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Comparative Study on the Pharmacokinetics of *Ginkgo biloba* Extract between Normal and Diabetic Rats by HPLC-DAD

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***SUMMARY*. A new HPLC-DAD method has been developed and validated for the simultaneous analysis of five flavonoids (rutin, quercitrin, quercetin, kaempferol, and isorahamnetin) in rat plasma. Sample pretreatment consisted of a liquid–liquid extraction. The five flavonoids was separated on a kromasil C18 column and recorded at 350 nm. The greatest resolution was achieved with methanol-0.1% formic acid gradient at a flow rate of 1.0 mL min^{-1}. The correlation coefficients for all the calibration plots ($r > 0.999$) showed good linearity over the range tested. The relative standard deviation of the method was less than 7% and 10% for intra and inter-day assays respectively, and average recovery was between 77.2 and 99.2%. The detection limits of this method were between 0.006 and 0.02 μg mL^{-1}. The method has been successfully applied to determine drug concentrations in normal and diabetic rat plasma samples and pharmacokinetic of *Ginkgo biloba* extract.**

INTRODUCTION

Ginkgo biloba is an ancient Chinese phytomedicine which was used to treat various ailments including circulatory and demential disorders. Standardized leaf extracts have been proved to be clinically effective in the treatment of Alzheimer's disease, depression, impotency, memory impairment, peripheral vascular disease, intermittent claudication, vertigo and tinnitus, Diabetes mellitus (DM) [1-5]. The positive effects of *Ginkgo bibola* extracts (EGb) are thought to result from the synergistic action of two distinct groups of compounds, the flavonoids and triterpene lactones [6]. The flavonoids are responsible for the free radical scavenging effects of *Ginkgo biloba* [7-9], while the ginkgolides are potent anti-platelet factor (PAF) antagonists [10].

DM is a serious health problem affecting millions of individuals worldwide. By the year 2025, the World Health Organization (WHO) predicts that 300 million people will have diabetes mellitus [11]. It is showed that some changes of many enzymes and transporters concerned with metabolism and disposal of drug have taken place in organism under pathologic state of DM. The expression of cytochrome P450 of liver microsomes showed marked change in the body of DM rat induced by streptozotocin (STZ), such as significant advance of expression level of messenger ribonucleic acid (mRNA) of CYP1A2, CYP2B1/2, CYP2E1, and CYP3A2/3, striking decrease of expression of CYP2C11 and significant reduction of expression of its mRNA [12]. Price discovered that metabolic glucuronic acidification of rats with DM induced by STZ increased strikingly [13]. Watanabe K presented that expression of Peptide Transporter 1 (PepT1) increased notably with DM rats. Thus, pharmacokinetics of drug is probably influenced under pathologic state of DM [14].

Many scholars have studied flavonoids in biological specimen [15-19], but there was little attention paid to the simultaneous determination of the concentrations of selected flavonols (rutin, quercitrin, quercetin, kaempferol, and isorahamnetin) in diabetic rat plasma and their pharma-

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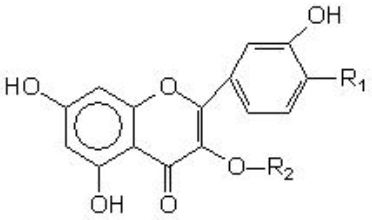
cokinetic after intravenously administrated EGb. There are literatures published the pharmacokinetic of EGb in rabbits by calculating the total flavonol glycoside content from the aglycone concentration after acid hydrolysis or total peak area of EGb in plasma [20-21], but rutin, quercitrin, and other flavonols might be effective components in EGb for the therapy of DM [22-29] and simultaneous determination of several effective components *in vivo* through optimizing the extraction method and chromatographic conditions might be suitable for the study of pharmacokinetic properties of EGb. Meanwhile, the pharmacokinetic of EGb should be different in normal and diabetic rat.

Thus, the objectives of this study were to develop and validate a simple and rapid RP-HPLC-DAD method for analyzing five effective flavonoids in normal and diabetic rat plasma after intravenous administration of EGb and to investigate the difference in the pharmacokinetic of EGb in normal and diabetic rats.



Flavonol	R_1	R_2
Rutin	OH	Rutinose
Quercitrin	OH	Rhamnose
Quercetin	OH	H
Kaempferol	H	H
Isorhamnetin	OCH_3	H

Figure 1. Chemical structure of five flavonoids. R_1 = OH, R_2 = rutinose: Rutin. R_1 = OH, R_2 = rhamnose: Quercitrin. R_1 = OH, R_2 = H: Quercetin. R_1 = R_2 = H: Kaempferol. R_1 = OCH_3, R_2 = H: Isorhamnetin.

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade-methanol was obtained from Fisher Scientific (Fisher Scientific, USA). Rutin, quercitrin, quercetin, kaempferol, and isorhamnetin (see Fig. 1) reference standard were purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPP, Beijing, China).The purity (more than 99.5%) of these reference standards was assumed as provided by the suppliers. Water was prepared by Automatic double purified water distilling apparatus (Shanghai Yarong Biochemical Factory, China).The mobile phase was degassed by Ultrasonic Generator (Wuxi Ultrasonic Generator Electronic Equipment Company, China) and filtered by 0.45 um filter (Autoscience Instrument Co. Ltd, China). EGb used in this study was obtained from Xuzhou Enhua Pharmaceutical Factory (Xuzhou, China). EGb injection (20 mg mL^{-1}, dissolved with 30% PEG-400 water solution) was prepared by our laboratory. Streptozotocin (STZ, Lot No. P7993b) was purchased from Biomol Research Lab (Plymouth Meeting, PA, USA). All other chemicals were of analytical grade.

Chromatography

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, DGU-20A degasser, FCV-10ALVP, SCL-10AVP system controller, Rheodyne 7725 injector with a 20 µL loop and a SPD-20AVP Diode array detector. System control and data analyses were carried out using LCsolution software (Shimadzu). Separation have been done on a kromasil C18 column (5 µm particle size, 250 mm x 4.6 mm i.d. Dalian Elite company, China) using a one step linear gradient. Mobile phase A (methanol) and B (0.1% formic acid) ratios changed as follows: 0~5 min, 35~40%A; 5~40 min, 40~50%A; 40~50 min, 50~60%A; 50~55 min, 60~65%A. The total run time was 55 min at a flow rate of 1 $mL.min^{-1}$. The eluent was monitored by a diode array detector and the detection wavelength was set at 350 nm. The sample injection volume was 20 µL and the column temperature was 35 °C.

Preparation of standard stock solutions

Individual standard stock solutions of the reference compounds were prepared by accurately dissolving 5.05 mg rutin, 4.83 mg quercitrin, 5.05 mg quercetin, 1.23 mg kaempferol, and 0.98 mg isorhamnetin into 25 mL methanol respectively. These solutions were stored at 4 °C away from light and were found to be stable for at least 1 month. Working standard solutions for spiking plasma were freshly prepared by diluting the stock solution with methanol-0.1% formic acid (70:30, v/v) to appropriate concentrations.

The samples for plasma standard calibration curves were prepared by spiking the blank rat plasma (100 µL) with 100 µL of the appropriate working solution to yield the following concentrations: rutin 0.2525~20.2 µg mL^{-1}, quercitrin at

0.1208~9.66 μg mL^{-1}, quercetin at 0.1008~20.16 μg mL^{-1}, kaempferol at 0.031~2.46 μg mL^{-1}, isorhamnetin at 0.098~7.84 μg mL^{-1}. Quality control samples used for the study of intra-day and inter-day accuracy and precision, extraction recovery and stability were prepared in the same way as the calibration samples.

Plasma sample preparation

One hundred microliter plasma was aliquoted in a centrifuge tube, and then spiked with acetone-diethyl ether (1:14, v/v) 1.5 mL by vortex mixing for 1 min. The mixture was centrifuged at 14,000 rpm for 15 min to separate water phase from the organic phase (I). The water phase was spiked with 1 mL methanol by vortex mixing for 1 min and centrifuged at 14,000 rpm for 15 min to separate protein from the organic phase (II). The organic phase (I) and the organic phase (II) were put together and transferred to a clean centrifuge tube. This mixture was evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted with 1 mL methanol-0.1% formic acid (70:30, v/v) and filtrated by using 0.45 μm cellulose acetate membrane. An aliquot of 20 μL of the solution was injected into the chromatographic system.

Validation of the assay method

Calibration

The plasma samples for standard cure were prepared as described in plasma sample preparation. An aliquot of 20 μL of the resulting solution was injected into HPLC system, and each concentration was analyzed for five times. Based on the peak area, calibration lines of peak area versus analyte concentrations were plotted.

Detection wavelength and specificity

The rat plasma samples were detected at 254 nm and 350 nm by Diode array detector under the chromatography conditions as described in chromatography system. Interference of endogenous substance in rat plasma was investigated.

The specificity of the method was investigated by comparing the chromatograms of blank plasma samples from different rats with that of blank plasma spiked with standard solution and the samples collected after intravenous administration of EGb injection.

Recovery

Recovery was calculated by comparing the peak areas of the extracted quality control samples with that of the unextracted standard solutions containing the equivalent amount of analytes.

Precision

Spiked rat plasma samples with different concentrations of rutin, quercitrin, quercetin, isorahamnetin, and kaempferol were used for precision studies. The intra-day precision was calculated by analyzing five replicates of control samples within a day. The inter-day variability was estimated by analyzing samples on five separate days. The relative standard deviation (RSD) was used to estimate the precision.

Sensitivity

The limit of detection (LOD) was defined as the lowest concentration of the drug resulting in a signal-to-noise ratio of 3:1. The limit of quantification (LOQ) was defined as the lowest concentration of spiked plasma samples that can be determined with sufficient precision and accuracy, i.e., RSD. less than 15%.

Stability

Stability of five flavonoids in rat plasma was examined in two ways: (1) The stability of analytes in the plasma stored at –20 °C was determined in 0, 1, 2, 3, 7 days. (2) The stability of the analytes after extraction from plasma and dissolving in methanol-0.1% formic acid (70:30, v/v) at 4 °C away from light was determined at 0, 24 and 48 h.

Pharmacokinetic studies

Healthy male Sprague-Dawley rats with an average weight of 200 g were provided by Xuzhou Medical College (Xuzhou, China) following the Guiding Principles for Care and Use of Laboratory Animals of Xuzhou Medical College. Diabetes mellitus was induced in the male Sprague-Dawley rats by i.p. injection of 60 mg kg^{-1} of the beta-cell toxin STZ (dissolved in pH 4.5 citrate buffer immediately before the injection). Meanwhile controlled normal standard rats were received 6 mL kg^{-1} citrate buffer. The induction of the diabetic state was confirmed by the blood glucose level measurement on the third day after STZ administration. The rats with fasting blood glucose concentrations at 14 mmol L^{-1} were applied to research. The controlled normal standard and diabetic animals housed in the barrier environment refer to breed specific pathogen-free grade animals, and they were allowed free access to food and water.

EGb was given to diabetic and normal

Sprague-Dawley rats (n=10) by intravenous administration at the dosage of 20 mg kg^{-1}. Blood samples (0.5 mL) were collected by vena caudalis at 1, 2, 3, 5, 10, 15, 30, 60, 90, 120, 180, and 240 min post-intravenous administration and were immediately centrifuged (14000 rpm for 10 min) to separate the plasma fractions. The plasma samples were treated according to the procedure described in plasma sample preparation. And then 20 μL of the above solution was injected under the optimum conditions mentioned earlier.

RESULTS AND DISCUSSION

In addition to the determination of effective components in traditional preparation medicines, it is more important to clarify the destiny of effective components *in vivo*-the pharmacokinetic profiles. Optimizing the extraction method and chromatographic conditions should be a quick and simple way to study the pharmacokinetic properties of several effective components in one preparation *in vivo*. Due to the obvious difference in polarity and concentration among rutin, quercitrin, quercetin, kaempferol, and isorhamnetin, simultaneous determination of the five flavonoids in plasma becomes difficult in a single HPLC run. Although there are literatures concerning the determination of rutin, quercitrin, quercetin, kaempferol, and isorhamnetin simultaneously in Ginkgo bibola extract solid oral dosage or independently in plasma using HPLC with relatively fine sensitivity [30], the methods may not be appropriate for the study of the traditional Chinese preparations *in vivo* for it's low drug concentration and interference of plasma endogenous substance The major contribution of the present HPLC method is to develop a simple extraction method and a gradient elution program to reduce the running time and reach satisfactory specificity and sensitivity for rutin, quercitrin, quercetin, kaempferol, and isorhamnetin in diabetic rat plasma at the same time after intravenously administrated with EGb.

Selection of the detection wavelength and study on the specificity

In order to obtain the optimal detection wavelengths for the chromatography separation, the separated result of rutin, quercitrin, quercetin, kaempferol, and isorhamnetin was obtained at 254 nm and 350 nm by diode array detector. The results of different detection wavelength showed that the absorbance peaks of five flavonoids were different. Although they all had the best or better absorbance peaks at 254 nm, interference of endogenous substance from rat plasma in the preparation have the same chromatographic behavior as rutin (see Figure 2C and 2D). Therefore, the Diode array detector was set at the wavelength of 350 nm in this assay.

The representative RP-HPLC profiles of a

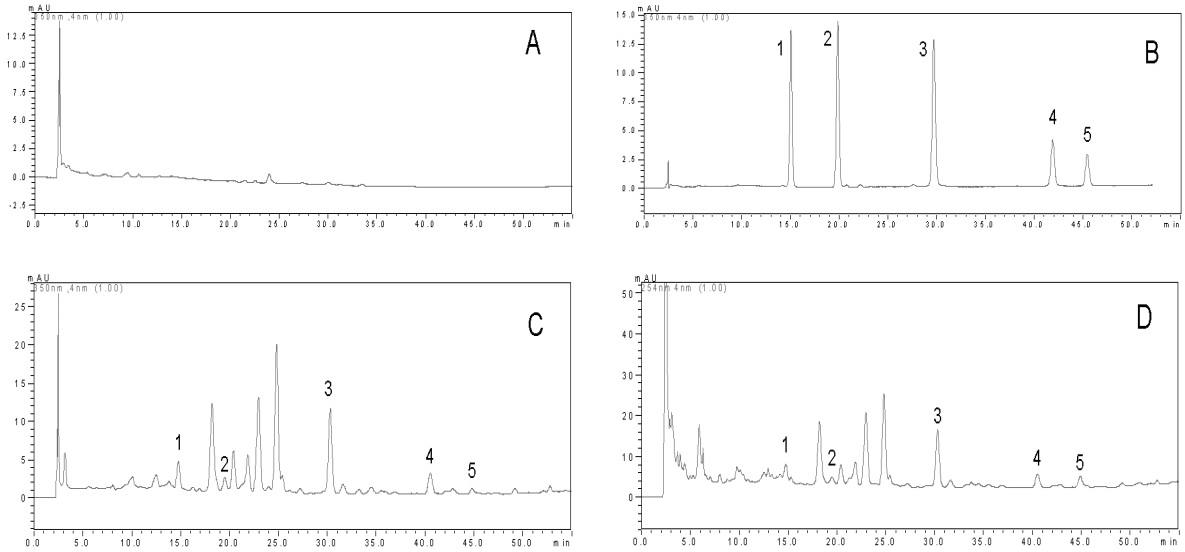


Figure 2. Chromatograms of diabetic rat plasma samples. (**A**) Blank diabetic rat plasma detected at 350 nm; (**B**) Blank diabetic rat plasma spiked with Rutin, Quercitrin, Quercetin, Kaempferol and Isorhamnetin detected at 350 nm; (**C**) Diabetic rat plasma sample at 10 min after intravenous administration of *Ginkgo biloba* extract injection detected at 350 nm; (**D**) Diabetic rat plasma sample at 10 min after intravenous administration of *Ginkgo biloba* extract injection detected at 254 nm. (1: Rutin; 2: Quercitrin; 3: Quercetin; 4: Kaempferol; 5: Isorhamnetin).

blank plasma sample (Fig. 2A), a blank plasma sample spiked with standard solution (Fig. 2B) and plasma sample at 10 min after intravenous administration of EGb injection (Fig. 2C) were shown in Fig.2. No interference was observed under the assay conditions. The peaks of the analytes in the plasma were identified by comparing their retention time with that of the standard and further confirmed by their on-line UV spectra.

Sample preparation

In this study, several solvent systems, such as 10% trichloroacetic acid, methanol, acetonitrile, acetone, acetoacetate, and acetone-diethyl ether with different composition ratios were tested for extraction of rutin, quercitrin, quercetin, kaempferol, and isorhamnetin from rat plasma. Among them best extraction and precision of quercetin, kaempferol, and isorhamnetin were observed when a mixture of acetone-diethyl ether (1:14, v/v) was chosen. Therefore, the rat plasma was extracted with methanol and acetone-diethyl ether (1:14, v/v) respectively and the two extracted solution were put together for simultaneous determination of rutin, quercitrin, quercetin, kaempferol, and isorhamnetin.

Linearity and sensitivity

The calibration curve of each compound was established by injecting eight different concentrations consisting of five flavonoids, a good correlation was found between peak areas *(y)* and concentrations *(x)*. The results of each compound, such as linearity, linear range, limit of detection (LOD), limit of quantification (LOQ) and correlation coefficient (R), were summarized in Table 1. The experimental results showed that the LODs of each flavonoid were very low (0.006–0.02 μg mL^{-1}), which indicated that this method was highly sensitive.

Precision

Analytical precision data were shown in Table 2. The precisions of rutin, quercitrin, quercetin, kaempferol, and isorhmnetin calculated as the relative standard deviation (RSD) at various concentrations were lower than 7% and 10% for intra-day and inter-day assays, respectively.

Recovery

The accuracy of the method, in terms of recovery efficiency, is a measure of the response of the analytical method to the entire quantity of the analyte contained in a sample. The recovery of the five flavonoids in rat plasma samples was calculated and the results were shown in Table 2 with the mean values of five replicate injections. The recoveries for the five flavonoids were between 72.2% and 99.2% in rat plasma (see Table 2). From the results of recoveries, it can be concluded that the present method for the analysis of five flavonoids in plasma has a good accuracy.

Storage stability

The stability experiments were aimed to testing all possible conditions that the samples might experience after collection and prior to the analysis. So the stability of five flavonoids in rat plasma samples was investigated. The results of storage stability were summarized in Table 3. The stability test indicated that all five flavonoids were stable for at least 7 days in plasma at -20 °C. In addition, the five flavonoids were also stable in prepared samples for 48 h demonstrating the stability of the analytes in 2 days when placed at 4 °C away from light before analysis.

Pharmacokinetic studies

The validated HPLC-DAD method has been

Analyte	**Linear range (μg mL^{-1})**	**Regression equation**	**Correlation coefficient**	**LOD** [a] **(μg mL^{-1})**	**LOQ** [b] **(μg mL^{-1})**
Rutin	0.126~20.2	Y = 9181x-23.51	0.9995	0.01	0.04
Quercitrin	0.121~9.66	Y = 11276x-440.88	0.9999	0.02	0.07
Quercetin	0.101~20.2	Y = 41571x-3742.1	0.9977	0.006	0.02
Kaemferol	0.031~2.46	Y = 39793x-592.53	0.999	0.02	0.07
Isorhamnetin	0.098~7.84	Y = 25563x+35.203	0.9995	0.02	0.08

Table 1. Linearity and sensitivity of detection for five flavonoids in rats plasma by RP-HPLC (n^c = 5). [a]LOD: Limit of detection. [b]LOQ: Limit of quantitation. [c]n: Number of determination.

Analyte	Nominal Concentration (μg.mL^{-1})	Recovery (n = 5)		Precision R.S.D [a] (%) (n = 5)	
		Mean (%)	R.S.D. (%)	Intra-day	Inter-day
Rutin	10.1	89.6	5.3	1.6	4.7
	2.53	80.5	7.7	2.3	4.1
	0.253	73.2	7.5	2.5	9.1
Quercitrin	4.83	87.2	4.5	2.9	9.3
	1.21	77.9	5.1	1.2	7.9
	0.121	76.3	6.3	3.9	8.5
Quercetin	10.1	83.1	4.1	2.4	9.2
	2.52	84.9	6.2	5.1	7.4
	0.252	72.2	5.2	6.1	8.2
Kaemferol	1.23	99.2	4.2	1.1	4.9
	0.308	84.3	5.3	2.6	6.8
	0.031	72.5	8.4	2.8	8.3
Isorhamnetin	3.92	86.3	3.9	3.3	6.6
	0.98	85.7	5.1	3.4	4.4
	0.098	72.3	7.9	2.9	8.6

Table 2. Extraction recoveries and intra-day and inter-day (5 separate days) precision for the determination of five flavonoids in rat plasma. [a] RSD Relative standard deviation.

Analyte	In plasma			In prepared samples	
	Nominal concentration (μg mL^{-1})	Mean detected concentration (μg mL^{-1})	Variation coefficient (%)	Mean detected concentration (μg mL^{-1})	Variation coefficient (%)
Rutin	10.1	10.08	2.3	9.98	1.9
	2.53	2.41	6.5	2.49	3.5
	0.253	0.227	7.1	0.226	4.8
Quercitrin	4.83	4.72	4.2	4.59	3.8
	1.21	1.09	7.1	1.16	2.8
	0.121	0.123	5.2	0.123	2.2
Quercetin	10.1	9.93	1.9	9.92	2.7
	2.52	2.30	3.5	2.24	2.4
	0.252	0.254	3.5	0.261	2.1
Kaempferol	1.23	1.17	2.5	1.26	2.6
	0.308	0.273	3.6	0.303	4.9
	0.031	0.038	4.3	0.030	3.2
Isorhamnetin	3.92	3.88	4.3	3.90	1.5
	0.98	0.832	7.1	0.908	3.8
	0.098	0.092	5.1	0.086	5.6

Table 3. The stability of five flavonoids in plasma stored at –20 °C was determined during 7 days as well as in prepared samples stored at 4 °C away from light during 48 h (n = 3).

successfully used to simultaneously determine the concentrations of rutin, quercitrin, quercetin, kaempferol, and isorhamnetin in plasma samples obtained after intravenous administration of 20 mg.kg^{-1} of EGb injection to rats. The chromatograms generated from the above rat plasma were shown in Figure 2, respectively. It showed that there was no obvious interference on the analysis of five flavonoids in rat plasma matrixes. The concentration of five flavonoids was calculated according to the previously established linear regression equations in Table 1, and the mean plasma concentration–time profiles of the five flavonoids were shown in Figs. 3-7. The

Parameters	Units	Rutin		Quercitrin		Quercetin		Kaemperol		Isorhamnetin	
		N [a]	D [b]	N	D	N	D	N	D	N	D
AUC(0–*t*)	mg.h.L^{-1}	60.43	30.34**	55.29	40.49**	3.24	1.71**	0.97	0.42*	4.62	2.08**
AUC(0–∞)	mg.h.L^{-1}	65.59	53.20	66.79	44.17**	4.13	2.46**	1.03	0.51	4.79	2.14**
MRT(0–*t*)	h	0.69	0.74	1.45	0.98**	0.56	0.46*	0.77	0.76	0.62	0.63
*t*1/2	h	1.69	7.76	1.66	1.26*	3.84	3.84	1.18	1.32	1.21	1.07
Cl [c]	L.h^{-1}.kg^{-1}	0.66	0.83	0.69	0.93**	9.94	16.86**	26.62	81.01**	9.24	19.13**
V [d]	L.kg^{-1}	1.58	7.31*	1.44	1.69	53.04	83.89	79.51	154.81**	15.49	29.07*

Table 4. Pharmacokinetic parameters of five flavonoids in normal and diabetic rat (mean, n = 10). N [a]: normal rat. D [b]: diabetic rat. Cl [c]: clearance. V [d]: apparent volume of distribution. *p<0.05 or **p<0.01 vs Normal rat.

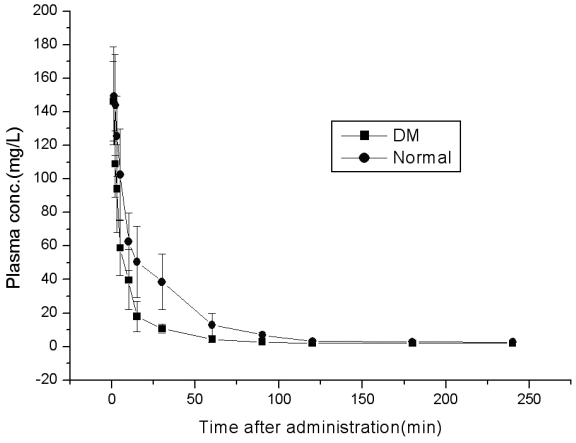


Figure 3. Plasma concentration *versus* time profiles of rutin in normal and diabetic rats (n=10) following intravenous administration of *Ginkgo biloba* extract injection at a single dose of 20 mg kg^{-1}.

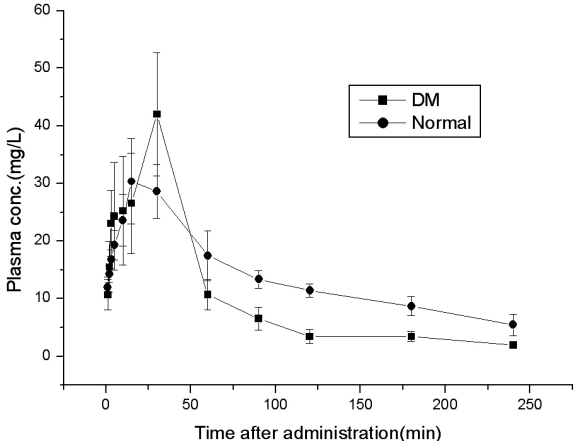


Figure 4. Plasma concentration *versus* time profiles of quercitrin in normal and diabetic rats (n=10) following intravenous administration of *Ginkgo biloba* extract injection at a single dose of 20 mg kg^{-1}.

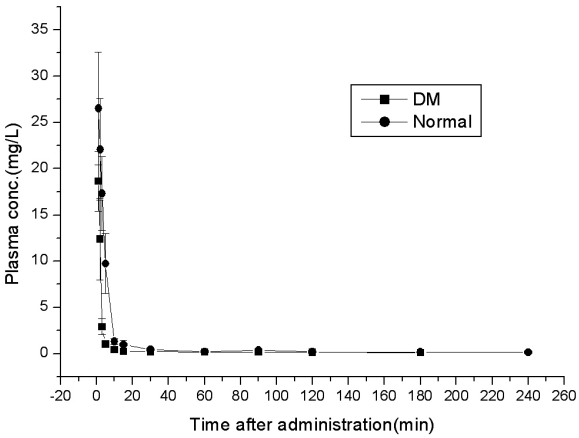


Figure 5. Plasma concentration *versus* time profiles of quercetin in normal and diabetic rats (n=10) following intravenous administration of *Ginkgo biloba* extract injection at a single dose of 20 mg kg^{-1}.

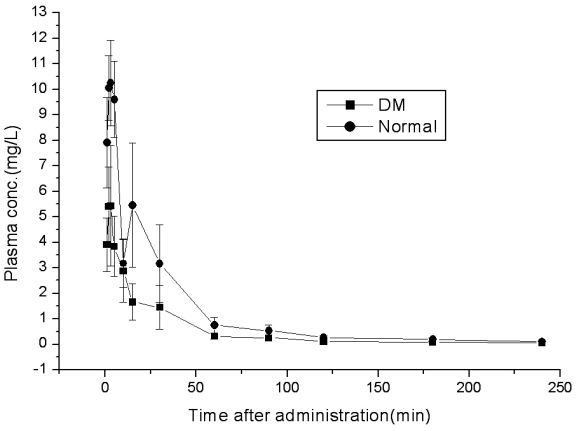


Figure 6. Plasma concentration *versus* time profiles of kaempferol in normal and diabetic rats (n=10) following intravenous administration of *Ginkgo biloba* extract injection at a single dose of 20 mg kg^{-1}.

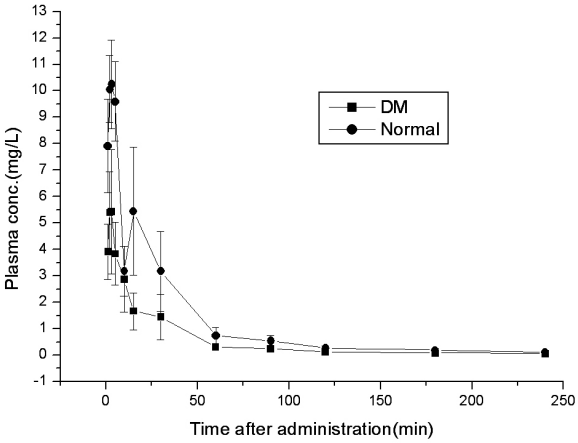


Figure 7. Plasma concentration *versus* time profiles of isorhamnetin in normal and diabetic rats (n=10) following intravenous administration of *Ginkgo biloba* extract injection at a single dose of 20 mg kg^{-1}.

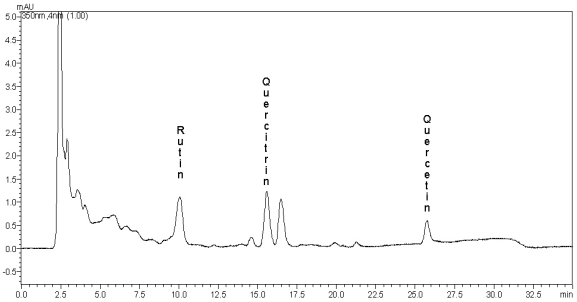


Figure 8. Chromatogram of rat plasma following intravenous administration of rutin. (Chromatography conditions: mobile phase **A** (methanol) and **B** (0.1% formic acid) ratios changed as follows: 0~10 min, 40~45% A; 10~25 min, 45~55% A; 25~26 min, 55~40% A; 26~35 min, 40% A. Other conditions were same with that of *Ginkgo biloba* extract).

pharmacokinetic parameters from two-compartment model analysis (Drug and Statistics for Windows) were summarized in Table 4.

The concentration of five flavonoids in diabetic rat plasma was significantly lower than that of normal rat plasma (AUC (0-*t*) in Table 4, $p < 0.01$ or 0.05), suggesting that clearances of the respective five ingredients in rat plasma increased in pathologic conditions. But this did not reduce half-life for each flavonoid (Table 4).

We found that Cmax of quercitrin appeared at 20~30 min in concentration-time profile of quercitrin (Fig. 4) after intravenously administration of EGb. This may result from biotransformation of rutin or other flavonoids. In order to confirm our suspect, we investigated the biotransformation of rutin in rat plasma by HPLC-

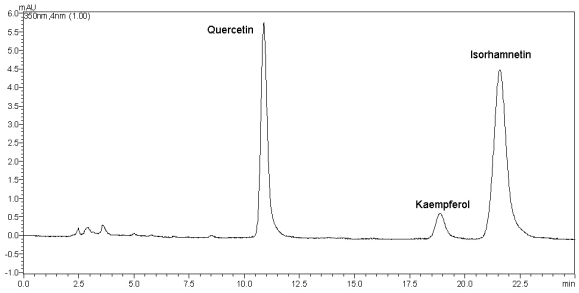


Figure 9. Chromatogram of rat plasma following intravenous administration of quercetin. (Chromatography conditions: mobile phase **A** (methanol) and **B** (0.1% formic acid) was 50 and 50. Other conditions were same with that of *Ginkgo biloba* Extract).

DAD after intravenous administration of rutin. We found chromatogram of rutin, quercitrin, and quercetin in rats plasma by comparing with chromatogram of standard reference substances (Fig. 8). This suggested that biotransformation of rutin resulted in change of concentration-time profile of quercitrin.

The results of pharmacokinetics revealed that there were second peaks in profiles of kaempferol and isorhamnetin after intravenous administration of EGb (Figs. 6 and 7), which was not according with pharmacokinetic of intravenous administration. Chen *et al*. reported that isorhamnetin was found in rats plasma after oral administration quercetin [31]. In order to investigate transformation of quercetin further, we observed the change of rat plasma by HPLC-DAD after intravenous administration of quercetin. The result revealed that quercetin, kaempferol, and isorhamnetin were detected in rat plasma by comparing with chromatogram of standard reference substances (Fig. 9). Therefore, we suspected that the second peak of kaempferol and isorhamnetin resulted from the transformation of quercetin.

CONCLUSIONS

In summary, the RP-HPLC method mentioned here represents an excellent technique for simultaneous separation and determination of five flavonoids in normal and diabetic rat plasma after the intravenous administration of EGb, and has the advantages of good sensitivity, precision and reproducibility. In this method, a simple sample preparation of rat plasma samples was employed; it is easily adaptable for many laboratories with commonly available RP-HPLC equipment. Especially, it was successfully applied to determine drug concentrations in

normal and diabetic rat plasma samples that had been intravenously administered with EGb, and it will play a reference role on the determination of flavonoids in other medicinal plants, pharmaceutical preparations or clinic analysis. The pharmacokinetic parameters of EGb in diabetic rat were apparently different from that in normal rat.

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