phase was a gradient of acid water and methanol containing 0.1% (*v/v*) formic acid, time was 15min.

Results: From the analysis of the experimental results, it can be seen that the plasma concentrations of procyanidin B_2, procyanidin B_3, catechin and epicatechin in AD rats were significantly higher than those in normal rats ($P < 0.05$). And after comparison, it also can be seen that the content of epicatechin was the highest in rats given procyanidin, followed by catechin, procyanidin B_2, procyanidin B_3.

Conclusion: We found that the absorptions of these analytes in AD group were more than in normal group, provides an analytical basis for the treatment of AD with procyanidins.

Key words: Grape seed procyanidins, UPLC-MS/MS, pharmacokinetics, Alzheimer's disease, plasma concentration, gavage administration

1. INTRODUCTION

The main component of grapes is a large seed amount of phenolic compounds. Procyanidins exist in many kinds of plants, but the content of Procyanidins is higher in grape seeds, and cheap and easy to get [1]. Procyanidins include two types, catechin type and procyanidins type. Such as catechin, epicatechin and gallic acid ester are all through the C_4 - C_8 and/or C_4 - C_6 hydroxy flavane-3-alcohol polymer composed of unit is connected [2, 3]. In aspects of anti-arteriosclerosis, anti-mutation, anti-inflammatory, detumescence, cardiovascular protection, anti-cancer and free radical scavenging, procyanidins play an important role. In the protection of human health, procyanidins had a positive effect [4]. As the in-depth study of procyanidins, some researchers found that procyanidins had stronger abilities to anti-oxidation and scavenge free radicals because of containing many electronic hydroxyl portions. And studies have shown *in vivo* experiment that thoes abilities of procyanidins were more strong compared to vitamin C and vitamin E [5].

Alzheimer's Disease (AD) is a central neurodegenerative disease characterized by progressive dementia. Its nosogenesisn is very complex. Besides the classic β-Amyloid (Aβ) and the Tau protein phosphorylation hypothesis, another that due to the oxidative stress in the brain of AD patients [6, 7]. And studies have shown that oxidative stress contributes to the production of Aβ and neuroinflammation, they could increases the permeability of the Blood-Brain Barrier (BBB) leading to the production of Aβ [8, 9]. Some researches have shown that procyanidins can inhibit the production of Aβ, which can reduce the neurotoxicity of Aβ-induced, play a neuroprotective role [10, 11]. Moreover, the combined use of catechin and anthocyanin were more obvious [12]. Especially procyanidins also play an important role in the anti-inflammatory process [13, 14]. Growing research suggests that grapes and wines are a beneficial effect for human health, which also have confirmed procyanidins for chronic diseases, neurodegenerative diseases and cardiovascular diseases oneself have a positive effect [15].

Although, the pharmacological effects of GSP have been well studied currently, there are still few reports on pharmacokinetics of these active compounds between normal and AD rats after oral administration of GSP. In particular, the comparison between procyanidin monomers and dimers was even rare. Comparison of several components of GSP *in vivo* and *in vitro* has also received little attention. Therefore, for its further development and utilization, we obtained the original anthocyanin products after purification of dried grape seeds, determined the purity of procyanidins and the content of four components in vitro, including catechin(A), epicatechin(B), procyanidin B2(C), procyanidin B3(D) **Fig. 1**. In order to investigate the possible pharmacokinetic differences between GSP in control and AD rats, to fill the pharmacokinetic gap of GSP in normal and AD mice.And also compare the *in vitro* content of procyanidins with the utilization *in vivo*, in order to better study the procyanidins to improve the main components of AD.

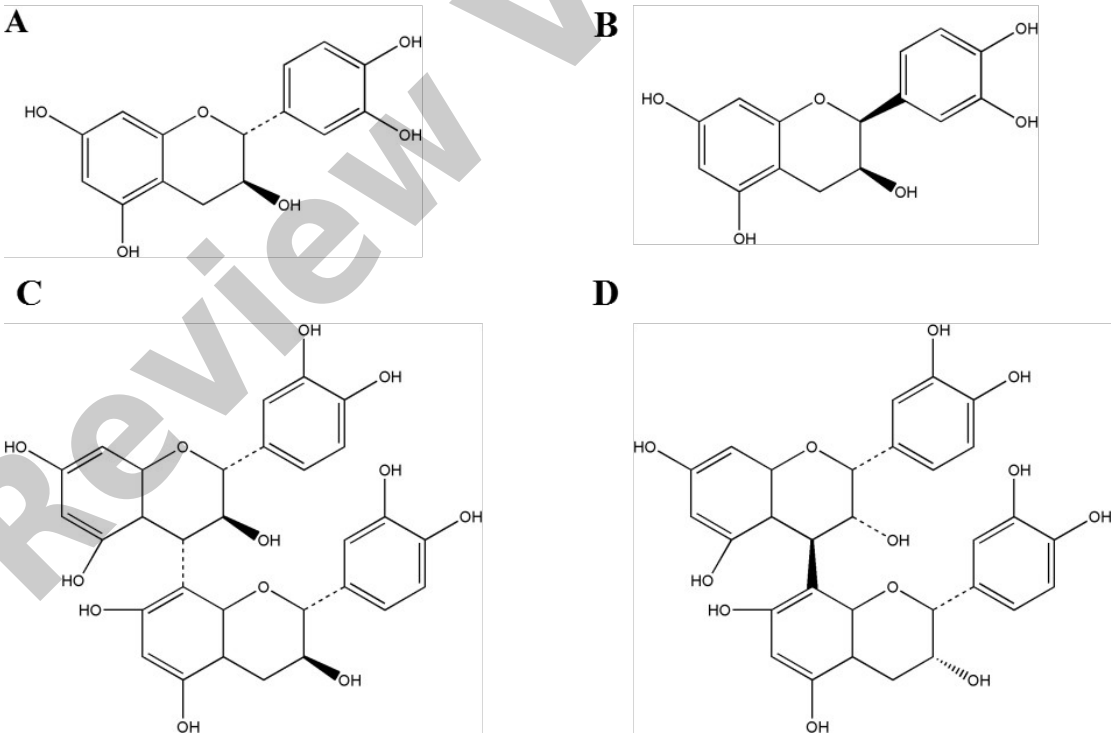

Fig. 1 Chemical structures of catechin (A), epicatechin (B), procyanidin B_2 (C), procyanidin B_3 (D).

2. MARERLALS AND METHODS

2.1. Chemicals and reagents

The reference standards of extract GSP (purity 95%) were purchased from the Tianjin Jianfeng Natural Product R&D Co.Ltd. (Tianjin, China). Reference standard procyanidin B_2 (purity98%), procyanidin B_3 (purity 98%), epicatechin (purity98%), catechin (purity 98%) and quercetin (IS, purity 98%) were all purchased from Dalian Meilunbio.Co.Ltd. (Dalian, China). $A\beta_{1-42}$ was purchased from Sigma-Aldrich (St Louis, MO, USA). Methanol and formic acid of HPLC grade were provided by Shandong Yuwang Industrial Co.Ltd. (Shandong, China). ultrapure water equipment was purchased from Shanghai Yarong Biochemical Instrument Factory (Shanghai,China). All other chemicals and reagents were analytical grade. Placed the collected plasma in the 1.5 mL EP tube of heparinization.

2.2. Instrumentation and UPLC-MS/MS conditions

The main parameters of the liquid chromatograph (Agilent 1290 Ultra Performance Liquid Chromatograhy, USA) were set as follows: with an on-line degasser, an autosampler and a column temperature controller were used for all analyses. Chromatographic column used was C_{18} column (100×2.1 mm,1.8μm, Agilent,USA). The mobile phase consisted: Mobile phase A: methanol, Mobile phase B:0.1% formic acid aqueous solution; delivered at a flow rate of 0.5 mL/min; The injection volume was kept at 5.0 μL. The column temperature was set at 35℃. Gradient elution according to the following gradient procedure：Mobile phase A: 0-5min (11%), 5-9min (11%-35%), 9-15min (35%- 90%).

The main parameters of the mass spectrometry (Agilent 6430 Triple Quadrupole Mass Spectrometer, Santa Clara, CA, USA) were set as follows: Electrospray Ionization (ESI) in negative mode with Multiple Reaction Monitoring (MRM) approach was used. capillary voltage 3.5kV; atomizer voltage 35 psi; source temperature 150℃; desolvation temperature 350℃ and Nitrogen was used as the

sheath and auxiliary gas. Analysis was carried out by Selected Ion Monitoring (SIM) mode for procyanidin B_2 [M−H]-m/z 577.4, procyanidin B_3 [M−H]−m/z 577.7, catechin [M−H]−m/z 289.3, epicatechin [M−H]−m/z 289.4 and IS [M−H]−m/z 301.4.

2.3 Preparation of GSP extract

The dried grape seed (150kg) was degreased, extracted and refluxed, and purified by ADS-8 resin column to obtain 1400g of procyanidin extract. The content of procyanidins extracted from the procyanidins obtained through the method of vanillin-HCl method was determined to be 2.8% (procyanidins(g) / grape seed (g), purity of 90%. And UPLC was used methanol-0.1% phosphoric acid solution as mobile phase to determine the content of procyanidin B_2, procyanidin B_3, epicatechin, and catechin. Among them, the contentin the effective parts($mg \cdot g^{-1}$) of procyanidin B_2, procyanidin B_3, catechin and epicatechin in procyanidins were **10.59**, **7.575**, **41.60**, **50.23**, respectively. The results indicated that both the content of the grape seeds and the content in the effective parts were determined, in which the content of epicatechin were the highest, followed by catechin, procyanidin B_2, and procyanidin B_3.

2.4. Preparation of calibration and quality control samples

Prepared a series of mixed working standards having 4–10,000$ng \cdot mL^{-1}$ for procyanidin B_2 and epicatechin, 3-8000$ng \cdot mL^{-1}$ for procyanidin B3, 5-12,000$ng \cdot mL^{-1}$ for catechin were obtained by diluting a mixture of the stock solutions with methanol. The stock solution were then serially diluted with methanol.Procyanidin B_2 and epicatechin (4, 40, 200, 1000, 2500, 5000 and 10,000 $ng \cdot mL^{-1}$); procyanidin B_3 (3, 30, 150, 800, 2000, 4000 and 8000$ng \cdot mL^{-1}$); catechins (5, 50, 250, 1200, 3000, 6000 and 12,000$ng \cdot mL^{-1}$) for drawing standard curve and were stored at 4℃. Prepared by adding 10μL of the mixed working standard solution to blank plasma, the concentrations of simulated plasma biological samples were the same as the concentration of standard solutions. Quality Control (QC) samples. Procyanidin B_2 and epicatechin were made at 40, 1000 and 5000$ng \cdot mL^{-1}$, procyanidin B_3 were made at 30, 800 and 4000$ng \cdot mL^{-1}$, catechin were made at 50, 1200 and 6000$ng \cdot mL^{-1}$. Stock solutions of IS was prepared in methanol at 4000$ng \cdot mL^{-1}$.

2.5. Animals and treatment

Twelve male Wistar rats were assigned randomly into two groups, each group with 6 rats. The rat models were established,and administrated with 500mg·mL^{-1} water solution of GSP by intragastric administration until collecting blood. The process of making the rats of AD model worked the following way: the rats were anesthetized with 3.5% chloral hydrate at the dose of 10mg·mL^{-1}, after that fixed in the rat brain stereotaxis. The amyloid $A\beta_{1\text{-}42}$ (10μg) was injected into the unilateral ventricle of the rats, according to rat brain stereogram (AP: –3.0 mm ML:±2.2 mm,DV: –3.0mm), and the injection rate was 0.2 mg·mL^{-1}. The normal group were injected with the same amount of saline in the same area, keeping the needle in rat brain about 5min after the injection with chloral hydrate, ensure the solution was fully dispersed, then the needle was slowly withdrawn and suture wounds. Penicillin was injected into the muscles for 3 days. The water maze test were carried out later 5 days, and both take the results of the escape latency in the last day of the five days' training period and the swimming time after the removal the target quadrant in the sixth day test with parameters to evaluate rat models were successful or not. And biosample collection the blood samples were collected from the suborbital vein into heparinized tubes(1.5mL) before administration and 0.08, 0.17, 0.33, 0.75, 1, 2, 4, 6, 8 and 10 h after dosing, and then immediately centrifuged at 3000 rpm for 10 min. The plasma samples were stored at −80℃ and analyzed.

2.6. Sample processing

100μL of rat plasma sample with 10μL of IS (4000ng·mL^{-1}) and 10μL methanol were added to the 1.5 mL EP tube and then mixed well.400μL methanol as the protein precipitation solution was added, then the centrifuge tube was vortexed for 2 min. After a centrifugation at 12,000 rpm for 15 min, the supernatant from each sample was transferred to another new tube and nitrogen flow to dryness. The residue was reconstituted in 100μL of the mobile phase, then filtered through a 0.2μm filter and 5μL of which was used for UPLC–MS/MS analysis.

2.7. Method validation

Validation according to the Bioanalytical Method Validation Guide (US Food and Drug Administration, 2018), including selectivity, linearity, accuracy, precision, recovery, matrix effects, and stability.

2.7.1. Selectivity

Five kinds of detection ions were used for quantitative analysis by ionization of procyanidin B_2, procyanidin B_3, catechin, epicatechin, IS according to the above liquid chromatography and mass spectrometric conditions. Analyzing five sources of 100μL blank rat plasma without internal standard.

2.7.2. Linearity

The Calibration curves were prepared and was established by using a series of standard plasma samples as described in 2.4. The linearity of each calibration curve was determined by plotting the peak area ratio of each analyte to IS versus the concentrations of the analyte. A leastsquares linear regression method ($1/x^2$) was fitted to determine the slope, intercept and correlation coefficient of linear regression equation.

2.7.3. lower limit of quantification(LLOQ)

LLOQ was defined as the lowest concentration on the calibration curve, RSD should be less than 20%, and its accuracy should be achieved 80%~120% of the true concentration.

2.7.4. Precision and accuracy

The precision and accuracy were assessed by QC samples at low, medium and high concentrations. Low, medium and high QC samples at concentration were analyzed on the same day with six replicates at each concentration per occasion to determine the intra-day accuracy and precision. The inter-day accuracy was evaluated by analyzing QC samples at three levels on three consecutive days.

2.7.5. Extraction recovery and matrix effect

The extraction recoveries of three QC levels concentrations were assayed by comparing the individual peak areas of procyanidin B_2, procyanidin B_3, catechin and epicatechin in the regular pretreated QC samples with those from postextracted blank plasma spiked with the analytes at the same concentration. The recovery of the IS was

determined in the same way at the concentration. The matrix effect was determined by comparing the standard peak areas ofthe analytes dissolved with the blank matrix extract against those dissolved with mobile phase at corresponding concentrations. Both the precision (RSD) of extraction recovery and matrix effect should be no more than 15%.

2.7.6. Stability

The stability of procyanidins in rat plasma was evaluated using three QC levels in six replicates. The evaluating index were set as follows: 8 h storage at room temperature; three freeze; −20℃ to room temperature; 10d storage at room temperature, frozen (−4℃) for 12h.

2.8. Data analysis

The pharmacokinetic parameters were calculated by Phoenix WinNonlin 6.0 software (Shenyang Pharmaceutical University). Statistical analyses of pharmacokinetic parameters were performed by SPSS 16.0 (Statistical Package for the Social Science) with $P<0.05$ as the minimal level of significance. Data are expressed as the mean±s.d. Statistical analysis was performed by an analysis of variance (ANOVA) with $P<0.05$ as the minimal level of significance.

3. RESULTS

3.1 .Optimization of UPLC-MS/MS and sample treatment method

The positive and negative selection of the scanning mode, the determination of precursor ions, the deletion of product ions, and the optimization of other conditions were vital to the quality of the liquid, since procyanidins were multiple hydroxyl compounds. That was,the procyanidin B_2, procyanidin B_3, catechin, epicatechin and IS were all polyhydroxy compounds. Therefore, their detection in the negative ion mode was better response than that in the positive ion mode and the measurement was less easily interfered with by impurities in the blood sample. So we chose the negative ion mode to get stronger ion fragmentation. The remaining two important parameters for the fragmentor voltage and the collision energy. The Fragmentor voltage was the capillary outlet voltage, optimized it was to ensure that the highest transmission

efficiency of the precursor ion, and the collision energy was the key of the product ions optimization.

We according to the Selected-Ion Monitoring (MS2 SIM) acquisition mode and the Product Ion Scanning acquisition mode, the ratio of the parent molecule ion mass charge ratio (m/z) of the analyte to the product ion mass charge ratio (m/z) was determined as follows: the detection ion pairs of procyanidin B_2 (m/z):577.4→ 425.5, the fragmentor voltage was 170V, collision energy was 15; procyanidin B_3 detection ion pairs (m/z) was: 577.7→425.7, fragmentor voltage was 150, collision energy was 15; the detection ion pair (m/z) of catechin was 289.3→245.3, the fragmentor voltage was 140, and the collision energy was 20, epicatechin detection ion pairs (m/z) was: 289.4→245.4, the fragmentor voltage was 150, the collision energy was 19 and the detection ion pair (m/z) of quercetin was 301.4→151.2, the fragmentor voltage was 170, and the collision energy was 23.

During the course of the experiment,we screened and mingling of various types and ratios of mobile phases, including acetonitrile-water, acetonitrile-0.1% formic acid water, methanol-water, and methanol-0.1% formate acid water, for different mobile phase types in the ion response, it was finally determined that methanol-0.1% formic acid water was used as a mobile phase for gradient elution, and elution time was 15 min.

The pretreatment of samples is also critical for the UPLC-MS/MS analysis. Currently, the biological sample preparation methods include precipitation proteins, liquid-liquid extraction, and extracted with solid phase extraction. In the preliminary experiment, three the preparation methods were done to pretreated the sample with methanol, acetonitrile precipitation proteins and liquid-liquid extraction with ethyl acetate. Experimental result found that the methanol precipitated protein had better experimental results than the other two methods.

3.2 Method validation

3.2.1. Selectivity

In this study, under the current optimal conditions, we didn't observe significant

interference peaks from endogenous substances during the same elution time. Typical mass spectra have blank plasma samples from rat, procyanidin B_2 plasma samples, procyanidin B_3 plasma samples, catechin plasma samples, epicatechin plasma samples, quercetin plasma samples, and plasma sample taken 0.75h after the administration. These spectrograms are shown in **Fig. 2 and 3**.

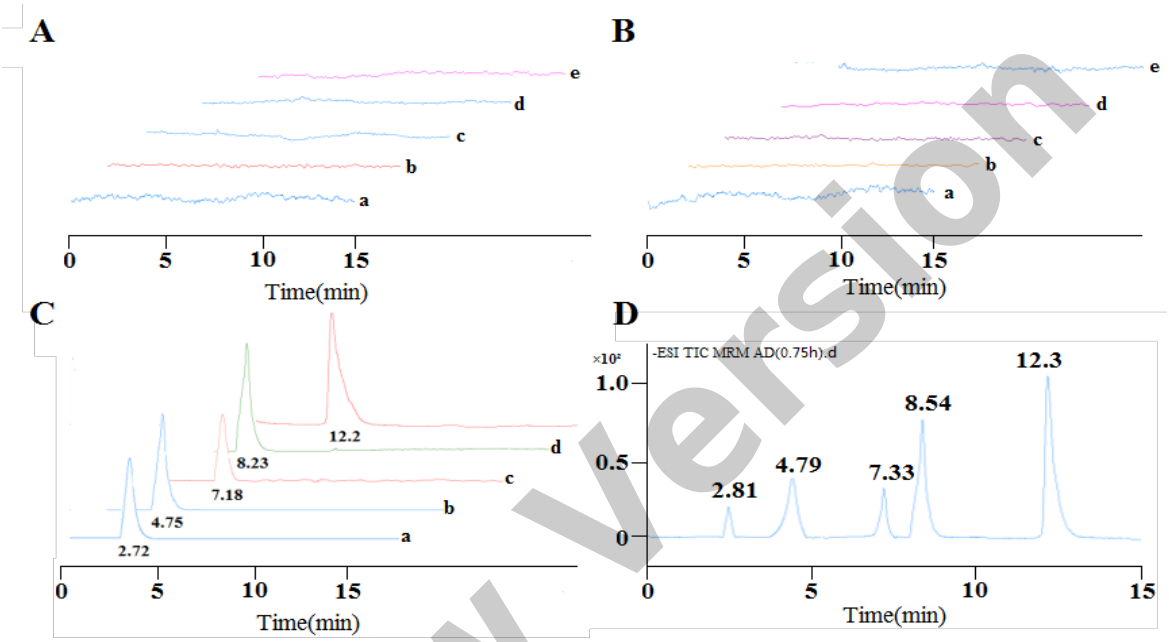

Fig. 2 Plasma chromatogram of epicatechin and internal standard: blank plasma sample of norma (A); blank plasma sample of normal (B); blank plasma sample (C); plasma sample from rat 0.75 h after procyanidin administration (D); The blank plasma sample included:blank plasma of procyanidin B_3 (a); blank plasma of catechin (b); blank plasma of procyanidin B_2 (c); blank plasma of epicatechin (d); blank plasma of IS (e).

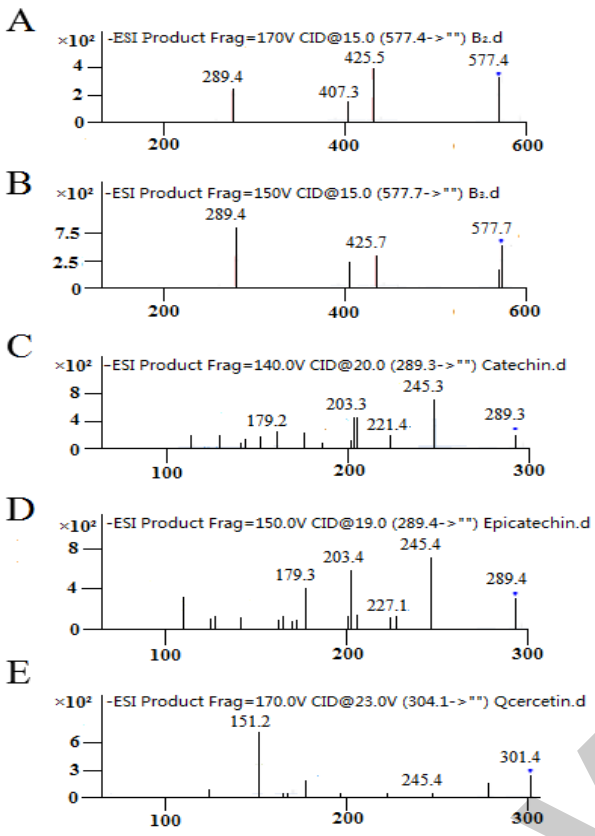

Fig. 3 Plasma mass spectrum of epicatechin and internal standard: blank plasma sample containing procyanidin B_2 (A); blank plasma sample containing procyanidin B_3 (B); blank plasma sample containing catechin (C); blank plasma sample containing epicatechin (D); blank plasma sample containing IS (E).

3.2.2. Standard curve and LLOQ

The best linear fit and least-square residual for the calibration curve were achieved with 1/2 weighting factor. The calibration curves showed good linearity of procyanidin B_2 has a concentration range of 4 to 10,000 ng·mL^{-1}, procyanidin B3 for 3 to 8000ng·mL^{-1}, catechin for 5 to 12,000ng·mL^{-1}, epicatechin for 4 to 10,000ng·mL^{-1}. The typical calibration curves were as follows: procyanidin B_2 for y=0.02738x+0.000367 (r^2= 0.9983), procyanidin B_3 for y=0.0812x+0.000524 (r^2=0.9928), catechin for y=0.09027x+0.00015(r^2=0.9973), epicatechin for y=0.4837x+ 0.00 8624 (r^2=0.9974), and LLOQ of quantification was: procyanidin B_2 and epicatechin were 4 ng·mL^{-1}; procyanidin B_3 was 3ng·mL^{-1}, and catechin was 5ng·mL^{-1}.

3.2.3. Accuracy and precision

The precision and accuracy data of procyanidins are shown in Table 1. All the results of the tested samples meet the analysis requirements normally within the range (RSD%: <15%;RE%: ±15%). The results were shown, which also shows that the method has good precision and accuracy.

3.2.4. Extraction recovery and matrix effect

The extraction recovery rate and matrix effect of procyanidins as shown in **Table 1**. We can see that the extraction recovery rates of procyanidin B_2, procyanidin B_3, catechin, epicatechin and IS ranged from 91% to 80%, which conform to the requirements of in vivo analysis. The matrix effect was less than 10%, it indicates that there is no significant matrix effect, which accords with the requirement of *in vivo* analysis.

Table 1 The extraction recovery and matrix effect of procyanidin and quercetin in different samples.The accuracy and precision of the analytical method (n = 6).

Different samples	Added conc. (ng/mL)	Intra-day RSD (%)	Inter-day RSD(%)	Accuracy RE(%)	Recovey (%)	Matrix effect (%)
Procyanidin B_2	40	6.8	9.9	87.4	85.2±9.2	93.2±1.1
	1000	2.6	2.9	104.0	90.7±2.7	97.5±5.9
	5000	4.3	1.7	101.0	89.2±6.9	86.1±3.9
Procyanidin B_3	30	8.6	6.3	98.9	90.1±4.4	97.5±6.5
	800	3.2	3.0	101.0	92.1±4.9	104.0±2.4
	4000	2.3	8.5	93.1	94.8±1.9	96.1±4.5
Catechin	50	6.4	5.8	97.9	93.5±5.3	95.8±4.8
	1200	4.7	4.6	96.0	89.4±3.9	96.4±5.1
	6000	1.5	7.6	100.0	88.3±3.5	92.7±7.9
Epicatechin	40	7.3	3.3	94.6	93.4±7.9	94.1±6.9
	1000	5.1	2.2	97.9	91.1±5.5	99.2±4.2
	5000	1.4	6.5	98.2	86.7±7.1	109.7±2.5

3.2.5. Samples stability

The results of stability of specimens as illustrated in **Table 2**. The results in the table show that there is no significant degradation under experimental conditions, thus

this does verify that the method was suitable for the routine pharmacokinetic analysis.

Table 2 Stability of procyanidin assay (n = 6).

	Spiked (ng/mL)	**Room temperature for 8 h**		**Three freeze and thaw cycles**		**Frozen for 10 d**		**4℃ in autos 12 h in proces**
		RE(%)	RSD(%)	RE(%)	RSD(%)	RE(%)	RSD(%)	RE(%)
Procyanidin	40	3.2	8.0	-1.8	4.0	5.4	9.3	1.3
B_2	1000	1.6	4.8	-1.9	4.7	5.1	5.6	-0.9
	5000	-2.1	5.4	5.7	6.1	-2.8	6.7	-0.4
Procyanidin	30	5.9	7.5	0.8	4.2	6.6	8.3	1.6
B_3	800	3.1	5.3	-1.3	6.5	-4.1	5.3	-0.6
	4000	2.3	2.6	2.9	5.7	-2.5	4.1	1.4
Catechin	50	-1.9	8.8	1.9	8.1	3.1	3.6	-0.6
	1200	0.5	5.7	2.6	6.9	-2.6	7.9	4.2
	6000	-1.1	6.1	-4.1	5.3	1.8	8.3	3.8
Epicatechin	40	4.2	7.1	-0.2	4.1	2.2	5.0	-1.3
	1000	2.3	2.8	1.3	5.3	6.3	9.6	1.8
	5000	5.2	8.5	4.2	6.2	3.8	5.5	2.9

3.4. Analysis of blood concentration in rat

According to the results of the water maze after modeling as shown, the date of escape latency of the last day of the training period was the escape latency of rats in AD group was significantly higher than the normal group. There was a significant difference (34.08±4.29s *vs* 14.33±1.75s, $p < 0.05$); and in the swimming time after the removal the target quadrant, AD group in the target quadrant of the swimming time were significantly lower than that of the normal group (15.17±2.62s *vs* 40.98±6.55 s, $p < 0.05$). So we know that $A\beta_{1\text{-}42}$ can cause reduce to spatial recognition abilities and memory impairment in brain of rats, leading to AD, this confirmed that successful modeling. The experimental results were shown in **Fig. 4**.

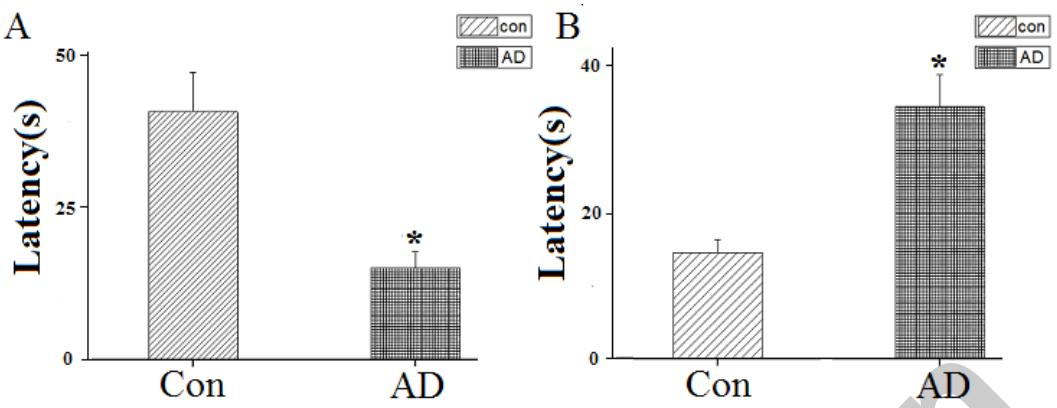

Fig. 4 spatial learning abilities and memory effect in the control rat and the AD rat: Escape latency of rats in water maze (A); the swimming time after the removal the target quadrant experiment (B). Error bars indicate SEM,*$P \leq 0.05$ by a two-tailed, unpaired Student's t-test.

3.5.Comparison of pharmacokinetics in AD and control in rats

Many studies have shown that there was a difference for the same drug in pharmacokinetics between the normal people and the diseased,Because the absorption and utilization of drugs in the body were different [16]. In this study, the pharmacokinetics of procyanidins in rat plasma was studied by UPLC-MS/MS, the pharmacokinetic parameters of procyanidins (procyanidin B_2, procyanidin B_3, catechin, epicatechin) in rats were shown in **Table 3,** and the average plasma concentration time curve was shown in **Fig.5**. Comparing the control group and the AD group by statistical analysis indicated that significant differences in $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and C_{max} of GSP were present between control and AD groups. In the AD group, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max} and T_{max} were higher than the control group, respectively. Indicating that GSP has more favorable absorption and slow elimination characteristics in the AD group.

Table 3 Pharmacokinetic parameters of procyanidin in rats.*P < 0.05 compared with control group (mean±SD; n = 6).

Parameters	Procyanidin B_2	Procyanidin B_3	catechin	epicatechin
control group				
$AUC_{(0-t)}$(h*ng/ml)	1343±345.1	853.5±237.8	2458±600.9	6057±1172
$AUC_{(0-\infty)}$(h*ng/ml)	1366±346.7	1074±349.1	2640±727.3	6178±1041
$MRT_{(0-t)}$(mg/L*h)	3.00±0.42	2.66±0.28	3.03±0.36	3.16±0.34
T_{max}(h)	0.75±0.00	0.79±0.10	0.75±0.00	0.75±0.00
$T_{1/2}$(h)	1.68±1.17	1.60±0.57	1.54±0.50	1.78±0.53
C_{max}(h)	379.7±66.54	354.9±70.99	728.6±87.86	1510±177.1
AD group				
$AUC_{(0-t)}$(h*ng/ml)	1739±261.1*	1228±338.7*	2974±604.3*	8043±1632*
$AUC_{(0-\infty)}$(h*ng/ml)	1767±262.4*	1267±378.2*	3442±705.9*	8483±1665*
$MRT_{(0-t)}$(mg/L*h)	3.14±0.33	2.81±0.16	3.10±0.36	3.24±0.16
T_{max}(h)	0.85±0.27*	0.88±0.14	0.75±0.00	0.83±0.13
$T_{1/2}$(h)	1.30±0.48	1.51±0.87	1.73±0.82	2.08±0.82
C_{max}(h)	457.6±51.29*	370.1±72.8	743.4±112.8	1969±212.7*

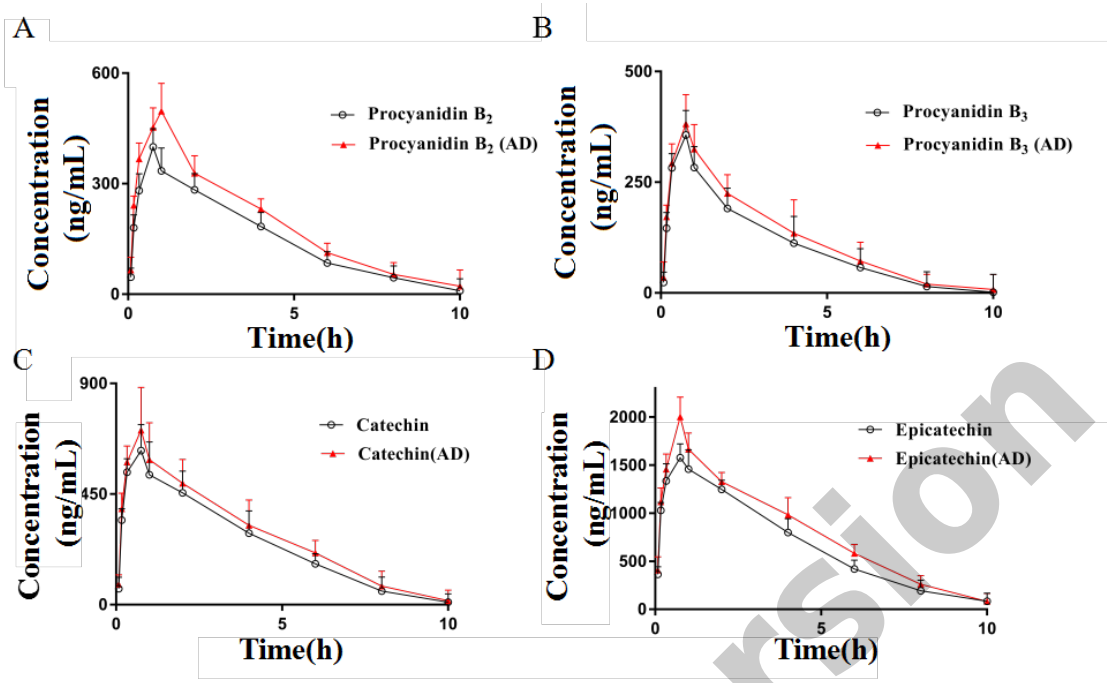

Fig. 5 Plasma concentration–time curves of Procyanidin B_2 (A); Procyanidin B_3 (B); Catechin (C); Epicatechin (D), in rat plasma after oral administration of extract of procyanidin at 500mg·kg^{-1} to rats in normal and AD groups. Each point represents the mean±S.D (n = 6).

4. DISCUSSION

The results showed that the absorption of GSP in AD rats was higher than that in control group, and the distribution and elimination process were slower. As shown in Table 3, by comparing C_{max} with *AUC*, it was found that in control and AD group, the absorption of epicatechin was higher than the other three substances. And in the comparison of its pharmacokinetic parameters (including $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, C_{max}, $T_{1/2}$, and *MRT*), it can be found that compared with the control group, the $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, C_{max}, $T_{1/2}$ of AD group were significantly different (p <0.05); The results showed that the absorption of procyanidins in AD rats was higher than that in control group, and the distribution and elimination process were slower.

Found in the study of GSP. Found in the study of GSP，it is very stable in the gastrointestinal environment, Proanthocyanidin oligomers (trimer to hexamer) are hydrolyzed to a mixture of epicatechin monomers and dimers, thereby enhancing their

potential for absorption in the small intestine [17]. It indicates that most of the ingested procyanidins can reach the small intestine intact and can be used for absorption or metabolism [18, 19], as well as significant degradation occurred in the cecum and colon [20]. In addition, prcyanidins can improve intestinal flora, reduce intestinal and systemic inflammatory diseases, repair intestinal mucosal damage, and restore normal metabolism [21]. Therefore, regulation of intestinal flora may be one of the mechanisms which GSP impacts metabolic health [22]. And procyanidins can be degraded into phenyl valerolactone and phenolic acid by colonic microbiota [23]. It transfers the bacterial population in the gastrointestinal tract of rats, causing major bacteria to transform into tannin-resistant Gram-negative Enterobacteriaceae and Bacteroides species, and promotes the growth of Lactobacillus/Enterococcus and reduces in vitro fermentation. During the process of Clostridium solani and human fecal microbiota, such changes may affect the bioavailability of proanthocyanidins [24, 25]. And studies have shown that the important contribution of gut microbiota to the protective activity of GSP in AD [26]. In patients with AD, the intestinal flora may also be disordered [27, 28, 29], which may also lead to higher bioavailability of grape seed proanthocyanidins in the AD group. In addition, AD has been shown to cause a decrease in endothelial tight junction proteins in the gut and brain [30], increasing the permeability of intestinal epithelial cells and the BBB [31]. It may also be one of the reasons why the AD group has high bioavailability of GSP.

CONCLUSION

In combination with the results of this experiment, the bioavailability of GSP in the AD group is higher than that of the normal rats. The result may be an increase in gastrointestinal permeability and degradation of the intestinal flora in AD patients.Moreover, the absorption of catechins and epicatechins *in vivo* is better than that of procyanidin dimers, which may be related to the degradation of procyanidins *in vivo*, provide new ideas for the study of improving the symptoms of AD. It has laid the scientific foundation for its further application in the food, health products and

pharmaceutical industries. And it could be used as a potential drug for the prevention and even treatment of AD.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments that described in the present study were performed in adherence to the Shenyang Pharmaceutical Universityanimal experiment center guidelines and approved by the Animal Ethics Committee.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study.

The animal experiments were performed in accordance with institutional guidelines of the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical Universityanimal, China.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

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