

Pharmacokinetic profile of diosgenin and trigonelline following intravenous and oral administration of fenugreek seed extract and pure compound in rabbit

Neda Mohamadi, Fariba Sharififar, Mehdi Ansari, Mostafa Pournamdari, Mahdiah Rezaei & Navid Hassanabadi

To cite this article: Neda Mohamadi, Fariba Sharififar, Mehdi Ansari, Mostafa Pournamdari, Mahdiah Rezaei & Navid Hassanabadi (2020): Pharmacokinetic profile of diosgenin and trigonelline following intravenous and oral administration of fenugreek seed extract and pure compound in rabbit, Journal of Asian Natural Products Research

To link to this article: <https://doi.org/10.1080/10286020.2020.1769609>



Published online: 25 May 2020.



Submit your article to this journal [↗](#)



View related articles [↗](#)



View Crossmark data [↗](#)



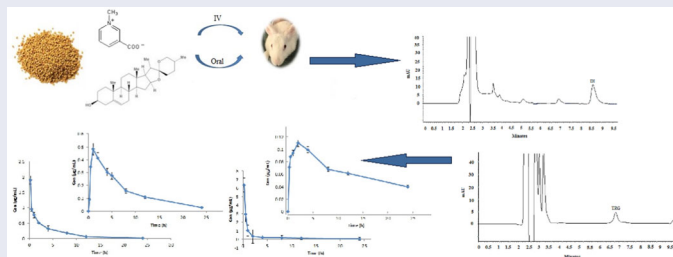
Pharmacokinetic profile of diosgenin and trigonelline following intravenous and oral administration of fenugreek seed extract and pure compound in rabbit

Neda Mohamadi^a, Fariba Sharififar^b, Mehdi Ansari^c , Mostafa Pournamdari^d , Mahdieh Rezaei^e and Navid Hassanabadi^e

^aPharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; ^bHerbal and Traditional Medicines Research Center, Department of Pharmacognosy, Kerman University of Medical Sciences, Kerman, Iran; ^cDepartment of Pharmaceutics, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran; ^dDepartment of Drug and Food Control, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran; ^eDepartment of Clinical Science, Faculty of Veterinary Medicine, Shahid Bahonar University, Kerman, Iran

ABSTRACT

Diosgenin (DI) and trigonelline (TRG) are the main bioactive compounds of fenugreek seed. A micro-extraction method using magnetic nanocomposites coupled with ion-pairing reversed phase HPLC (RP-HPLC) method was used to determine the analytes in rabbit plasma. The main PK parameters after oral administration of pure DI and the extract were: C_{max} : 0.48, 0.057 $\mu\text{g/ml}$; t_{max} : 1, 2 h; and $t_{1/2\beta}$: 6.23, 15.04 h, respectively. Pure TRG and the extract PK parameters were: C_{max} : 0.121, 0.081 $\mu\text{g/ml}$; t_{max} : 1.30, 1.30 h; and $t_{1/2\beta}$: 20.06, 24.70 h, respectively. Generally, the PK profile of the two compounds was best fitted on the two-compartment model.



ARTICLE HISTORY

Received 28 March 2019
Accepted 12 May 2020

KEYWORDS

Fenugreek; trigonelline; diosgenin; pharmacokinetics; magnetic nanoparticles

1. Introduction

Plant-derived bioactive compounds possess the potential to be used as drugs; and nowadays, many phytochemicals are used as a raw material in pharmaceutical and cosmetic industries and as a dietary supplement in the treatment of various diseases.

CONTACT Mehdi Ansari  mansari@kmu.ac.ir  Departments of Pharmaceutics, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran; Mostafa Pournamdari  m_pournamdari@kmu.ac.ir  Department of Drug and Food Control, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran

© 2020 Informa UK Limited, trading as Taylor & Francis Group

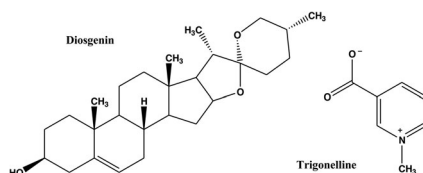


Figure 1. Chemical structures of DI and TRG.

Salim et al. reported that 88 pure chemicals isolated from 72 medicinal plants have been introduced into modern medical therapy up to 2008 [1].

Trigonella foenum-graecum L. belongs to Fabaceae family with many potentially therapeutic compounds [2]. The fenugreek seed contains several alkaloids among which trigonelline (TRG) as a hydrophilic alkaloid (Figure 1) comprise the most considerable one. Many biological effects of TRG such as antidiabetic, antihypertensive, hypolipidemic, neuroprotective, and antioxidant activities with effective doses have been reported for the treatment of numerous diseases [3–6]. Diosgenin (DI) is a phytosteroid sapogenin (Figure 1) found in the fenugreek seeds. It is used as an alternative medicine to treat various disorders such as diabetes, hypercholesterolemia, cancer, hormonal disorders, and inflammation. DI is not synthesized in the mammalian body or does not convert into steroid products; but it acts as a primary intermediate for the synthesis of new steroids [7].

In PK studies of phytochemicals, it is required to utilize a precise method of analytical separation to remove the interfering compounds. Two main extraction methods including liquid–liquid extraction (LLE) and solid-phase extraction (SPE) [8, 9] have been previously reported for DI and TRG. SPE and LLE as pre-analysis treatment techniques are time-consuming and need relatively large amounts of the sample and organic solvents [10]. Selective separation of DI by molecularly imprinted polymers has been reported also [11].

Magnetic nanoparticles (MNPs) have been recently introduced as a novel approach for bio-extraction [12, 13]. MNPs alone [14] and combined with selective sorbents [15, 16] have been reported for extraction of various analytes from different complicated matrices.

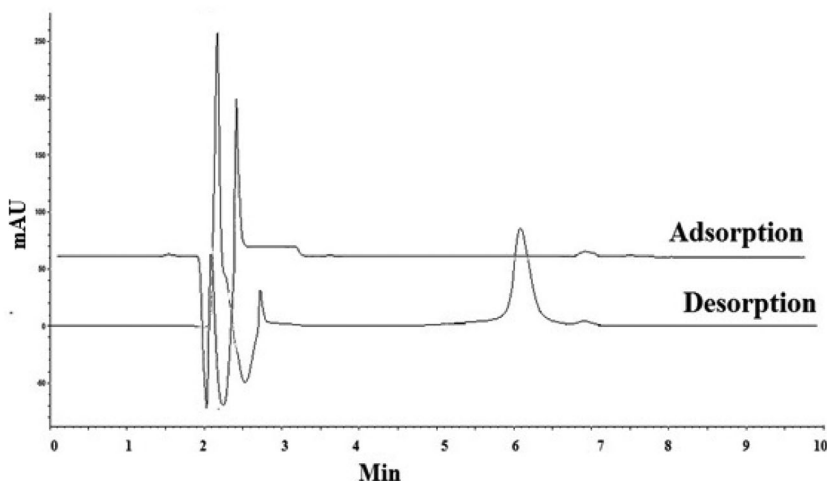
There are some reports for individual PK study of TRG and DI in rat [8, 17], mice [18], and rabbit [19]; while there has been no report of this kind for DI in rabbit. Hence, the objective of the current study was to develop a micro-extraction method using bentonite/ β -cyclodextrin coated MNPs coupled with the ion-pairing reversed phase HPLC (RP-HPLC) method to determine DI and TRG. These extraction and analysis procedures were used successfully for PK study of the analytes in rabbit after intravenous and oral administration of pure compounds and fenugreek seed extract.

2. Results and discussion

Three methods of protein precipitation including the addition of organic solvents (MeCN), chaotropic salts (ammonium chloride), and organic acids (TCA 20% v/v) were examined to have an efficient pre-analysis preparation of rabbit plasma samples (Table 1). Results showed that higher recoveries for TRG and DI could be achieved by the addition of TCA 20% (more than 95% of DI and less than 30% of TRG) in comparison with the addition of acetonitrile and ammonium chloride. Due to the low recoveries of TRG, synthesized magnetic nanocomposites as a solid phase

Table 1. Extraction of DI and TRG from plasma in the presence of different precipitants.

Compounds	Recovery (%)		
	Acetonitrile	Ammonium chloride 5%	Trichloroacetic acid 20%
DI	0	0	96 ± 1.0
TRG	0	0	25 ± 1.5

**Figure 2.** Adsorption and desorption of TRG.

extracting material was applied to improve the extraction of the analyte. Results of the adsorption and desorption procedure of TRG showed a significant increase above 82% for TRG recoveries (Figure 2).

2.1. Method validation

2.1.1. Specificity

The retention times of DI and TRG were 8.6 and 6.8 min (Figures 3–4). The typical chromatograms of blank rabbit plasma sample (Figures 3 and 4A), a rabbit plasma spiked with pure analytes (Figures 3 and 4B), a rabbit plasma sample after oral administration of analytes (Figures 3 and 4C), and a rabbit plasma sample after oral administration of fenugreek extract have been shown in Figures 3 and 4D. No interfering peak was observed in the blank plasma sample at the retention time of two analytes.

2.1.2. Linearity, LOD, and LLOQ

The calibration curves were linear, with a high correlation coefficient, over the concentration range of 20–8000 ng/ml and 50–4000 ng/ml for TRG. DI regression equation was $Y = 92.74 \times 1 - 1416.5$ ($R^2 = 0.9993$) and TRG equation was $Y = 145.75 \times 2 - 7727.9$ ($R^2 = 0.9930$), where X_1 and X_2 are DI and TRG concentrations in ng/ml, respectively, and Y is the peak area under the curve of analytes.

The limit of detection (LOD), LOQ, and lower limit of quantification (LLOQ) were 1.07, 3.24, and 20 ng/ml for DI and 1.30, 4.20, and 50 ng/ml for TRG,

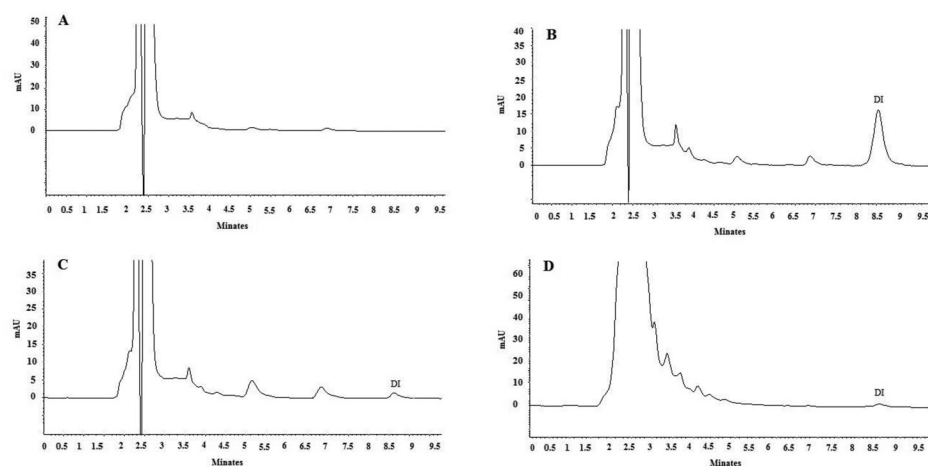


Figure 3. Typical chromatograms of a blank rabbit plasma sample (A), a rabbit plasma sample spiked with pure DI (B), a rabbit plasma sample 30 min after oral administration of pure DI (25 mg/kg) (C), and a rabbit plasma sample 30 min after receiving oral doses of 500 mg/kg extract (D).

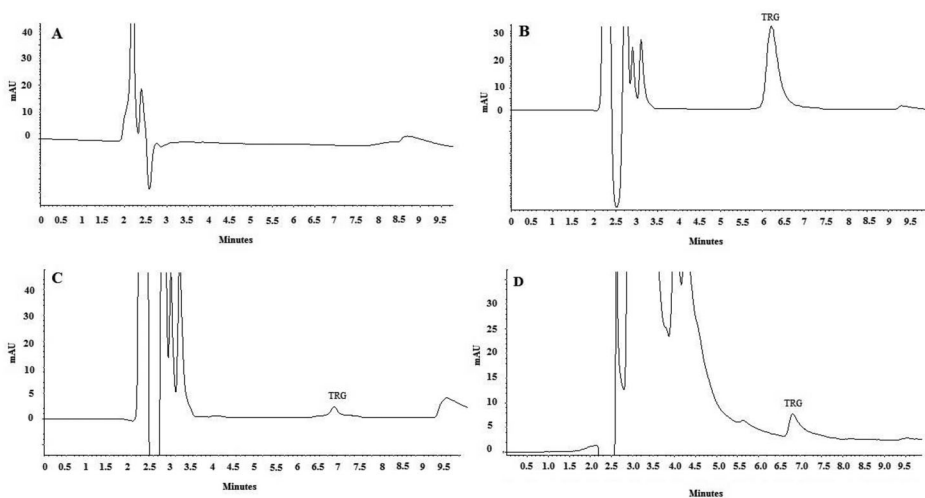


Figure 4. Typical chromatograms of a blank rabbit plasma sample (A), a rabbit plasma sample spiked with pure TRG (B), a rabbit plasma sample 30 min after oral administration of pure TRG (10 mg/kg) (C), and a rabbit plasma sample 30 min after receiving oral doses of 500 mg/kg extract (D).

respectively. Acceptable linearity of the method in a wide range indicates it could be applied to evaluate low and high concentrations of analytes in plasma samples.

2.1.3. Precision and accuracy

The intra- and inter-day precisions, designated with relative standard deviation (RSD%), of the developed method for DI ranged from 1.1 to 10% and for TRG ranged from 1.3 to 9.5%. The accuracy of the method for all calibration points ranged from

Table 2. Stability results for TRG and DI in rabbit plasma ($n = 4$).

Compounds		Short term		Long term		Freeze–thaw	
	Conc (ng/ml)	Conc found ng/ml (mean $\pm$ SD)	RSD%	Conc found ng/ml (mean $\pm$ SD)	RSD%	Conc found ng/ml (mean $\pm$ SD)	RSD%
TRG	50	56.50 $\pm$ 1.18	2.09	55.30 $\pm$ 1.50	2.7	56.03 $\pm$ 1.96	3.5
	500	471.42 $\pm$ 11.78	2.5	466.22 $\pm$ 8.08	1.7	470.66 $\pm$ 11.37	2.4
	2000	2017.10 $\pm$ 98.42	4.72	2111.6 $\pm$ 80.29	3.8	2006.40 $\pm$ 25.51	1.2
	20	21.80 $\pm$ 1.59	7.33	21.10 $\pm$ 1.39	6.59	20.9 $\pm$ 0.56	2.71
DI	500	480.00 $\pm$ 17.61	3.67	485.00 $\pm$ 20.95	4.32	489.00 $\pm$ 8.06	1.65
	4000	3920 $\pm$ 59.58	1.52	3720.00 $\pm$ 74.77	2.01	4020.01 $\pm$ 46.23	1.15

90.2 to 102.8% for DI and 99.6 to 104.0% for TRG. These data indicated that the method was repeatable and accurate for the quantitative determination of the analytes.

2.1.4. Recovery

The recovery of DI at concentrations of 20, 500, and 4000 ng/ml were 82.1 ± 11.2 , 94.8 ± 8.6 , and $95.8 \pm 6.3\%$, respectively; and the respective TRG recoveries at concentrations of 50, 500, and 2000 ng/ml were 113 ± 12.0 , 103 ± 9.8 , and $114 \pm 10.5\%$. The obtained data of this criterion were also satisfaction for the extraction and determination of the two analytes.

2.1.5. Stability

The stability assessment showed that the analytes were chemically unchanged for 4 h at ambient temperature, for 14 days at -20°C and three freeze–thaw cycles. The variations of analytes were not more than 10% at three studied concentrations. Short- and long-term stability evaluation exhibited no obvious loss of the substances during the period of the pharmacokinetic study (Table 2).

2.1.6. Pharmacokinetic study

The developed method was successfully applied to quantify DI and TRG in rabbit plasma after oral and intravenous administration of pure compounds and oral administration of ethanolic extract of fenugreek seeds. Rabbit plasma samples were drawn at the above-mentioned intervals in a period of 24 h, and then they were extracted and analyzed to determine the DI and TRG concentrations. The mean plasma concentration–time profiles of pure DI and TRG were, respectively, shown in Figure 5, demonstrating that both components were fitted to the extravascular two-compartment open model. All PK parameters were presented in Tables 3 and 4. It was found that DI was rapidly absorbed after a single oral administration of 25 mg/kg of the pure compound and 500 mg/kg of the extract (equivalent to 3.3 mg/kg DI), with peak plasma concentrations of 1 and 2 h, respectively. As it can be seen in Table 3, TRG was well absorbed and reached its peak plasma concentration within 1.50 h following 10 mg/kg of oral administration of pure TRG and 500 mg/kg of the extract (equivalent to 4.5 mg/kg TRG; Figure 5). The main PK parameters of oral administration of pure DI and the extract (based on DI) were as follows, respectively: $t_{\max}$: 1 and 2 h; $t_{1/2\alpha}$: 1.52 and 1.19 h^{-1} ; $t_{1/2\beta}$: 6.23 and 15.04 h^{-1} ; k_{10} : 0.16 and 0.18 h^{-1} ; k_{12} : 0.17 and 0.20 h^{-1} ; k_{21} : 0.32 and 0.14 h^{-1} ; AUC_{0-24} : 4.28 and $2.64\text{ }\mu\text{g/ml h}$; Vd_{ss} : 6.8 l/kg. The main parameters

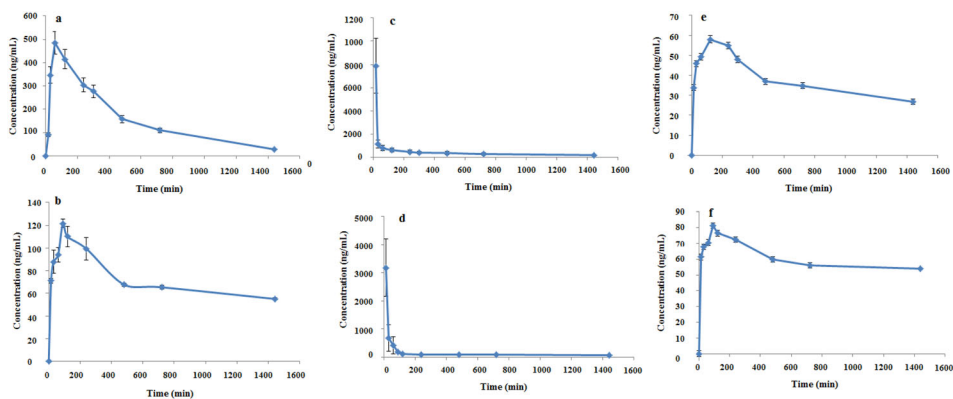


Figure 5. Mean plasma concentration–time curves of Pure DI in rabbit after administration of single intravenous (2.5 mg/kg) (a) and oral (25 mg/kg) (c) doses. Mean plasma concentration–time curves of Pure TRG in rabbit after administration of single intravenous (10 mg/kg) (b) and oral (10 mg/kg) (d) doses. Mean plasma concentration–time curves of DI (e) and TRG (f) in rabbit after administration of fenugreek seed extract (500 mg/kg).

Table 3. Pharmacokinetic parameters of the DI in rabbit plasma after administration of pure form and ethanolic extract of *T. foenum-graecum* ($n = 4$).

Parameter	Unit	DI (pure) oral	DI (extract)
k_a	h^{-1}	1.44	1.10
C_{max}	$\mu g/ml$	0.48	0.057
t_{max}	h	1	2
$t_{1/2\alpha}$	h	1.52	1.19
$t_{1/2\beta}$	h	6.23	15.04
CL	l/h	5.85	2.50
k_{10}	h^{-1}	0.16	0.18
k_{21}	h^{-1}	0.32	0.14
k_{12}	h^{-1}	0.17	0.22
Vd_{ss}	l/kg	2.75	–
AUC^{0-24}	$\mu g/ml \ h$	4.28	2.64
F	%	7.10	–

Table 4. Pharmacokinetic parameters of TRG in rabbit plasma after administration of pure form and ethanolic extract of *T. foenum-graecum* ($n = 4$).

Parameter	Unit	TRG (pure) oral	TRG (extract)
k_a	h^{-1}	1.40	0.89
C_{max}	$\mu g/ml$	0.121	0.081
t_{max}	h	1.30	1.30
$t_{1/2\alpha}$	h	2.00	3.85
$t_{1/2\beta}$	h	20.06	24.70
CL	l/h	1.32	1.15
k_{10}	h^{-1}	0.047	0.041
k_{21}	h^{-1}	0.24	0.11
k_{12}	h^{-1}	0.083	0.040
Vd_{ss}	l/kg	8.61	–
AUC^{0-24}	$\mu g/ml \ h$	7.59	3.92
F	%	79.8	–

k_a is the absorption rate constant; k_{10} is the elimination rate constant from the central compartment; k_{12} is the transfer rate constant from the central compartment to the peripheral compartment; k_{21} is the transfer rate constant from the peripheral compartment to the central compartment; $t_{1/2\alpha}$ is the absorption half time for the central compartment; $t_{1/2\beta}$ is the absorption half time for the peripheral compartment; CL is the total clearance; t_{max} is the time to peak concentration; C_{max} is peak concentration; Vd_{ss} is the volume of distribution during β phase. $AUC_{0-24} \ h$ is the areas under the plasma curve from 0 to 24 h, respectively. F is bioavailability.

after oral administration of pure TRG and the extract (based on TRG) were respectively as follow: $t_{\max}$: 1.30 and 1.30 h; $t_{1/2\alpha}$: 2.00 and 3.85 h⁻¹; $t_{1/2\beta}$: 20.06 and 24.70 h⁻¹; k_{10} : 0.047 and 0.041 h⁻¹; k_{12} : 0.083 and 0.040 h⁻¹; k_{21} : 0.24 and 0.11 h⁻¹; AUC₀₋₂₄: 7.59 and 3.92 µg/ml h; Vd_{ss}: 8.61 l/kg. The PK study of the compounds has enabled us to understand and compare the absorption, metabolism, and bioavailability of the compounds in pure and in the extract forms. PK properties of DI in rabbit after oral administration were characterized as rapid absorption with a short residence time of about 6 h and poor absolute bioavailability. TRG showed a rapid absorption rate with a middle residence time of about 24 h with high bioavailability. In the current study carried out on rabbit, the concentration-time curves of TRG after oral and IV administration were best fitted on a two-compartment open model, whereas Cheng and colleagues study on rat [17] showed that oral and IV concentration-time profiles of this compound were best fitted on a one-compartment open model. Okawara *et al.* reported due to low lipid solubility of DI, it cannot pass the highly lipophilic membranes of the cells. Therefore, it is distributed throughout the body in the extracellular compartment. In this study, the absolute oral bioavailability of DI was as low as 7.10% which is compatible with previous studies reported by Okawara [20]. According to their report, this may be related to the high lipophilic characteristics of DI that exhibits poor permeability across intestinal epithelial cells. They suggested that β-cyclodextrin improved the bioavailability of DI to 45% in rats [21]. Low bioavailability of phytosterol has been observed in human plasma as well [22]; they reported that just 0.6–7.5% of orally administered phytosterol was transported through gut epithelial cells *in vivo*. In the present study, DI solubility has been increased by solving the analyte in ethanol. So its absorption rate increased and during 1 h reached to the maximum concentration. Its low bioavailability may be due to its metabolism in the liver before adequate plasma concentrations. The bioavailability of TRG in rabbits was quite high with a value of 79.8%. TRG is well absorbed and could easily pass through the intestinal wall. Cheng *et al.* reported that the absolute bioavailability of pure TRG was 57.37% in rats, whereas that of TRG in methanol extract of coffee beans was 64.42% [17]. Bioavailability refers to the extent and rate at which the active drug or metabolite enters systemic circulation, thereby accessing the site of action. The bioavailability of a drug is largely determined by the properties of the dosage form, which depend partly on its design and manufacture. Differences in bioavailability among formulations of a given drug can have clinical significance; thus, knowing whether drug formulations are equivalent is essential. Orally administered drugs must pass through the intestinal wall and then the portal circulation to the liver; both are common sites of first-pass metabolism (metabolism that occurs before a drug reaches systemic circulation). Thus, many drugs may be metabolized before adequate plasma concentrations are reached. Low bioavailability is most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs. If the drug does not dissolve readily or cannot penetrate the epithelial membrane, time at the absorption site may be insufficient. In such cases, bioavailability tends to be highly variable as well as low. Steady state volume of distribution (Vd_{ss}) is one of the basic PK parameters of drug candidates. Size, pH gradients, the polarity of the molecule, and tissue protein binding play dominant roles in determining Vd_{ss} [23]. Neutral molecules like DI which do not have any acid or base ionizable groups mainly bind to

lipoproteins and a lesser extent to albumin. Increasing lipophilicity may increase the volume of distribution. Zwitterion compounds are likely to bind to most plasma proteins. Specific structural effects on binding are frequently observed among these drugs, especially when the strongest acid or base groups are separated by 4–7 atoms. The PK parameters for both analytes were changed when they gave in the form of extract. Variation of PK parameters in two pure and extract group may be due to the presence of molecules with similar structures that they can competitively inhibit the binding, excretion, or metabolism of the main component [23].

In summary, the current study aimed to investigate the pharmacokinetic properties of DI and TRG in rabbit following intravenous and oral administration of the pure compounds and fenugreek seed extract. A micro-extraction method using MNPs containing bentonite/ β -cyclodextrin adsorbents coupled with the ion-pairing RP-HPLC method was used to determine the analytes in plasma. Fitting data on compartmental models demonstrated that the PK profile of the two compounds was best fitted on two-compartment model with, rapid absorption, middle elimination but poor bioavailability for DI and rapid absorption, elimination and high bioavailability for TRG.

3. Experimental

3.1. Materials and reagents

All reagents used in this study were of analytical grade. Standard TRG (purity > 93.0%), DI and 1-octanesulfonic acid (OSA) were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile (MeCN) and formic acid were obtained from Duksun Company (Seoul, South Korea) and Merck (Darmstadt, Germany), respectively. Deionized water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

3.2. Preparation of the plant extract

The dried powder of Fenugreek seed was extracted with 80% ethanol by the maceration method (72 h). The solution was filtered and then evaporated to dryness to yield nearly a dry solid.

3.3. Synthesis of bentonite nanocomposite

Bentonite nanocomposites were synthesized by an unpublished method. Briefly, an amount of 1.07 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was dissolved in ethanol/deionized water mixture (4:1 v/v %) and then 1.50 g of the bentonite was added and mixed in an ultrasonic shaker. In another vessel, 0.61 g sodium borohydride (NaBH_4) was dissolved in 100 ml of deionized water, and it was gradually added to the aqueous bentonite/ Fe^{2+} mixture. The precipitate was washed with absolute ethanol and dried in the oven at 50 °C. Next, 0.4 g of magnetic bentonite and 0.4 g β -cyclodextrin were added to 200 ml deionized water. The mixture was placed in a hot water bath at 60 °C for 3.5 h while stirring. Finally, nanocomposites were separated using a paper filter and dried and kept in the oven at 50 °C until use [24].

3.4. Extraction of analytes from plasma

A volume of 200 μ l rabbit plasma was vortex-mixed with 200 μ l trichloroacetic acid (TCA) 20% (as protein precipitant) and then 2 mg nanocomposite was added into the mixture. The mixture was then shaken for 10 min and centrifuged at 7000 rpm for 10 min. Finally, 50 μ l of the supernatant was injected into the HPLC system to determine DI. Desorption of TRG from the precipitate remained from the previous stage. A volume of 500 μ l 1 M NaCl was added to the magnetic nanocomposite pellet. The mixture was kept in a hot water bath at 90 °C for 15 min while stirring and centrifuged at 7000 rpm for 10 min. A volume of 50 μ l of the supernatant was injected into the HPLC system to determine TRG.

3.5. Analytical procedure

The analyses were performed using a Smartline[®] HPLC system (Knauer, Berlin, Germany) consisted of pump 1000, PDA-UV detector 2600, manager 5000, autosamplers 3950, membrane vacuum degasser and column temperature compartment. Separations were performed on a Waters[®] C18 column (250 $\times$ 4.6 mm, 4 μ m; Waters, Milford, MA, USA). The software ChromGate[®] version 3.1.7 (Knauer[®], Germany) was utilized for data acquisition and processing. The mobile phase consisted of H₂O:MeCN (77:23 v/v%) with 0.1% formic acid and 4 mM OSA as an ion-pairing reagent. Isocratic elution was performed at a flow rate of 1 ml/min in a 10 min run time. A volume of 50 μ l of the standards and samples were injected automatically into the column which was kept at 25 °C. The PDA detector was set at 232 and 297 nm for TRG and DI, respectively.

3.6. Validation

3.6.1. Calibration curve

Plasma working calibration standards were prepared at concentrations of 20, 50, 500, 1000, 2000, and 4000 ng/ml for DI and 50, 500, 1000, 2000, and 4000 ng/ml for TRG by spiking appropriate volumes of secondary and tertiary standards into rabbit plasma.

3.6.2. LOD, LOQ, and LLOQ

To calculate LOD and LOQ, eight analytical blanks were analyzed and the signal from noise observed at or close to the retention time (t_R) of the analyte was measured and considered as a criterion. Formulas I and II were used to calculate these two parameters.

$$(I) \text{ LOD} = 3.3SD_{\text{blank}}/\text{Slope}, \quad (II) \text{ LOQ} = 10 SD_{\text{blank}}/\text{Slope},$$

where SD blank is the standard deviation of analytical blanks; Slope is the slope of the standard calibration curve.

To determine LLOQ, DI, and TRG concentrations in the lower part of the linear range of the calibration curve, reliable precision (RSD) were used.

3.6.2. Precision and accuracy

The precision of the method was evaluated at two levels by triplicate analyses of all standard calibration points in one day for an intra-day level and in 3 different days for the intermediate level. RSD% of the analyses was the criterion for assessment of the precision.

3.6.3. Extraction recovery

Recovery of DI and TRG was determined by comparing peak areas of the extracted analytes from spiked plasma (S1) with peak areas of DI and TRG standard solutions (S2). This procedure was repeated six times at three concentration levels; and the recovery of extraction was calculated as: "Recovery percentage = $S1/S2 \times 100$ ".

3.6.4. Selectivity and specificity

To study whether endogenous plasma components interfere with the chromatographic peaks of two analytes or not, four different blank rabbit plasma samples were analyzed using the proposed extraction and HPLC methods, and then the potential interfering peaks around $t_{R(DI)}$ and $t_{R(TRG)}$ was assessed in the resulted chromatograms.

3.6.5. Stability experiments

Stability experiments were performed to evaluate the stability of the analytes in the plasma samples under different conditions. The stability of two analytes in plasma during the sample preparation was evaluated after 4 h storage at room temperature. For the long-term stability study, the plasma samples were analyzed after 14 days of storage at -20°C and after three freeze-thaw cycles (-20°C to room temperature as one cycle). The limit for acceptable stability was considered within $\pm 15\%$ of the peak area of freshly prepared standards.

3.7. Animal treatment

The pharmacokinetic study protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (No. IR.kmu.rec.1396.2511). Healthy male New Zealand white rabbits (supplied by the Animal Center of Kerman University of medical science) weighing between 2.3 and 3.0 kg and kept under controlled conditions at 25°C with a 12 h dark-light cycle was entered into the studies. The rabbits had free access to food and water up to 12 h before the experiments. Four rabbits were used in each group. For intravenous (IV) administration, DI and TRG were dissolved in ethanol 30% and distilled water, respectively. The solutions were filtered by a 0.2 micron filter membrane. To calculate tonicity, the following equation was used:

$$E = 17 \frac{L_{iso}}{MW}$$

In which, E is the sodium chloride equivalent, L_{iso} is Vant's Holff constant that for molecules of univalent such as TRG can be assumed as 3.4 and for a nonelectrolyte like DI is equal to 1.9 and MW is the molecular weight of TRG and DI that are equal to 137.18 and 414.63 g/mole, respectively. E values calculated for TRG and DI are 0.421 and 0.078, respectively. In the IV injection solution TRG concentration was 30 mg/

0.5 ml that is equal to 6 g/dl. In the case of TRG it means that its concentration is equal to $6 \times 0.421 = 2.53$, which is very greater than 0.9% means is hyperosmolar. This solution is hyperosmolar, however, the volume that injected to the bloodstream is negligible compared to the total blood volume of rabbit (about 60 ml/kg) and the injection of low volumes of intravascular hypertonic solutions is popular. In the case of DI, the IV injection solution concentration was 7.5 mg/0.5 ml which is equal to 1.5 g/dl. It means that its concentration is equal to $1.5 \times 0.078 = 0.12$, which is very smaller than 0.9% means is hypotonic. In this situation, it can be concluded that because the injection volume is very smaller than total blood volume of rabbit (0.5 ml compared to 150 ml), therefore, the tonicity adjustment of the solution was unnecessary.

The IV dose of DI was 2.5 mg/kg and that of TRG was 10 mg/kg. For oral administration, the rabbits were fed with aqueous solutions of TRG and fenugreek extract and ethanolic solution of DI using insulin syringes. The oral doses of DI, TRG, and the extract were 25, 10, and 500 mg/kg (equivalent to 4.5 mg/kg TRG and 3.3 mg/kg DI), respectively.

3.8. Pharmacokinetic assay

Blood samples (350 μ l) were taken from the marginal veins of rabbits' ear and collected in heparin-containing tubes. Blood samplings were done at 0 (before administration), 15, 30, 60, 120, 240, 300, 480, 720, and 1440 min after DI treatment and at 0 (before administration), 15, 30, 60, 90, 120, 240, 480, 720, and 1440 min after TRG treatment. Samples were centrifuged at 5000 rpm for 10 min at room temperature and plasma samples were aliquoted and kept at -20°C until analysis. The PK parameters were calculated for each subject by the Monolix (version 2018R1) software.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by Kerman University of Medical Science, Kerman, Iran [Grant Number 96000999].

ORCID

Mehdi Ansari  <http://orcid.org/0000-0003-4637-553X>

Mostafa Pournamdari  <http://orcid.org/0000-0001-8636-9869>

References

- [1] A.A. Salim, Y.W. Chin, and A.D. Kinghorn, *Bioactive Molecules and Medicinal Plants*. (Springer, Berlin, Heidelberg, 2008), p. 24.
- [2] M. Pournamdari, A. Mandegary, F. Sharififar, G. Zarei, R. Zarehshahi, A. Asadi, and M. Mehdipour, *J. Diet. Suppl.* **15**, 98 (2018).

- [3] N. Mohamadi, F. Sharififar, M. Pournamdari, and M. Ansari, *J. Diet. Suppl.* **15**, 207 (2018).
- [4] T.M. Khan, D.B.C. Wu, and A.V. Dolzhenko, *Phytother. Res.* **32**, 402 (2018).
- [5] F. Sharififar, P. Khazaeli, and N. Alli, *Iran. J. Pharm. Sci.* **5**, 157 (2009).
- [6] M. Ansari, P. Sadeghi, H. Mahdavi, M. Fattahi-Dolatabadi, N. Mohamadi, A. Asadi, and F. Sharififar, *JCIM* **16**, 1 (2019).
- [7] J. Raju, and C.V. Rao, *Bioactive Compounds in Phytomedicine*. (InTech, Shanghai, China, 2012), pp. 125–142.
- [8] L. Xu, Y. Liu, T. Wang, Y. Qi, X. Han, Y. Xu, J. Peng, and X. Tang, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **877**, 1530 (2009).
- [9] H.Q. Pang, Y.P. Tang, Y.J. Cao, Y.J. Tan, Y. Jin, X.Q. Shi, S.L. Huang, D.Z. Sun, J. Sun, Z.S. Tang, and J.A. Duan, *J. Chromatogr. B* **1061–1062**, 372 (2017).
- [10] N. Manousi, G. Raber, and I. Papadoyannis, *Separations* **4**, 18 (2017).
- [11] C. Zhai, Q. Lu, X. Chen, Y. Peng, L. Chen, and S. Du, *J. Chromatogr. A* **1216**, 2254 (2009).
- [12] K.C. de Souza, G.F. Andrade, I. Vasconcelos, I.M. de Oliveira Viana, C. Fernandes, and E.M.B. de Sousa, *Mater. Sci. Eng. C Mater. Biol. Appl.* **40**, 275 (2014).
- [13] M. Dastkhooon, M. Ghaedi, A. Asfaram, M. Arabi, A. Ostovan, and A. Goudarzi, *Ultrason. Sonochem.* **36**, 42 (2017).
- [14] A. Bhati, R.P. Desai, and C. Ramchand, *J. Pharm. Biomed. Anal.* **143**, 277 (2017).
- [15] A. Badruddoza, A. Tay, P. Tan, K. Hidajat, and M. Uddin, *J. Hazard. Mater.* **185**, 1177 (2011).
- [16] L. Fan, C. Luo, M. Sun, H. Qiu, and X. Li, *Colloids Surf. B Biointerfaces* **103**, 601 (2013).
- [17] Z.X. Cheng, W.J. Jun, L.Z. Qiu, and L. Na, *Chin. J. Nat. Med.* **11**, 164 (2014).
- [18] D. Szczesny, E. Bartosińska, J. Jacyna, M. Patejko, D. Siluk, and R. Kaliszan, *Biomed. Chromatogr.* **32**, e4054 (2018).
- [19] H. Zhao, Y. Qu, X. Wang, X. Lu, X. Zhang, and M. Hattori, *Acta Pharm. Sin. B* **38**, 279 (2003).
- [20] M. Okawara, F. Hashimoto, H. Todo, K. Sugibayashi, and Y. Tokudome, *Int. J. Pharm.* **472**, 257 (2014).
- [21] M. Okawara, Y. Tokudome, H. Todo, K. Sugibayashi, and F. Hashimoto, *Biol. Pharm. Bull.* **36**, 36 (2013).
- [22] I. Epriliati, and I.R. Ginjom, *Phytochemicals—A Global Perspective of their Role in Nutrition and Health*. (InTech, Shanghai, China, 2012), pp. 1–32.
- [23] D.A. Smith, K. Beaumont, T.S. Maurer, and L. Di, *J. Med. Chem.* **58**, 5691 (2015).
- [24] T. Shahwan, Ç. Üzümlü, A.E. Eroğlu, and I. Lieberwirth, *Appl. Clay Sci.* **47**, 257 (2010).