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Research Article

Comparative Pharmacokinetic Study of Mangiferin After Oral Administration of Pure Mangiferin and US Patented Polyherbal Formulation to Rats

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Abstract. The US patented polyherbal formulation for the prevention and management of type II diabetes and its vascular complications was used for the present study. The xanthone glycoside mangiferin is one of the major effector constituents in the *Salacia* species with potential anti-diabetic activity. The pharmacokinetic differences of mangiferin following oral administration of pure mangiferin and polyherbal formulation containing *Salacia* species were studied with approximately the same dose 30 mg/kg mangiferin and its distribution among the major tissue in Wistar rats. Plasma samples were collected at different time points (15, 30, 60, 120, 180, 240, 360, 480, 600, 1,440, 2,160, and 2880 min) and subsequently analyzed using a validated simple and rapid LC-MS method. Plasma concentration *versus* time profiles were explored by non-compartmental analysis. Mangiferin plasma exposure was significantly increased when administered from formulation compared to the standard mangiferin. Mangiferin resided significantly longer in the body (last mean residence time (MRT_{last})) when given in the form of the formulation (3.65 h). C_{max} values of formulation (44.16 μg/mL) administration were elevated when compared to equivalent dose of the pure mangiferin (15.23 μg/mL). Tissue distribution study of mangiferin from polyherbal formulation was also studied. In conclusion, the exposure of mangiferin is enhanced after formulation and administration and could result in superior efficacy of polyherbal formulation when compared to an equivalent dose of mangiferin. The results indicate that the reason which delays the elimination of mangiferin and enhances its bioavailability might the interactions of the some other constituents present in the polyherbal formulation. Distribution study results indicate that mangiferin was extensively bound to the various tissues like the small intestine, heart, kidney, spleen, and liver except brain tissue.

KEY WORDS: bioavailability; mangiferin; pharmacokinetics; polyherbal formulation; tissue distribution.

INTRODUCTION

A novel polyherbal formulation composed of *Salacia oblonga*, *Salacia roxburghii*, *Garcinia indica*, and *Lagerstroemia parviflora* was developed and US-patented by Dubey *et al.* (1). This polyherbal formulation has been used for the management of type II diabetes and its vascular complications associated with diabetes mellitus. *Salacia* species used in this formulation have been used in Ayurvedic medicine for diabetes since antiquity and have been extensively consumed in Japan, the USA, and other countries as a food supplement for the prevention of obesity and diabetes (2). Modern pharmacological studies have revealed that *Salacia* roots modulate multiple targets: peroxisome proliferator-activated receptor-alpha-mediated lipogenic gene transcription, angiotensin II/angiotensin II type 1 receptor, alpha-glucosidase, aldose reductase, and pancreatic lipase (3). These multi-target actions may mainly contribute to *Salacia*-species-induced improvement of type 2 diabetes and obesity-associated hyperglycemia, dyslipidemia, and related cardiovascular complications seen in humans and rodents. The results of bioassay-guided identification indicate that mangiferin, salacinol, kotalanol, and kotalagenin 16-acetate are, at least in part, responsible for these multi-target regulatory activities of *Salacia* species (3). However, the pharmacokinetics of this formulation containing *Salacia* species have not been investigated to date, which is not conductive to further research and development of this prescription.

Mangiferin (xanthone glycoside), the main component of *Salacia* species, was the main effective constituent of this formulation for the management of diabetes. Mangiferin has anti-oxidant, anti-diabetic, neuroprotective, gastroprotective, and immunomodulatory activities (4–7). The chemical

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structure of mangiferin is shown in Fig. 1. Previous investigations stated that the mangiferin has low bioavailability and poor absorption (8,9). However, it was well known that synergy of two or more drugs was one of the characteristics of the traditional system of medicines. The complexity of herbal extracts, some ingredients in the formulation may affect the pharmacokinetics of mangiferin, which was a primary active compound in this formulation. Several pieces of literature reveal that there was an increase in the bioavailability of the principle compound from the formulation when compared with the isolated compound or from the extract form. For example, the C_{max} and area under the zero moment curve of paeoniflorin were enhanced after oral administration of Shuang-Dan prescription when compared with extract of Cortex Moutan (10). The bioavailability of wogonoside was increased after oral administration of Huangqin-Tang decoction compared to the Huangqin decoction alone (11). Conversely, there have been some reports of the negative influence of herbal drug compatibility on the pharmacokinetics of active compounds in the formula. Chinese traditional medicine Shuang-Huang-Lian reduced the bioavailability of baicalein, and gentiopicroside absorption was significantly reduced by other components used in the Longdan Xiegan Tang (12,13). Recently, there has been increased focus on the scientific evaluation of formula compatibility through pharmacokinetic studies. Based on these reports, it was important to investigate the pharmacokinetic behaviors of mangiferin following administration of standard mangiferin alone and mangiferin from this formulation.

The present study therefore aimed to compare the pharmacokinetics of mangiferin in rats after oral administration of mangiferin alone and formulation containing the equivalent weight of the standard mangiferin dose. The results of this study will be useful to explain and predict a variety of events related to the efficacy and toxicity of drugs and to provide a firm basis for the design of dosage regimens in pharmacological experiments and clinical applications.

MATERIALS AND METHODS

Chemicals and Reagents. Mangiferin was isolated from the stem bark of *Mangifera indica* in our laboratory which was compared with standard mangiferin purchased from the Sigma-Aldrich Co. (MO, USA). The purity of mangiferin was 99.04%. Gallic acid (99.98% purity) was purchased from the Sigma-Aldrich Co. (MO, USA). Deionized water was produced by the Milli-Q system (Massachusetts). Acetonitrile (HPLC grade—Merck Co., India) and formic acid (Rankem, India) were used for LC-MS analysis. The above patent polyherbal formulation batch number SRM001 was purchased from M/s Varanasi Bioresearch (Varanasi, India).

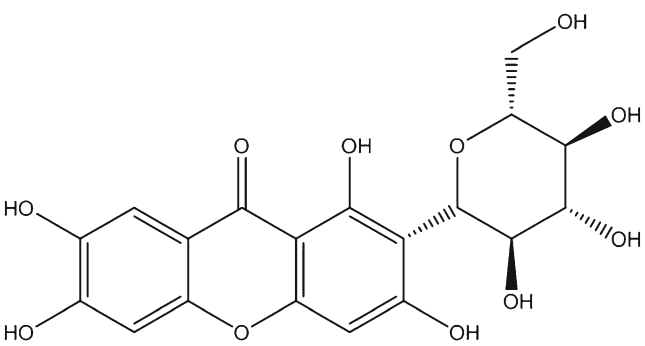

Fig. 1. Chemical structure of mangiferin

Plant Material

The bark of *M. indica* was collected in Chennai, India, in 2012. The plant material was identified by Dr. P. Jayaram, Director, Plant Anatomical Research Centre (PARC), Tambaram. The voucher specimen was filled in our laboratory. Mangiferin was isolated from the mangiferin bark using column chromatography process. The isolation procedure and characterization of mangiferin were provided in the supplementary data.

Determination of Mangiferin in Polyherbal Formulation Containing Two Species of *Salacia*

The concentration of mangiferin was detected by an HPLC diode array detector (DAD) developed and validated in our laboratory. The polyherbal formulation powder equivalent to 100 mg of the *S. oblonga* was weighed accurately in 10 mL of acetonitrile and water (1:1). One milliliter of solution was diluted to 10× water with vortex for 1 min. The diluted solution was then centrifuged at 18,000 rpm for 10 min and filtered using 0.2-μm microporous membrane before injection. An aliquot of 20 μL was injected in the LC-DAD system for determination of mangiferin in polyherbal formulation. Liquid chromatographic separation was achieved by instrumentation conditions. The content of mangiferin was 0.817% *w*/*w* in the polyherbal formulation. The chemical profiling and quantification of the other biomarkers from this formulation like ellagic acid and hydroxycitric acid were carried using HPLC method, Kammalla et al. (14).

Preparation of Mangiferin Standard Solution and Quality Control Samples

The stock solution of mangiferin and internal standard (IS) (gallic acid) was prepared in the mixture of acetonitrile and water (50:50, *v*/*v*). The stock solution of the standard was further diluted in acetonitrile–water (50:50, *v*/*v*) to produce the combined standard working solutions at concentrations of 0.4 to 16.0 μg/mL of mangiferin. A quantity of gallic acid was dissolved in a mixture of acetonitrile and water (50:50, *v*/*v*) to prepare the IS solution with a concentration of 400 ng/mL. For the validation of the method, three concentration levels of the standard solution containing mangiferin (0.4, 16, and 64 μg/mL) were used for preparing quality control (QC) plasma samples, and all solutions were stored at 4°C until needed.

Animal Models

Male Wistar rats (250±20 g) were supplied by the Kings Institute (Chennai, India). The experimental protocol number IAEC 152/2011 was approved by the Institutional Animal Ethics Committee of Sri Ramaswamy Memorial (SRM) College of Pharmacy, SRM University, for the use of

experimental animals, and all animal studies were carried out according to the CPCSEA Guidelines. The animals were maintained under standard laboratory conditions on a 12-h light/dark cycle and were fed standard rat chow and water *ad libitum*. The rats were fasted overnight before the experiments, and food was returned 2 h after dosing. Water was available *ad libitum* throughout the experiments.

Pharmacokinetic Study

For pharmacokinetic study, a single dose of mangiferin (30 mg/kg dissolved in normal saline) and polyherbal formulation containing an equivalent dose of 30 mg/kg mangiferin were orally administered to two groups (n=6). The dose of the mangiferin was selected from the literature reports by Muruganandan *et al.* (15). They showed that rats exhibit behavioral signs of depression when mangiferin was administrated with the doses beyond 100 mg/kg through intraperitoneal administration. Guha *et al.* showed pharmacological effects when mangiferin was administered at the dose of 10 mg/kg through intraperitoneal route (16). Muruganandan *et al.* also reported that mangiferin at the dose of 10 and 20 mg/kg did not show any gross sign of toxicity (15). Both preparations of pure mangiferin and polyherbal formulation were administered as solutions by oral gavage at a volume of 5 mL/kg utilizing feeding needle. Blood samples (300 μL) were collected from fossa orbitalis vein puncturing by using a capillary tube into heparinized centrifuge tubes at 15, 0, 60, 120, 180, 240, 360, 480, 600, 1,440, 2,160, and 2,880 min after single oral administration. All the collected blood samples were centrifuged at 4,000 rpm for 10 min, and the separated plasma samples were frozen in polypropylene tubes at −20°C prior to analysis.

Tissue Distribution Studies

To study the tissue distribution of mangiferin from formulation, three groups of male Wistar rats (n=3 per group) were orally administered at a single dose of 30 mg/kg equivalent weight of mangiferin of polyherbal formulation. The groups were euthanized by decapitation at 0.5, 1.5, and 3 h post-dosing, respectively. The tissues, including the heart, liver, brain, lung, kidney, stomach, spleen, and small intestine, were excised immediately and thoroughly rinsed with physiological saline solution. Then, the tissues were weighed and homogenized in acetonitrile solution twice the weight of the tissue. The obtained tissue homogenates were immediately stored at −20°C until analysis.

Plasma Sample Preparation

Frozen plasma samples were thawed at room temperature and treated as follows: 100 μL of IS solution (400 ng/mL), 100 μL of the working solution for the calibration curve and QC samples, and 200 μL of acetonitrile were added to 100-μL plasma samples. The mixture was vortexed for 5 min and centrifuged at 12,000 rpm for 10 min. The supernatant was transferred into a clean Eppendorf tube and evaporated to dryness under the stream of nitrogen. The residue was dissolved in 200 μL of the acetonitrile–water (50:50, v/v), vortexed, and centrifuged at 12,000 rpm for 15 min. The supernatant was passed through 0.2-μm membrane filter, and 20 μL of the filtrate solution was injected for the LC-MS analysis.

Tissue Sample Preparation

To study tissue distribution, small slices of tissues were individually homogenized with twofold volume of acetonitrile solution. A 200-μL homogenate was an aliquot in a centrifuge tube and then spiked with 20 μL IS. The mixture was vortexed for 30 s and then centrifuged at 6,000 rpm for 10 min. The supernatant was transferred to a clear Eppendorf tube, and 20 μL of the filtrate solution was injected into the LC-MS system for analysis.

Determination of Mangiferin in Rat Plasma and Tissue Samples by LC-MS

A Shimadzu LC-MS 2020 (Shimadzu, Japan) equipped with a binary solvent delivery system, column compartment, and photo diode array detector (PDA) was used for all analysis. The chromatographic separation was performed on a Phenomenex C_{18} column (i.d. 250 mm×4.6 mm, 5 μm), and the column oven temperature was set at 30°C. A linear gradient elution of eluents A (water containing 0.1% formic acid) and B (acetonitrile) was used for separation. The elution programer was optimized and conducted as follows: a linear gradient of 10–23% B with range of 0.01–15.00 min, then holding this mobile phase ratio for 5 min, a linear gradient of 23–45% B with range of 20–25 min, and a linear gradient of 45–10% B with the range of 25-30 min. This was followed by a 6-min equilibration period prior to the injection of each sample, and the flow rate was kept at 0.5 mL/min.

The instrument was operated by switching electrospray ionization (ESI) source in positive and negative ionization modes in a single run. The ESI was performed using nitrogen gas to assist nebulization (the flow rate was set at 1.5 L/min), capillary voltage at 1.6 kV, and temperatures of curved desolvation line (CDL) and heat block at 250 and 300°C were used. All instrumentation data were collected and synchronized by the Lab solutions software (Version 7.1) from Shimadzu.

Validation of LC-MS Method

The current LC-MS assay was validated for specificity, linearity, precision, accuracy, and matrix effects. The specificity was determined by analyzing six blank plasma samples. There was no interference from endogenous or exogenous materials observed at the retention time in the ion channel of either the analytes or the IS.

The calibration plasma and tissue homogenate samples were prepared by spiking 100 μL of working solution and 100 μL of IS solution to 100 μL of blank plasma, and then, the samples were treated according to the sample preparation. QC samples were prepared and assayed along with calibration curve samples. The calibration curve consisted of six concentration levels, and each concentration was prepared and assayed on three separate days. The calibration curves were constructed by the plot of the peak area of the analyte *versus* concentrations of the calibration standards and described in

the form of $y=a+bx$. The concentrations of the analytes in unknown samples were assessed by interpolation from the calibration curve. The limit of quantitation (LOQ) of the assay was defined as the lowest quantifiable concentration of the standard curve (LOQ, S/N=10). The limit of detection (LOD) was defined as the detectable amount (LOD, S/N=3).

The intra and interday precision was defined as the relative standard deviation (RSD), and the accuracy was assessed by comparing the measured concentration with its true value. The accuracy and precision were assessed by determining QC samples at three different validation batches. The intrabatch QC samples were prepared for six replicates. The acceptable intra and interday precision should be less than 15%, and the acceptable accuracy should be less than within 15% for all QC samples.

The matrix effect at three QC concentrations was assayed in sets of six replicates. The extraction recoveries of analytes at three QC levels were evaluated by determining the peak area ratios of the analytes in the post-extraction spiked samples to that acquired from pre-extraction spiked samples. The matrix effects were studied by comparing the peak areas of the analytes dissolved in the pretreated blank plasma with that of pure standard solution containing equivalent amounts of the analytes. The matrix effect was implied if the ratio was less than 85% or more than 115%.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by a noncompartmental approach. The area under the concentration *versus* time curve (AUC_{0-t}) was calculated by trapezoidal rule from zero to the last measured concentration. The noncompartmental model was based on the statistical moment theory in which AUC and area under the first moment curve were calculated by the following equations:

$$\text{Area under the zero moment curve} = \int_{t=0}^{t=\infty} t0\, Cp\, dt = \int_{t=0}^{t=\infty} Cp\, dt = AUC$$

$$\text{Area under the first moment curve} = \int_{t=0}^{t=\infty} t1\, Cp\, dt = \int_{t=0}^{t=\infty} t\, Cp\, dt = AUMC$$

The average time for the residence of all the drug molecules in the body which was called mean residence time (MRT) was calculated by the equation below:

$$MRT = \frac{AUMC}{AUC}$$

The pharmacokinetic parameters, such as maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max}), were directly obtained from the plasma concentration–time plots. The elimination rate constants (K_e) were determined by the linear regression analysis of the logarithmic transformation of the last four data points of the curve (17). The area under the concentration *versus* time curve from zero to infinity ($AUC_{0-\infty}$), total clearance (CL), and the volume of distribution (V_d) of mangiferin were calculated with the following equations:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/k_c$$
$$\text{CL} = \text{Dose}/AUC_{0-\infty}$$
$$Vd = \text{Dose}/Cp_0$$

where C is the drug concentration in plasma, CL is the total body clearance of the drug from the plasma, and Cp_0 is the drug blood concentration at time zero. Initial drug concentration was calculated from the best fitted line and is back extrapolated to the y-axis.

The y-intercept was taken as an initial drug concentration (Cp_0). The values were calculated by Microsoft Excel (Microsoft, Seattle, Washington, USA). Statistical significance was assessed by an unpaired Student's t test, and the significance level of $P<0.05$ was adopted for all statistical comparisons. All results were expressed as the arithmetic mean ± standard deviation (SD).

Wagner Nelson Method

It was a method that involves mathematical calculation of the cumulative amount of drug absorbed at each time point A_a after drug administration and the total amount of the drug absorbed $A_{a\infty}$. The fraction $A_a/A_{a\infty}$ represents the fraction of the absorbed dose that is already absorbed at each time point. The fraction remaining to be absorbed can be examined to determine the nature of the absorption process (18).

A plot of the fraction of the drug remaining to be absorbed *versus* time can be used to determine the order of the absorption process and to calculate the absorption rate constant for the mangiferin from the treated group of animals.

RESULTS

Method Validation

For the determination of mangiferin concentration in rat plasma, an LC-MS method was developed and validated. Due to the anticipated similarity in the extraction recovery, gallic acid was chosen as an internal standard for the determination. The developed method was evaluated by analyzing individual blank plasma samples from different sources for its specificity. There was no interference from endogenous components that were found in all samples at the same mass transitions and retention times as an analyte. The typical chromatograms of standard mangiferin, blank plasma, spiked plasma with mangiferin concentration of 320 ng/mL, standard mangiferin-treated group plasma sample of 30-min time point, formulation-treated group plasma at 90-min time point, and small intestine tissue homogenate after 30 min of formulation treated are shown in Fig. 2a–f.

The standard curves exhibited excellent linearity over a range of 0.4–64 μg/mL for the analyte with coefficients of

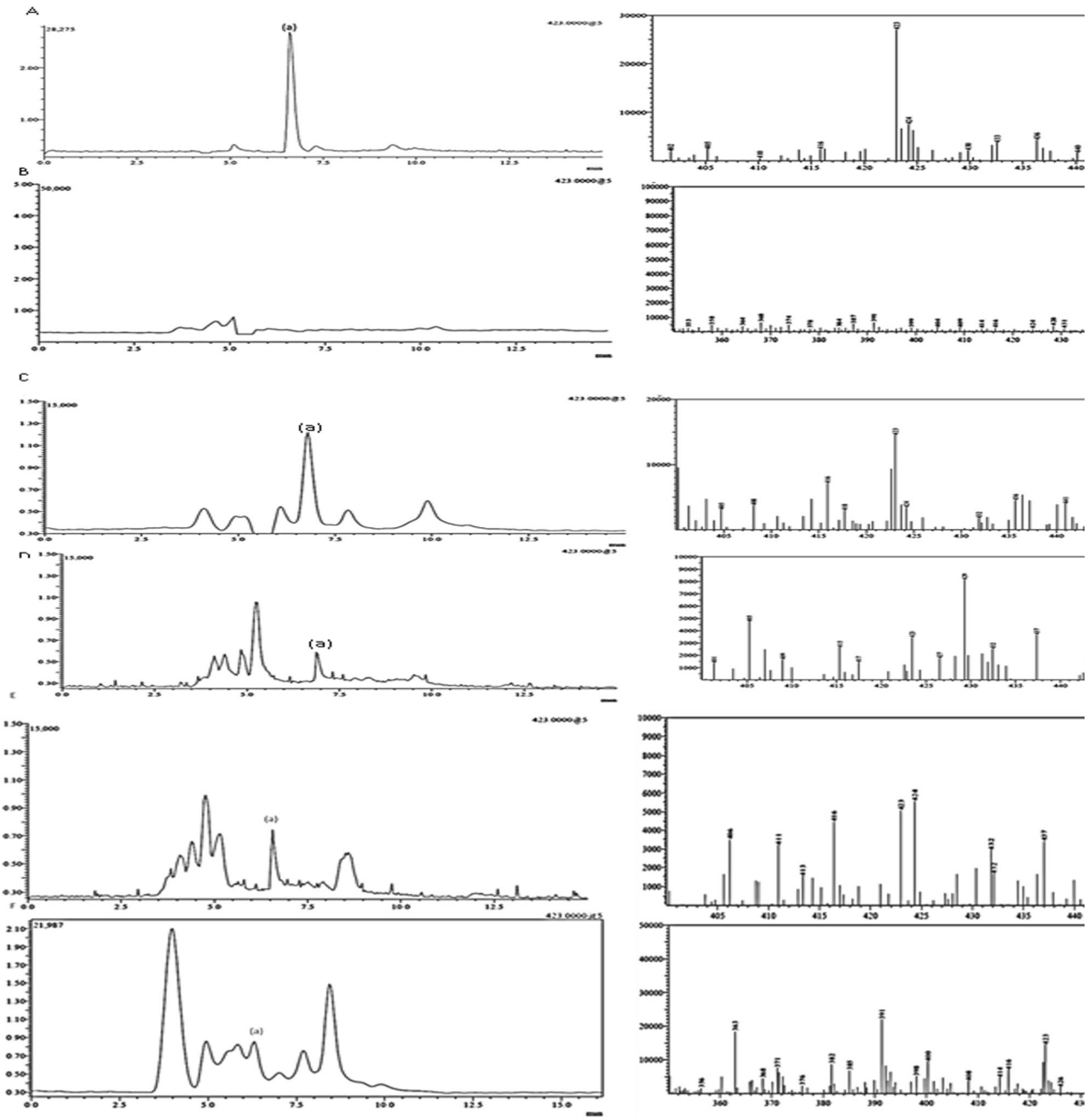

Fig. 2. SIM-mode positive-LC-MS chromatogram (*a*) mangiferin 423 (M^+ ion) peak of **a** standard mangiferin, **b** blank plasma, **c** spiked plasma with mangiferin concentration of 320 ng/mL, **d** standard-mangiferin-treated group plasma sample of 30-min time point, **e** formulation-treated group plasma at 90-min time point, and **f** small intestine tissue homogenate after 30 min of formulation treatment

correlation (r) greater than 0.99. The lower limit of detection of the method was 1.2 μg/mL (signal to noise>3). The validated parameters are depicted in Table I. For all the concentration QC samples, the interday and intraday precision (RSD) of the method was determined to be <15%, and accuracy was <110.0% (Table II). Extraction recovery was determined at three different concentrations and IS (0.4 μg/mL) by comparing the pre- and post-extraction spikes for the QC samples. The results showed that the extraction recoveries of mangiferin were in the range of 80–95%. The validated method was successfully applied to the pharmacokinetic comparative study of the mangiferin in rat plasma after the oral administration of standard mangiferin and polyherbal formulation.

Table I. Validation Parameters

Parameters	
Regression equation	$y=180.39\times-2856.7$
R^2	0.9954
Linearity range (μg/mL)	0.4-64
LOD (μg/mL)	1.2
LOQ (μg/mL)	3.0

Table II. Precision and Accuracy of Mangiferin in Rat Plasma

	Intrabatch (n=5)			Interbatch (n=10)		
Nominal concentration (μg/mL)	Measured concentration (μg/mL)	Precision (%)	Accuracy (%)	Measured concentration (μg/mL)	Precision (%)	Accuracy (%)
0.4	0.461	9.63	104.02	0.472	6.06	109.29
16	16.42	2.56	102.14	16.48	1.57	104.05
64	67.66	8.30	105.73	64.07	3.26	101.42

Pharmacokinetic Analysis

The mean plasma concentration *versus* time profiles of mangiferin after oral administration are shown in Fig. 3, and the pharmacokinetic parameters are summarized in Table III. After oral administration, standard mangiferin was absorbed rapidly from the gastrointestinal tract, detected in plasma from the first blood sampling time (15 min), and rapidly reached T_{max}. On the other hand, mangiferin from the formulation was slowly absorbed from the formulation when compared with standard alone. The rate of elimination of mangiferin alone (0.41/h) was comparatively low with the formulation (0.50/h). The average residence time (MRT) of molecules from the standard mangiferin and polyherbal formulation was 3.21 and 3.65 h, respectively. There was a higher volume of distribution (V_d) when it was administered with mangiferin alone (7.21 mL) that shows that it has the greater binding capacity with the tissue and plasma proteins. Total body clearance (CL_T) of mangiferin from the formulation (6.35 μg/mL/h) was higher than that of the mangiferin alone (1.59 μg/mL/h).

Tissue Distribution Studies

The results of mangiferin tissue distribution from polyherbal formulation among the major tissues of rats are listed in Table IV. A higher amount of mangiferin was distributed among the heart, small intestine, and stomach. Also, it was in detectable limits among the kidney, liver, and lungs. But, very trace amounts were distributed to the spleen. It could be seen that the concentration in the brain that was under the detectable limits proves that mangiferin could not cross the blood–brain barrier.

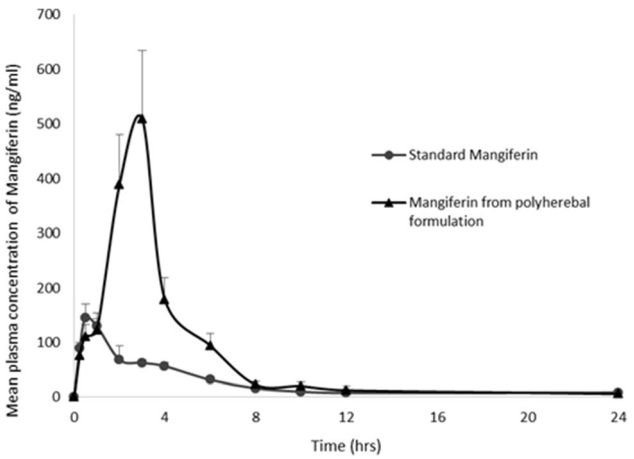

Fig. 3. Mean plasma concentration (mean±SD) *versus* time (h) profile of standard mangiferin and mangiferin from polyherbal formulation

DISCUSSION

Like many other traditional medicinal herbs, *Salacia* species and related proprietary products have been used clinically to treat diabetes mellitus for thousands of years. Although salacinol and kotanolol were considered as active ingredients in *Salacia* species, they are minor components in these herbs. Mangiferin was a major active ingredient in many herbs such as *S. oblonga*, *M. indica*, *Folium pyrroisa*, *Swetia chirata*, and *Rhizoma anemmarrhenae*. Most researchers focused on the pharmacokinetics of mangiferin, e.g., in rat plasma or human plasma after oral administration of pure mangiferin (19). For the modernization of herbal medicine, study of the pharmacokinetics was an important aspect (20). The main strategy was to choose one or more effective ingredients as the targeted components to represent the pharmacokinetics of an entire prescription. In the present study, mangiferin was chosen as a target compound. Also, it has been reported that the mangiferin was considered a suitable quality control parameter for the *Salacia* species and its products (21). A simple and sensitive LC-MS method was developed and validated for the pharmacokinetic study of mangiferin after oral administration of pure mangiferin and polyherbal formulation.

The pharmacokinetic results showed that mangiferin was rapidly absorbed in to the portal vein after oral administration of mangiferin alone with greater rate of absorption constant and higher volume of distribution. Rate of absorption constant determines the rate of drug absorption from the site of administration and the rate of appearance of the mangiferin in the systemic circulation to produce the pharmacological effect;

Table III. The Pharmacokinetic Parameters of Rat Plasma after Oral Administration of Mangiferin and Polyherbal Formulation at the Dose of 30 mg/kg Mangiferin

Parameter	Mangiferin (mean±SD, n=6)	Formulation (mean±SD, n=6)
C_{max} (μg/mL)	15.23±6.00	44.16±23.12***
T_{max} (h)	0.67±0.29	3.00±0.50**
$T_{1/2}$ (h)	1.06±0.95	1.89±2.45
$AUC_{0-\infty}$ (μg h/mL)	155.03±17.98	187.53±48.76*
Ka (/h)	0.56±0.24	0.36±0.16
Ke(/h)	0.41±0.16	0.50±0.15*
CL_T (μg/mL/h)	1.59±0.25	6.35±1.32*
Vd(mL)	7.21±1.54	1.86±0.46*
MRT (h)	3.21±1.41	3.65±1.38

Values are expressed as mean±SD
*P<0.05 *versus* oral administration of standard mangiferin;**P<0.01 *versus* oral administration of standard mangiferin, ***P<0.001 *versus* oral administration of standard mangiferin

Table IV. Distribution of Mangiferin in Different Tissues after Oral Administration of Polyherbal Formulation

Major tissues	Concentration (ng/mL) at different intervals of time		
	30 min	90 min	180 min
Heart	203.0±3.6	526.9±6.1	321.5±2.4
Lungs	40.0±1.9	60.0±3.3	12.7±1.1
Liver	78.9±1.1	75.5±3.4	128.5±2.0
Brain	0.5±0.1	1.1±0.1	0.9±0.2
Kidney	23.1±3.0	75.2±0.2	143.3±0.8
Small intestine	120.9±1.3	223.8±7.6	754.0±25.3
Stomach	332.9±18.4	75.6±0.3	55.2±1.1
Spleen	21.7±1.9	27.3±1.1	36.3±0.8

Values are expressed as mean±SD

then, faster drug absorption results in faster onset of drug effect which was required for the rapid therapeutic effect to treat an acute condition. In the chronic disease conditions like diabetes, there should be a slower absorption to maintain an effective plasma mangiferin concentration for a long time after drug administration. This could be achieved only when it was given with polyherbal formulation. Higher volume of distribution has the higher affinity toward the tissue or plasma proteins. Mangiferin has been reported earlier for its higher binding affinity for the plasma proteins which causes the higher volume of distribution and slower rate of elimination. Compared to mangiferin given alone, many parameters of mangiferin pharmacokinetics, C_{max}, T_{max}, $T_{1/2}$, MRT, and $AUC_{0-\infty}$, were significantly different from those of the standard-treated group.

The significant decrease in the AUC suggests that a smaller amount of mangiferin was absorbed after oral administration of mangiferin alone. This phenomenon will be caused by the poor absorption of mangiferin from the intestine, and the unabsorbed fraction may be degraded by intestinal bacteria. Also, there will be the possibility of hydrolyzing the molecule by the gastrointestinal enzymes. When it was administered in the form of formulation, there will be an increase of the AUC, which indicated that higher amount of the mangiferin was absorbed from the formulation. The increased AUC of mangiferin after administration of formulation might be because of the presence of other biomarkers in the formulation, which shows the greater binding effect to the human serum albumin. Ellagic acid is one of the other major constituents present in the formulation that may be showing a better binding effect with human serum albumin (22). If the same saturable enzymes metabolize other constituents and mangiferin, saturation of those enzymes occurs at comparatively lower levels of mangiferin. Hence, first-pass effect and the metabolism were decreased, and thus, plasma levels of mangiferin were higher from the formulation. According to the prior phytochemical study and from patent details, many other glycosides were separated and identified in the polyherbal formulation. Thus, the competitive inhibition between mangiferin and other glycosides might reduce the degradation and increase the concentration of mangiferin in the intestine, which finally enhance the bioavailability of mangiferin. However, it was to be considered that mangiferin having a higher tissue binding capacity showing greater volume of distribution correlates to the decrease in the rate of elimination and total body clearance. Due to the complexity of the other constituents in the formulation, the principle constituent will be decreasing its binding affinity toward the plasma proteins and increasing its free drug concentration which shows the therapeutic effects and gets rapidly eliminated. Also, there will always be a strong correlation between plasma drug concentration and the intensity of its therapeutic and toxic effects (23). For example, the C_{max} and AUC of visnagin were increased after oral administration of *Ammi visnaga* extract compared with a single constituent (24). The bioavailability of ephedrine was increased after combination of *Ramulus cinnamomi*, *Semen armeniacea*, and *Radix glycyrrhizae* with *Herba ephedra* (25). Increasing attention was currently being paid to scientific evaluation of formula compatibility by means of pharmacokinetic study.

Tissue distribution of the mangiferin was investigated in rats following a single oral dose of polyherbal formulation (30 mg/kg equivalent to mangiferin). Mangiferin concentration among the major the tissues of Wistar rats is depicted in Fig. 4. The results indicated that mangiferin underwent a rapid and wide distribution into tissues within the time course examined. The highest concentration of mangiferin was detected in the small intestine (754.2 ng/mL of homogenate). After 30 min, the higher amount of drug was present in the stomach. The levels of mangiferin in spleen were much lower than that in other tissues. On the other

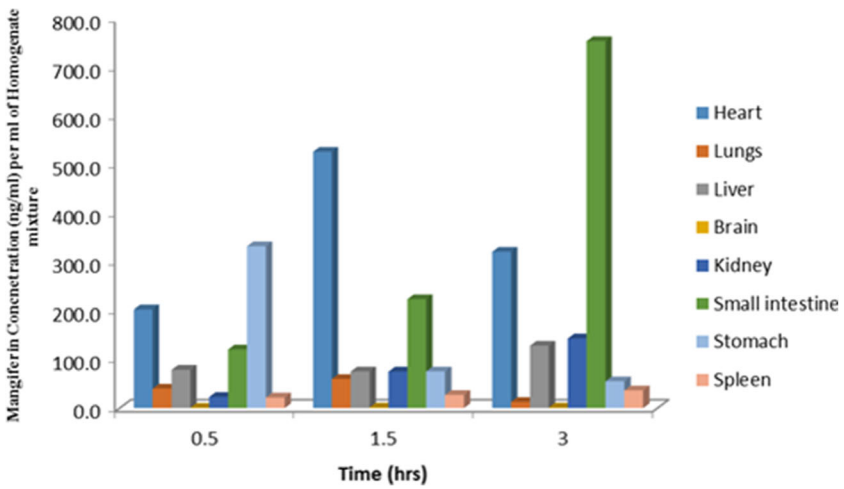

Fig. 4. Tissue distribution of mangiferin from polyherbal formulation among major tissues of rat

hand, mangiferin could hardly accumulate in the brain as the levels were well below the LOQ. Due to the hydrophilic character, it was difficult to pass the blood–brain barrier. Mangiferin was effectively distributed into the extravascular system of the rat body with substantial disposition in the kidney and liver, suggesting that the renal and hepatic metabolism was the primary elimination route. The rapid and wide distribution of mangiferin into the tissues implied that polyherbal formulation could show comprehensive pharmacological effects on the diverse organs throughout the body. These results may be helpful to understand the pharmacological effects of mangiferin from this formulation as well as its toxicity (26).

On the whole, all obtained pharmacokinetic data indicated that mangiferin from this formulation displayed ideal pharmacokinetics in rat plasma and tissues. Since this formulation was patented for the management of diabetes and also, mangiferin possess anti-diabetic activity, it would be very useful and meaningful to obtain its pharmacokinetic information for better understanding when, where, and how this compound from this formulation can be effective. The present pharmacokinetic study was therefore believed to provide a deeper understanding of pharmacological and toxic properties of mangiferin. Our further work will focus on elucidating the possible metabolites of mangiferin from this formulation and its dose–response relationship. Due to its ideal pharmacokinetic characteristics, mangiferin could be tentatively used as a marker of quality control of *Salacia* species and *Salacia*-species-based herbal medicines.

CONCLUSION

In conclusion, the pharmacokinetic parameters of the mangiferin from the polyherbal formulation have been varied when compared with pure mangiferin. There was a statistically significant difference ($P<0.05$) in the C_{max}, $AUC_{0-\infty}$, and CL_T parameters of mangiferin administered orally among the rats with the polyherbal formulation. Based on the result obtained, it may be concluded that the intradrug interactions could account for the different pharmacokinetic behaviors of mangiferin. Also, tissue distribution study results reveal that mangiferin could be rapidly and widely distributed into tissues and does not cross the BBB in rats. The findings might help to provide useful evidence for the clinical applications of this polyherbal formulation.

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Conflict of Interest The authors declare no conflict of interest.

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