In silico analysis and experimental validation of active compounds from fructus *Schisandrae chinensis* in protection from hepatic injury

S. Y. Wang^{*,a}, L. L. Fu^{†,a}, S. Y. Zhang^{†,a}, M. Tian[†], L. Zhang^{*}, Y. X. Zheng^{*}, J. H. Wang^{*}, J. Huang^{*} and L. Ouyang[‡]

^{*}School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, 110016, China, [†]College of Life Sciences, Sichuan University, Chengdu, 610064, China and [‡]State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, 610041, China

Received 7 August 2014; revision accepted 10 September 2014

Abstract

Objectives: The aim of this study was to explore mechanisms by which fructus *Schisandrae chinensis* (Wuweizi) is able to reveal its protective capacity against hepatocyte injury.

Materials and methods: Identification of candidate small molecular compounds was performed by text-mining, extraction and isolation, reverse-docking, network construction, molecular docking and molecular dynamics (MD) simulation. *In vitro* cytological examination and western blotting were used to validate efficacy of selected compounds.

Results: We analyzed chemical composition of fructus *Schisandrae chinensis* and constructed protein–protein networks of key targets. Networks of miRNA–protein were constructed. Molecular docking and MD simulation results supported good interaction between selected compound 11/12 and GBA3/SHBG. Further *in vitro* examination divulged molecular mechanisms involved.

Conclusions: *In silico* analysis and experimental validation together demonstrated that compound 11/12 of fructus *Schisandrae chinensis* targetted GBA3/SHBG in hepatocytes. Hopefully this will shed light on exploration of its complex molecular mechanisms.

Correspondence: L. Ouyang, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China. Tel.: +86-28-85503817; Fax: +86-28-85503817; E-mail: klivis@163.com; J. Huang, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China. Tel.: +86-24-23993994; Fax: +86-24-23993994; E-mail: 13504051049@163.com; and J.H. Wang, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China. Tel.: +86-24-23993994; Fax: +86-24-23993994; E-mail: 15999290001@163.com.

^aThese authors contributed equally to this work.

Introduction

Fructus *Schisandrae chinensis* (Wuweizi), fruit of *Schisandra chinensis* (Turcz.) Baill, is a traditional Chinese medicine, officially listed as a tonic in the Chinese Pharmacopoeia (1). It was first recorded in the ancient pharmaceutical book “Shen Nong Ben Tsao Ching” as being a superior drug. It has been used for 2000 years, its curative properties being associated with the five traditional tastes, sweet, sour, bitter, pungent and salty. Sour and salty supposedly exert their effects on liver and kidney, bitter and pungent influence heart and lungs, and sweet affects the stomach (2–4). In the long-term, Wuweizi has been claimed to displayed prominent hepatoprotective properties (5), as is ascribed to a variety of chemical constituents. It has been found that it contains lignans, flavonoids, triterpenoids, essential oils, polysaccharides, organic acids and other constituents (6–10). Many reports have suggested protective effects of Wuweizi on the liver to be attributed to its lignan and flavonoid constituents, particularly dibenzocyclooctadiene-type lignans. Schisandrin, schisantherin A, deoxyschisandrin and schisandrin B, have exhibited potent hepatoprotective effects to CCl₄-induced hepatic injury in rats by down-regulation of alanine aminotransferase and aspartate aminotransferase levels (5). In addition, schisandrin A and schisandrin B inhibit activity of CYP3A in rat liver microsomes being both time- and concentration-dependent (9). Deoxyschisandrin has a synergistic effect with tacrolimus, when it used to prevent allograft rejection after liver transplantation, by increasing blood level of tacrolimus, in liver transplant patients (11–13).

Thus, hepatic injury protective effects of fructus *Schisandrae chinensis* seem to be clear. However, its pharmacodynamic material basis and protein interactions still need far more research as its related pathways and mechanisms are far from resolved. In this study, we

gathered some hundreds of small molecule compounds and performed reverse docking networking by *in silico* analysis. We then computationally constructed PPI network of target proteins. Also, combined with prediction of target miRNAs, potential targets (with which compounds interact), were demonstrated. Experimental validation such as MTT and Western blotting were performed to verify the active substance basis of the protection from hepatic injury, of fructus *Schisandrae chinensis*.

Materials and methods

Predictive targets of small molecule compounds

Target proteins of the small molecule compounds, were obtained from the similarity ensemble approach (SEA) (<http://sea.bkslab.org/>) (14). For searching for data from SEA dock, we developed an assistant tool to simplify the process. Copying and pasting such target information is laborious and the assistant tool is able view source code of the results page and test whether content matches the pattern set. If the match is successful, the program copies it into a buffer before filing it. With the help of this tool, the task became inputting a compound and clicking the button. Function of target proteins was screened on Universal Protein Resource (UNIPROT) (15) (<http://www.uniprot.org>). We selected target proteins involved in disease pathogenesis in the liver.

Construction of the target PPI network

In this study, we computationally constructed the PPI network of target proteins. We used PrePPI, a database of predicted and experimentally determined human protein–protein interactions (PPIs) (16,17). Predicted interactions were assigned a likelihood using a Bayesian framework that combines structural, functional, evolutionary and expression information. The unified conceptual framework of PPI network was integrated by Cytoscape (18).

Targeted microRNA prediction

As available prediction methods have strongly varying degrees of sensitivity and specificity, a combination of methods was assumed, to mitigate the problem of presentation of false positives and false negatives, and only accounted for interactions that were predicted by several algorithmically different methods. We utilized miRWalk, a database of predicted and validated microRNA targets (19) (<http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/>). Predicted result from 6 databases,

Diana-MicroH (20), miRanda (21), miRDB (22), miR-Walk, PITA and Targetscan (23) are utilized in miR-Walk. Targets per miRNA by miRWalk are predicted on Promoter, 5'UTR, CDS and 3'UTR of all human genes.

Molecular docking

We selected effective small molecule compounds which already had been reported as ligands, and 3 proteins as receptors. Before docking, we downloaded initial three-dimensional geometric co-ordinates of X-ray crystal structures of compounds and proteins from Protein Data Bank (<http://www.pdb.org/pdb/home/home.do>). Molecular docking was performed using UCSF DOCK6.5 program, with DOCK algorithm to address rigid body docking, by superimposing the ligand on to a negative image of the binding pocket (24–27).

Molecular dynamics simulations

Refinement of 3D structures of complexes was performed using 10 ns molecular dynamic (MD) simulations. MD simulation of a complex was carried out with GROMACS 4.5.4 (28) package using GROMOS96 43a1 force field (29). Lowest binding energy (most negative) docking conformation generated by CDOCKER module embedded in Accelrys Discovery Studio 3.5 was taken as initial conformation for MD simulation. Proteins topology parameters were created using Gromacs program and topology parameters of ligands were built by Dundee PRODRG server. The complex was immersed in a cubic box of simple point charge water molecules. Eight and eleven sodium counter-ions were added by replacing water molecules, to ensure overall charge neutrality of the receptor simulated system respectively. To release conflicting contacts, energy minimization was performed using the steepest descent method of 5000 steps, followed by conjugate gradient method, for 5000 steps. MD simulation studies consisted of equilibration and production phases. To equilibrate the system, the solute was subjected to position-restrained dynamics simulation (NVT and NPT) at 300 K for 300 ps. Finally, the full system was subjected to MD production run at 300 °K and 1 bar pressure 5000 ps. For analysis, atom coordinates were recorded every 0.5 ps during MD simulation.

Preparation of rat hepatocytes

Untreated male rats (250–300 g) ages ranging between 10 and 12 weeks, were used throughout the study. They were allowed tap water and standard granulated diet *ad libitum*, and were maintained under standard light (12/12-h light/dark), temperature (22 ± 2 °C) and

relative humidity ($50 \pm 10\%$). After sacrifice, hepatocytes were sampled and prepared by two-stage collagenase perfusion, containing calcium ions (30), and a cell suspension was obtained by filtration and centrifugation.

Hepatoprotective assay against alcohol or D-GalN induced damage

Hepatocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) heat-inactivated foetal bovine serum and incubated in a humidified incubator with 5% CO₂. They were then transferred to 96-well plates at approximately 5.0×10^4 cells/ml. Cytotoxicity induced by a test compound was measured using the MTT assay as follows: hepatocytes were cultured in DMEM in the presence of 50 μ M test compound for 24 h, then 10 μ l MTT (5 mg/ml) was added to cells in each well. After 4 h culture, medium was removed, and blue formazan crystals that had formed were dissolved in dimethyl sulphoxide. Absorbance of formazan generated from MTT was measured at 570 nm (Bio-Rad Model 680; Bio-Rad, Hercules, CA, USA). Hepatocyte injury was induced by D-GalN (alcohol) in the following manner: after hepatocytes had been incubated for 3 h with 50 μ M test compound was added, dissolved in DMSO (50 mM); cells were then cultured with D-GalN 30 mM (alcohol 1.4 M). After 24 h, hepatocyte viability was detected using the MTT assay. Hepatoprotective effect of the test compound was assessed by cell viability assay and expressed as percentage protection (31–36).

Results

Small-molecule compound screening and target protein prediction

To further verify mechanisms of fructus *Schisandrae chinensis*, we selected 142 reported small molecule compounds from PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>). 420 small molecule target compounds were obtained from SEA. From the results so far, we observed that proteins were targeted by more than one compound. Then, we selected 2 target proteins, GBA3_HUMAN and SHBG_HUMAN, involved in liver disease pathogenesis, depending on function data obtained from UNIPROT. The protein-compound interaction network was constructed using these data (Fig. 1). As available, prediction methods have strongly varying degrees of sensitivity and specificity regarding GBA3 and SHBG. GBA3 is a cytosolic beta-glucosidase probably involved in intestinal absorption and metabolism of dietary flavonoid glycosides. SHBG functions as an androgen transport protein, but may also be involved in receptor-mediated processes. It is a liver-secreted plasma glycoprotein that binds sex steroids. According to traditional Chinese medicine, these proteins contribute to recovery from hepatic injury. Also, we extracted, separated and identified 12 small molecule compounds of fructus *Schisandrae chinensis* (Table 1), of which we found compounds 11 and 12 to target GBA3 and SHBG respectively. As they are specific components of fructus *Schisandrae chinensis*, it is they that might be surmised to have effected the protection from hepatic injury.

Figure 1. Target protein-compound interaction networks. (a) GBA3 protein-compound interaction network. (b) SHBG protein-compound interaction network.

Table 1. Separated and identified compounds of fructus *Schisandrae chinensis*

No.	Name	Structure	Iden. Method
1	wuweizisu C		¹ H-NMR ¹³ C-NMR CD
2	gomisin A		¹ H-NMR ¹³ C-NMR CD
3	(+)-gomisin M ₁		¹ H-NMR ¹³ C-NMR CD
4	(-)-gomisin N		¹ H-NMR ¹³ C-NMR CD
5	(+)-gomisin M ₂		¹ H-NMR ¹³ C-NMR CD
6	γ-schizandrin		¹ H-NMR ¹³ C-NMR CD
7	schizandrin		¹ H-NMR ¹³ C-NMR CD

Table 1b. continued

No.	Name	Structure	Iden. Method
8	(-)-gomisin B		¹ H-NMR ¹³ C-NMR CD
9	β-sitosterol		co-TLC co-mp
10	Spiro[cyclohexane-2-methylene-6,6-dimethyl-1,2'-bicyclo[3.2.0]hept-6'-en-1'-one]		¹ H-NMR ¹³ C-NMR HSQC HMBC
11	Compound 11		¹ H-NMR ¹³ C-NMR HSQC HMBC NOESY HRESI-MS CD
12	Compound 12		¹ H-NMR ¹³ C-NMR HSQC HMBC ¹ H- ¹ H COSY NOESY HRESI-MS

PPI networks of GBA3 and SHBG

Based on PrePPI, the set of true-positive gene pairs was constructed. Physical PPIs of GBA3_HUMAN and SHBG_HUMAN are derived from it, including 5 and 370 protein pairs respectively (Fig. 2). With the networks, we illuminated more molecular mechanisms in protection from hepatic injury.

Targeted microRNA prediction

On the basis of GBA3 and SHBG, we used miRWalk to predicted their miRNAs respectively. Two hundred and

four targeted miRNAs of GBA3_HUMAN were predicted from 5 databases (miRanda, miRDB, miRWalk, PITA and Targetscan). We then integrated these predicted consensus results that 13 miRNAs; hsa-mir-34a, hsa-miR-548c-3p, hsa-miR-640, hsa-miR-1236, hsa-miR-449a, hsa-miR-34c-5p, hsa-miR-548d-3p, hsa-miR-449b, hsa-miR-409-3p, hsa-miR-653, hsa-miR-140-5p, hsa-miR-330-3p and hsa-miR-522, originated from all of them (Fig. 3a). Twenty-five targeted miRNAs of SHBG_HUMAN were predicted from 3 databases (Diana-MicroH, miRanda and miRWalk). Ten are; hsa-mir-330, hsa-mir-326, hsa-mir-148b, hsa-mir-486,

Figure 2. Target PPI networks. (a) GBA3 PPI network. (b) SHBG PPI network.

Figure 3. Target microRNA prediction. (a) GBA3 microRNA prediction. (b) SHBG microRNA prediction.

hsa-mir-637, hsa-mir-661, hsa-mir-760, hsa-mir-23a, hsa-mir-148a and hsa-mir-152 were from 2 databases (Fig. 3b).

Molecular docking and molecular dynamics simulations

From small molecule compounds targeting GBA3, compound 11 was selected for virtual screening with

Figure 4. Target binding analysis. (a, b) Binding modes of compound 11 and compound 12 in GBA3 and SHBG respectively. (c, d) Time-dependence RMSDs of compounds 11 and 12 in 10 ns MD simulations.

molecular docking simulations, due to its effect on hepatic fibrosis. For SHBG, compound 12 was selected, for its hepatic mitochondrial membrane lipid peroxide, and effects of superoxide anion-free radical and antiviral activities. Results of molecular docking indicated that they were both top-scored for target proteins, meaning they indeed interacted with the targets. MD simulations of compound 11/12 and GBA3/SHBG were carried out, to further confirm precise binding mechanisms and interaction stability. As shown in Fig. 4, RMSD of compound 11 remained stable through the whole simulation. Compound 12 reached equilibrium after 700 ps simulation. Generally, maximum RMSD for all cases was limited to 0.3 nm, indicating stabilities of dynamic equilibria of both were reliable. This thus, provides a reliable basis for further analysis (Fig. 4).

Effect of compounds 11 and 12 on disease protection in rat hepatocytes

All three groups were treated with alcohol or D-GalN, which lead to death of hepatocytes. Inhibition ratio of control groups was much higher than of the experimental group. Yet, compounds 11 and 12 reduced inhibition ratio and indicated their protective effect on hepatic injury. When treated with alcohol, inhibition ratio of the control group was 68.0%, experimental group with compound 11 was 46.0% and of compound 12 was 36.3%. When treated with D-GalN, inhibition ratio of the control group was 67.4%, experimental group with compound 11 was 41.2% and compound 12 was 47.4% (Fig. 5a,b). Both GBA3 and SHBG were highly expressed when hepatocytes were injured, but

Figure 5. Experimental verification of targets. (a, b) Damage reduction effects of compound 11 and 12 on primary hepatocytes. (c, d) Primary rat liver cell GBA3 and SHBG western blotting (1) Con. (2) Alcohol (3) Alcohol+compound 11/compound 12 (4) GalN (5) GalN+compound 11/compound 12.

compounds both 11 and 12 can reduced this expression (Fig. 5c,d).

Discussion

As a traditional Chinese medicine, fructus *Schisandrae chinensis* has been accustomed to having great value in protection against hepatic injury. However, as is well-known, traditional Chinese medicine has complex chemical components. It is valuable and essential to identify which small molecule compounds of fructus *Schisandrae chinensis* are active, and relationships amongst their biological targets. In this study, we collected some hundreds of compounds with structural diversity, and separated and identified 12 of them. These were major parts of fructus *Schisandrae chinensis*. After reverse-docking, we predicted target proteins to reveal effects of each compound. Key targets able to protect against hepatic injury were selected according to online databases. The results indicated that compounds 11 and 12 interacted with the targets. We also constructed GBA3 and SHBG-related PPI network to analyze molecular mechanisms involved. Also, we predicted miRNAs based on networks, which may expound more potential regulatory mechanisms in protection against hepatic injury.

To further confirm precise binding mechanisms, molecular docking and molecular dynamic analyses were carried out. With the computational results, we experimentally identified effects of two predicted compounds,

11 and 12. Combined with protective effects on hepatocytes, mechanisms of fructus *Schisandrae chinensis* in protection against hepatic injury became more clear and definite. *In silico* analysis and experimental validation of the compounds shed light on complicated mechanisms involved, which may further disclose signalling pathways in protection against hepatic injury (37,38).

Acknowledgements

This work was supported by grants from the Key Projects of the National Science and Technology Pillar Program (no. 2012BAI30B02), National Natural Science Foundation of China (nos U1170302, 81160543, 81260628, 81303270 and 81172374), and Shenyang Science and Technology Project (F12-157-9-00).

References

- 1 Chinese Pharmacopoeia Commission (2010) Pharmacopoeia of the People's Republic of China Version (2010). *Chin. Med. Sci.* **1**, 1–500.
- 2 Panossian A, Wikman G (2008) Pharmacology of *Schisandra chinensis* Bail.: an overview of Russian research and uses in medicine. *J. Ethnopharmacol.* **118**, 183–212.
- 3 Xu M, Wang G, Xie H, Huang Q, Wang W, Jia Y (2008) Pharmacokinetic comparisons of schizandrin after oral administration of schizandrin monomer, fructus *Schisandrae* aqueous extract and Sheng-Mai-San to rats. *J. Ethnopharmacol.* **115**, 483–488.

- 4 Zhai HQ, Hhang JR, Gao MC, Liu Y, Zhang SF, Wang XH *et al.* (2014) Comparative study between *Ephedra sinica* Stapf and fructus *Schisandrae chinensis* on ET-1 and 6-keto-prostaglandin F1 α in rats with idiopathic pulmonary fibrosis. *Genet. Mol. Res.* **13**, 3761–3771.
- 5 Xie Y, Hao HP, Kang A, Liang Y, Xie T, Sun SQ *et al.* (2011) Integral pharmacokinetics of multiple lignan components in normal, CCl₄-induced hepatic injury and hepatoprotective agents pretreated rats and correlations with hepatic injury biomarkers. *J. Ethnopharmacol.* **131**, 290–299.
- 6 Huang J, Wang JH (2014) CEMTDD: Chinese Ethnic Minority Traditional Drug Database. *Apoptosis* **19**, 1419–1420.
- 7 Ouyang L, Luo Y, Tian M, Zhang SY, Lu R, Wang JH, Kasimu R, Li X (2014) Plant natural products: from traditional compounds to new emerging drugs in cancer therapy. *Cell Prolif.* **47**, 506–515.
- 8 Ma CH, Liu TT, Yang L, Zu YG, Chen XQ, Zhang L *et al.* (2011) Ionic liquid-based microwave-assisted extraction of essential oil and biphenyl cyclooctene lignans from *Schisandra chinensis* Baill fruits. *J. Chromatogr. A* **1218**, 8573–8580.
- 9 Li XN, Lei C, Yang LM, Li HM, Huang SX, Du X *et al.* (2012) Three new aryl-naphthalene lignans from *Schisandra propinqua* var. *Sinensis* *Fitoterapia* **83**, 249–252.
- 10 Li WL, Xin HW, Su MW, Xiong L (2010) Inhibitory effects of schisandrin A and schisandrin B on CYP3A activity. *Methods Find. Exp. Clin. Pharmacol.* **32**, 163–169.
- 11 Xie SA, Li GY, Huang J, Zheng YX, Wang HY, Zhang K *et al.* (2013) A new flavanol from *Cynomorium songaricum*. *J. Asian Nat. Prod. Res.* **15**, 413–416.
- 12 Jiang W, Wang X, Kong L (2010) The effect of deoxyschisandrin on blood tacrolimus levels: a case report. *Immunopharmacol. Immunotoxicol.* **32**, 177–178.
- 13 Lu Y, Chen DF (2008) Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*. *J. Chromatogr. A* **1216**, 1980–1990.
- 14 Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoi-chet BK (2007) Relating protein pharmacology by ligand chemistry. *Nat. Biotechnol.* **25**, 197–206.
- 15 O'Brien KP, Remm M, Sonnhammer EL (2005) Inparanoid: a comprehensive database of eukaryotic orthologs. *Nucleic Acids Res.* **33**, D476–D480.
- 16 Zhang QC, Petrey D, Deng L, Qiang L, Shi Y, Thu CA *et al.* (2012) Structure-based prediction of protein-protein interactions on a genome-wide scale. *Nature* **490**, 556–560.
- 17 Zhang QC, Petrey D, Garzón JI, Deng L, Honig B (2013) Genome network medicine: new diagnostics and predictive tools. *Nucleic Acids Res.* **41**, D828–D833.
- 18 Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D *et al.* (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504.
- 19 Dweep H, Sticht C, Pandey P, Gretz N (2011) miRWalk – database: prediction of possible miRNA binding sites by “walking” the genes of 3 genomes. *J. Biomed. Inform.* **44**, 839–837.
- 20 Maragkakis M, Alexiou P, Papadopoulos GL, Reczko M, Dalamagas T, Giannopoulos G *et al.* (2009) Accurate microRNA target prediction correlates with protein repression levels. *BMC Bioinformatics* **10**, 295.
- 21 Betel D, Wilson M, Gabow A, Marks DS, Sander C (2008) MicroRNA target predictions: the microRNA.org resource: targets and expression. *Nucleic Acids Res.* **36**, D149–D153.
- 22 Wang XW (2008) miRDB: a microRNA target prediction and functional annotation database with a wiki interface. *RNA* **14**, 1012–1017.
- 23 Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **19**, 92–105.
- 24 Li GY, Gao HY, Huang J, Lu J, Gu JK, Wang JH (2014) Hepatoprotective effect of *Cichorium intybus* L., a traditional Uighur medicine, against carbon tetrachloride-induced hepatic fibrosis in rats. *World J. Gastroenterol.* **20**, 4753–4760.
- 25 Lang PT, Brozell SR, Mukherjee S, Pettersen EF, Meng EC, Thomas V *et al.* (2009) DOCK 6: combining techniques to model RNA-small molecule complexes. *RNA* **15**, 1219–1230.
- 26 Dolinsky TJ, Czodrowski P, Li H, Nielsen JE, Jensen JH, Klebe G *et al.* (2007) PDB2PQR: expanding and upgrading automated preparation of biomolecular structures for molecular simulations. *Nucleic Acids Res.* **35**, W522–W525.
- 27 Fu LL, Liu J, Chen Y, Wang FT, Wen X, Liu HQ *et al.* (2014) *In silico* analysis and experimental validation of azelastine hydrochloride (N4) targeting sodium taurocholate co-transporting polypeptide (NTCP) in HBV therapy. *Cell Prolif.* **47**, 326–335.
- 28 Hess B, Kutzer C, van der Spoel D, Lindahl E (2008) GROMACS 4: algorithms for highly efficient, load-balanced and scalable molecular simulation. *J. Chem. Theory Comput.* **4**, 435–447.
- 29 Berendsen HJC, Postma JPM, van Gunsteren WF, Dinola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* **81**, 3684–3690.
- 30 Bonney VR, Becker JE, Walker PR, Potter VR (1974) Primary monolayer cultures of adult rat liver parenchymal cells suitable for study of regulation of enzyme synthesis. *In Vitro* **9**, 399–413.
- 31 Gao HY, Li GY, Lou MM, Li XY, Wei XY, Wang JH (2012) Hepatoprotective effect of matrine salvianolic acid B salt on carbon tetrachloride-induced hepatic fibrosis. *J. Inflamm.* **9**, 16–24.
- 32 Tezuka Y, Kasimu R, Li JX, Basnet P, Tanaka K, Namba T *et al.* (1998) Constituents of roots of *Salvia deserti* Schang. (Xinjiang-Danshen). *Chem. Pharm. Bull.* **46**, 107–112.
- 33 Wu YH, Hao BJ, Cao HC, Xu W, Li YJ, Li LJ (2012) Anti-hepatitis B virus effect and possible mechanism of action of 3,4-O-dicaffeoylquinic acid *in vitro* and *in vivo*. *Evid. Based Complement. Alternat. Med.* **2012**, 2.
- 34 Pan SY, Yu Q, Zhang Y, Wang XY, Sun N, Yu ZL *et al.* (2012) Dietary Fructus *Schisandrae* extracts and fenofibrate regulate the serum/hepatic lipid-profile in normal and hypercholesterolemic mice, with attention to hepatotoxicity. *Lipids Health Dis.* **11**, 120.
- 35 Yan F, Zhang QY, Jiao L, Han T, Zhang H, Qin LP *et al.* (2009) Synergistic hepatoprotective effect of *Schisandrae* lignans with *Astragalus* polysaccharides on chronic liver injury in rats. *Phyto-medicine* **16**, 805–813.
- 36 Ko KM, Ip SP, Poon MK, Wu SS, Che CT, Ng KH *et al.* (1995) Effect of a lignan-enriched fructus *schisandrae* extract on hepatic glutathione status in rats: protection against carbon tetrachloride toxicity. *Planta Med.* **61**, 134–137.
- 37 Wang H, So PK, Yao ZP (2014) Direct analysis of herbal powders by pipette-tip electrospray ionization mass spectrometry. *Anal. Chim. Acta* **809**, 109–116.
- 38 Huang X, Song F, Liu Z, Liu S, Ai J (2011) Comprehensive quality evaluation of Fructus *Schisandrae* using electrospray ionization ion trap multiple-stage tandem mass spectrometry coupled with chemical pattern recognition techniques. *Analyst* **136**, 4308–4315.