JOMH
Journal of Men's Health

Original Article
DOI: 10.15586/jomh.v16iSP1.235

COMPARATIVE PHARMACOKINETIC AND BIOAVAILABILITY STUDIES OF MONOTROPEIN, KAEMPFEROL-3-*O*-GLUCOSIDE, AND QUERCETIN-4'-*O*-GLUCOSIDE AFTER ORAL AND INTRAVENOUS ADMINISTRATION OF MOTILIPERM IN RATS

Bo Ram Choi[1†], Jae Sung Pyo[2†], Mi Seon Yeo[2], Min-Gul Kim[3], Yu Seob Shin[6], Yu Seob Shin[1], Sung Won Lee[4], Chul Young Kim[5], Insuk So[7], Hye Kyung Kim[2‡], Jong Kwan Park[6‡]

[1]Jaseng Spine and Joint Research Institute, Jaseng Medical Foundation, Seoul, Republic of Korea.

[2]College of Pharmacy and Brain Busan 21 program, Kyungsung University, Busan, Republic of Korea

[3]Department of Pharmacology, School of Medicine, Jeonbuk National University, Jeonju, Republic of Korea

[4]Department of Urology, Samsung Medical Center, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[5]College of Pharmacy, Hanyang University, Ansan, Republic of Korea

[6]Department of Urology, Jeonbuk National University and Research Institute of Clinical Medicine of Jeonbuk National University-Biomedical Research Institute and Clinical Trial Center of Medical Device of Jeonbuk National University Hospital, Jeonju, Republic of Korea

[7]Department of Physiology and Biophysics, Seoul National University College of Medicine, Seoul, South Korea.

[†]These authors contributed equally to this work.

[‡]Hye Kyung Kim and Jong Kwan Park contributed equally to this work.

Corresponding Authors: Hye Kyung Kim: fiona30@ks.ac.kr; **Jong Kwan Park**: rain@jbnu.ac.kr

Submitted: 03 March 2020. Accepted: 20 June 2020. Published: 24 July 2020.

ABSTRACT

Background and Objective

This study has evaluated the pharmacokinetic parameters and bioavailabilities of monotropein, kaempferol-3-O-glucoside, and quercetin-4'-O-glucoside after administration of MOTILIPERM in rats.

Material and Methods

Following the administration of MOTILIPERM, the plasma concentrations of each compound in rats were simultaneously determined by using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results

The pharmacokinetic parameters of monotropein in rats were AUC_{inf} 20,020.44±3944.67 and 11,915.53±1190.91 min·ng/mL and C_{max} 286.99±38.37 and 56.23±9.02 ng/mL for intravenous and oral administration, respectively. The pharmacokinetic parameters of kaempferol-3-*O*-glucoside in rats were AUC_{inf} 287.86±126.17 min·ng/mL and not estimated; C_{max} 5.80±1.87 and 1.24±0.41 ng/mL for intravenous and oral administration, respectively. The pharmacokinetic parameters of quercetin-4'-*O*-glucoside in rats were AUC_{inf} 511.38±248.11 and 481.44±65.72 min·ng/mL; C_{max} 10.72±2.70 and 2.83±0.34 ng/mL for intravenous and oral administration, respectively.

Conclusion

The absolute bioavailabilities of monotropein and quercetin-4'-*O*-glucoside for oral administration were evaluated and calculated as 3.0 and 4.7%, respectively. The absolute bioavailability of kaempferol-3-*O*-glucoside was not calculated because the elimination rate constant could not be estimated. These results may be applied to the basic data in a further study in order to develop functional foods or herbal medicinal products.

Key Words: *bioavailability; kaempferol-3-O-glucoside; monotropein; pharmacokinetic; quercetin-4'-O-glucoside*

INTRODUCTION

Male infertility is a major clinical health issue.[1] About 15% of couples are infertile and 40–50% of these cases are related to male factor.[2] Several studies demonstrated that oral medicines and herbal medicines have been proposed to improve male fertility potential.[3,4] MOTILIPERM is a drug under development by the Dong-A Pharmaceutical Company (Gyeonggi-do, Korea) for the treatment of male infertility. It was prepared through a mixture of three medicinal herb extracts the roots of *Morinda officinalis* How (Rubiaceae), the seeds of *Cuscuta chinensis* Lamark (Convolvulaceae), and the outer scales of *Allium cepa* Linnaeus (Liliaceae).

M. officinalis is a small vine bush plant that is widely found in China, Australia, the Pacific Islands, and other tropical and subtropical regions.[5] The dried roots *of M. officinalis,* locally known as "bajitian," are a well-known traditional oriental medicine.[6] *M. officinalis* has traditionally been used to treat impotence, high blood pressure, osteoporosis, respiratory problems, and inflammatory diseases.[7] Recently, a number of biologically active components have already been isolated from *M. officinalis* roots, such as carbohydrate constituents, anthraquinones, β-sitosterol, rotungenic, iridoid glycosides lignans, and triterpenoids.[8] Monotropein, the main iridoid glycoside isolated from the roots of *M. officinalis* has been reported to have antinociceptive and anti-inflammatory properties.[9,10]

Tu-Si-Zi, which is derived from the seed of *C. chinensis*, is one of the important Chinese traditional medicines widely used to treat deficiencies in the kidney and liver, improve sexual function, and prevent cardiovascular diseases.[11] Previous studies have demonstrated that *C. chinensis* has anticancer, hepaprotective, and neuroprotective effects as well as immunostimulatory activity.[12,13] The active compounds of *C. chinensis* include flavonoids, polysaccharides, lignans, steroids, and others.[14] The ethyl acetate fraction of *C. chinensis* extract showed antioxidant effects with kaempferol-3-*O*-glucoside.[15] Kaempferol-3-*O*-glucoside, the major compound of flavonoids from the seeds of *C. chinensis*, is a traditional herbal medicine that is commonly used as a liver and kidney tonic.[16]

Onion (*A. cepa*) of the Alliaceae family is one of the oldest cultivated plants and is widely

cultivated all over the world.[17] A number of studies have shown that the biological activities of *A. cepa* extract may be beneficial for human health.[18] *A. cepa* extracts have also been found to reduce total blood cholesterol and to have antiasthmatic, anti-inflammatory, and antithrombotic activities due to the inhibition of cyclooxygenase.[17,19] *A. cepa* is a major source of valuable phytonutrients such as flavonoids, fructooligosaccharides, and other sulfur compounds.[20] Olsson et al. have suggested that quercetin-4'-*O*-glucoside and quercetin-3,4-di-glucoside are the two major flavonoids in *A. cepa*.[19] Quercetin-4'-*O*-glucoside is the major flavonoid found in the extracts of onion outer scales that have traditionally been considered waste including and possess antioxidant activity.[21]

In our previous studies, MOTILIPERM were reported to increase the sperm motility in a rat model of cisplatin, finasteride, and varicocele-induced infertility.[22–24] The present study was undertaken to evaluate the pharmacokinetic parameters and bioavailabilities of monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside after oral and intravenous administration of MOTILIPERM in rats.

MATERIALS AND METHODS

Chemicals and Reagents

MOTILIPERM is a mixture of the ethanol extracts of three medicinal herbs (the roots of *M. officinalis*, the seeds of *C. chinensis*, and the outer scales of *A. cepa*). These three extracts were mixed at the ratio of 10:1:10 (w/w/w). Herbs were ground and extracted with ethanol under reflux for 3 h at 70–80°C three times. The combined filtrate was concentrated in a rotary evaporator, freeze dried, and then stored at −20°C until required. This extract was dissolved in sterile normal saline and administrated orally with a Zonde needle (JD-S-124, Jeungdo, Seoul, Korea). The contents of monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside in MOTILIPERM were determined to be 0.67%, 0.04%, and 0.36% (w/w), respectively, by high-performance liquid chromatography (HPLC). The three standards (monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The purity of each standard was more than 99%. Internal standard (desipramine-d_3) was obtained from Cerilliant (Round Rock, Texas, USA). Formic acid and ammonium formate were also purchased from Sigma-Aldrich. Acetonitrile (ACN) was obtained from Honeywell (HPLC Grade, NJ, USA). HPLC-grade water was obtained from Tedia (Fairfield, OH, USA).

Animals

Male Sprague-Dawley rats (300–330 g) were purchased from the KOATECH Animal Company (Gyeonggi-do, Korea). All animal procedures in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Jeonbuk National University and were approved by the Jeonbuk National University Laboratory Animal Center of the Institutional Animal Care and Use Committee (cuh-IACUC-2016-13). All possible efforts were made to minimize animal suffering. All rats were housed in a controlled environment for 1 week; the rats were fed with standard rat chow and had access to water. They were maintained in an animal facility under constant environmental conditions (room temperature 20±2°C, relative humidity 50±10%, and a 12 h light--dark cycle).

In Vivo Pharmacokinetic Study in Rats

Twelve male Sprague-Dawley rats were enrolled in each group for the pharmacokinetics of MOTILIPERM. In Group 1, rats were intravenously administered 20 mg/kg MOTILIPERM into the penis vein. A number of researchers maintained that the penis vein injection for male rats is much easier, more quick, and simpler to accomplish than tail vein injection.[25,26] In Group 2, rats were orally administered 400 mg/

kg MOTILIPERM by oral gavages. Upon the induction of anesthesia with ketamine (30 mg/kg) plus Rompun® (3 mg/kg), a midline incision was made in the neck region, and the jugular vein as well as the contralateral carotid artery were cannulated with PE-50 tubing and flushed with physiologic saline solution containing heparin (10 IU, JW Pharmaceutical, Seoul, Korea). Following intravenous or oral administration, aliquots of 250 μL blood samples were collected in heparinized 1.5 mL polythene tubes at different time intervals postdosing (0, 5, 10, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 600 min). Plasma was separated by centrifugation at 3000 rpm for 30 min and then stored at −60°C until analysis.

Sample Preparation

Plasma proteins were precipitated by adding acetonitrile (400 μL) containing internal standard (IS), desipramine-d_3 (0.5 μg/mL) 25 μL. The sample was mixed using a vortex mixer for 1 min and then centrifuged at 15,000 rpm for 10 min. The supernatant was transferred into another clean glass tube and evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was finally reconstituted with 50 μL of methanol, and an aliquot (5 μL) was subjected to liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.

LC-MS/MS Conditions

LC-MS/MS analysis was performed using an Agilent 1290 infinity UHPLC (Agilent Technologies, Santa Clara, CA, USA) with AB Sciex Qtrap® 4000 MS/MS (Sciex, Foster City, MA, USA). The samples were separated by a Kinetex® Biphenyl 100Å LC column (100 × 2.1 mm, 2.6 μm). The mobile phase consisted of 2 mM ammonium formate containing 0.2% formic acid in D.W. (A) and 2 mM ammonium formate containing 0.2% formic acid in ACN (B) at a flow rate of 400 μL/min. The following gradients of mobile phases were used: 0–1 min, B 2%; 1–12 min, B 2–100%; 12–15 min, B 100%; 15–15.1 min, B 100–2%; and 15.1–17.5 min, B 2%.

The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode. High-purity nitrogen served as both the nebulizing and drying gas. The other parameters of the mass spectrometer were set as follows: drying gas flow, 10 L/min; source temperature, 550°C; nebulizer gas pressure, 40 psi; capillary voltage, 5000 V; and curtain gas pressure, 20 psi.

The analytes were quantified by MRM to product ion transitions and parameters (Table 1): monotropein, *m/z* 408.3→211.0 with declustering potential (DP) 46 V and collision energy (CE) 17 eV; kaempferol-3-*O*-glucoside, *m/z* 447.1→283.8 with

TABLE 1 Optimized Mass Spectrometry Parameters

Analytes	RT	Q1 (Da)	Q3 (Da)	Time (ms)	DP	EP	CE	CXP
Monotropein 1	0.71	408.3	211.0	50	46	10	17	36
Monotropein 2			193.0	50	46	10	23	34
Kaempferol-3-*O*-glucoside 1	5.79	447.1	283.8	50	−96	−10	−38	−14
Kaempferol-3-*O*-glucoside 2			254.9	50	−96	−10	−56	−13
Quercetin-4'-*O*-glucoside 1	4.59	465.2	303.0	50	106	10	31	16
Quercetin-4'-*O*-glucoside 2			228.9	50	106	10	71	38
Desipramine-d_3 1	5.97	270.2	75.0	50	106	8.5	25	4
Desipramine-d_3 2			193.1	50	106	8.5	51	4

RT = retention time; DP = declustering potential; EP = entrance potential; CE = collision energy; CXP = collision cell exit potential.

DP -96 V and CE -38 eV; quercetin-4'-*O*-glucoside, *m*/*z* 465.2→303.0 with DP 106 V and CE 31 eV; and IS, desipramine-d_3, *m*/*z* 270.2→75.0 with DP106 V and CE 25 eV, respectively. Data acquisition was performed with Analyst® 1.6 software (AB Sciex, USA).

Method Validation

Method validation was carried out in terms of precision, accuracy, linearity, matrix effect, recovery, and analyte stability. Calibration curves were prepared at the concentrations of 0.1, 0.2, 0.5, 1, 2, 10, 50, and 100 ng/mL under plasma concentrations for monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside, respectively. These standards were spiked in 100 μL plasma. The biologic samples were stored at −20°C.

The precision and accuracy were evaluated both intraday and interday and stated as the coefficient of variation (CV). Stability was determined as the ability of each analyte to maintain its chemical properties in a biometric matrix. To measure stability, three concentrations of each compound were prepared in three conditions (short-term, long-term, and three freeze-thaw cycle). The short-term stabilities of the plasma samples were studied for periods of 7 h in room temperature (25°C). The samples of freeze and thaw stability were stored at −20°C for 3 day freeze and thaw cycles. The long-term stability was examined after 3 weeks of storage at −20°C. Three conditions of the spiked samples of standard and IS were analyzed in the autosampler.

The matrix effects and recoveries of the biologic samples were investigated with five different sources of blank rat plasma at three concentrations. For matrix effects, the peak areas of the plasma samples spiked after deproteinization were compared with the peak areas of the neat standard. The recovery was determined by comparing the peak areas of the plasma samples spiked before and after deproteinization.

Pharmacokinetic Assessments

The individual pharmacokinetic parameters were obtained through noncompartmental methods using Phoenix® WinNonlin® (Pharsight Corporation, Mountain View, CA, USA). The maximum plasma concentration (C_{max}) and T_{max} (time to reach C_{max}) were obtained directly from the observed values. The area under the curve (AUC) from time 0 to the last measurable time (AUC_{last}) was calculated using the linear trapezoidal rule. AUC_{inf} was calculated using the following equation: $AUC_{last} + (C_{last}/k_e)$, where C_{last} is the last measureable concentration and the elimination rate constant (k_e) describes the rate of decrease in concentration per unit time, which is estimated from the log-linear terminal part of the concentration–time curve as the slope of the natural logarithm of the concentration against time. The elimination half-life ($t_{1/2}$) of a substance is the amount of time required for its plasma concentration to decrease by 50%, calculated as $\ln(2)/k_e$. The plasma concentrations for monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside that were below the limit of quantification following drug administration were assigned a value of 0 if collected prior to C_{max}, and were treated as missing values if collected after C_{max}. The pharmacokinetic parameters were summarized as the mean±SD, except for T_{max}, for which the median (minimum ~ maximum) values are given. The absolute bioavailability was calculated by the dose-normalized ratios of AUC_{inf} following oral administration divided by the intravenous $AUC_{inf.}$

RESULTS

Method Validation

Figure 1 shows the MRM chromatograms of three standards (monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside) and IS (desipramine-d_3) for blank plasma (Figure 1a), blank plasma spiked 50 ng/mL (Figure 1b), intravenous sample plasma (Figure 1c), and oral sample plasma (Figure 1d).

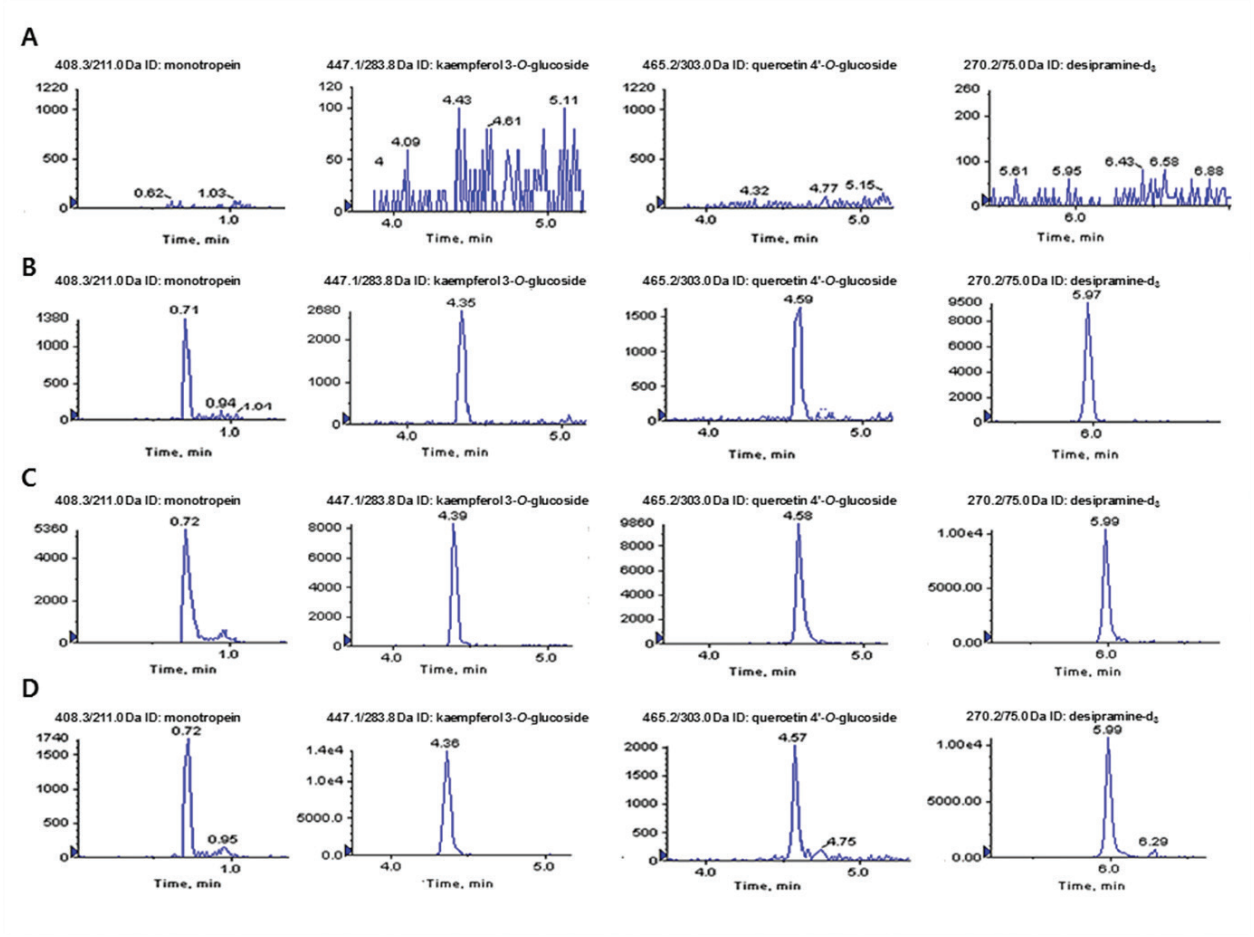

FIG. 1 Multiple reaction monitoring (MRM) chromatograms of three standards (monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside) and IS (desipramine-d_3). (a) Blank plasma. (b) Blank plasma spiked 50 ng/mL. (c) Intravenous sample plasma. (d) Oral sample plasma.

The lower limit of detection (LLOD) was set at 0.03 ng/mL for kaempferol-3-*O*-glucoside and 0.2 ng/mL for monotropein and quercetin-4'-*O*-glucoside, with a signal to noise (S/N) ratio of 3. The lower limit of quantification (LLOQ) was set at 0.1 ng/mL for kaempferol-3-*O*-glucoside and 0.5 ng/mL for monotropein and quercetin-4'-*O*-glucoside, with an S/N ratio of 10.

The calibration curves were characterized by six points (kaempferol-3-*O*-glucoside: 0.1, 0.2, 0.5, 1, 10, and 50 ng/mL; monotropein and quercetin-4'-*O*-glucoside: 0.5, 1, 2, 10, 50, and 100 ng/mL). The regression equation was $y=0.0096x-0.0019$ ($R^2=0.9999$, n=4) for monotropein, while for kaempferol-3-*O*-glucoside, the regression equation was $y=0.0063x-0.0018$ ($R^2=0.9998$, n=4) and for quercetin-4'-*O*-glucoside, the regression equation was $y=0.0075x+0.0003$ ($R^2=1$, n=4). The calibration curves showed good linearity in the studied range. The correlation coefficients were higher than 0.9998 for all of the samples. The intraday and interday precision and accuracy were each evaluated at three concentrations. All CV values of precision and accuracy

were lower than 15% in the low, medium, and high concentrations. The results are shown in Table 2.

The matrix effects of monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside were: monotropein 106.18±10.47%, 110.87±4.04%, and 100.97±1.86% at 0.5, 10, and 100 ng/mL concentrations, respectively; kaempferol-3-*O*-glucoside 102.53±3.77%, 106.57±5.34%, and 100.18±5.97% at 0.1, 1, and 10 ng/mL concentrations, respectively; and quercetin-4'-*O*-glucoside 103.93±1.89%, 101.23±3.21%, and 101.08±0.68% at 0.5, 10, and 100 ng/mL concentrations, respectively.

The mean recoveries of monotropein (100.85–110.15%), kaempferol-3-*O*-glucoside (96.14 ~ 100.57%), and quercetin-4'-*O*-glucoside (100.58–103.16%) at low, medium, and high concentrations are summarized in Table 3.

TABLE 2 Accuracy and Precision of Monotropein, Kaempferol-3-*O*-glucoside, and Quercetin-4'-*O*-glucoside

Compounds	Concentration	Accuracy (%)		Precision (CV, %)	
	(ng/mL)	Intraday*	Interday**	Intraday*	Interday**
Monotropein	0.5	112.66	101.05	11.4	12.35
	10	97.85	109.04	9.27	8.87
	100	89.79	99.72	9.45	9.96
Kaempferol-3-*O*-glucoside	0.1	96.27	93.26	1.77	4.89
	1	102.85	101.72	3.00	5.50
	10	97.18	96.81	2.48	2.39
Quercetin-4'-*O*-glucoside	0.5	102.21	107.46	14.32	14.73
	10	102.87	109.89	6.61	6.93
	100	96.69	103.70	8.05	10.56

**Five times per day. **Five times analysis of standards per day for 3 days.*
CV = coefficient of variation.

TABLE 3 Matrix Effects and Recovery at LOQ, Medium and High Concentration

Compounds	Concentration (ng/mL)	Mean Matrix Effect (%)	CV, %	Mean Recovery (%)	CV, %
Monotropein	0.5	106.18	10.47	110.15	11.14
	10	110.87	4.04	100.85	0.18
	100	100.97	1.86	101.04	0.35
Kaempferol-3-*O*-glucoside	0.1	102.53	3.77	100.57	5.27
	1	106.57	5.34	96.53	2.33
	10	100.18	5.97	96.14	2.85
Quercetin-4'-*O*-glucoside	0.5	103.93	1.89	103.10	2.71
	10	101.23	3.21	103.16	1.54
	100	101.08	0.68	100.58	0.35

LOQ = limit of quantification; CV = coefficient of variation.

The stabilities of monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside are shown in Table 4. Regarding short-term stability, monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside were all stable after 7 h of storage at room temperature. For measuring long-term stability, the three standards were measured after 3 weeks of storage at −20°C. The 3 day freeze/thaw cycle stabilities in plasma were all stable for 3 days.

Pharmacokinetic Assessments

The mean plasma concentration–time profiles of monotropein (Figure 2a), kaempferol-3-*O*-glucoside (Figure 2b), and quercetin-4'-*O*-glucoside (Figure 2c) following the intravenous administration of MOTILIPERM 20 mg/kg and oral administration of MOTILIPERM 400 mg/kg were obtained in Figure 2. The pharmacokinetic parameters of monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside are summarized in Table 5. The mean±SD values of monotropein for C_{max}, AUC_{last}, AUC_{inf}, and $t_{1/2}$ following the intravenous administration of MOTILIPERM at 20 mg/kg were 286.99 ± 38.37 ng/mL, 19,180.83±4003.93 min·ng/mL, 20,020.44±3944.67 min·ng/mL, and 184.07±135.05 min, respectively. The mean±SD values of monotropein for C_{max}, AUC_{last}, AUC_{inf}, and $t_{1/2}$ following the oral administration of MOTILIPERM at 400 mg/kg were 56.23±9.02 ng/mL, 10,343.57±1135.94 min·ng/mL, 11,915.53±1190.91 min·ng/mL, and 236.40±23.85 min, respectively. The mean±SD values of kaempferol-3-*O*-glucoside for C_{max}, AUC_{last}, AUC_{inf}, and $t_{1/2}$ following the intravenous administration of MOTILIPERM at 20 mg/kg were 5.80±1.87 ng/mL, 217.68±97.15 min·ng/mL, 287.86±126.17 min·ng/mL, and 71.48±30.64 min, respectively. The mean±SD values of kaempferol-3-*O*-glucoside for C_{max} and AUC_{last} following the oral administration of MOTILIPERM at 400 mg/kg were 1.24±0.41 ng/mL and 68.89±33.12 min·ng/mL, respectively. The AUC_{inf} and $t_{1/2}$ of kaempferol-3-*O*-glucoside were not calculated because the elimination rate constant could not be estimated. The mean±SD values of quercetin-4'-*O*-glucoside for C_{max}, AUC_{last}, AUC_{inf}, and $t_{1/2}$ following the intravenous administration of MOTILIPERM

TABLE 4 Stabilities of Short, Long, and Freeze/Thaw

Compounds	Concentration (ng/mL)	Short Term (RSD %)	Long Term (RSD %)	Freeze/Thaw Cycle (RSD %)
Monotropein	0.5	3.37	3.66	1.25
	10	1.16	1.03	1.21
	100	2.18	2.22	2.01
Kaempferol-3-*O*-glucoside	0.1	1.43	3.60	1.50
	1	2.51	4.28	3.31
	10	0.66	1.47	2.26
Quercetin-4'-*O*-glucoside	0.5	3.80	1.42	1.14
	10	1.72	1.76	3.81
	100	4.34	2.55	3.90

Short term = 7 h at 25°C; Long term = 3 weeks at −20°C; Freeze/thaw cycle = 3 days at 25°C/−20°C; RSD % = intra- and interday precisions.

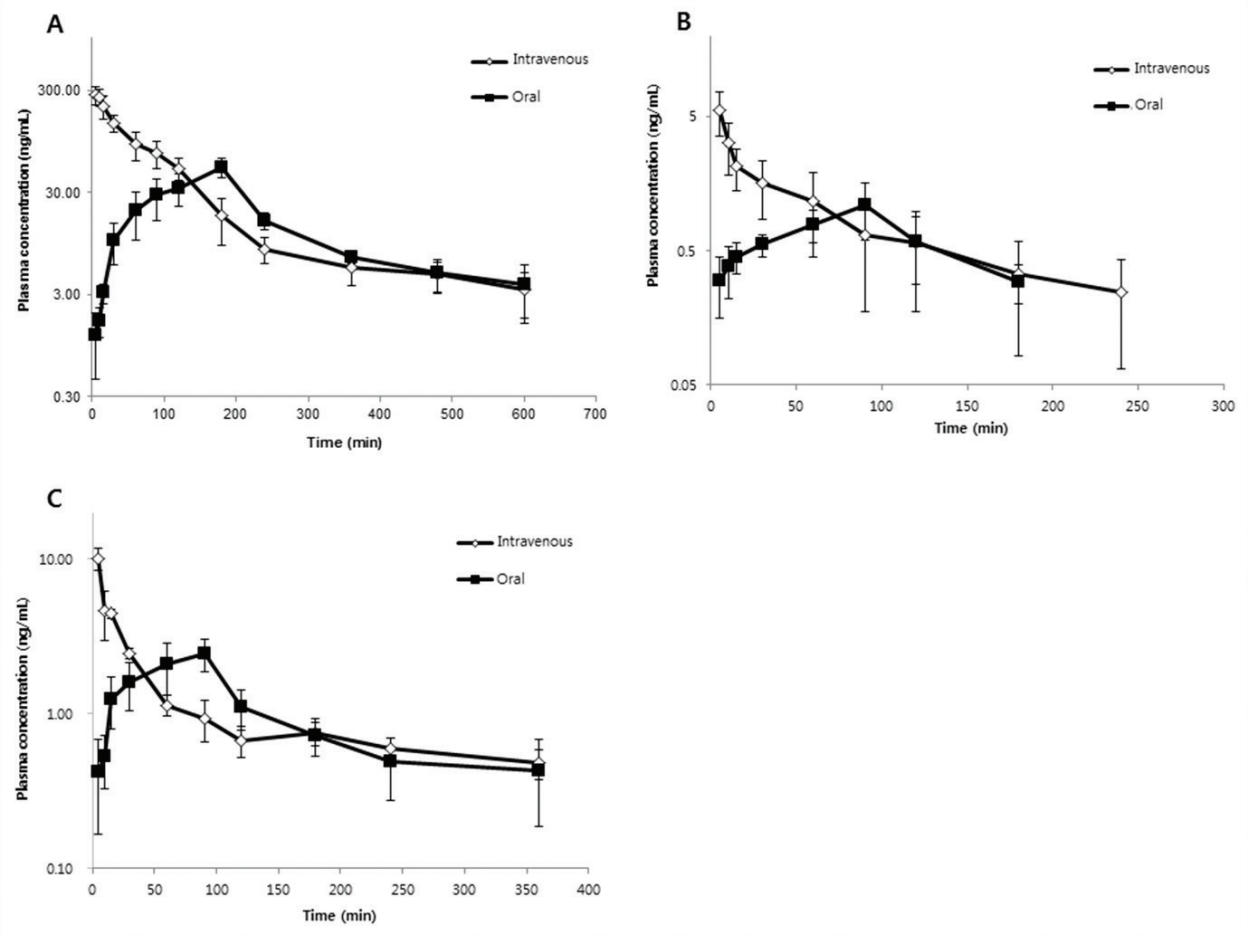

FIG. 2 Mean plasma concentrations versus time profiles of (a) monotropein, (b) kaempferol-3-*O*-glucoside, and (c) quercetin-4'-*O*-glucoside in rats following the intravenous administration of MOTILIPERM at 20 mg/kg and oral administration of MOTILIPERM at 400 mg/kg.

at 20 mg/kg were 10.72±2.70 ng/mL, 368.91±78.0 5 min·ng/mL, 511.38±248.11 min·ng/mL, and 140.40±182.15 min, respectively. The mean±SD values of quercetin-4'-*O*-glucoside for C_{max}, AUC_{last}, AUC_{inf}, and $t_{1/2}$ following the oral administration of MOTILIPERM at 400 mg/kg were 2.83±0.34 ng/mL, 354.63±32.29 min·ng/mL, 481.44±65.72 min·ng/mL, and 125.46±57.15 min, respectively. The absolute bioavailabilities of monotropein and quercetin-4'-*O*-glucoside for oral administration were 3.0% and 4.7%, respectively.

DISCUSSION

The pharmacokinetic parameters of MOTILIPERM after oral and intravenous administration to rats including bioavailability were revealed in the present study. These parameters are valuable in further study related to its toxicity in blood levels and determination of optimum dosage regimens.

Several studies have shown that oral and intravenous administration is adopted in most of the pharmacokinetics and bioavailability studies.[27–29]

TABLE 5 Pharmacokinetic Parameters for Monotropein, Kaempferol-3-*O*-glucoside, and Quercetin-4'-*O*-Glucoside in Rats after Intravenous Administration of MOTILIPERM at 20 mg/kg and Oral Administration of MOTILIPERM at 400 mg/kg

Pharmacokinetic Parameters	Intravenous Administration	Oral Administration
Monotropein		
C_{max} (ng/mL)	286.99±38.37	56.23±9.02
AUC_{last} (min·ng/mL)	19,180.83±4003.93	10,343.57±1135.94
AUC_{inf} (min·ng/mL)	20,020.44±3944.67	11,915.53±1190.91
T_{max} (min)*	5	180
$t_{1/2}$ (min)	184.07±135.05	236.40±23.85
Clearance (mL/min)	2.10±0.38	70.91±7.26
Volume of Distribution (mL)	556.47±423.38	24,093.12±2696.55
Absolute Bioavailability (%)	--	3.0
Kaempferol-3-*O*-glucoside		
C_{max} (ng/mL)	5.80±1.87	1.24±0.41
AUC_{last} (min·ng/mL)	217.68±97.15	68.89±33.12
AUC_{inf} (min·ng/mL)	287.86±126.17	NE**
T_{max} (min)*	5	90 (60–90)
$t_{1/2}$ (min)	71.48±30.64	NE**
Clearance (mL/min)	10.11±4.65	NE**
Volume of Distribution (mL)	896.83±356.66	NE**
Absolute Bioavailability (%)	--	NE**
Quercetin-4'-*O*-glucoside		
C_{max} (ng/mL)	10.72±2.70	2.83±0.34
AUC_{last} (min·ng/mL)	368.91±78.05	354.63±32.29
AUC_{inf} (min·ng/mL)	511.38±248.11	481.44±65.72†
T_{max} (min)*	5 (5–10)	90 (60–90)
$t_{1/2}$ (min)	140.40±182.15	125.46±57.15†
Clearance (mL/min)	50.35±20.50	943.876±129.49
Volume of Distribution (mL)	6647.87±4718.49	163,945.2±58,326.99
Absolute Bioavailability (%)	--	4.7

Values are the arithmetic means±SD.

**Median (minimum ~ maximum); **NE (Not estimated): the elimination rate constant cannot be estimated; †n=6: the elimination rate constant of one rat cannot be estimated.*

C_{max} = maximum plasma concentration; AUC_{last} = area under the curve from time 0 to the last measurable time; AUC_{inf} = calculated using the following equation $AUC_{last} + (C_{last}/k_e)$; T_{max} = time to reach, $C_{max} = t_{1/2}$, the elimination half-life.

Therefore, the bioavailability of MOTILIPERM is determined as the ratio of the normalized area under plasma concentration–times curves after oral and intravenous dosing, although bioavailability determined in this way is usually very variable.

MOTILIPERM was prepared through a mixture of three medicinal herb extracts of the roots

of *M. officinalis*, the seeds of *C. chinensis*, and the outer scales of *A. cepa*. A number of chemical constituents have been isolated from herb extracts. We selected active compounds (monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside) of each herb extracts because it was possess bioactivities effect. Monotropein has been reported to have anti-inflammatory and antinociceptive properties.[9,10] Kaempferol-3-*O*-glucoside is a traditional herbal medicine that is commonly used as a kidney and liver tonic.[16] Quercetin-4'-*O*-glucoside have been possess antioxidant activity.[21] Several studies on the quantification of monotropein from *M. officinalis* using HPLC have been published.[30,31] According to a prior pharmacokinetic study, many other compound were separated *C. chinensis*, such as hyperin, chlorogenic acid, neochlorogenic acid, p-coumaric acid, astragalin, and isoquercitrin.[29] But there is not any study about the pharmacokinetic and bioavailability of quercetin-4'-*O*-glucoside *in vivo*. We selected MOTILIPERM concentration because its doses of 200 mg/kg orally administered once daily for 28 days significantly improved infertility in a rat model of varicocele-induced infertility.[23] According to a prior study, there was a 10-fold difference between oral and intravenous concentration.[32,33] Plasma concentration versus time profiles of kaempferol-3-O-glucoside in rats were not estimated for oral administration 200 mg/kg because it mixed at the ratio of 1. Therefore, we used orally administered 400 mg/kg.

Infertility affects 9%–15.8% of the general population and male factors contribute to about approximately half.[34,35] There are no oral medicines and herbal medicines for the treatment of male infertility.[36] Our previous study reported that MOTILIPERM acted to improve sperm motility by reactive oxygen species-dependent endoplasmic reticulum stress pathway and decreasing apoptosis in a rat infertility model.[22–24] To the best of our knowledge, this is the first report presenting the pharmacokinetic parameters of MOTILIPERM.

CONCLUSIONS

In the present study, an LC-MS/MS method for the simultaneous detection of MOTILIPERM was developed and fully validated. This method was also successfully applied to a pharmacokinetic study on oral and intravenous administration. This method potentially offers a sort of platform for the clinical study of MOTILIPERM. The pharmacokinetic parameters and bioavailabilities of monotropein, kaempferol-3-*O*-glucoside, and quercetin-4'-*O*-glucoside were also reported here for the first time. The results may be effective for promoting the further study of functional foods or herbal medicinal products and may also provide valuable knowledge for pharmacokinetic investigation.

FUNDING

This study was supported by grants from the Korean Healthcare Technology R&D Project, Ministry for Health, Welfare, & Family Affairs, Republic of Korea (HI14C0018 and HI17C0982).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The Korean Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the Korean Healthcare Technology R&D Project, Ministry for Health, Welfare, & Family Affairs, Republic of Korea. All researchers are receiving support from this grant, but there are no financial or other potential conflicts of interest to declare.

REFERENCES

1. Makker K, Agarwal A, Sharma R. Oxidative stress & male infertility. Indian J Med Res 2009;129(4):357–67.
2. Dohle G, Colpi G, Hargreave T, et al. EAU guidelines on male infertility. Eur Urol 2005; 48(5):703–11. https://doi.org/10.1016/j.eururo.2005.06.002
3. Bae WJ, Ha U, Choi JB, et al. Protective effect of decursin extracted from *Angelica gigas* in male infertility via Nrf2/HO-1 signaling pathway. Oxid Med Cell Longev 2016;2016:1–9. https://doi.org/10.1155/2016/5901098
4. Clark NA, Will M, Moravek MB, et al. A systematic review of the evidence for complementary and alternative medicine in infertility. Int J Gynaecol Obstet 2013;122(3):202–6. https://doi.org/10.1016/j.ijgo.2013.03.032
5. Zhang Z, Zhang Q, Yang H, et al. Monotropein isolated from the roots of *Morinda officinalis* increases osteoblastic bone formation and prevents bone loss in ovariectomized mice. Fitoterapia 2016;110:166–72. https://doi.org/10.1016/j.fitote.2016.03.013
6. Yoshikawa M, Yamaguchi S, Nishisaka H, et al. Chemical constituents of Chinese natural medicine, *Morindae Radix*, the dried roots of *Morinda officinalis* How: Structures of morindolide and morofficinaloside. Chem Pharm Bull (Tokyo) 1995;43(9):1462–5. https://doi.org/10.1248/cpb.43.1462
7. Zhu M, Wang C, Wang X, et al. Extraction of polysaccharides from *Morinda officinalis* by response surface methodology and effect of the polysaccharides on bone-related genes. Carbohydr Polym 2011;85(1):23–8. https://doi.org/10.1016/j.carbpol.2011.01.016
8. Yang Z, Hu J, Zhao M. Isolation and quantitative determination of inulin-type oligosaccharides in roots of *Morinda officinalis*. Carbohydr Polym 2011;83(4):1997–2004. https://doi.org/10.1016/j.carbpol.2010.11.006
9. Choi J, Lee KT, Choi MY, et al. Antinociceptive, anti-inflammatory effect of Monotropein isolated from the root of *Morinda officinalis*. Biol Pharm Bull 2005;28(10):1915–8. https://doi.org/10.1248/bpb.28.1915
10. Shin JS, Yun KJ, Chung KS, et al. Monotropein isolated from the roots of *Morinda officinalis* ameliorates proinflammatory mediators in RAW 264.7 macrophages and dextran sulfate sodium (DSS)-induced colitis via NF-kappaB inactivation. Food Chem Toxicol 2013;53:263–71. https://doi.org/10.1016/j.fct.2012.12.013
11. Yen FL, Wu TH, Lin LT, et al. Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis*. Food Chem 2008;108(2):455–62. https://doi.org/10.1016/j.foodchem.2007.10.077
12. Umehara K, Nemoto K, Ohkubo T, et al. Isolation of a new 15-membered macrocyclic glycolipid lactone, Cuscutic Resinoside a from the seeds of *Cuscuta chinensis*: A stimulator of breastcancer cell proliferation. Planta Med 2004;70(4):299–304. https://doi.org/10.1055/s-2004-818939
13. Bao X, Wang Z, Fang J, et al. Structural features of an immunostimulating and antioxidant acidic polysaccharide from the seeds of *Cuscuta chinensis*. Planta Med 2002;68(3):237–43. https://doi.org/10.1055/s-2002-23133
14. Wang Z, Fang JN, Ge DL, et al. Chemical characterization and immunological activities of an acidic polysaccharide isolated from the seeds of *Cuscuta chinensis* Lam. Acta Pharmacol Sin 2000;21(12):1136–40.
15. Donnapee S, Li J, Yang X, et al. *Cuscuta chinensis* Lam.: A systematic review on ethnopharmacology, phytochemistry and pharmacology of an important traditional herbal medicine. J Ethnopharmacol 2014;157:292–308. https://doi.org/10.1016/j.jep.2014.09.032
16. Yen FL, Wu TH, Lin LT, et al. Nanoparticles formulation of *Cuscuta chinensis* prevents acetaminophen-induced hepatotoxicity in rats. Food Chem Toxicol2008;46(5):1771–7. https://doi.org/10.1016/j.fct.2008.01.021
17. Marotti M, Piccaglia R. Characterization of flavonoids in different cultivars of onion (*Allium cepa* L.). J Food Sci 2002;67(3): 1229–32. https://doi.org/10.1111/j.1365-2621.2002.tb09482.x

18. Wiczkowski Wa, Nèmeth K, Buciñski A, et al. Bioavailability of quercetin from flesh scales and dry skin of onion in rats. Pol J Food Nutr Sci 2003;12(53):95–9.
19. Olsson ME, Gustavsson KE, Vagen IM. Quercetin and isorhamnetin in sweet and red cultivars of onion (*Allium cepa* L.) at harvest, after field curing, heat treatment, and storage. J Agric Food Chem 2010;58(4):2323–30. https://doi.org/10.1021/jf9027014
20. Slimestad R, Fossen T, Vagen IM. Onions: A source of unique dietary flavonoids. J Agric Food Chem 2007;55(25):10067–80. https://doi.org/10.1021/jf0712503
21. Singh BN, Singh BR, Singh RL, et al. Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. Food Chem Toxicol 2009;47(6):1161–7. https://doi.org/10.1016/j.fct.2009.02.004
22. Soni KK, Zhang LT, You JH, et al. The effects of MOTILIPERM on cisplatin induced testicular toxicity in Sprague-Dawley rats. Cancer Cell Int 2015;15:121. https://doi.org/10.1186/s12935-015-0274-1
23. Soni KK, Zhang LT, Choi BR, et al. Protective effect of MOTILIPERM in varicocele-induced oxidative injury in rat testis by activating phosphorylated inositol requiring kinase 1α (p-IRE1α) and phosphorylated c-Jun N-terminal kinase (p-JNK) pathways. Pharm Biol 2018;56(1):94–103. https://doi.org/10.1080/13880209.2017.1421672
24. Soni KK, Shin YS, Choi BR, et al. Protective effect of DA-9401 in finasteride-induced apoptosis in rat testis: Inositol requiring kinase 1 and c-Jun N-terminal kinase pathway. Drug Des Devel Ther 2017;11:2969–79.
25. Tsuruma T, Sahara H, Takenouchi M, et al. Synthetic sulfonolipids deduced from sulfonoquinovosyl diacylglycerols of sea urchin reduces hepatic ischemia-reperfusion injury in rats. Transplant Proc 2004;36(7):1965–9.
26. Suyama Y, Handa O, Naito Y, et al. Mucus reduction promotes acetyl salicylic acid-induced small intestinal mucosal injury in rats. Biochem Biophys Res Commun 2018;498(1):228–33. https://doi.org/10.1016/j.bbrc.2018.02.202
27. Mekjaruskul C, Jay M, Sripanidkulchai B, et al. Pharmacokinetics, bioavailability, tissue distribution, excretion, and metabolite identification of methoxyflavones in Kaempferia parviflora extract in rats. Drug Metab Dispos 2012;40(12):2342–53.
28. Li X, Wang G, Sun J, et al. Pharmacokinetic and absolute bioavailability study of total panax notoginsenoside, a typical multiple constituent traditional chinese medicine (TCM) in rats. Biol Pharm Bull 2007;30(5):847–51.
29. Liu J, Zou S, Liu W, et al. An established HPLC-MS/MS method for evaluation of the influence of salt processing on pharmacokinetics of six compounds in cuscutae semen. Molecules 2019;24(13):2502.
30. Li C, Dong J, Tian J, et al. LC/MS/MS determination and pharmacokinetic study of iridoid glycosides monotropein and deacetylasperulosidic acid isomers in rat plasma after oral administration of *Morinda officinalis* extract. Biomed Chromatogr 2016;30(2):163–8. https://doi.org/10.1002/bmc.3532
31. Zhou Q, Yan H, Li R, et al. Quantitative determination of monotropein in rat plasma and tissue by LC–MS/MS and its application to pharmacokinetic and tissue distribution studies. Rev Bras Farmacogn 2018;28(4):451–6.
32. Li L, Brunner I, Han AR, et al. Pharmacokinetics of α-mangostin in rats after intravenous and oral application. Mol Nutr Food Res 2011;55(S1):S67–74. https://doi.org/10.1002/mnfr.201000511
33. Wang F, Cao J, Hao J, et al. Pharmacokinetics, bioavailability and tissue distribution of geniposide following intravenous and peroral administration to rats. Biopharm Drug Dispos 2014;35(2):97–103.
34. Lee HW, Kil KJ, Lee MS. Ginseng for improving semen quality parameters: A systematic review. World J Mens Health 2020;38:1–10. https://doi.org/10.5534/wjmh.190125
35. Yoon YE, Kim TY, Shin TE, et al. Validation of SwimCount™, a novel home-based device that detects progressively motile spermatozoa:

Correlation with world health organization 5th semen analysis. World J Mens Health 2020;38: 191–7. https://doi.org/10.5534/wjmh.180095

36. Park HJ, Koo YK, Park MJ, et al. Restoration of spermatogenesis using a new combined herbal formula of epimedium koreanum nakai and angelica gigas nakai in an luteinizing hormone-releasing hormone agonist-induced rat model of male infertility. World J Mens Health 2017;35: 170–7. https://doi.org/10.5534/wjmh.17031

J Mens Health Vol 16(SP1):e57-e70; 24 July 2020