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Article

Pharmacokinetics and pharmacodynamics of Astragali Radix-Corni Fructus Herb-pair Extract, in kidney-yin deficiency model

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To investigate the pharmacokinetics and pharmacodynamics of Astragali Radix-Corni Fructus herb-pair in kidney-yin deficiency model, which was made by continuously oral gavage of thyroxine. A simple and rapid LC-MS/MS method was developed and validated for the determination of loganin and morroniside in rat plasma and used for the pharmacokinetics study. The kidney-yin deficiency significantly changed the $AUC_{(0-\infty)}$, C_{max} and CLz/F of loganin and morroniside. The $T_{1/2z}$ of morroniside increased significantly in the kidney-yin deficiency rats. For the pharmacodynamics study, the liver index, kidney index, and ALT, TBIL, UREA, CREA level in the kidney-yin deficiency mice were examined. The herb-pair had been proved to be effective for the treatment of kidney-yin deficiency by affecting the liver, kidney, ALT, UREA and CREA, which showed positively correlated with the dose. The pharmacokinetics and pharmacodynamics studies in the pathological status could offer more valuable information for the future application of Astragali Radix-Corni Fructus herb-pair.

Keywords: Astragali Radix-Corni Fructus herb-pair extract. Kidney-yin deficiency model. LC-MS/MS. Pharmacodynamics. Pharmacokinetics.

INTRODUCTION

Under the pressure of fast-paced life, modern people face with the problems of poor health and the unhealthy lifestyles cause body fluid consumption, dry mouth, irritability, flushing, dreams and other symptoms of kidney-yin deficiency. As known to all, the traditional Chinese medicine (TCM) is widely used as therapeutic products for kidney-yin deficiency. However, there are hardly any herbal drugs used solely in the practice of TCM. The herb-pair, which embodies the characteristics of the compatibility of TCM, is the dominant administration mode.

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Astragali Radix-Corni Fructus, a typical herb-pair, is successfully used for the treatment of diabetic nephropathy. Astragali Radix, the dried root of *Astragalus membranaceus* (Fisch.) Bge or *Astragalus membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao (Fu *et al*., 2015), exhibits a variety of biological activities such as immune regulation (Bedir *et al*., 2000), anti-inflammatory, anti-allergic (Duan *et al*., 2014), antioxidant (Shahzad *et al*., 2016), etc. In recent years, many experimental studies have reported that Astragali Radix and its active extracts have therapeutical effect on diabetic nephropathy because of its renal protective effect (Duan *et al*., 2016). Corni Fructus is the dried sarcocarp of *Cornus officinalis* Sieb. et Zucc (Xiong *et al*., 2015). Various pharmacologic studies have indicated that Corni Fructus has therapeutic effects on anti-neoplastic (Yamabe *et al*., 2007), antimicrobial (Park *et al*., 2013), inhibiting lipid metabolism (Xiong, Li, Zhang, 2016), preventing renal damage (Yokozawa *et al*.,

2008) and so on. Loganin (Figure 1 (I)) and morroniside (Figure 1 (II)) are the main active ingredients from Astragali Radix-Corni Fructus herb-pair, which is the rich source of iridoid glycosides.

In the preliminary experiment, the Astragali Radix-Corni Fructus herb-pair has been proved to be effective for the treatment of kidney-yin deficiency. The primary objective of the currently reported study was to investigate the pharmacokinetics and pharmacodynamics of Astragali Radix-Corni Fructus herb-pair extract in kidney-yin deficiency model. For the pharmacokinetics studies, a HPLC-MS/MS method for the determination of loganin and morroniside in rat plasma was established and validated and it was successfully used to investigate the pharmacokinetics of loganin and morroniside in normal and kidney-yin deficiency rats. For the pharmacodynamics studies, the liver index, kidney index, the ALT, TBIL, UREA and CREA level in kidney-yin deficiency mice were examined. This research might be useful in further application of the Astragali Radix-Corni Fructus herb-pair.

MATERIAL AND METHODS

Chemicals and reagents

Morroniside was extracted and purified from sarcocarp of C. officinalis by the department of pharmacology, Xuanwu Hospital of Capital Medical University and its purity was over 98.5% by HPLC analysis. Loganin (Lot 111640-201606) and peoniflorin (Lot 110736-201539, internal standard, IS, Figure 1 (III)) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. HPLC-grade methanol (Lot 14045085) and acetonitrile (Lot 12105748) were obtained from Tedia (Fairfield, OH, USA). All other chemicals were of analytic grade or better. Astragali Radix-Corni Fructus extract (1:2, Lot SFS170110, the contents of loganin and morroniside were 4.86 mg/g and 6.35 mg/g, respectively) was purchased from Shaanxi Sciphar Natural Products Co., Ltd.. Liu Wei Di Huang Wan (Lot 161215), used as positive agent, was obtained from Zhongjing Wanxi Co., Ltd..

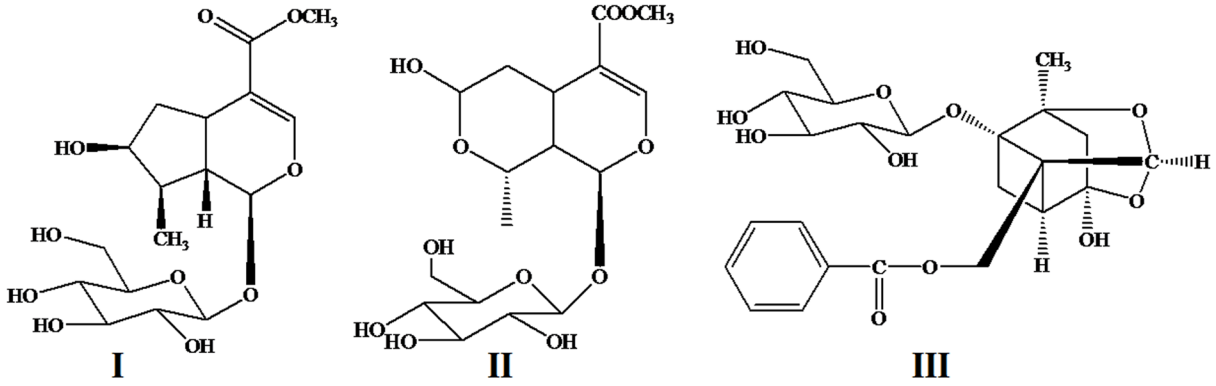

FIGURE 1 - The chemical structures of loganin (I), morroniside (II), and IS (III).

Animals

Ten male SD rats weighing 180 - 220g and fifty male ICR mice weighing 18 - 22g were obtained from Shandong Laboratory Animal Center of Shandong Academy of Medical Sciences [Certificate no. SCXK (Shandong) 2014-0007]. Rats and mice were housed in cages with 12h day light 12h^{-1} night cycle, a temperature of 25 ± 2 °C and relative humidity of 65 ± 5%. All animal were fed with standard diet and had free access to water. All the animal welfare and experimental procedures were approved by the Animal Ethics Committee at School of Pharmacy and Pharmaceutical Sciences & Institute of Materia Medica, Shandong First Medical University & Shandong Academy of Medical Sciences (Jinan, China), which was strictly in accordance with the principles of laboratory animal care (NIH publication no. 85-23, revised 1985).

Equipment and LC–MS/MS conditions

The pharmacokinetics studies were carried out on a TSQ Quantum Ultra LC-MS/MS system with an electrospray ionization source (ESI, Thermo Scientific, USA). The chromatographic separation was carried out at 25 °C on a Hypersil GOLD C18 (50 mm × 2.1 mm, 3 μm, Thermo) column. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.4 mL min^{-1}. The gradient was as follows: 0 min 5% B, 0.2 min 5% B, 1 min 100% B, 1.01 min 5% B, 2 min 5% B, stop. The parameters in the source were set as follows: spray voltage 3250 V, nebulization temperature 200 °C, sheath gas pressure 45 Arb, auxiliary gas pressure 10 Arb, capillary temperature 350 °C. The selected reaction monitoring (SRM) mode of m/z 413.1/219.1 $[M+Na]^+$ for loganin, m/z 428.9/267.0 $[M+Na]^+$ for morroniside, and m/z 502.9/341.1 $[M+Na]^+$ for IS at positive ionization mode were used as quantitative analysis.

Plasma sample preparation

A simple standard protein precipitation method was applied. Frozen plasma samples were thawed at room temperature and vortexed for 60 s. 50 μL of rat plasma was mixed with 150 μL of IS solution (1000 ng mL^{-1} in methanol) and vortex-mixed for 60 s. After centrifuging at 14000 rpm for 10 min at 4 °C, 10 μL of the supernatant was injected onto the LC-MS/MS system for analysis (Zhao *et al*., 2016).

Preparation of calibration standards and quality control samples

The appropriate amounts of loganin and morroniside were accurately weighed and dissolved in methanol to prepare the stock solution, respectively, and then serially diluted with methanol to obtain the working solutions.

The diluted solutions were prepared by spiking appropriate amounts of the standard solutions in blank rat plasma at final concentrations of 5.0, 25, 100, 500, 2500, 5000 ng mL^{-1} for both loganin and morroniside. The quality control (QC) samples were prepared in blank plasma at four different concentration levels, lower limit of quantification (LLOQ, 5.0 ng mL^{-1}), low QC (LQC, 10 ng mL^{-1}), medium QC (MQC, 200 ng mL^{-1}), and high QC (HQC, 4000 ng mL^{-1}), for both loganin and morroniside.

Methodological validation

A method validation of the loganin and morroniside assay in rat plasma was performed according to the China Food and Drug Administration guidelines for pre-clinical pharmacokinetics studies with respect to selectivity, linearity, precision, accuracy, extraction recovery and matrix effect.

Blood sampling

Ten SD rats were randomly assigned to two groups (n = 5). Normal rats and kidney-yin deficiency rats were employed to investigate the pharmacokinetics of loganin and morroniside in Astragali Radix-Corni Fructus herb-pair. The kidney-yin deficiency rats were given thyroxine at the dose of 0.5 g kg^{-1} for 7 consecutive days by oral administration. The rats in normal control groups were given same volume of purified water under the same conditions. On day 8, all rats were fasted overnight with free access to water for at least 12 h and then the rats were received a single oral dose of 9 g kg^{-1} of Astragali Radix-Corni Fructus herb-pair extract. About 150 μL of blood samples were collected before and at 5, 15, 30, 45, 60, 90, 120, 240, 360, 480 and 720 min after administration and the heparin was used as anticoagulant. All the blood samples were centrifuged at 3000 rpm for 15 min and the plasma was immediately separated and frozen at -20 °C until analyzed.

Pharmacodynamics experiment

Fifty mice were used to explore the effect of Astragali Radix-Corni Fructus herb-pair on the kidney-yin deficiency and randomly divided into normal control group, model control group, Liu Wei Di Huang Wan (positive control) group, Astragali Radix-Corni Fructus herb-pair low-dose group and high-dose group. Mice of all groups except the normal control group were modeled by giving thyroxine at the dose of 0.3 g kg^{-1}

for 30 consecutive days orally. Simultaneously, the Liu Wei Di Huang Wan group (0.9 g kg^{-1}), herb-pair low-dose group (6 g kg^{-1}) and high-dose group (9 g kg^{-1}) were administrated accordingly. The normal control group and model control group were given same volume of purified water under the same conditions. On day 31, all mice were fasted overnight with free access to water for at least 12 h and weighed. About 1 mL of blood sample was collected for determining the level of ALT, TBIL, UREA and CREA in serum. The livers and kidneys were excised from the mice, rinsed in ice-cold saline, blotted, and weighed immediately. The collected tissues were used for calculating the liver and kidney indexes (the ratio of liver or kidney weight to body weight) (Ren *et al*., 1999).

Data analysis

Pharmacokinetic parameters were estimated by a noncompartmental method using the Drug and Statistics 2.0 (DAS 2.0) software package (Mathematical Pharmacology Professional Committee of China, Shanghai, China). Statistical analyses were performed using the SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). Student's *t* test was used to compare the two groups. A value of $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Method validation

The selectivity was tested by comparing the chromatograms of the blank plasma sample, blank plasma spiked with loganin, morroniside and IS and plasma sample collected 1 h after oral administration. No interferences from endogenous substances were observed around the retention regions of loganin, morroniside or IS. The retention times of loganin, morroniside and IS were 1.44, 1.26 and 1.84 min, respectively (Figure 2).

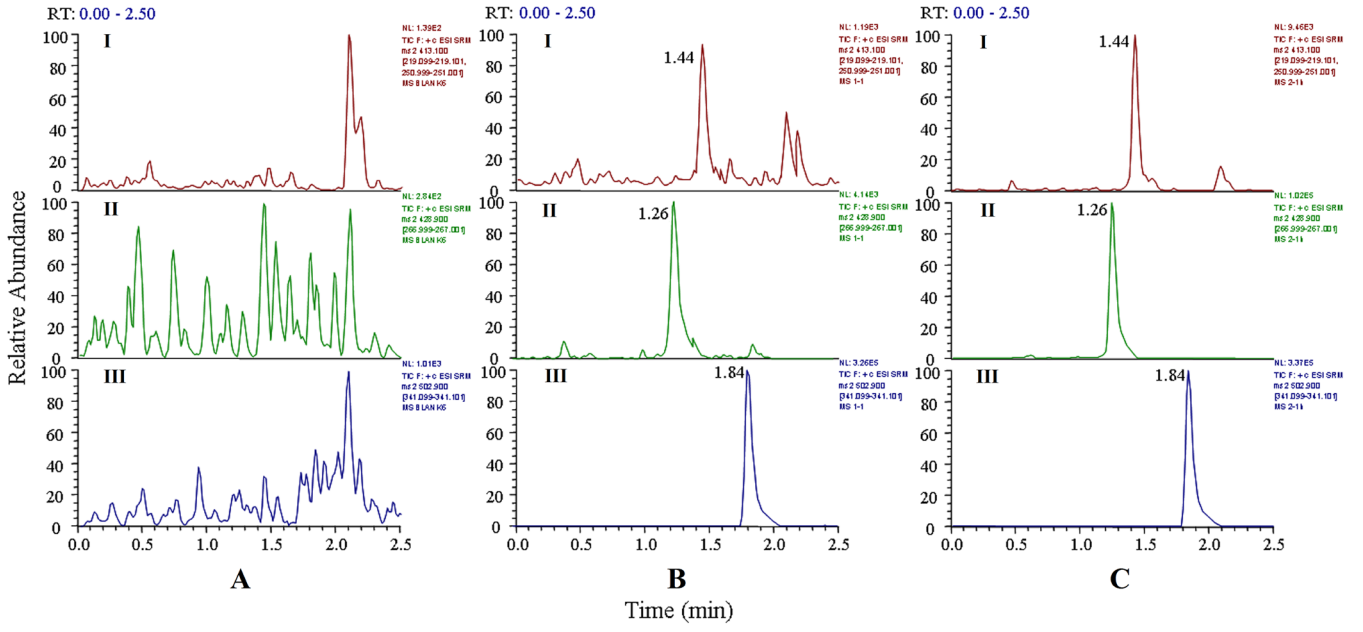

FIGURE 2 - SRM chromatograms for loganin (I), morroniside (II), and IS (III) in rat plasma. (A) Chromatographic profile of blank rat plasma; (B) Chromatographic profile of rat plasma spiked with loganin (I, 5 ng mL^{-1}), morroniside (II, 5 ng mL^{-1}), and IS (III); (C) Chromatographic profile of a plasma sample 1 h after administration of the herb-pair to a rat.

The linear ranges of loganin and morroniside in rat plasma were both from 5.0 to 5000 ng mL^{-1}. The regression equations in rats plasma were $Y = 2.02 \times 10^{-3} + 1.39 \times 10^{-4} X$ ($r^2 = 0.9952$) and $Y = 2.62 \times 10^{-3} + 1.48 \times 10^{-3}$

X (r^2 = 0.9967) for loganin and morroniside, respectively, where *Y* meant the peak area ratio of analytes to IS and *X* equalled the nominal concentration of analytes. In the present study, the precision (RSD) of LLOQ (5.0 ng mL^{-1}) was within ± 15% from the theoretical value.

The results of accuracy and precision calculated by determining QC samples at four concentration levels (LLOQ, LQC, MQC and HQC) are presented in Table I. The results were completely within the acceptance limits (± 15%).

TABLE I - The intra- and inter-day precision and accuracy of the analytes in rat plasma (mean ± SD, 3 days, 6 replicates per day)

Analytes	Spiked conc. (ng mL^{-1})	Intra-day (*n* = 6)			Inter-day (*n* = 18)		
		Measured conc. (ng mL^{-1})	Precision (RSD, %)	Accuracy (%)	Measured conc. (ng mL^{-1})	Precision (RSD, %)	Accuracy (%)
Loganin	5	5.07 ± 0.18	3.51	101.38	——	——	——
	10	9.96 ± 0.61	6.09	99.55	10.06 ± 0.51	5.07	100.56
	200	190.38 ± 8.92	4.69	95.19	201.05 ± 14.20	7.06	100.52
	4000	3566.20 ± 164.33	4.61	89.16	3765.08 ± 248.47	6.60	94.13
Morroniside	5	4.79 ± 0.17	3.59	95.72	——	——	——
	10	8.93 ± 0.46	5.21	89.27	9.57 ± 0.68	7.10	95.72
	200	200.46 ± 6.47	3.23	100.23	200.95 ± 5.10	2.54	100.47
	4000	4305.96 ± 155.67	3.61	107.65	4323.71 ± 112.72	2.61	108.09

The extraction recoveries and matrix effects of the analytes in rat plasma are shown in Table II. The mean recoveries of loganin and morroniside were both more than 74% at three concentration levels (LQC, MQC and HQC). The matrix effect values obtained from the analytes at three concentration levels were in the ranges of 91.68 - 98.58%. The results suggested that the extraction recovery was within the acceptable range and there was no obvious matrix effect on loganin and morroniside in this assay.

TABLE II - Recovery and matrix effect data of the analytes in rat plasma (*n* = 3)

Analytes	Concentration (ng mL^{-1})	Recovery (%)		Matrix effect (%)	
		mean ± SD	RSD	mean ± SD	RSD
Loganin	10	82.71 ± 4.11	4.97	96.66 ± 2.23	2.30
	200	74.59 ± 2.30	3.08	94.80 ± 1.51	1.60
	4000	83.03 ± 1.55	1.87	93.57 ± 0.55	0.58
Morroniside	10	92.34 ± 0.54	0.59	91.68 ± 8.70	9.48
	200	96.43 ± 2.35	2.44	98.58 ± 1.91	1.93
	4000	90.46 ± 1.31	1.45	94.63 ± 2.48	2.62

Pharmacokinetics study

The validated HPLC-MS/MS method for simultaneous determination of loganin and morroniside in rat plasma was successfully applied to the pharmacokinetics studies of Astragali Radix-Corni Fructus herb-pair. The mean plasma concentration-time curves of loganin and morroniside following single oral administration in rats are presented in Figure 3 and Figure 4, respectively. The pharmacokinetics parameters including $AUC_{(0-\infty)}$, $MRT_{(0-\infty)}$, $T_{1/2}$, T_{max}, C_{max}, CLz/F and Vd are summarized in Table III. All results were expressed as arithmetic mean ± standard deviation (SD). After oral administration of the Astragali Radix-Corni Fructus herb-pair extract, the $AUC_{(0-\infty)}$ of loganin and morroniside in the kidney-yin deficiency rats were 64533.66 ± 15739.10 ng mL^{-1} min and 101707.83 ± 55294.20 ng mL^{-1} min, compared with 40593.03 ± 8952.08 ng mL^{-1} min and 39356.46 ± 8206.326 ng mL^{-1} min in normal rats ($P < 0.05$). The kidney-yin deficiency increased the C_{max} of loganin and morroniside ($P < 0.05$). The CLz/F decreased by 36.36% ($P < 0.05$) and 75.18% ($P < 0.01$) in the kidney-yin deficiency rats for loganin and morroniside, respectively. In addition, the $MRT_{(0-\infty)}$ and $T_{1/2z}$ of morroniside in the kidney-yin deficiency rats increased by 67.66% and 117.27% ($P < 0.05$), respectively. Based on the results, the pharmacokinetics characteristics of loganin and morroniside from the Astragali Radix-Corni Fructus herb-pair extract were significantly changed in the kidney-yin deficiency rats compared with that in normal rats.

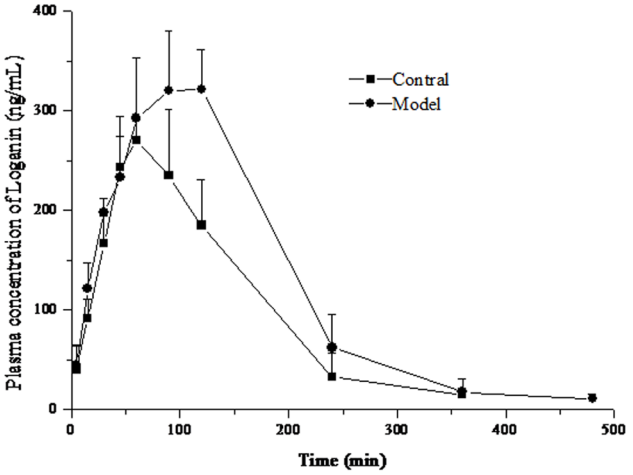

FIGURE 3 - Plasma concentration-time profiles of loganin in normal control and kidney-yin deficiency rats ($n = 5$).

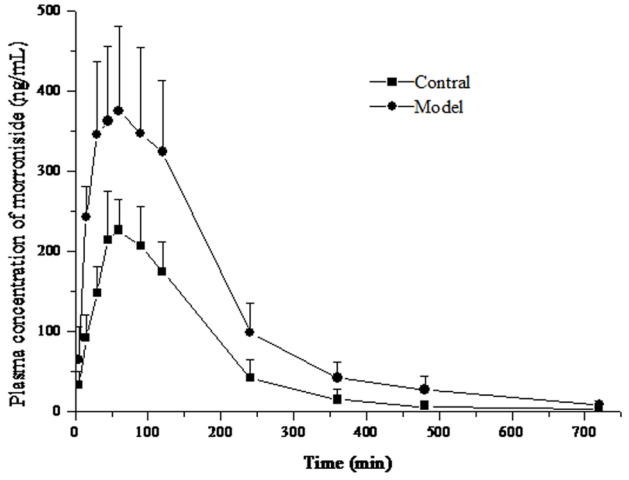

FIGURE 4 - Plasma concentration-time profiles of morroniside in normal control and kidney-yin deficiency rats ($n = 5$).

TABLE III - Main pharmacokinetics parameters for loganin and morroniside in rat plasma after oral administration of Astragali Radix-Corni Fructus herb-pair in normal control and kidney-yin deficiency rats ($n = 5$)

Parameters	Unit	Normal control group		Kidney-yin deficiency group	
		Loganin	Morroniside	Loganin	Morroniside
$AUC_{(0-\infty)}$	ng mL^{-1} min	40593.03 ± 8952.08	39356.46 ± 8206.326	64533.66 ± 15739.10*	101707.83 ± 55294.20*
$MRT_{(0-\infty)}$	min	114.46 ± 27.62	127.93 ± 30.64	131.85 ± 14.34	214.49 ± 85.94
$T_{1/2z}$	min	63.14 ± 21.82	70.77 ± 16.23	69.89 ± 14.42	153.76 ± 51.55*
T_{max}	min	63.75 ± 18.88	71.25 ± 22.50	96.00 ± 25.10	69.00 ± 29.24
C_{max}	ng mL^{-1}	292.70 ± 33.43	250.50 ± 43.44	346.02 ± 32.11*	387.35 ± 98.21*
CLz/F	L min kg	1.10 ± 0.21	2.74 ± 0.59	0.70 ± 0.15*	0.68 ± 0.29**

Data were expressed as mean ± S.D. *$P < 0.05$, **$P < 0.01$ compared with normal control group

Pharmacodynamics study

The liver index, kidney index, the serum ALT, TBIL, UREA and CREA concentrations were determined in all groups as shown in Table IV. The kidney-yin deficiency mice showed increased values of liver index, kidney index, ALT, UREA and CREA concentrations when compared to normal mice ($P < 0.01$). Compared with model control group, Liu Wei Di Huang Wan decreased liver index, kidney index ($P < 0.05$), ALT ($P < 0.01$), UREA ($P < 0.01$) and CREA ($P < 0.05$) level. Astragali Radix-Corni Fructus herb-pair decreased liver index and UREA level in both low and high dose groups as compared with model control group (no significant difference). Meanwhile it decreased kidney index and CREA level in both low and high dose groups as compared with model control group, although significance existed only in high dose group ($P < 0.05$). In addition, Astragali Radix-Corni Fructus herb-pair could significantly decrease the ALT in both two dose groups ($P < 0.05$).

TABLE IV - Effects of Astragali Radix-Corni Fructus herb-pair on the liver index and kidney index, ALT, TBIL, UREA and CREA ($n = 10$)

Groups	Doses	Liver index (%)	Kidney index (%)	ALT (U L^{-1})	TBIL (μmol L^{-1})	UREA (mmol L^{-1})	CREA (μmol L^{-1})
Normal control group		4.27±0.37	1.15±0.08	41.65±6.75	0.95±0.38	5.86±0.89	13.78±3.32
Model control group		5.09±0.82**	1.50±0.16**	74.30±13.68**	1.12±0.90	7.32±1.19**	19.10±2.74**
Liu Wei Di Huang Wan group	0.9 g kg^{-1}	4.55±0.36$^{\Delta}$	1.32±0.15**$^{\Delta}$	43.31±15.17$^{\Delta\Delta}$	1.00±0.52	6.04±0.61$^{\Delta\Delta}$	15.52±1.68$^{\Delta}$
Herb-pair low-dose group	6 g kg^{-1}	4.60±0.33*	1.48±0.17**	56.64±13.44**$^{\Delta}$	1.03±0.75	7.00±0.73**	17.87±2.21**
Herb-pair high-dose group	9 g kg^{-1}	4.52±0.49	1.42±0.19**$^{\Delta}$	52.49±9.02**$^{\Delta}$	1.17±0.40	6.47±0.83	15.75±1.28$^{\Delta\Delta}$

Data were expressed as mean ± S.D. *$P < 0.05$, **$P < 0.01$ compared to normal control group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ compared to model control group

CONCLUSIONS

A simple and rapid LC-MS/MS method was established and validated for the quantification of loganin and morroniside in rat plasma, which was successfully used for the pharmacokinetics researches of loganin and morroniside from the Astragali Radix-Corni Fructus herb-pair in the kidney-yin deficiency model. The kidney-yin deficiency could significantly change the pharmacokinetics characteristics of loganin and morroniside in rats. The Astragali Radix-Corni Fructus herb-pair had effects on kidney-yin deficiency by affecting the liver, kidney, ALT, UREA and CREA, which showed positively correlated with dose. The pharmacokinetics and pharmacodynamics studies in the pathological status could offer more valuable information for the future application of Astragali Radix-Corni Fructus herb-pair.

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