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ORIGINAL ARTICLE

The influence of *Eruca sativa* (Arugula) on pharmacokinetics of Sildenafil in rats

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Abstract

OBJECTIVES: A drug like Sildenafil is commonly used for the treatment of erectile dysfunction. *Eruca sativa* is known as a garden plant used in folk medicine to enhance the sexual desire in males. Nevertheless, the interaction of Sildenafil and *Eruca sativa* was not studied. In the current study, we aimed to examine the influence of *Eruca sativa* on Sildenafil pharmacokinetics in rats.

STUDY DESIGN: A crossover experiment with washout period of two weeks was conducted. To one group of animals, *Eruca sativa* was given as food and a drinking solution to rats for 12 hours before the day of the experiment. On the day of the experiment, the same group received 5 ml (50 mg/ml) orally and a half an hour later animals received 1 ml Sildenafil citrate (2.85 mg/kg) oral administrated to the study group. The other group of rats only received Sildenafil. Two-weeks later a cross-over design on the same animals was conducted. Blood samples were collected from optical vein on different time intervals, samples were analyzed using validated (HPLC-UV) method.

RESULTS AND CONCLUSION: Pre-administration of *Eruca sativa* has increased Sildenafil C_{max} from 226.72 to 345.25 ng/ml, ($p<0.05$). In addition, the AUC of Sildenafil has significantly increased when it was pre-administered with *Eruca sativa* (550.59 vs. 916.48 ng/ml*hr). Our findings suggest that co-administration of *Eruca sativa* with Sildenafil enhances the pharmacokinetics of Sildenafil in rats plasma.

INTRODUCTION

Sildenafil citrate, a 5-phosphodiesterase inhibitor, also known as (Viagra), is a synthetic drug that is commonly prescribed to treat erectile dysfunction (ED) (Osterloh 2004). Erectile dysfunction is a common chronic sexual disorder in men defined as the persistent inability to achieve and maintain a penile erection (Selvin *et al.* 2007). It affects males of all ages; incidence rates are significantly higher at ages over 40 (Chen *et al.* 2015). Erectile dysfunction is a widespread condition with a markedly negative impact on quality of life. The Global Online Sexuality Survey (GOSS) for the year 2011

demonstrated that 33.7% of the United States population suffers from ED. Meanwhile, a higher prevalence of 47% in the Middle East was demonstrated with respect to Worlds Standard Population (Shaeer & Shaeer 2012).

Pharmacological action of Sildenafil is mediated through its selective inhibitory effect on the catalytic site of Phosphodiesterase type 5 (PDE-5) (Ralph & McNicholas 2000). Catalytic enzyme PDE-5 (cGMP) and is abundant in vascular smooth muscle cells specifically in the penile arteries. In healthy individuals, sexual stimuli induce the release of Nitric Oxide (NO), accumulation of NO results in the activation of guanylyl cyclase enzyme in the vascular smooth muscle result in the elevation of intracellular cGMP. In the case of ED patients, Sildenafil blocks PDE-5-induced degradation of cGMP promotes the accumulation of cGMP levels, and its signaling pathway, thus enhancing penile erection (Ralph & McNicholas 2000). Clinically, sildenafil citrate is rapidly absorbed after oral administration with a mean absolute pharmacokinetics of 41%. The absorption time ranges from 30 minutes up to 1 hour 60 minutes average T_{max} (Gupta *et al.* 2005). Several publications reported Sildenafil pharmacokinetics are altered in response to food-drug or drug-drug interactions (O'rourke & Xiong-Jing 2000; Cheitlin *et al.* 1999; McLeod *et al.* 2002).

Eruca sativa, also known as Arugula or Garden Rocket, in Arabic called (Jarjeer), is a cruciferous plant with methylthiobutylisothiocyanate as the major active component (Miyazawa *et al.* 2002). Garden rocket consumption has become increasingly popular worldwide; it is a valuable source of vitamins and vital antioxidants such as carotenoids, and polyphenols (Melchini & Traka 2010). It is also rich in glucosinolates and flavonols, which hold therapeutic properties such as diuretic, digestive, tonic, laxative and stimulant (Michael *et al.* 2011; Bell & Wagstaff 2014). In addition, *Eruca sativa* has been shown to enhance sexual stimulation and performance in rats; albeit it remains unknown whether *Eruca sativa* has a synergistic effect on the efficacy of sexual performance-targeted drugs.

Recently, drug-drug and drug-food interactions are being in the center of our interest (Shaikhli *et al.* 2015; Mallah *et al.* 2014; Tbeekh *et al.* 2014; Tamimi *et al.* 2014; Awad *et al.* 2016). The use of herbs and natural products to enhance libido and sexual performance has been the quest of mankind since ancient times (Ratnasooriya & Fernando 2008).

Today, with the advancement of pharmaceutical science and the emerging interest in natural remedies, as well as, the combination of natural products and pharmacological agents is used to enhance the pharmacological action of sexual performance-targeted drugs.

The current study was conducted to investigate the effect of food-drug interaction on the pharmacokinetics of Sildenafil in rats plasma. The pharmacokinetics was estimated by comparing both pharmacokinetics of Sildenafil alone and pharmacokinetics of Sildenafil after *Eruca sativa* ingestion.

MATERIAL & METHODS

Chemicals and reagents

All chemicals and reagents from commercial sources were used as instructed. Sildenafil citrate and carbamazepine were provided by (The Jordanian Pharmaceutical Manufacturing Company and United Pharmaceuticals, Amman-Jordan) with a chemical purity of 99%. Methanol and acetonitrile, advanced gradient grade, were obtained from (Fischer scientific, US), Triethylamine was from (TEDIA, US) and Phosphoric acid was from (Medex, UK). *Eruca sativa* was obtained from local market.

Eruca sativa preparation

Eruca sativa was obtained from local market in Amman, Jordan. *Eruca sativa* leaves were washed and dried on a large plate. *Eruca sativa* was given to animals as dried leaves and as solution prepared freshly before administration. The *Eruca sativa* solution was prepared by placing 50 g in a beaker containing 1.0 L of hot water, incubated for 6 hours for proper extraction, and then filtered.

Animal model

Female Sprague–Dawley rats (200–250 g) were obtained from the animal house of Applied Science University (Amman-Jordan). They were placed in air-conditioned environment (20–25 °C) and exposed to a photoperiod cycle (12 hours light/12 hours dark) daily, the rats were fasted overnight before drug administration. All animal procedures were followed based on the guidelines of FELASA (Federation of European Laboratory Animal Science Association) and the study protocol was approved by the ethical committee of the High Research Council, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.

Study design

Female rats were randomly divided into two groups, 12 rats for each group. In the first period of the experiment group A received sildenafil aqueous solution alone, while group B received sildenafil solution with *Eruca sativa*. Twelve hours before sildenafil administration, Group B rats were left with *Eruca sativa* leaves as food supply and bottles of *Eruca sativa* solution (50 mg/ml). Approximately, each rat from group B consumed 20 g of *Eruca sativa* leaves and 10 ml of the solution. On the day of experiment and before the administration of sildenafil solution, 5.0 ml of *Eruca sativa* solution was directly provided to the studied rats. As for Sildenafil, Sildenafil citrate 81mg of Sildenafil citrate was dissolved in 100 ml of distilled water, and then diluted to 0.57 mg/ml before administration. Sildenafil citrate was administered in a dose of 2.85 mg/kg. After a 2-week washout period, a cross-over design was performed on the same animals.

Blood samples were withdrawn into heparinized tubes at the following time intervals: (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 4.0, and 6.0) hours. Plasma samples of sildenafil

were harvested after centrifugation of the blood samples at 5000 rpm for 10 min and stored at –50 °C.

Instrumentation and chromatographic conditions

The HPLC-UV system (FINNIGAN SURVEYOR) consist of pump (LC Pump Plus), auto-sampler Plus, UV-VIS Plus Detector, Hypersil Thermo Electron Corporation, temperature controlled column compartment, degasser module and ChromQuest software 4.2.34 Solvent delivery systems.

Chromatographic separation was carried out at room temperature on a reversed phase Sepax GP-C18, (150×4.6 mm, 5 µm) column, a mobile phase consisting of acetonitrile (60%) and water (40%) with 675 µl Triethylamine/1L, pH=7.0 adjusted by phosphoric acid. UV detection at 234 nm wavelength and carbamazepine was used as internal standard. The flow rate was 1 ml/min with an injection volume of 25 µl.

Calibration standard and quality control sample preparation

Standard samples and quality control samples were prepared to cover calibration range (20–500 ng/ml). The stock solution of Sildenafil (1000 µg/ml) which was obtained by dissolving 10 mg of Sildenafil working standard in 10 ml methanol. The serial dilution give standard concentrations of (20, 50, 100, 200, 300, 400 and 500) ng/ml. The quality control samples were prepared to give low, medium and high concentrations corresponding to (60, 250 and 425) ng/ml.

Sample preparation

Rat plasma (100 µl) and 150 µl of internal standard 20 ng/ml carbamazepine in acetonitrile were placed in Eppendorf tube, vortexed for 1 min and centrifuged for 10 min at 5000 rpm. The supernatant was collected in another Eppendorf tube and centrifuged for 10 min at 12000 rpm. Thereafter, the supernatant was transferred to the autosampler vial and 25 µl was injected into HPLC system.

Method validation

The developed method was validated in terms of accuracy, precision, stability, recovery and linearity in accordance with EMA guideline (Use 2011). The acceptable values of accuracy and precision are below 15% except at the LLOQ, for which accuracy and precision are less than 20%. The linearity of the plotted curve was calculated through evaluation of the correlation coefficient (R^2), which should be more than 0.98 (EMEA 2012).

Pharmacokinetic parameters

The pharmacokinetic parameters were determined using the non-compartmental method. The area under the curve (AUCs), the maximum concentration of drug (C_{max}) and time to achieve C_{max} (T_{max}) were calculated using Winnonlin software V 5.2.

Statistics

The statistical analysis was conducted using SPSS 19 software, statistical significance of the results was analyzed at $p<0.05$ and $p<0.01$ using Student's t-test.

RESULTS

Validation results

Representative HPLC chromatograms of blank, LLOQ, and STD high samples are shown in Fgures 1–3, respectively. The values of accuracy and precision are

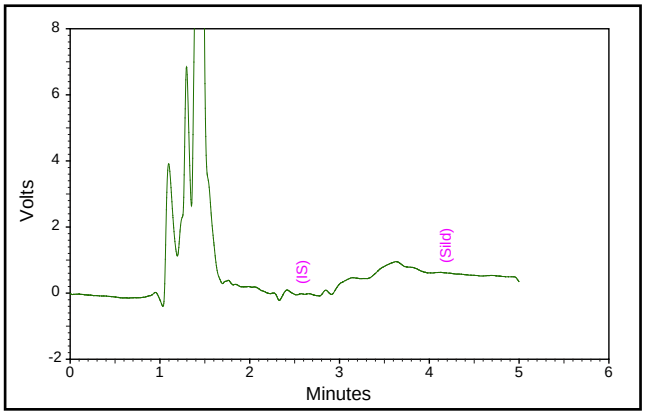

Fig. 1. Representative chromatogram of blank plasma sample

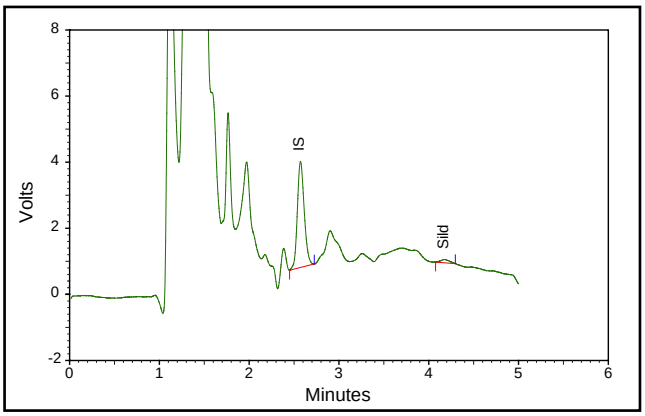

Fig. 2. Sildenafil representative chromatogram of plasma concentration 20 ng/ml

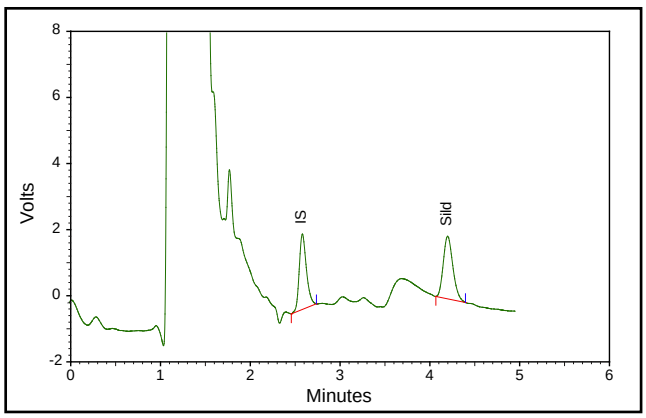

Fig. 3. Representative chromatogram of plasma samples containing 500 ng/ml of Sildenafil

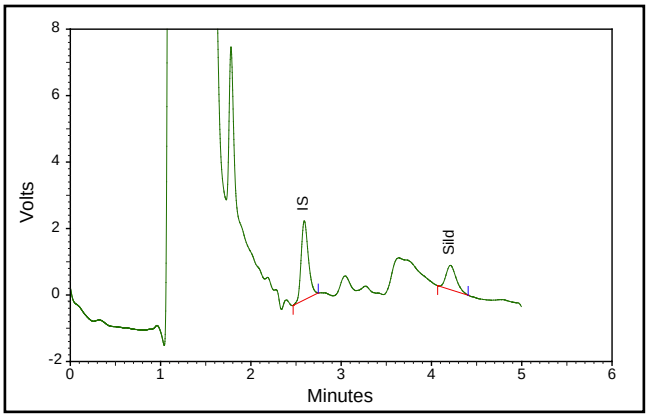

Fig. 4. Chromatogram of plasma samples with Sildenafil 0.5 hours post adminstration for group 1A.

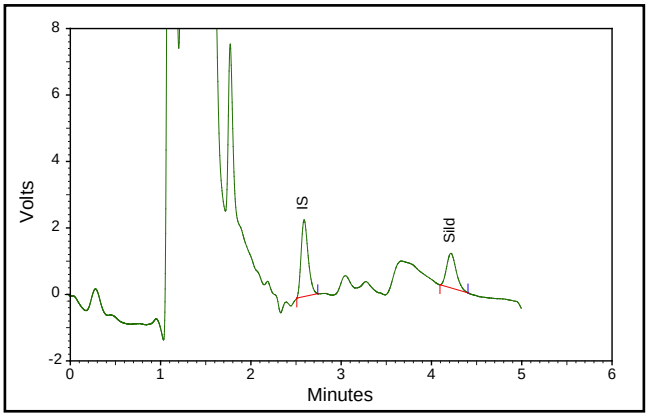

Fig. 5. Chromatogram of plasma samples containing Sildenafil and *Eruca sativa* 0.5 hour post adminstration for group 1B.

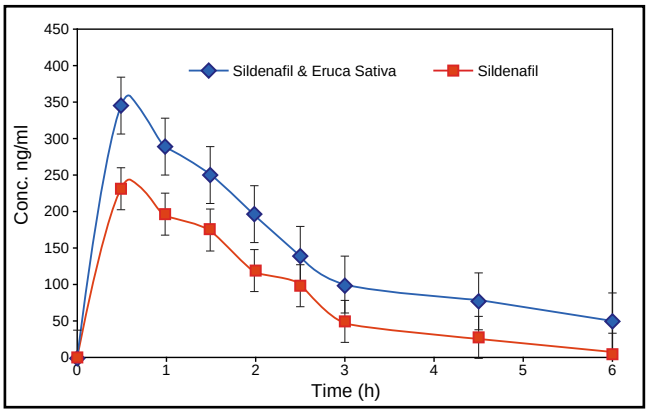

Fig. 6. Mean Plasma Sildenafil concentration profile following oral ingestion and combined with *Eruca sativa* (n=12).

illustrated in Table 1. The run time for the developed method was 5 minutes and the retention times of Sildenafil and IS were observed at 4.2 and 2.7 minutes, respectively.

The effect of Eruca sativa on the pharmacokinetic of Sildenafil

Representative HPLC chromatograms of Sildenafil with and without *Eruca sativa* at 0.5 h are shown in Figures 4 and 5, respectively. Plasma profile of Sildenafil was constructed with and without *Eruca sativa* as shown in Figure 6.

The mean pharmacokinetic parameters of Sildenafil citrate in the plasma of rats after the admission of Sildenafil alone or with *Eruca sativa* were depicted in Table 2 . In Sildenafil fed rats, Sildenafil plasma level has increased rapidly in less than 1 hour, T_{max} and C_{max} were 0.5 h and 226.72 ng/ml, respectively (Table 2). At the end of infusion, the plasma concentration of Sildenafil has declined with $T_{½}$ of 1.78 hours to reach a minimum concentration of (22.09 ng/ml) after 6 hours. In the Sildenafil and *Eruca sativa* fed rats, Sildenafil plasma level was significantly higher than Sildenafil levels alone C_{max} 345.25 ng/ml (Figure 5). Meanwhile, the time required to achieve the C_{max} was similar in both conditions without a significant difference in T_{max} =0.5, whereas, the time required to eliminate the Sildenafil from plasma has increased $T_{1/2}$=2.3 hours.

DISCUSSION

The pharmacokinetic parameters of Sildenafil in this study were consistent with previous studies (Mallah *et al.* 2016). The pharmacokinetics of Sildenafil as a therapeutic drug with a narrow safety spectrum is an era of investigation for several scientist and institutions (Kandeel *et al.* 2001). Nevertheless, *Eruca sativa* is known to have an aphrodisiac and sexual stimulating properties (Barillari *et al.* 2005). In the current study, as a consequence of administration of *Eruca sativa* with Sildenafil; an increment was noticed in levels of Sildenafil in the rat's plasma in the first half hour after ingestion. Statistical analysis using Student's t-test has revealed a significant effect of *Eruca sativa* on the pharmacokinetics of Sildenafil when compared with Sildenafil parameters alone in a proportion of 67% and 53% ($p<0.05$)

Tab. 1. Inter-day precision and accuracy for the quality control samples of Sildenafil in the three days of validation.

	LLOQ (ng/ml)	QC low (ng/ml)	QC.mid (ng/ml)	QC. high (ng/ml)
CV%	2.27	3.21	1.95	2.56
Accuracy%	104.55	95.67	98.45	102.1

Tab. 2. Pharmacokinetic parameters of Sildenafil citrate following oral administration. Geometric means are presented for C_{max} and AUC.

Drug	C_{max} (ng/ml)	T_{max} (hour)	AUC	$T_{0.5}$
Sildenafil	226.72	0.5	550.5934	1.7858
Sildenafil + *Eruca sativa*	345.25	0.5	916.4846	2.3015
p-value	0.031		0.001	

in AUC and C_{max} values, respectively. This increase in the absorption may be attributed to the effect of *Eruca sativa* on the gastric system through decreasing the pH of the stomach and protecting the mucosal barrier of the gastric system by a gastro-protective effect against generated Oxygen Reactive Species (Alqasoumi *et al.* 2009). There is a great impact on the interactions associated with gastric acid secretion which may increase or reduce the pharmacokinetics of certain drug's (Schmidt & Dalhoff 2002; Nekvindova & Anzenbacher 2007). Gastric pH is a vital contributing factor to drugs pharmacokinetics (Martinez & Amidon 2002). Previously, *Eruca sativa* has been shown to reduce gastric acid secretion in rats, thus, reducing gastric acidity and increase pH levels (Alqasoumi *et al.* 2009). However, the exact physiological effect of *Eruca sativa* on gastric acidity varies from one case to another. This makes it difficult to reason the underlying mechanism of *Eruca sativa* increasing Sildenafil pharmacokinetics. As a rational thinking, we expect that alteration of gastric pH to less acidic condition increases the absorption process (Nichols *et al.* 2002) through enhancing the non-ionization state for sildenafil citrate. In addition, the decrease of the stomach emptying time will increase the pharmacokinetics of Sildenafil especially if there is a high-fat diet (Nichols *et al.* 2002). Another possible explanation is the effect of the phytochemicals of *Eruca sativa* as flavonoids, sterols and/or triterpenes, quercetin, quercetin derivatives and Sulfhydryl compounds on the inhibition of p-glycoprotein efflux pump (Alqasoumi *et al.* 2009). The presence of those phytochemicals and compounds in *Eruca sativa* may contribute, at least in part, to the increased absorption of sildenafil citrate. For instance, flavonoid has a vital role in the increase of drug levels in different occasions (Mallah *et al.* 2016). Grapefruit juice inhibits the p-glycoprotein pump as talinolol increased five folds increase in the drug when administered with GFJ (Kirby & Unadkat 2007). Pamelo and star fruits contain agents which inhibit the activity of Cytochrome p450 3A4 (Dresser *et al.* 2002). Eventually, biochemical agents harbored in fruits and vegetables influence the drug pharmacokinetic in the biological systems.

CONCLUSION

Herein we report a significant increase in rat plasma concentration of sildenafil in presence of *Eruca sativa*. Both, Sildenafil and *Eruca sativa* are used by men seeking for better sexual performance. Therefore, some precautions needed to be implemented when sexual stimulants co-administrated with Sildenafil.

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Conflict of interest

The authors declare that they have no conflict of interest.

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