2017 International Conference on Medicine Sciences and Bioengineering (ICMSB 2017)
ISBN: 978-1-60595-508-7

Pharmacokinetic Profile of Calceolarioside A Administration to Mice

Gang XUE[1,a], Yu-Juan CHEN[1,b,*], Xiang-Nan MENG[2,c] and Cheng-Hong MA[1,d]

[1]School of Life Science and Technology, Changchun University of Science and Technology, Changchun, China

[2]Gene Science Pharmaceuticals Co., Ltd. (GenSci), Changchun, China

[a]919161507@qq.com, [b]jychenzxcv@163.com, [c]mengxiangnan@gensci-china.com, [d]2369520253@qq.com

*Corresponding author

Keywords: Pharmacokinetic, Calceolarioside A, Mouse, Serum.

Abstract. Calceolarioside A has a potential effect of anti-food allergy, which was isolated from the bark of *Fraxinus mandshurica* Rupr. In this article, to study the pharmacokinetic of Calceolarioside A in mice, HPLC was used to determine it in the mice serum at different time after oral administration of Calceolarioside A (group A) and butanol extract (group B, contain the same concentration of Calceolarioside A). The Pharmacokinetic parameters were analyzed by PK-solver. As the results showed, the C_{max} was 661.19±104.07 ng/mL, T_{max} was 1h, AUC_{0-t} was 1958.76±442.18 ng/mL·h, $T_{1/2}$ was 4.46±0.79 h in group A. And in group B, the C_{max}, T_{max}, AUC_{0-t} and $T_{1/2}$ were 1624.92±702.35 ng/mL, 1h, 5854.52±1596.86 ng/mL·h, 2.81±0.65 h, respectively. In conclusion, this method is successfully applied in the pharmacokinetic profile of Calceolarioside A in mice serum.

Introduction

Calceolarioside A is a phenylethanoid glycoside. Most of phenylethanoid glycosides are isolated from herbs and they have exhibited convincing bioactivities, such as hepatoprotective Activities [1, 2] improve glucose tolerance in mice [3], melanogenesis inhibitory activity [4], antioxidant and anti-HBV activity [5], anti-fatigue, neuroprotective, and anti-inflammatory effects in both in vivo and in vitro studies [6].

Calceolarioside A can be isolated from the bark and leaves of *Fraxinus mandshurica* Rupr, which is a traditional Chinese herb medicine. The bark has been widely used for treatment of inflammatory, urinary retention, fever and so on in Chinese folk medicine [7]. *Fraxinus mandshurica* Rupr can alleviate the pain, and significantly inhibit acute and chronic inflammatory reaction [8]. Some compounds have been isolated from the bark of *Fraxinus mandshurica* Rupr, such as calceolarioside A, calceolarioside A-2′-*α*-*L*-rhamnopyranoside, homovanillyl alcohol-4′-glycoside, ligstroside, balanophonin, fraxinol, isofraxidin, fraxetin, aesculetin, mandshurin, isofraxidin-β-D glucoside, fraxin and isoscopoletin-β-D-glucoside [9-11].

The extraction from the leaves of *Fraxinus mandshurica* Rupr and Calceolarioside A could inhibit the IgE (Immunoglobulin E) production in U266 cells [12], it suggests that Calceolarioside A could potentially serve as anti IgE-mediated food allergy. However, there

was no data reported pharmacokinetic of Calceolarioside A, neither oral or inject in animal. In this paper, to study on the pharmacokinetic of Calceolarioside A in mice, experimental mice were given Calceolarioside A and butanol extracts (extract from the bark of *Fraxinus mandshurica* Rupr.) by intragastrical administration. Then the mice sera were determined by HPLC.

Results and Discussion

Pharmacokinetic method Validation HPLC (High Performance Liquid) has been used as an important and useful analytical method in life science for its sensitivity, low detection limitation, fast analysis, selectivity and specificity. The goal of this study was to develop a simple, sensitive, reliable, repeatable and rapid method to determine Calceolarioside A from mice serum samples and analysis the pharmacokinetic of Calceolarioside A in mice.

Selectivity specificity The analysis of Calceolarioside A by HPLC was highly selective with no interfering compounds, and the chromatograms were showed in Fig.1, Calceolarioside A and the internal standard Calceolarioside B were separated well, R (the degree of separation) >1.5. The limit of detection was 10 ng/mL (RSD≥5%), N/S (the value of signal to noise ratio) ⩾3.

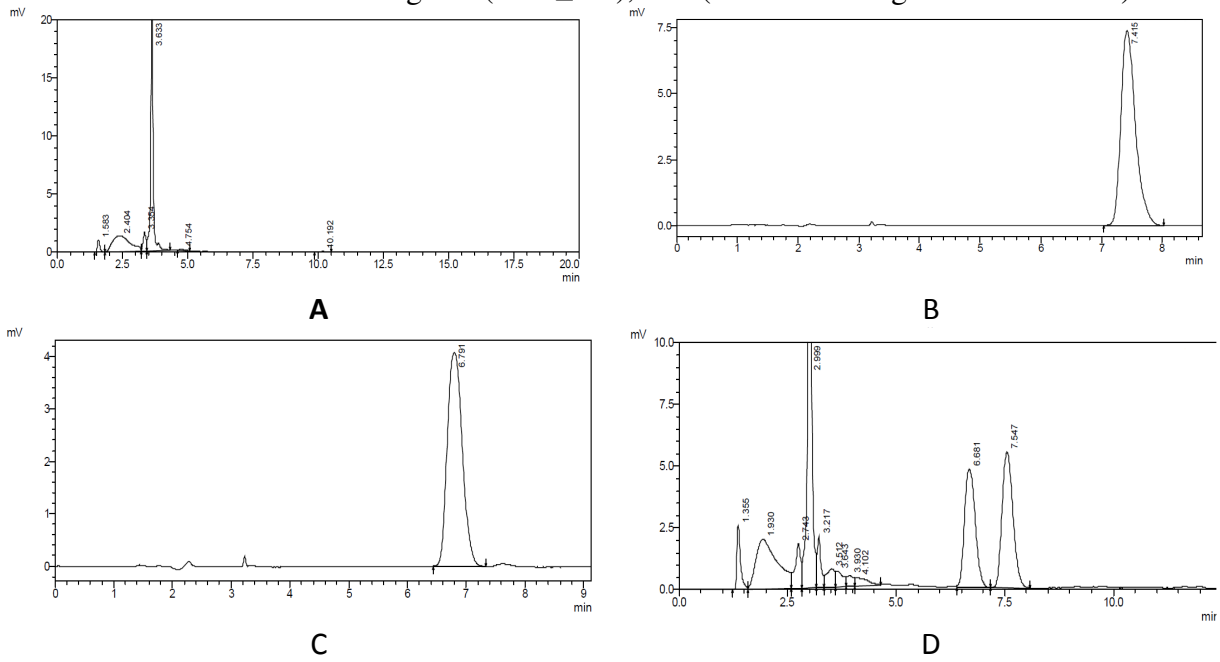

Figure 1. HPLC chromatograms of Calceolarioside A and the internal standard Calceolarioside B.A is the blank serum; B is the standard of Calceolarioside A (t_R =7.415 min); C is the internal standard Calceolarioside B (t_R =6.791 min); D is the mice serum with administration Calceolarioside A (t_R =7.547 min) and extract with internal standard Calceolarioside B (t_R =6.681 min)

Accuracy and Calibration curve Day and intra-day variations were used to validate the accuracy. The accuracy of the method was expressed in terms of relative standard deviation (RSD). The RSD of day and intra-day variations were 2.7% and 3.9%, respectively. Calibration curve was generated to confirm the linear relationship between the peak area of Calceolarioside A /IS and the concentration of Calceolarioside A/IS in the test samples. The standard curve was

obtained as following: y=1.2445x-0.1262, R^2=0.9982. The calibration curve showed good linearity over the range 50-2500 μg/L for Calceolarioside A.

Recovery The extraction recovery was calculated by comparing the peak area of Calceolarioside A and internal standard extracted from mice serum samples and determined by HPLC. The average of extraction recoveries of Calceolarioside A was determined during 90%-105%.

These results showed that HPLC was simple, fast, accurate, reliable and repeatable to analysis Calceolarioside A in the mice serum samples.

Pharmacokinetic study After oral administration of butanol extract and Calceolarioside A, serum samples were collected at different time. Pharmacokinetic analysis was performed using the PK-solver program [13]. The concentration-time curves of butanol extract and Calceolarioside A in mice serum were shown in Fig.2. The main pharmacokinetic parameters are shown in Tab.1 C_{max} (Highest Concentration) of Calceolarioside A in group B was 2.5 times as much as that in group A, and the AUC_{0-t} (Area under curve) in group B was 3 times higher than that in group A. Maybe there were some compounds in the butanol extract which were useful to promote the absorption of Calceolarioside A under oral administration. Synergistic effects were widely used in Traditional Chinese Medicine [14, 15].

As showed in Fig. 2, there were two peaks in concentration-time curve of Calceolarioside A in mice serum; it was possible due to distribution and re-absorption. Therefore, it needed further study to determine the concentration of Calceolarioside A in mice tissues.

Table 1. The Pharmacokinetic parameters of Calceolarioside A in mice following oral administration.

Group	C_{max}(ng/mL)	T_{max}(h)	AUC_{0-t}(ng/mL·h)	$T_{1/2}$(h)
A: Calceolarioside A	661.19±104.07	1	1958.76±442.18	4.46±0.79
B: Butanol extract	1624.92±702.35	1	5854.52±1596.86	2.81±0.65

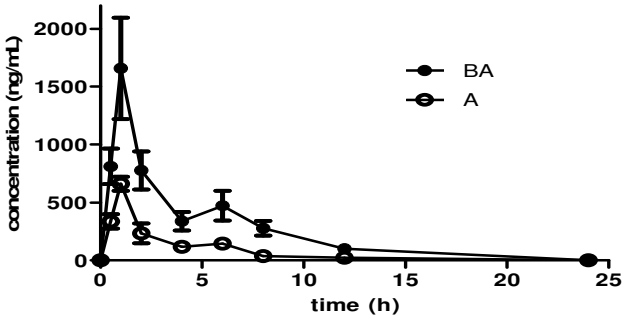

Figure 2. Concentration-time curve of Calceolarioside A in mice serum
A: oral administration of Calceolarioside A alone (50 mg/kg/mice), BA: oral administration of butanol extracts (517mg/kg/mice, containing the same dose of Calceolarioside A as A).

Materials and Methods

Materials Calceolarioside A and was prepared as previously reported [7]. Butanol extract contained the phenylethanoid glycosides (such as Calceolarioside A) extracted by butanol form the bark of *Fraxinus mandshurica* Rupr. The internal standard Calceolarioside B was obtained from Wuhan ChemFaces. Ethanol, ethyl acetate and other chemical reagents were obtained from Beijing Chemical Works.

Animals 20 Swiss mice (20±2g) were purchased from the Animal Experiment Centre of Jilin University (Changchun, China). The mice were housed in groups of five with a standard mouse diet and water, and fasted for 12 hours before Calceolarioside A and butanol extract given. 18 mice were random allocation to 2 groups (group A and group B, 9 mice in each group) and were labeled from 1 to 9 in each group. To collect the blank sera, the remaining two mice were sacrificed without oral neither Calceolarioside A nor butanol extract.

HPLC The concentrations of Calceolarioside A in serum samples were assayed by Shimadzu HPLC (Japan) with LC-20AD double pump, SIL-20A auto-injector, CTO-20A column temperature tank, Inertsil ODS-SP column (5μm, 4.6×250mm), SPD-20A UV detector. The mobile phase was methane and 0.1% phosphoric acid solution (40:60 V/V) and the flow rate was 1.0mL/min. The column temperature was 40°C. The detection wavelength was 350 nm.

Prepare of standard solution The standard stock solution was prepared by dissolved 5mg Calceolarioside A in 100 mL methanol to yield a concentration of 50 mg/L. The internal standard was Calceolarioside B, which was also dissolved by methanol, and the final concentration was 1 g/L. All stock solutions were kept at 4°C before use.

Administration and serum samples preparation In group A, Calceolarioside A was administered intragastrically at a dose of 50 mg/kg/mice on study hour 0. In group B, butanol extract was administered intragastrically at the dose of 517mg/kg/mice contained the same concentration of Calceolarioside A. Blood samples (100 μL) were collected from the facial vein of mice. In both groups, mice labeled 1, 2 and 3 were collected blood at 0 h (prior to administration), 2h, 8h; mice labeled 4, 5 and 6 were collected blood at 0.5h, 4h and 12h; mice labeled 7, 8 and 9 were collected blood at 1h, and 6h and 24h. Keep the blood samples at room temperature for 0.5 h, then the blood samples were centrifuged for 10min at 4,000rpm. The supernatant sera were collected and add the same volume methane. All of the mixtures were mixed by vortexing for 1min, then collect the supernatant and dried by nitrogen gas. The samples were then reconstituted into 50 μL of methane and analyzed by using HPLC.

Pharmacokinetic method validation *Selectivity and specificity* The selectivity of the method was determined by five mouse blank serum samples eluting with Calceolarioside A, and the internal standard. Chromatographic peaks of Calceolarioside A and the internal standard were identified based on their retention times (t_R).

Calibration curve and Accuracy The concentrations of Calceolarioside A which were added to these blank mice serum samples were 50, 100, 500, 1,000, 2000, 2,500 μg/L, respectively, and the IS (Calceolarioside B) were added as 100 μg/L in each mice serum samples. The calibration curve was constructed using vales ranging from 50 μg to 2,500 μg/L of Calceolarioside A in blank mouse serum, and determined six times on the same day for intraday and different days for the interday accuracy test.

Recovery The concentrations of Calceolarioside A at 100 and 500 μg/L were added to mouse blank serum samples respectively. After the serum samples preparation processing and the recovered Calceolarioside A was determined by HPLC.

Pharmacokinetic study The concentrations of Calceolarioside A in the serum of treated group of mice were evaluated using the equation from the standard curves. Pharmacokinetic analysis was performed using PK-solver program.

Statistics

The Pharmacokinetic parameters were analyzed by PK-solver, and other data were analyzed by Microsoft Office Excel 2007 and GraphPad Prism 5.

Acknowledgment

All the authors thank Changchun University of Science and technology for financial support (NO. XJJLG-2016-10).

References

[1] Q. Ma, Y. Guo, W Liu, Z. Wang, W. Mao, X. Zhang, L. Yu, Q. Yang, R. Wei. Phenylethanoid Glycosides from Houttuynia cordata and Their Hepatoprotective Activities. Chem. Nat. Compd. 52 (2016) 761-763.

[2] T. Shen, X. Li, W. Hu, L. Zhang, X. Xu, H. Wu, L. Ji. Hepatoprotective effect of phenylethanoid glycosides from Incarvillea compacta against CCl4-induced cytotoxicity in HepG2 cells. J. Korean. Soc. Appl. Biol. Chem. 58 (4) (2015) 617-625.

[3] T. Morikawa, K. Ninomiya, M.Imamura, J. Akaki, S. Fujikura, Y. Pan, D. Yuan, M. Yoshikawa, X. Jia, Z. Li, O. Muraoka. Acylated phenylethanoid glycosides, echinacoside and acteoside from Cistanche tubulosa, improve glucose tolerance in mice. J. Na.t Med. 68(3) (2014) 561-566.

[4] T. Morikawa, K. Ninomiya, H. Kuramoto, I. Kamei, M. Yoshikawa, O. Muraoka. Phenylethanoid and phenylpropanoid glycosides with melanogenesis inhibitory activity from the flowers of *Narcissus tazettavar. chinensis.* J. Nat. Med. 70 (1) (2016) 89-101.

[5] X.L. Zhou, Q.W. Wen, X. Lin, S.J. Zhang, Y.X. Li, Y.J. Guo, R.B. Huang. A new phenylethanoid glycoside with antioxidant and anti-HBV activity from Tarphochlamys affinis. Arch. Pharm. Res. 37 (5) (2014) 600-605.

[6] S.P. You, J. Zhao, L. Ma, M.Tudimat, S.L. Zhang, T. Liu. Preventive effects of *phenylethanol glycosides* from *Cistanche tubulosa* on bovine serum albumin-induced hepatic fibrosis in rats. DARU. J. Pharm. Sci. 23 (2015) 52.

[7] Y.J. Chen, H.G. Zhang, X. Li. Phenylethanoid glycosides from the bark of Fraxinus mandshurica. Chem. Nat. Compd. 45 (3) (2009) 330-332.

[8] Y.J. Chen, H.G. Zhang, H.T. Wang, L.P. He, F.X. Li. Study on the Anti-inflammatory and Analgesic Effect of Fraxinus Mandshurica Rupr. Lishizhen Medicine and Materia Medica Research; 19 (4) (2008) 780-781

[9] Y.J. Chen, H.T. Wang, L.P. He, H.G. Zhang, J.L. Yin. Study on Chemical Constituents from Bark of *Fraxinus mandschurica* Rupr. Chin. Pharm. J. 44(15) (2009) 1133-1135.

[10] Y.J. Chen, H.T. Wang, L.P. He, H.G. Zhang, J.L. Yin. Study on the Chemical Constituents from the Bark of *Fraxinus mandshurica* Rupr. Lishizhen Medicine and Materia Medica Research. 20(9) (2009) 2146-2147.

[11] I. Kostova, T. Iossifova. Chemical components of Fraxinus species. Fitoterapia. 78 (2007) 85-106.

[12] Y. Chen, G. Xue, F. Liu, X. Gong, Immunosuppressive effect of extracts from leaves of Fraxinus MandshuricaRupr. Bioengineered. 8 (3) (2016) 212-216.

[13] Y. Zhang, M. Huo, J. Zhou, S. Xie, PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput. Methods. Prog. Biomed. 99 (2010) 306-314.

[14] D.L. Wang, X.G. Lu; W.X. Guo, T. Chen, Y. Song, Z.W. Fan. The effect of Chinese Herbal Medicine on Hemorrhagic Shock: A systematic review and meta-analysis. Complement .Ther. Me. 29 (2016) 78-88.

[15] Y.G. Zhang, J.E. Wuman, Energy theory of drug and cooperation mechanism of energy for active ingredients of Chinese materia medica, Chin. Traditl. Herbal. Drugs, 34 (10) (2003) 865-868.