International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.68.75

Research Article
Pharmacokinetics Study of Seven Lignans in Alzheimer's Rats

[1]Wang Xiao-tong, [2]He Fan and [3]Sun Xiao-ling

[1]Liaoning University of Traditional Chinese Medicine, Key Laboratory of Ministry of Education for TCM Viscera-State Theory and Applications, 110847 Shenyang, China
[2]Department of Chinese Medicine Analysis, Liaoning University of Traditional Chinese Medicine, 77 Life One Road, DD port, 116600 Dalian, China
[3]Institute of Traditional Chinese Medicine, Liaoning Institute for Drug Control, 7 Congshan West Road, 110036 Shenyang, China

Abstract

Background and Objective: *Schisandra chinensis* (*S. chinensis*) (Turcz., Bail.), is Chinese traditional herbal medicine and the schisandra lignans are the major constituents of *S. chinensis* responsible for the nephroprotective effect. However, there was no study done on pharmacokinetics of schisandra lignans in Alzheimer's disease (AD) rat plasma after oral *Schisandra chinensis* extract (SCE). The aim of this study was to investigate the pharmacokinetic profiles of 7 lignans after oral administration of SCE and compared the difference of the pharmacokinetics profiles between normal and Alzheimer's disease rats. **Materials and Methods:** The plasma concentrations of 7 lignans were determined by using a simple and rapid high-performance liquid chromatography. All the rats were divided randomly into two groups (Alzheimer's and normal groups). Each group received oral administration of 0.1 g kg^{-1} SCE. The relevant pharmacokinetic parameters were calculated by using the computer program DAS 2.0. **Results:** The results showed that 7 lignans of *Schisandra chinensis* could be detected in rat plasma after oral administration of SCE to rats. Gomisin D and schisantherin A had shown better absorption than other lignans. Oral administration of SCE, AD rats showed better absorption than normal rats. **Conclusion:** It can be concluded that the pharmacokinetics properties of 7 lignans differed between Alzheimer's rats and normal rats, including $AUC_{(0-t)}$ and C_{max} ($p<0.05$).

Key words: *Schisandra chinensis*, lignans, pharmacokinetic, high-performance liquid chromatography, Alzheimer's disease

Received: June 02, 2017 Accepted: September 11, 2017 Published: December 15, 2017

Citation: Wang Xiao-tong, He Fan and Sun Xiao-ling, 2018. Pharmacokinetics study of seven lignans in Alzheimer's rats. Int. J. Pharmacol., 14: 68-75.

Corresponding Author: He Fan, Department of Chemistry of TCM, Liaoning University of Traditional Chinese Medicine Tel: +86 0411 85890137

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alzheimer's disease (AD), is a progressive neurodegenerative disease with cognitive dysfunction due to the presence of many senile plaques, neurofibrillary tangles in the brain and it is the most common form of dementia[1]. The pathophysiology of AD is very complex, various deranged mechanisms such as Aβ production, mitochondrial dysfunction, hormonal imbalance, chronic oxidative stress, calcium mishandling, neurofibrillary tangles accumulation, inflammation and genetic components, may contribute to the disease process[2]. D-galactose is a kind of reducing sugar which serves as a feasible brain aging model for animals. Injection of D-galactose can lead to a progressive deterioration in learning and memory ability. Nonetheless, the underlying mechanism of D-galactose-induced brain aging remains unclear. Injection of D-galactose on long term basis in mice could lead to impairments in antioxidant capacity[3], mitochondrial function[4], calcium homeostasis[5], inflammatory response[6], neuronal apoptosis[7] and cholinergic degeneration[8] in the brain.

Schisandra sphenanthera, the dried fruit of *Schisandra chinensis* Bail, is Chinese traditional herbal medicine and recorded in the Chinese Pharmacopoeia as a tonic, sedative and astringent[9]. It has been traditionally used as tonic, sedative, antidiabetic, hepatoprotective and also for the treatment of chronic asthma, spontaneous sweating, palpitation, diabetes, insomnia and forgetfulness in Eastern Asian countries. SCE may be used in the prevention and treatment of Alzheimer's disease. The major constituents of SCE are lignan-type molecules which might be responsible for pharmacological effects of SCE as aforementioned[10,11]. In particular, among the lignans isolated from SCE, the dibenzylcyclooctadiene-type lignans including schisandrin A, schisandrin B, schisandrin C, gomisin A, schisantherin A, schisantherin B and their analogs[11,12] which are known to ameliorates neurodegeneration with cognitive impairment in mice and primary mouse neuronal cells.

Up to now, there have been many reports for SCE dealing with the development of analytical methods including a simultaneous quantitation of several lignans. However, there was no simultaneous analytical method in biological samples for sever lignans from SCE and also no study on pharmacokinetics in Alzheimer's disease plasma. Therefore, this study was in order to develop a sensitive, simple and accurate HPLC method to simultaneously determine the concentration of seven compounds in normal and Alzheimer's diseased rat plasma. And the purpose was to investigate and compare the pharmacokinetic parameters of seven lignin compounds after oral administration of SCE.

MATERIALS AND METHODS

Materials and chemicals: The fruits of *Schisandra chinensis* (Turcz.) Bail. were purchased from the Dandong, China in September, 2015 and identified by Prof. Wang Bing, Liaoning University of Traditional Chinese Medicine. A voucher specimen (M 203) was deposited at the Herbarium of the Department of Pharmacy, Liaoning University of TCM, China. Schisandrol A, schisandrol B, schisandrin A, schisandrin B, schisantherin A were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Schisandrin C was purchased from the Chengdu Must Bio-technology Co., LTD (Chendu, China). The purities of gomisin D and arctigenin (internal standard) were extracted and purified in the authors' laboratory. The purities of arctigenin and gomisin D were determined to be up to 98% by HPLC. The solvents (HPLC grade) used for chromatographic analysis were purchased from Fisher Company Inc., USA. Deionized water was prepared in a Mill-Q academic water purification system (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade and provided by Kermel Chemical Co. (Tianjin, China).

Schisandra chinensis extract (SCE) preparation: Ninety five percent ethanol was added to the *S. chinensis* fruit. Circumfluent extraction was then performed for two times which lasted 1.5 h each time. The extraction was combined and vacuum dried. Accurately weighted SCE powder 1.5 g, dissolved with saline 20 mL, mixed into a solution of SCE of 0.075 g mL^{-1}.

Apparatus and chromatographic conditions: The concentrations of the schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C in plasma were assayed by using reverse-phase high performance liquid chromatography (Agilent 1200 series) equipped with a variable wave length UV detector and pump (Agilent model G1314A VWD). Separation was accomplished on a Welch Ultimate AQ-C_{18} column (150×4.6 mm, 5 μm particle size). The mobile phase was composed of acetonitrile (A): Water (B) (0→8 min, 40: 60, 8 →12 min, 50: 50, 12 →23 min, 75: 25, 23→27 min, 75: 25, v/v) at a flow rate of 1.0 mL min^{-1} with gradient elution. The column temperature was 30°C. The detector was set at 250 nm. The injection volume was 20 μL. The chromatographic run time for each analysis was 27.0 min.

Animals: Male wistar rats, weighed 200-250 g, were obtained from Liao Ning Chang Sheng Biotechnology Co., LTD (Benxi,

China). Animal welfare and experimental procedures were strictly in accordance with the Guide for the Care, Use of Laboratory Animals[13] and the related ethics regulations of Liaoning University of TCM. Rats were housed in an air-conditioned animal quarter at a temperature of 22±2°C and a relative humidity of 50±2%. All animals received food and water *ad libitum*. The animals were acclimatized to the facilities for 5 days and then fasted with free access to water for 24 h prior to each experiment.

Animal model: All rats were randomly split into 2 groups of ten rats each, a control group and a model group. AD models rats were established by intraperitoneal injection of D-galactose (D-gal) (63 mg kg^{-1} day) and administered intragastrically with aluminum chloride ($AlCl_3$) (28 mg kg^{-1} day) simultaneously for 105 consecutive days. And rats were treated with same volume of sterile saline in control group. Behavior evaluation (Morris Water Maze) were taken one week prior to sacrifice.

The stock solutions were prepared by dissolving 83.7 mg of schisandrol A, 78.1 mg of gomisin D, 85.5 mg of schisandrol B, 86.9 mg of schisantherin A, 80.6 mg of schisandrin A, 83.1 mg of schisandrin B, 81.6 mg of schisandrin C and 3.14 mg arctigenin (IS) in 10 mL methanol, respectively. A series of mixture standard working solutions were obtained by diluting the mixture of the stock standard solutions with methanol. The IS working solution was prepared by diluting the IS stock solution with methanol. All solutions were stored at 4°C.

Sample preparation: Plasma samples (200 µL) was spiked with 50 µL IS and the mixtures were extracted with 800 µL acetonitrile by vortex mixing for 3 min. After centrifugation at 4,000 rpm for 5 min, the solution was transferred to a polypropylene tube and dried under nitrogen gas at room temperature. The plasma residue was reconstituted in 50 µL of methanol, respectively. The injection volume was 20 µL for analysis.

Method validation: The validation had been performed according FDA guidelines.

Linearity and quantification: The method was fully validated for its specificity, linearity, lower limits of detection (LLOD), lower limits of quantification (LLOQ), accuracy and precision. The LLOD was determined during evaluation of the linear range of the calibration curve and was defined as the lowest concentration level resulting in a signal-to-noise ratio of 3:1. The LLOQ was determined as the lowest concentration of the analyte in rat plasma and tissue that could be quantified with an inter-assay relative standard deviation (%, RSD) lower than 20% and with accuracy rates between 80 and 120%.

Accuracy and precision: The precision and accuracy of the method was evaluated by analyzing QC samples with different concentrations. The intra-day variability was determined by assaying 5 replicates on the same day and the inter-day variability was determined by assaying 5 replicates on three consecutive days. Precision was defined as the coefficient of variation expressed as percentage. The accuracy of these samples was determined by comparing the calculated concentration obtained from the calibration curve with the known concentration.

Extraction recovery: Extraction recoveries from rat plasma was determined at three concentrations by comparing the peak areas extracted from rat plasma with those of the same quantities added to methanol.

Stability: Stability of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE in rat plasma was assessed with QC samples (n = 3) stored at -20°C for 30 days. Freeze-thaw stability of 7 compounds in rat plasma was investigated with QC samples (n = 3) subjected to three freeze/thaw cycles.

Pharmacokinetic study: The normal wistar rats (n = 10) and AD rats (n = 10) were assigned to receive SCE solution by oral administration at the dose of 0.5 g kg^{-1}. Serial blood samples (0.4 mL) were obtained *via* the rat's orbital vein at 0.5, 1.5, 3, 4, 5, 7, 9, 11 h after administration and were collected into heparinised centrifuge tubes. The blood samples were immediately centrifuged at 667 rpm for 10 min at room temperature. The plasma samples were analysed by the previously described methods.

Statistical analysis: The plasma of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE at different time points was evaluated by means of linear regression analysis. All data were calculated by using Microsoft Excel 2003 (Microsoft). The relevant pharmacokinetic parameters were calculated by using the

computer program DAS 2.0 (Chinese Society of Mathematical Pharmacology, Beijing, China) from the Chinese Pharmaceutical Association.

RESULTS AND DISCUSSIONS

HPLC assay: So far, there have been many reports on the development of SCE processing analysis[10-12]. But in the previous reports, only 3-4 components could be detected simultaneously in the biological samples[13-16]. Compare to them, the author has developed a method to detect seven compounds simultaneously in normal and Alzheimer's rat plasma.

The selectivity of the method was evaluated by analyzing blank plasma samples prior to administration. The chromatograms of the plasma were shown in Fig. 1. Arctigenin (IS), schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C were well separated at 4.73, 13.150, 14.753, 15.629, 20.832, 23.781, 25.182 and 26.822 min, respectively with no endogenous interference.

The linear calibration curves were obtained in the given concentration range of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE in plasma samples, respectively. The standard curves were fitted to a first-degree polynomial, Y = aX+b, where, Y was the peak area of one of 7 lignans/IS, a and b were constants and X was the concentration (μg mL^{-1}) one of 7 lignans. Calibration curves, correlation coefficients, LLOQ were listed in Table 1. In author's research, the LOQ of schisandrol A, schisandrin A and schisandrol B were 0.696, 0.670, 0.613 ng mL^{-1}, respectively. This method was more sensitive than the previous reports[14,15], which showed the LOQ of schisandrol A, schisandrin A and schisandrol B were 0.1, 0.2, 0.2 ng mL^{-1}, respectively.

The RSD for the intra-day (repeatability) and inter-day precision ranged from 1.41-13.3% for the QC standards. The percentage of schisandrol A, gomisin D, schisandrol B,

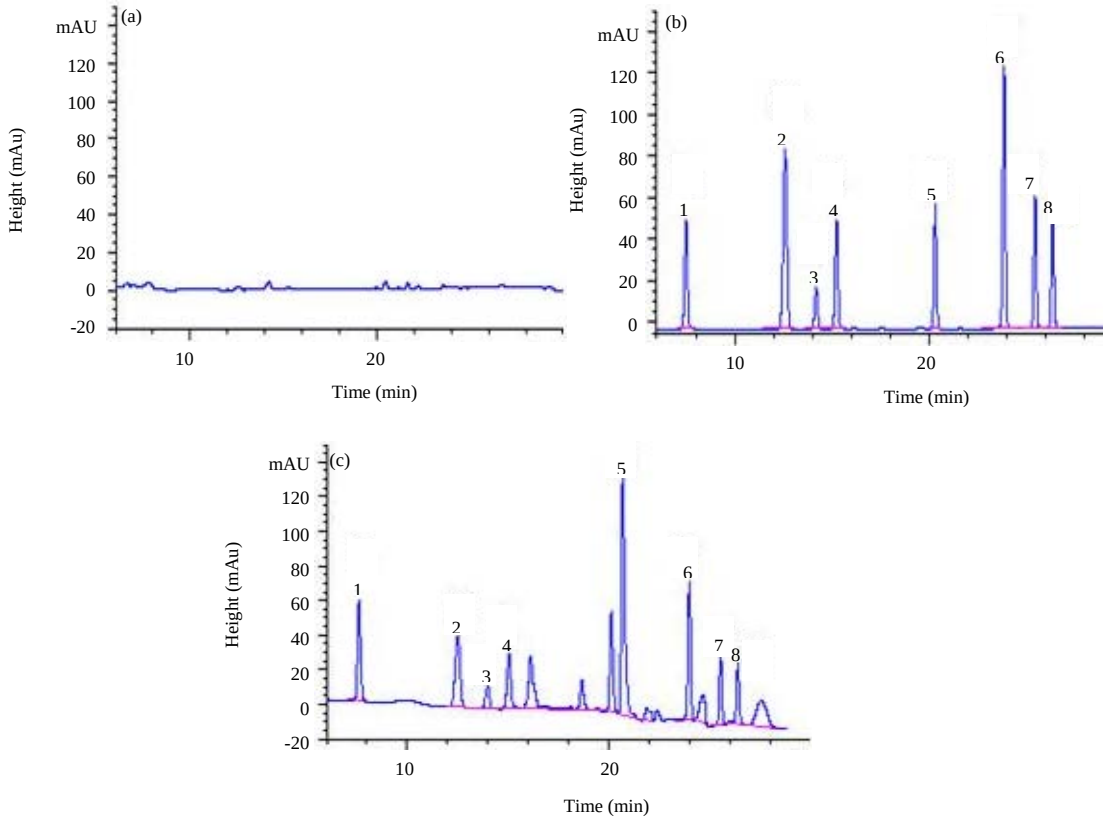

Fig. 1(a-c): Chromatograms of rat plasma samples, Arctigenin (IS), schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B, schisandrin C, (a) Blank plasma, (b) Blank plasma spiked with IS and 7 lignans and (c) Plasma sample obtained 2 h after oral administration of SCE

Table 1: Standard curves, regression equations, linear ranges LOD and LQD of 7 lignans of SCE in rat plasma

Analytes	Linearity	R	Range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Schisandrol A	y = 0.523X-0.660	0.994	2.09-83.7	2.09	0.696
Gomisin D	y = 0.150X-0.216	0.993	1.95-78.1	1.95	0.650
Schisandrol B	y = 0.280X-0.398	0.990	2.14-85.5	2.14	0.713
Schisantherin A	y = 0.279X-0.089	0.996	2.17-86.9	2.17	0.723
Schisandrin A	y = 0.570X-0.890	0.996	2.01-80.6	2.01	0.670
Schisandrin B	y = 0.297X-0.439	0.997	2.08-83.1	2.08	0.693
Schisandrin C	y = 0.242X-0.338	0.993	2.04-81.6	2.04	0.680

LOD: Limit of detection, LQD: Limit of quantification

Table 2: Pharmacokinetic parameters for 7 lignans in normal rats and AD rats (Mean±SD, n = 6) after a single oral administration of SCE

Parameters		$AUC_{(0-12)}$ (mg L^{-1}*h)	$AUC_{(0-\infty)}$ (mg L^{-1}*h)	Clz/F (L/h/kg)	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (mg L^{-1})
Schisandrol A	Normal	55.3±13.2	66.7±15.8	7.90±1.61	3.00±1.24	11.80±2.81	5.10±2.34
	AD	60.9±20.3	68.0±25.8	1.79±0.321	5.00±2.35	9.97±1.78	3.91±2.61
Gomisin D	Normal	90.2±22.5	111.3±31.3	4.54±1.62	5.00±1.80	7.90±2.00	9.40±2.10
	AD	110.2±10.3	135.2±0.321	0.361±0.123	5.50±2.31	6.80±3.21	12.00±4.31
Schisandrol B	Normal	33.0±9.60	40.2±10.3	14.10±3.81	3.00±1.71	7.82±2.40	3.90±1.60
	AD	40.2±12.8	45.3±11.4	1.37±0.621	1.75±0.621	6.53±1.23	2.84±0.521
Schisantherin A	Normal	103.6±19.3	126.6±30.3	4.31±0.931	7.00±1.80	12.30±2.90	5.30±1.51
	AD	123.4±33.6	158.3±51.3	2.15±0.821	4.50±1.62	13.10±2.00	7.66±2.51
Schisandrin A	Normal	15.5±3.83	20.4±4.82	2.13±0.824	5.00±1.80	12.10±2.90	1.70±0.30
	AD	18.7±1.25	35.7±5.78	3.14±1.62	5.00±1.80	7.92±2.35	1.92±0.321
Schisandrin B	Normal	16.1±3.82	23.3±7.50	4.01±1.23	3.00±0.90	10.00±2.82	1.82±0.421
	AD	19.0±2.24	24.1±3.58	6.71±1.62	4.00±0.124	9.71±2.35	2.25±0.327
Schisandrin C	Normal	17.6±4.80	28.2±7.60	5.91±1.73	6.00±1.80	10.20±2.30	2.09±0.613
	AD	21.1±1.32	40.3±11.4	7.04±1.62	3.25±1.83	9.42±2.58	2.66±1.130

TCM: Traditional Chinese Medicine, QC: Quality control, AUC: Area under the plasma level/time curve, AIC: Akaike's information criterion, C_{max}: Concentration maximum, T1/2: Terminal half-life

schisantherin A, schisandrin A, schisandrin B and schisandrin C for the plasma were between 86.2 and 100.8%, respectively. These data indicated that the sample preparation method was satisfied and resulted in no appreciable matrix effect for schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE and IS. The stability tests were designed by taking into account the anticipated conditions that real samples may experience. The RSD of the stability studies were 3.65-13.4%. This result agreed with many pharmacokinetic studies which reported in US National Research Council[13].

Pharmacokinetics of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C after oral administration of SCE.

The method presented here was successfully used to quantify the schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C in rat plasma after oral administration of SCE. The concentration-time profiles of the schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE were shown in Fig. 2. According to the F test and the AIC, a one-compartment pharmacokinetic model fitted best the plasma data of the schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE. The calculated pharmacokinetic parameters are listed in Table 2.

The non-compartmental model was applied to the pharmacokinetic evaluation of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C in AD rats and in normal rats. Schisandrol A, schisandrol B and schisandrin B exhibited a rapid and poor absorption phase followed by a sharp but lasting disappearance of gomisin D, schisantherin A, schisandrin A and schisandrin C. The concentration peak values of gomisin D and schisantherin A were much higher than others, indicating that liposoluble compounds were more easily absorbed by the body.

After oral administration of SCE, the T_{max} of schisandrol A, schisantherin A, schisandrin A in AD rats and normal rats were all about 6 h which conformed to many other studies reporting that the T_{max} was 6 h[16]. The AUC values, $T_{1/2}$ and C_{max} of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C in AD rats were higher than those in the normal rats. The value of AUC in (Table 2) were higher than other studies reporting that the AUC of Schisantherin A was 12.28 mg L^{-1}*h and schisandrol A 9.76 mg L^{-1}*h[16]. The results showed that the absorption of schisandrol A, gomisin D, schisandrol B, schisantherin A,

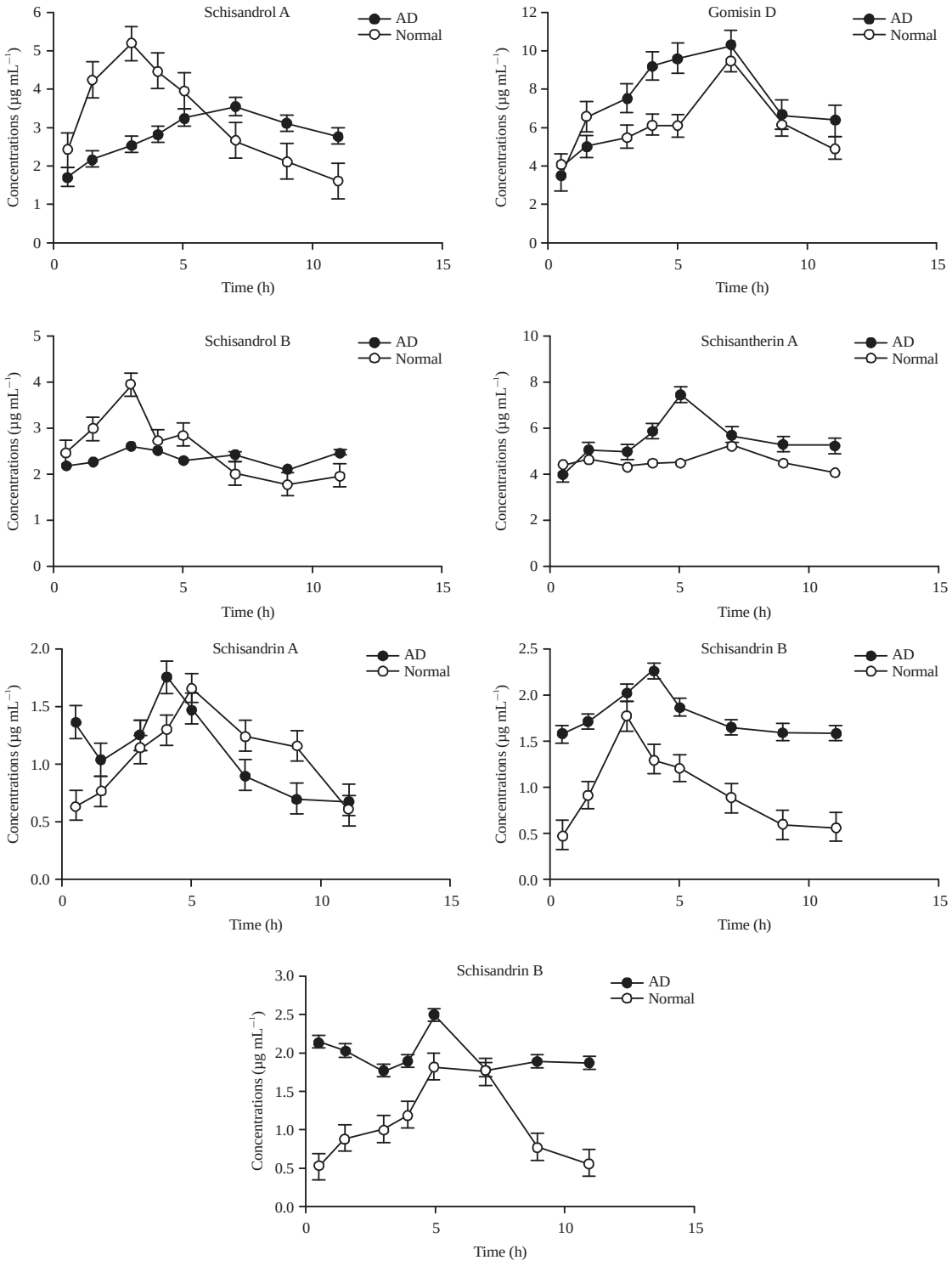

Fig. 2: Mean plasma concentration-time curves of 7 lignans after oral administration of SCE to normal rats and AD rats

schisandrin A, schisandrin B and schisandrin C in normal rats was lower than in AD rats but the clearance was higher than AD mice. It may be that the body environment in AD rats advanced the absorption and metabolism of drug. Therefore, the dosage needed to be adjusted appropriately according to practical applications, to achieve the desired therapeutic effect.

CONCLUSION

The schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C had been quantified by HPLC-UV. The validated method was simple, fast, reproducible and suitable for the research of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C in rat plasma with arctigenin as the internal standard. The assay utilized an acetonitrile extraction method and a reversed-phase separation with sufficient selectivity and sensitivity. The evaluation of the pharmacokinetics of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C will help further the understanding of their pharmacological activity and clinical use. The authors need to take caution when extrapolating PK and exposed data from healthy animals to diseased animals in designing pharmacological studies.

SIGNIFICANCE STATEMENTS

This study discovered the pharmacokinetic of seven lignin compounds after oral administration of SCE that can be beneficial for Alzheimer's disease. This study will help the researcher to uncover the critical areas of seven lignin compounds of SCE *in vivo* process in normal and model rats. Thus, the authors found out a new theory on plasma pharmacokinetics of schisandrol A, gomisin D, schisandrol B, schisantherin A, schisandrin A, schisandrin B and schisandrin C of SCE in rat.

ACKNOWLEDGMENTS

We thank the Project was supported by the Open fund of Key Laboratory of Ministry of Education for TCM Viscera-State Theory and Applications, Liaoning University of Traditional Chinese Medicine, the elitist funding of innovation in science and technology of Liaoning Provincial Department of Education (Grant No: LJQ2015071), Liaoning S and T project management system (Grant No; 201602495).

REFERENCES

1. Francis, P.T., A.M. Palmer, M. Snape and G.K. Wilcock, 1999. The cholinergic hypothesis of Alzheimer's disease: A review of progress. J. Neurol. Neurosurg. Psychiatry, 66: 137-147.
2. Kang, S.Y., K.Y. Lee, K.A. Koo, J.S. Yoon, S.W. Lim, Y.C. Kim and S.H. Sung, 2005. ESP-102, a standardized combined extract of *Angelica gigas*, *Saururus chinensis* and *Schizandra chinensis*, significantly improved scopolamine-induced memory impairment in mice. Life Sci., 76: 1691-1705.
3. Cui, X., P. Zuo, Q. Zhang, X. Li and Y. Hu *et al*., 2006. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration and oxidative damage in mice: Protective effects of R-alpha-lipoic acid. J. Neurosci. Res., 83: 1584-1590.
4. Long, J., X. Wang, H. Gao, Z. Liu and C. Liu *et al*., 2007. D-Galactose toxicity in mice is associated with mitochondrial dysfunction: protecting effects of mitochondrial nutrient R-alpha-lipoic acid. Biogerontology, 8: 373-381.
5. Lu, J., Y.L. Zheng, L. Luo, D.M. Wu, D.X. Sun and Y.J. Feng, 2006. Quercetin reverses D-galactose induced neurotoxicity in mouse brain. Behav. Brain Res., 171: 251-260.
6. Lu, J., D.M. Wu, Y.L Zheng, B. Hu and Z.F. Zhang *et al*., 2010. Ursolic acid attenuates D-Galactose-induced inflammatory response in mouse prefrontal cortex through inhibiting AGEs/RAGE/NF-κB pathway activation. Cerebral Cortex, 20: 2540-2548.
7. He, M., L. Zhao, M.J. Wei, W.F. Yao, H.S. Zhao and F.J. Chen, 2009. Neuroprotective effects of (-)-epigallocatechin-3-gallate on aging mice induced by D-galactose. Biol. Pharm. Bull., 32: 55-60.
8. Lei, M., Y. Su, X. Hua, J. Ding, Q. Han, G. Hu and M. Xiao, 2008. Chronic systemic injection of D-galactose impairs the septohippocampal cholinergic system in rats. Neuroreport, 19: 1611-1615.
9. Chinese Pharmacopoeia Commission, 2015. Pharmacopoeia of the People's Republic of China 2015. Chinese Pharmacopoeia Commission, China.
10. Liu, H., J. Zhang, X. Li, Y. Qi, Y. Peng, B. Zhang and P. Xiao, 2012. Chemical analysis of twelve lignans in the fruit of *Schisandra sphenanthera* by HPLC-PAD-MS. Phytomedicine, 19: 1234-1241.
11. Zhang, W.D., Q. Wang, Y. Wang, X.J. Wang, J.X. Pu, Y. Gu and R. Wang, 2012. Application of ultrahigh-performance liquid chromatography coupled with mass spectrometry for analysis of lignans and quality control of Fructus *Schisandrae chinensis*. J. Sep. Sci., 35: 2203-2209.
12. Liang, Y., H. Hao, L. Xie, A. Kang and T. Xie *et al*., 2010. Development of a systematic approach to identify metabolites for herbal homologs based on liquid chromatography hybrid ion trap time-of-flight mass spectrometry: Gender-related difference in metabolism of *Schisandra lignans* in rats. Drug Metab. Dispos., 38: 1747-1759.
13. National Research Council, 1996. National Science Education Standards. National Academy Press, Washington, DC.
14. Wu, X., Y. Zhou, F. Yin, G. Dai and L. Li *et al*., 2014. Comparative pharmacokinetics and tissue distribution of schisandrin, deoxyschisandrin and schisandrin B in rats after combining acupuncture and herb medicine (schisandra chinensis). Biomed. Chromatogr., 28: 1075-1083.

15. Kim, Y.J., H.J. Lee, C.Y. Kim, S.Y. Han, Y.W. Chin and Y.H. Choi, 2014. Simultaneous determination of nine lignans from *Schisandra chinensis* extract using ultra-performance liquid chromatography with tandem mass spectrometry in rat plasma, urine and gastrointestinal tract samples: Application to the pharmacokinetic study of *Schisandra chinensis*. J. Sep. Sci., 37: 2851-2863.

16. Wang, B.L., J.P. Hu, W. Tan, L. Sheng, H. Chen and Y. Li, 2008. Simultaneous quantification of four active schisandra lignans from a traditional Chinese medicine *Schisandra chinensis* (Wuweizi) in rat plasma using liquid chromatography/mass spectrometry. J. Chromatogr. B: Anal. Technol. Biomed. Life Sci., 865: 114-120.