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Article

Effect of herb-drug interactions of *Bacopa monnieri* Linn. (Brahmi) formulation on the pharmacokinetics of amitriptyline in rats

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Interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component. Experimental data on the pharmacokinetic interactions between herbal products and drugs are limited. This study attempted to investigate the effect of *Bacopa monnieri* Linn. (Brahmi) formulation on the pharmacokinetics of amitriptyline in rats. In this study, rats were randomly divided into two groups (n = 6 each) which were served as a control (amitriptyline alone) and treatment group (amitriptyline with *B. monnieri*), respectively. Rats in the treatment group received *B. monnieri* (31 mg/kg/day) whereas the control group received normal saline by oral gavage for seven days before a single intragastric administration of 25 mg/kg amitriptyline. Plasma concentrations of amitriptyline were measured up to 24 h after its administration by a developed and validated high-performance liquid chromatography method. Pretreatment with *B. monnieri* produced a significant increase in the maximum plasma concentration (C_{max}), area under the curve (AUC_{0-t}) and elimination half-life ($t_{1/2}$) of amitriptyline by 16.8%, 26.5%, and 15.5%, respectively, compared to amitriptyline alone. Moreover, oral clearance and volume of distribution (V_{ss}) were decreased by 26.2% and 15.5% respectively. This study concluded that *B.monnieri* significantly enhanced the oral bioavailability of amitriptyline in rats.

Keywords: Herbal drug interaction. *Bacopa monnieri*/effects. Amitriptyline/pharmacokinetics. CYP450. Bioavailability.

INTRODUCTION

Amitriptyline, a tricyclic compound, has been widely used globally for decades in the treatment of mental illnesses, especially depression. It still serves as one of the most commonly used antidepressants. (Leucht, Huhn, Leucht, 2012). Although the mode of action of amitriptyline is not evident, it is speculated to act by inhibiting the reuptake of serotonin–norepinephrine at the adrenergic nerve endings, thus disrupting the functions of these chemicals (Tatsumi *et al.*, 1997; Amin Bano, 2014). Amitriptyline is completely but slowly absorbed from the gastrointestinal tract after its oral uptake such that the peak plasma concentrations are achieved within 4 to 8 h of its administration. It has a systemic bioavailability ranging from 33 to 62% and is subjected to extensive hepatic pre-systemic elimination (Schulz *et al.*, 1983). Within the liver, the drug is primarily metabolized by the action of cytochrome P450 enzymes, namely, CYP2D6, CYP3A4, and CYP2C19 (Rudorfer, Potter, 1999). The significant adverse effects associated with therapeutic concentrations of amitriptyline are manifested in terms of a moderate therapeutic index; an overdose of it may prove to be dangerous.

Bacopa monnieri, a perennial herb, belongs to the family, Scrophulariaceae and is commonly known as Brahmi in the Ayurvedic system of medicine. It has been widely employed as a brain stimulator, antidepressant, and memory enhancer (Stough *et al.*, 2001). Various research studies, performed using the standardized extracts of *B. monnieri*, have demonstrated the herb to facilitate the processes of acquisition, retention, and retrieval of learned

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tasks. A study that evaluated the antidepressant potential of *B. monnieri* reported it exhibit a significant antidepressant activity in the most commonly used behavior paradigms in animal models of depression. These included forced swim test and learned helplessness test (Sairam *et al.*, 2002). Another study that employed different convulsive models in albino rats to investigate the anticonvulsant activity of an alcoholic extract of *B. monnieri* reported the presence of a broad spectrum of anticonvulsant profile of the extract in chemical, electrical, and hypoxic convulsions (Kaushik *et al.*, 2009). Moreover, a clinical trial conducted to assess the effects of administration of *B. monnieri* (300 mg/day) over a period of 12 weeks on memory performance reported the ability of the herb to significantly improve the memory acquisition and retention in individuals over the age of 55 years (Morgan, Stevens, 2010). Another study that analyzed the effects of a standardized *B. monnieri* (300 mg/day) on cognitive performance, anxiety, and depression in the elderly individuals concluded it to be a plant with a potential to safely enhance the cognitive performance in aging (Calabrese *et al.*, 2008). This hypothesis was also favored by the studies conducted in rodents where *B. monnieri* provided protection against age-related oxidative stress and some inflammatory conditions (Williams *et al.*, 2014). It is also known to enhance the lifespan during stress conditions (Phulara *et al.*, 2015). The above-mentioned findings have been strengthened by several clinical studies (Stough *et al.*, 2001; Roodenrys, 2002; Calabrese *et al.*, 2008) that ascribed the observed antidepressant effects to its phytochemical constituents, known as bacoside. Bacoside is a mixture of four triglycosidic saponins, namely, bacoside A3, bacopaside II, bacopaside X, and bacopasaponin C (Singh *et al.*, 1988; Dhawan, Singh, 1996; Russo, Borrelli, 2005).

B. monnieri has an extensive market both in India and outside. The neuropharmacological properties of *B. monnieri* (Russo, Borrelli, 2005; Calabrese *et al.*, 2008) considerably increase the chances of chronic or recurrent usage of the herb and its associated products by patients with mental illnesses. The prescription of therapeutic drugs to these patients significantly contributes to herb–drug interactions at the physiological levels. These interactions, in turn, may increase or decrease the pharmacological or toxicological effects of either component (Hu *et al.*, 2005). Inhibition or induction of hepatic and intestinal drug-metabolizing enzymes, particularly cytochrome P450 (CYP) and transporters (e.g., p-glycoprotein) act as the trigger for the interactions (Zhang *et al*., 2010a; Wanwimolruk, Prachayasittikul, 2014a; Wanwimolruk, Phopin, Prachayasittikul *et al.*, 2014b). However, there exists a paucity of literature on the pharmacokinetic interactions between herbal products and drugs. The present study attempted to investigate the effects of a commercial formulation of *B. monnieri* (Brahmi) on the pharmacokinetics of amitriptyline using rodents as the model system. An extensive review of the literature revealed the presence of several analytical methods, based on high-performance liquid chromatography (HPLC), for the estimation of amitriptyline and some of its metabolites in biological samples (Ghahramani, Lennard, 1996; Aymard *et al.*, 1997; Farag *et al.*, 2013). However, the major drawbacks of these procedures are complexity, time constraint, and limited sensitivity, leading to the requirement of a rapid, specific, and sensitive analytical method. The present study aimed to develop a highly sensitive reversed-phase high-performance liquid chromatography (RP-HPLC-UV) method, followed by its validation for the quantification of amitriptyline in rat plasma.

MATERIAL AND METHODS

Material

Amitriptyline and imipramine, as internal standards (ISs), were purchased from Sigma-Aldrich (Germany). High-performance liquid chromatography (HPLC) grade LiChrosolv methanol and LiChrosolv acetonitrile were from Merck (Darmstadt, Germany). Chemicals of highest available commercial purity were utilized. The commercial formulation of *B. monnieri* (Himalaya Drug Company, Bangalore, India) was procured from the local market. The HPLC-grade solvents were used for HPLC determinations. Milli-Q plus water (Millipore, Bedford, MA, United States) was used for all preparations. All other chemicals were of analytical grade.

Animals and study design

The study was conducted in accordance with current legislation on animal experiments as per Institutional Animal Ethical Committee (CPCSEA Approval no. 962/PO/Re/S/06/CPCSEA). Twelve male Wistar albino rats, weighing 225 to 250 g were provided by the animal house, Alwar Pharmacy College, Alwar, Rajasthan. The rats were housed under standard animal conditions with alternate 12 h of light and dark cycles in an environment maintained at a constant temperature prior to the study. Water was supplied *ad libitum*. Rats were randomly divided into two groups (n = 6 each); one served as the control (amitriptyline alone), and the other group served as the treatment group

(amitriptyline with *B. monnieri*). A randomized parallel design study was employed to study the pharmacokinetics of amitriptyline. Rats in the control group were provided normal saline orally (10 mL/kg) for 7 consecutive days followed by administration of amitriptyline (25 mg/kg bw p.o.) on day 8, 1 h after administration of normal saline. Amitriptyline was administered as an aqueous solution in 0.5% (w/v) sodium carboxy-methyl cellulose (CMC) at a dose of 25 mg/kg bw p.o. Rats in the treatment group were administered *B. monnieri* extract in 0.5% CMC at a dose of 31 mg/kg/day, p.o. in saline water (Singh *et al.*, 2013) for 7 consecutive days. The rats were made to fast for at least 12 h (overnight) with free access to water before the day of the experiment. On the morning of day 8, the last dose of *B. monnieri* was administered to the fasting rats. One hour after the administration of the last dose of *B. monnieri*, amitriptyline aqueous solution in 0.5% (w/v) CMC (25 mg/kg p.o.) was administered to these rats. The drug was administered via gastric gavage throughout the study.

Blood collection

A total of 1 mL of blood samples were collected into heparinized vacutainer tubes from the retroorbital plexus of each rat using capillary tubes before and after the administration of amitriptyline at 0, 1, 2, 4, 6, 8, 12, and 24 h. The blood samples were immediately centrifuged at 2,500×g for 10 min to separate the plasma that was stored at –80 ºC until analysis. To replace the fluid loss, equal volumes of normal saline were injected through the cannula in all experiments. The same sampling scheme was followed for rats in the treatment group to determine the plasma concentrations of amitriptyline and evaluate the effect of *B. monnieri* on amitriptyline disposition in rats.

Amitriptyline assay by HPLC Method

Instrumentations and chromatographic conditions

The high-performance liquid chromatography (Waters, 1525 Binary HPLC pump) employed was equipped with the Waters-2489 UV-visible detector and Waters-2707 Autosampler and operated by Breeze 2 service pack A (SPA) software, Waters Corporation. The chromatographic identification was conducted at ambient temperature. The mobile phase consisted of a v/v ratio of acetonitrile and potassium dihydrogen phosphate buffer (KH_2PO_4, 38:62), which was delivered at the isocratic condition with a flow rate of 1 mL/min. EC 150/4.6-NUCLEODUR Sphinx RP, 5 µm MACHERY-NAGEL column was used to elute amitriptyline at a wavelength (λ_{max}) of 254 nm. Different combinations of solvent systems of acetonitrile, buffer (KH_2PO_4): acetonitrile, and formic acid: water, and methanol: water: acetonitrile were tried to determine the optimum conditions for the separation and standardization of amitriptyline. The mobile phase consisted of acetonitrile and 70 mM KH_2PO_4 buffer. Its pH was adjusted to 4.5 by 85% orthophosphoric acid in a ratio of 38:62 (v/v). The selection of mobile phase was based on its ability to provide high resolution for amitriptyline with minimal tailing.

Sample preparation

The samples for analysis were prepared by the protein precipitation method. Plasma samples stored at around − 80˚C were thawed at room temperature and vortexed for 30 s to ensure homogeneity. The internal standard (8 µL, 100 µg/mL) was added to 200 µL of plasma sample, and 592 µL of acetonitrile was added to precipitate the protein from the sample. The samples were mixed gently for 1.5 min followed by centrifugation at 12,000 rpm for 10 min. After centrifugation, 700 µL of clear supernatant was transferred into HPLC vials. Finally, 25 µL of each sample was subjected to HPLC-UV analysis.

Calibration and control samples

The linearity of an analytical method refers to its ability to furnish test results that are directly proportional to the concentration of an analyte in the samples within a given range. In other words, linearity is the relationship between the concentration of analyte and assay measurement (ICH, 1996). We used correlation coefficient (R^2) obtained from the linear regression to demonstrate the linearity of the relationship between the peak area ratio and the concentration. The experiment was performed in triplicates with the concentration of analyte ranging from 0.125 to 50.0 µg/mL. The relative standard deviations were calculated for all the calibration curve slopes. The observed straight-line equation, $y = 0.0343 \times - 0.0026$ ($R^2 = 0.9994$) was used for the calculation of plasma concentrations of amitriptyline.

Validation and stability of HPLC method

The guidelines of the international conference on harmonization were employed to validate the HPLC method in terms of linearity, specificity, sensitivity,

precision, and accuracy (ICH, 1996). The robustness of the method was evaluated by intentionally introducing minor modifications in the mobile phase volume ratios. These included mobile phase having different compositions of acetonitrile-70 mM KH_2PO_4 (6038:62 ± 5 mL), alteration of the pH of buffer (4.5 ± 0.2), and slight changes in the isocratic flow rate (1.0 ± 0.2 mL/min) of the mobile phase. All the validation parameters were studied in triplicate (n=3) at a concentration of 20 µg/mL. The stability of amitriptyline in plasma at 4 and 20 ºC and after freeze–thaw cycles was determined.

Pharmacokinetic analysis

We utilized the non-compartmental analysis to determine the pharmacokinetic parameters of amitriptyline after oral administration. The maximum observed plasma concentration (C_{max}) and the time to reach this concentration (T_{max}) following oral administration were calculated from the observed data. The volume of distribution at steady state (V_{ss}) was calculated as ($AUMC_{0\text{-}inf}/AUC_{0\text{-}inf}$)*CL and total body clearance (CL) as dose/$AUC_{0\text{-}inf}$. The area under the plasma concentration versus time curve ($AUC_{0\text{-}t}$) was calculated by the trapezoidal rule with extrapolation to infinity. The linear regression analysis of the terminal portion of the log concentration–time data was utilized to calculate the apparent terminal elimination rate constant (K_{el} or λ_z) of amitriptyline. Amitriptyline apparent terminal elimination half-life ($t_{1/2}$) was calculated as (ln2)/λz, where λz is the elimination rate constant. Pharmacokinetic analysis was performed using the program, PKSolver for Microsoft Excel (Zhang *et al.*, 2010b).

Statistical data analysis

All statistical data were expressed as the mean ± standard deviation (SD). Student's *t*-test on log-transformed data was utilized for assessing the differences in the pharmacokinetic parameters of amitriptyline with and without *B. monnieri*. Data were considered statistically significant at a p<0.05. All calculations were performed using GraphPad Prism, version 3.00 for Windows (San Diego, CA, United States).

Herbal medicine and dose calculation

The recommended adult dose of *B. monnieri* extract containing 20% of total bacopasides was 300 to 400 mg daily. A dose equivalent to human dose was used as the animal dose of these herbs and was calculated using the following equation.

$$\text{Human equivalent dose (mg/kg)} = \text{Animal dose (mg/kg)} \times \frac{\text{Animal Km}}{\text{Human Km}}$$

The human dose was selected from authentic herbal textbook resources (Reagan-Shaw, Nihal, Ahmad, 2008). The rat dose was found to be 31 mg/kg/day (Singh *et al.*, 2013).

RESULTS AND DISCUSSION

HPLC method development and validation for amitriptyline assay

The mobile phase of HPLC column was selected on the basis of previously used and described methods available for amitriptyline (Shen *et al.*, 2010; Farag *et al.*, 2013). The KH_2PO_4 buffer was checked at different concentrations (0.02, 0.05, and 0.07 M) for determining its efficient working concentration. At lower concentrations, amitriptyline and IS did not result in the same retention time. At a buffer concentration of 0.07 M, the analytes were found to be well separated from plasma and hence it was chosen as the optimal buffer concentration. A range of buffer pH (3.0–7.0) was assayed to optimize the chromatographic separation. Optimal peak separation for amitriptyline and IS was produced using a pH value ranging between 3.0 and 7.0. However, when the spiked matrix samples were analyzed, the peaks from some of the plasma impurities matched with that of IS. A pH value of 4.5 was found to be optimal for the complete separation of amitriptyline and imipramine (IS); at this pH value, peaks were resolved well. The final mobile phase consisted of acetonitrile-KH_2PO_4 (0.07 M), pH 4.5 with 1.0 mL/min flow rate. This condition was found to be outstanding in terms of sensitivity and peak separation. The wavelengths checked in the present study were 230, 240, and 254 nm (Olsen, Sullivan, 1995). The wavelength of 254 nm was found to be optimal in terms of sensitivity for all the analytes; it also avoided the occurrence of numerous matrix impurities and exogenous substances.

Selectivity and specificity

The selectivity of any analytical method refers to the ability of the method to assess the analyte unequivocally in the presence of endogenous matrix compounds (i.e., plasma and proteins in this method). The chromatograms of plasma samples by using HPLC method are depicted in Figure 1. The symmetrical peaks were observed for amitriptyline with a retention

time of 6.15 min. There was no interference with the amitriptyline peak, and the overall chromatographic run time was 10 min. HPLC chromatograms of blank plasma (Figure 1A), plasma spiked with imipramine as IS 25 ng/mL (Figure 1B), plasma sample spiked with amitriptyline (20 µg/mL) with retention times of 6.15 min. Retention time of the IS was 4.52 min (Figure 1C). The plasma samples obtained from rat, administered with 25 mg/kg p.o. dose of amitriptyline (Figure 1D), were compared to show the selectivity of the adopted HPLC method. The retention times of amitriptyline and imipramine were approximately 6.15 and 4.52 min, respectively. The designed HPLC method was found to be considerably selective, reflected by the absence of the appearance of any other interfering peaks around the retention times due to endogenous matrix substances or metabolite effects during quantification of amitriptyline and imipramine (IS) in plasma samples.

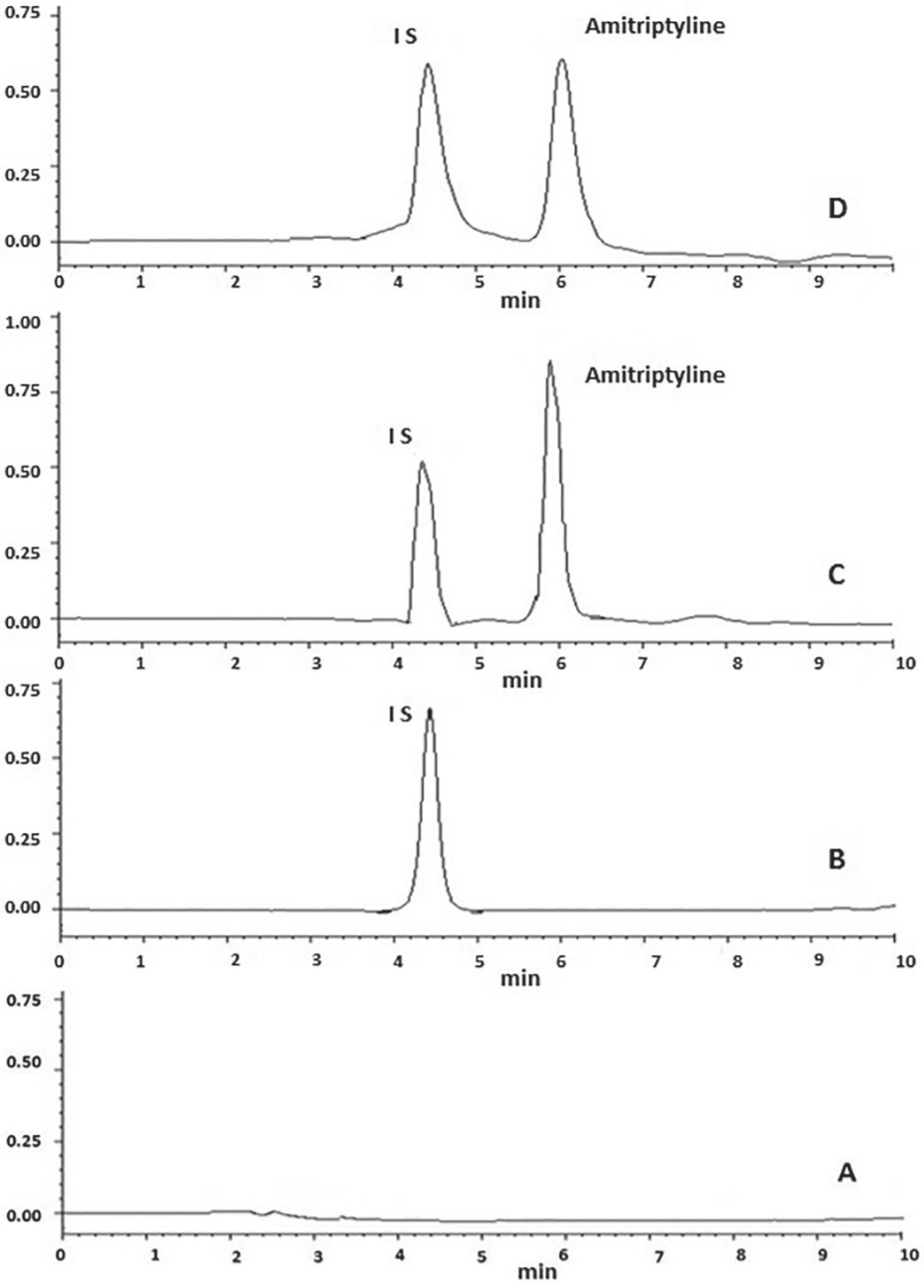

FIGURE 1 - HPLC chromatogram of blank plasma (A), plasma spiked with imipramine as internal standard (I.S) (B), plasma sample spiked with amitriptyline (20 µg/mL concentration) (R_t 6.15 min) and IS (R_t 4.5 min) (C), plasma samples obtained from rat, administered with 25 mg/kg, p.o. dose of amitriptyline (D).

Precision and accuracy of developed HPLC method

An evaluation of the concentrations of three levels, namely, LLOQ, MQC, and ULOQ indicated the relative standard deviation (% RSD) values to be satisfactory. The calculated percentage recovery of the MQC level was found to be 100.14 ± 0.15%. The recovery values obtained from the analysis of different known concentrations by the developed HPLC method ranged from 98.31 to 99.02, indicating the method to be highly accurate. The percentage recovery determined for the lowest concentration (LLOQ) and MQC were 98.31 ± 0.85 and 99.23 ± 0.15%, respectively, and that of the highest concentration (ULOQ) was 99.02 ± 0.54 % during the inter-day analysis of the samples. All the results are listed in Table I. The reproducibility and reliability of the method was evident by low values of % RSD for LLOQ, MQC, and ULOQ.

Sensitivity, Limit of Detection (LOD), and Limit of Quantification (LOQ)

The lower limit of quantitation (LLOQ) was defined as the concentration of the lowest non-zero calibration standard, which met the acceptance criteria for accuracy and precision. The signal-to-noise ratios of 3.3:1 and 10:1 were considered as LOD and LOQ, respectively, and were found to be 0.04334 and 0.13134 µg/mL, respectively.

Stability

Studies on the stability of the drug were performed to ensure reproducibility and reliability of the method. The amitriptyline stock solutions and its working stock solutions were stable at 4 °C for 3 months. The stability of the analyte in rat plasma was investigated under different storage conditions. It was found to be stable under the following conditions: at 10 °C for 24 h post-extraction, after three freeze and thaw cycles (from −20 to 25 °C) and at –80 °C for 90 days. The results of stability studies are summarized in Table II.

Recovery and matrix effects

The vortexing and ultracentrifugation were used for the extraction of the analyte and the IS. The percentage extraction recoveries of amitriptyline were found to be 65.25, 76.75, and 74.28% in the low (LQC), medium (MQC), and high (HQC) concentration quality control samples (n=6), respectively. The percentage extraction recovery of the IS was 61.65%. We did not detect any apparent matrix effect in the determination of amitriptyline by this method of analysis. The values for the three quality control samples, i.e., LQC, MQC, and HQC were reported to be 100.4, 100.13, and 100.29%, respectively.

Carryover test

The HPLC chromatograms obtained by using the presently developed method of a blank sample (IS without amitriptyline) were analyzed following six consecutive analyses of ULOQ samples. The analysis revealed no obvious carryover.

Pharmacokinetic analysis

The pharmacokinetic parameters of amitriptyline are summarized in Table III. The mean plasma concentrations versus time profiles of amitriptyline after oral administration with and without *B. monnieri* were characterized in rats and are presented in Figure 2. The peak plasma concentration (C_{max}) was significantly ($p< 0.05$) increased by 16.8% (2.86 ± 0.11 to 3.34 ± 0.06 µg/mL) in the presence of *B. monnieri*. Similarly, the area under the plasma concentration–time curve, $AUC_{0–24}$ and $AUC_{0\text{-inf}}$ significantly increased by 26.5% (29.34 ± 0.90 to 37.12 ± 0.62 µg·h/mL) and 36.7%

TABLE I - Inter- and intra-day precision and recovery as accuracy of quality control samples (mean ± SD, n = 3)

Parameters	Values	Inter-day (µg/mL)			Intra-day (µg/mL)		
		LLOQ (10)	MQC (30)	ULOQ (50)	LLOQ (10)	MQC (30)	ULOQ (50)
Precision	Mean ± SD	9.73 ± 0.03	28.96 ± 0.14	48.92 ± 0.98	9.65 ± 0.02	29.02 ± 0.16	49.06 ± 0.97
	%RSD	1.65	2.98	1.85	1.52	0.85	1.45
Recovery	Mean ± SD	9.95 ± 0.08	29.96 ± 0.04	49.82 ± 0.32	10.34 ± 0.02	30.02 ± 0.15	50.51 ± 0.25
	% Recovery	98.31	99.23	99.02	100.49	100.13	100.29
	%RSD	0.85	0.15	0.54	0.99	0.51	0.185

TABLE II - Results of stability study of QC samples at different conditions (mean ± SD, *n* = 3)

Stability test	Initial concentration ± SD (µg/mL)	Measured concentration ± SD (µg/mL)
Stock solution (4 °C for 3 months)	250.00	249.06 ± 1.26
Working solution (4 °C for 3 months)	30.00 (HQC sample)	29.56 ± 0.56
Post-extraction (10 °C for 24 h)	[a]58.83 ± 0.12	58.60 ± 0.49
	[b]29.25 ± 0.05	28.81 ± 0.45
	[c]9.15 ± 0.09	9.04 ± 0.16
Freeze-thaw (from -20 °C to 25 °C, 3 cycles)	[a]59.01 ± 1.08	58.83 ± 0.42
	[b]29.25 ± 1.12	29.11 ± 0.15
	[c]9.88 ± 1.01	9.33 ± 0.43
Long term (-80 °C for 90 days)	[a]58.54 ± 0.98	58.82 ± 0.27
	[b]29.42 ± 0.78	29.04 ± 0.22
	[c]9.02 ± 0.29	8.94 ± 0.089

Note: [a]values of ULOQ (60 µg/mL) and [b]values of MQC (30 µg/mL) samples and [c]values of LLOQ (10 µg/mL)

TABLE III - Pharmacokinetic parameters of amitriptyline control (alone) and co-administered with *B.monnieri* (mean ± SD, n = 6)

Pharmacokinetic parameters	Amitriptyline only (mean ± SD, *n* =6)	Amitriptyline with *B.monnieri* (mean ± SD, *n* =6)
[1]C_{max} (µg/mL)	2.86 ± 0.11	3.34 ± 0.06*
[2]T_{max} (h)	4.0 ± 0.00	4.0 ± 0.00
[3]K_{el} (h^{-1})	0.07 ± 0.0079	0.062 ± 0.0021*
[4]AUC_{0-24}(µg·h/mL)	29.34 ± 0.90	37.12 ± 0.62*
[5]AUC_{0-inf} (µg·h/mL)	38.38 ± 1.23	52.46 ± 1.73*
[6]$AUMC_{0-inf}$ (µg·h/mL)	617.77 ± 26.04	984.13 ± 63.33*
[7]$t_{1/2}$ (h)	9.63 ± 0.21	11.13 ± 0.39*
[8]V_{ss} (L/Kg)	9.05 ± 0.26	7.65 ± 0.07*
[9]CL (mL/h)	0.65 ± 0.02	0.48 ± 0.02*
[10]MRT (h)	16.09 ±0.27	18.74 ± 0.59*

*Significant difference from " Amitriptyline alone" group with t-test,*P ≤ 0.05, [1] maximum blood concentration (**C_{max}**) , [2] time of peak concentration(**T_{max}**), [3]elimination rate constant (**k_{el}**), [4] area under the concentration time profile curve until last observation (**AUC_{0-24}**), [5] area under the concentration time profile curve from time 0 to infinity (**AUC_{0-inf}**), [6] area under the first moment curve concentration time profile curve from time 0 to infinity (**$AUMC_{0-inf}$**) , [7] half-life ($t_{1/2}$), [8] Steady-State Volume distribution (**V_{ss}**),[9] Total clearance (**CL**), and [10] mean residence time (**MRT**)

(38.38 ± 1.23 to 52.46 ± 1.73), respectively, compared to the control group. However, there was no significant change in the time to reach the peak plasma concentration (T_{max}) of amitriptyline in the presence of *B. monnieri.* On the other hand, the calculated total oral clearance (CL) decreased by 26.2% (0.65 ± 0.02 to 0.48 ± 0.02 mL/h; $p<0.05$), while the estimated apparent oral volume of distribution (V_{ss}) at steady state decreased by about 15.5% (9.05 ± 0.26 to 7.65 ± 0.07 L/kg; $p<0.05$). Also, an increase of elimination half-life ($t_{1/2}$) from 9.63 ± 0.21 to 11.12 ± 0.39 h, which is about 15.5% as compared to the control group, was reported.

Our study revealed the potential of *B. monnieri* to significantly alter the oral pharmacokinetic profile of amitriptyline compared with the control. This is attributed to the considerable enhancement in the oral bioavailability of amitriptyline in rats. Various studies indicate the importance of herbal drugs to alter the expression of drug-metabolizing enzymes and membrane transporters after administration. Cytochrome P450 (CYPs) are the primary

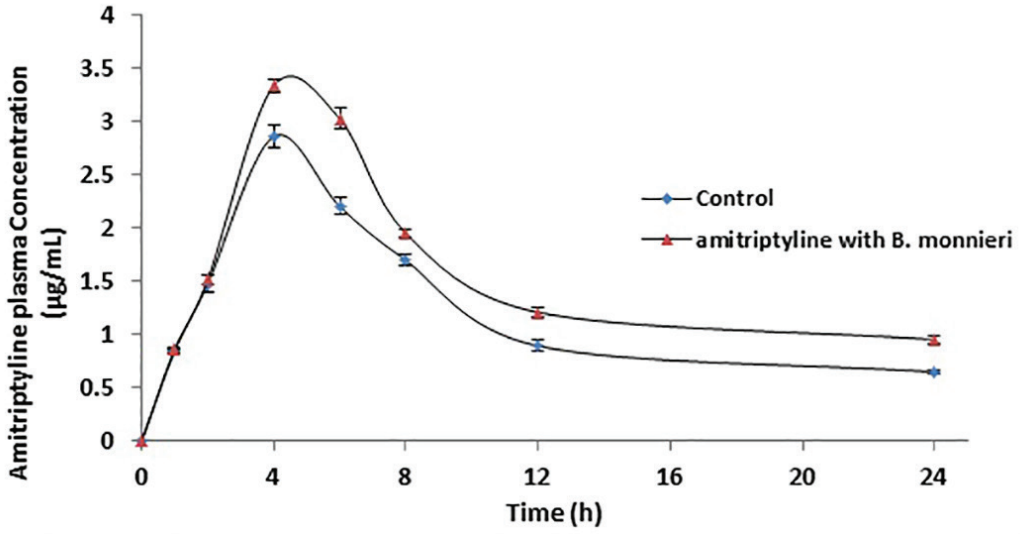

FIGURE 2 - Plasma concentration-time curve of amitriptyline (25 mg/kg, p.o) administered with and without *B.monnieri* (31 mg/kg/day, p.o) in rats (each data is mean ± SD, $n = 6$)

enzymes in the liver involved in the hepatic metabolism of most drugs. These comprise CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP2E1 (Badyal, Dadhich, 2001). Amitriptyline, after oral administration, is readily absorbed by the gastrointestinal tract, followed by its metabolism, majorly on its first pass through the liver. CYP2D6-, CYP3A4-, and CYP2C19-mediated *N*-demethylation into nortriptyline constitute the main pathway for its metabolism (Rudorfer, Potter, 1999). Recently, a study conducted to evaluate the effects of a standardized extract of *B. monnieri* on the expression and activity of hepatic and intestinal cytochrome P450 3A and P-glycoprotein (Pgp) in rats revealed that administration of *B. monnieri* for 7 days modulated the expression of CYP3A and Pgp. Administration of *B. monnieri* led to a reduced expression of CYP3A and concomitant decrease in the CYP3A-dependent testosterone hydroxylase catalytic activity in liver and intestine (Singh *et al.*, 2013). Another study reported similar findings that *B. monnieri* moderately inhibited CYP2C19, CYP2C9, CYP1A2, and CYP3A4 but weakly inhibited CYP2D6 (Ramasamy, Kiew, Chung, 2014). These findings indicated toward the possible potential of *B. monnieri* to contribute to herb–drug interactions when orally co-administered with drugs metabolized by CYP1A2, CYP3A4, CYP2C9, and CYP2C19. Based on this hypothesis, the plasma levels of drug administered concomitantly could be elevated by inhibition of drug-metabolizing enzymes to prolong the pharmacological effects of the drug, thereby increasing the incidence of drug-induced toxicity (Hu *et al.*, 2005). The enhanced bioavailability of amitriptyline observed in the current study might result from the inhibition of the metabolizing enzymes, CYP3A4 and CYP2C19, in the intestinal mucosa or liver by *B. monnieri.* Another reason for the inhibition of the CYP enzymes by *B. monnieri* could be the presence of other constituents in the extract, such as free aglycones, such as jujubogenin (Kawai *et al.*, 1974) and pseudojujubogenin (Kawai, Shibata, 1978), which are more lipophilic. The better lipophilic property of free aglycones enhances their binding to the CYP isoforms through hydrogen bonding, thereby resulting in stronger inhibitory effects (Ramasamy, Kiew, Chung, 2014). The increase in C_{max} and area under curve (AUC) and decrease in oral clearance may lead to increase in the total bioavailability of amitriptyline, leading to toxic effects. Hence, concomitant administration of *B. monnieri* preparations with drugs that are primarily cleared through CYP2C19-, CYP2C9-, CYP1A2-, and CYP3A4-mediated metabolism should be administered to the patients with increased vigilance .

CONCLUSION

The findings of the present study suggest that the medicinal herb, *B. monnieri* exhibit significant potential to alter pharmacokinetics of amitriptyline in rats. The herb–drug interaction was reflected by the *B. monnieri*-mediated increased intestinal absorption and reduced first-pass metabolism of amitriptyline in the intestine and liver through inhibition of CYP2C and CYP3A enzymes. The results obtained in the present study indicate toward exercising caution while administering a combination of

B. monnieri and amitriptyline. Consequently, concomitant use of *B. monnieri* and amitriptyline warrants close monitoring for possible drug interactions. Further confirmation of these results in humans would assist in evaluating the clinical consequences of such interactions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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