

Influence of Energy Drinks on Pharmacokinetic Parameters of Sildenafil in Rats

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Both sildenafil and RED BULL®(energy drink) are claimed to boost up energy. RED BULL®, one of the most commonly used energy drinks and its easily and widely available for daily use, the idea of this research work had arisen from observations about the concomitant use of sildenafil with RED BULL® by men seeking for better sexual performance. To study the effect of RED BULL® on the pharmacokinetic profile of sildenafil by using HPLC. The pharmacokinetic parameters (C_{max}, T_{max} and AUC) were determined in 10 rats following oral administration of 0.57 mg/ml sildenafil with and without RED BULL® in crossover design, to achieve this purpose, simple, rapid and accurate method for validation and determination of sildenafil in rat plasma in the presence of RED BULL® has been developed. This was performed using High-Performance Liquid Chromatography- Ultra Violet (HPLC-UV). The pharmacokinetic data showed that sildenafil plasma level was lower when combined with RED BULL®. According to the results obtained, maximum concentration (C_{max}) for sildenafil alone was (162.05 ng/ml) after 0.5 hours of administration. The C_{max} decreased to (44.68 ng/ml) after 0.5 hour of administration RED BULL® concomitantly with sildenafil which showed a significant effect on sildenafil plasma level (P<0.001). The area under the curve (AUC) decreased significantly from (370.53 ng/ml*hr) for sildenafil alone to (87.74 ng/ml*hr) when combined with RED BULL®. RED BULL® can alter sildenafil pharmacokinetic if they were taken together.

Keywords: Drug Interaction; Energy Drinks; Pharmacokinetic; Sildenafil; Validation.

Sildenafil citrate is an oral drug used as a therapy for erectile dysfunction¹. It is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 Hpyrazolo [4,3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate².

Sildenafil citrate structure is shown in figure1.

The mechanism of erection involves the release of nitric oxide (NO) in the Corpus cavernosum during sexual stimulation. The

NO activates the enzyme guanylate cyclase, which increases the levels of cyclic guanosine monophosphate (cGMP) ³.

The production of cGMP leads to relaxation of smooth muscles in the corpus cavernosum and increases blood flow into the penis ⁴.

Sildenafil citrate has no direct relaxant effect, but it increases the levels of cGMP by inhibiting phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP ⁵. Sildenafil is primarily metabolized by the CYP3A4 enzyme, which is the principal enzyme responsible for the oxidative metabolism of the majority of drugs ⁶. The interaction between sildenafil and other drugs that are also metabolized by CYP3A4 should be taken into consideration when drugs are prescribed to avoid undesired pharmacological effects ⁶⁻⁸.

RED BULL[®] is the best-selling energy drink in the United States of America and worldwide. It contains different ingredients such as sugar, caffeine, taurine, glucuronolactone

and B vitamins ⁹. Caffeine is probably the most common ingredient in RED BULL[®], as it acts as a stimulant ¹⁰. Taurine is a homocysteine derived amino acid, but not a constituent of proteins ¹¹. It is present freely in the intracellular fluid and distributed widely in the sarcoplasm of the cardiac tissues, hepatocytes, central nervous system, and retina. Taurine mediates the homeostasis of different physiological functions ¹² including osmoregulation, antioxidation, detoxification, neuromodulation, and brain and retinal development ¹³. Moreover, a number of studies have documented some pharmacological functions of taurine against congestive heart failure, liver disease, hyperlipidemia ¹⁴.

Several papers reported that Sildenafil pharmacokinetics are altered in response to food-drug (or drug-drug) interactions^{5,15-17}. It has been demonstrated the co-administration of sildenafil with potent CYP3A4 inhibitors such as azole antifungal agents, macrolide antibiotics, and protease inhibitors causes a significant increase in plasma sildenafil levels ^{18,19}. Another example of interaction is Sitaxentan (endothelin receptor antagonist), which when administered with sildenafil, demonstrated a weak, but a statistically significant interaction ²⁰. The effect of concomitant administration of caffeine with certain drugs used for cardiovascular, CNS, gastrointestinal, infectious, respiratory and skin disorders has been extensively studied as well ²¹, whereas, the effect of other ingredients in Red Bull has not been studied for the pharmacokinetic effect on sildenafil. Caffeine has been reported to interact with many medications such as certain selective serotonin reuptake inhibitors (particularly fluvoxamine), antiarrhythmics (mexiletine),

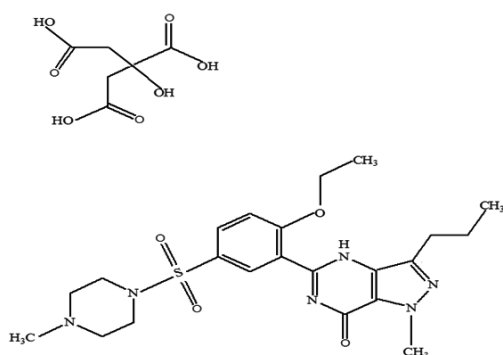


Fig. 1. Sildenafil citrate structure

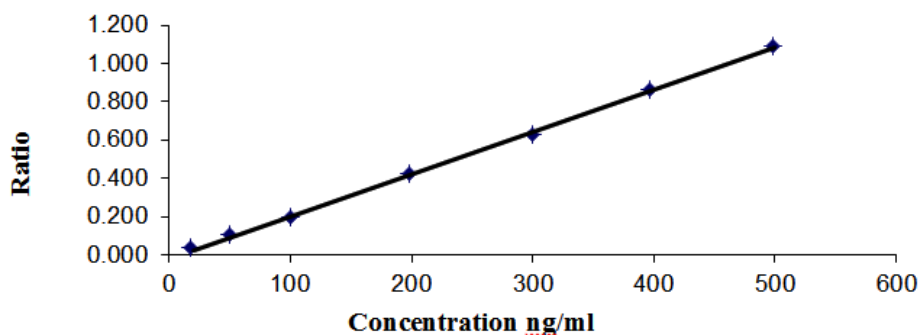


Fig. 2. The plot of calibration curve concentrations against the ratios

antipsychotics (clozapine), psoralens, idrocilamide and phenylpropanolamine, quinolones (enoxacin) and bronchodilators (furafylline and theophylline)¹⁰. Thus, pharmacokinetic interactions at the CYP1A2 enzyme level may cause toxic effects during concomitant administration of caffeine and certain drugs used for cardiovascular, CNS, gastrointestinal, infectious, respiratory and skin disorders⁹.

Recently, drug-drug and drug-food interactions are being the center of our interest²²⁻²⁶. The idea of this research work had arisen from observations of about the concomitant use of sildenafil with RED BULL[®] by men seeking for better aphrodisiac effect during sexual intercourse. Both Sildenafil and RED BULL[®] claimed to boost up energy. Since RED BULL[®] is one of the most commonly used energy drinks, it is easily and widely available for daily use. The current study was conducted to investigate the effect of RED BULL[®] ingestion on the pharmacokinetics of Sildenafil in rats plasma using HPLC in a crossover study.

MATERIALS AND METHODS

Reagents and materials

Deionized Water, Nano pure (Fischer Scientific), Rats plasma (from animal house in ASU)

Methanol, advanced gradient grade (Fischer scientific), Acetonitrile (ACN) (Fisher), Triethylamine (TEDIA), Phosphoric acid

(Medex), Sildenafil (from Dar al-Dawa pharma), Carbamazepine, EDTA, Distilled water.

Instrumentation

An HPLC (FinniganSurveyor) was used and composed of ChromQuest software 4.2.34, Solvent delivery systems pump (LC Pump Plus), Auto-sampler plus, UV-VIS plus Detector, Sepax GP-C18, (150 x 4.6 mm), 5µm.

Preclinical study and protocol

Adult female Sprague-Dawley laboratory rats were supplied by the animal house of Applied Science University. The average weight of these rats was about 240g (200g -260), and they were in healthy condition. They were placed in an air-conditioned environment (20-25 °C) and were exposed to 12 hours light /12 hours dark cycle daily.

All animals in the experiments were handled in compliance with FELASA guidelines (Federation of European Laboratory Animal Science Association) and the study protocol was approved by the research committee (February 2014) at the Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.

10 rats were included in the drug interaction study in a crossover study. The rats were divided into 2 groups (group A and group B), 5 rats each. In the first period of the experiment, group (A) had received sildenafil aqueous solution with a concentration of 0.57 mg/ml and a dose of 2.85/kg (200mg/70kg) alone as a single dose, while group (B) had received sildenafil aqueous solution as a single dose with RED BULL[®] (purchased from local market), respectively and spontaneously.

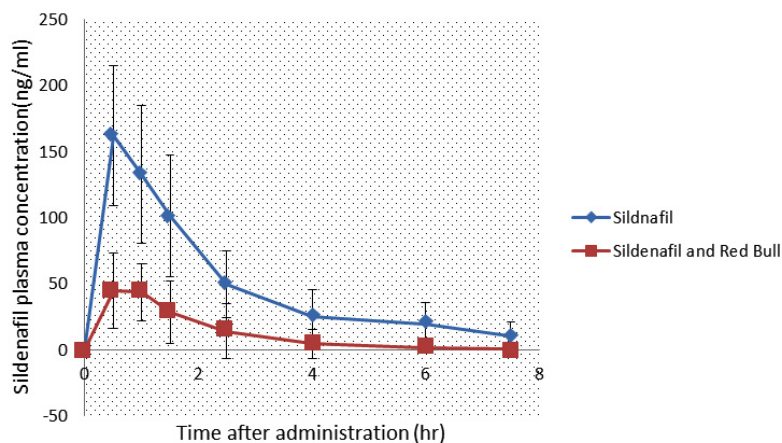


Fig. 3. Mean plasma sildenafil concentration in female rats with time after drug administration, compared to its combination with RED BULL[®]. Each data point represents the mean (n=10)

Washing out period of 2 weeks was implemented, after which the test items were switched between the 2 groups, Group (A) received sildenafil solution and RED BULL® while group (B) received sildenafil solution alone.

The dose used is corresponding to 200 mg for human of 70 kg weight, 200 mg is the double dose of sildenafil regular dose. This dose was chosen to clarify any possible interaction, as it found to be used in the previous clinical trial²⁷.

The least weight of rats was 200g and the concentration was calculated based on this weight. Rats weighing 200 mg took 1 ml of the solution, and then other rats took more than 1ml according to their weights.

The rats which were aimed to take RED BULL® in each part of the experiment were supplied with 15 ml, 16 hours before sildenafil solution administration. They received another 5ml of RED BULL® orally after the administration of sildenafil solution.

A syringe with a specific needle was used for the oral administration of both sildenafil and RED BULL® solutions. For rats which were aimed to take sildenafil solution alone, drinking water was administrated insufficient amount all the time.

Blood samples were taken from the rats' optical vein at the following time points: (zero, 0.5, 1, 1.5, 2.5, 4, 6, 7.5) hours.

Blood samples were drawn into EDTA-containing microtubes. The microtubes were immediately centrifuged for 10 minutes at 5,000 rpm and separated plasma was transferred into labeled Eppendorf tubes then stored at -20 °C until analysis.

Preparation of solutions

Preparation of sildenafil solution for oral use 0.081 g of sildenafil citrate (equals to 0.057 g sildenafil) was dissolved in 100 ml of distilled water, vortex to have complete dissolution to get a concentration of 0.57 mg/ml.

Preparation of stock solution of sildenafil

An equivalent weight of 10 mg of sildenafil working standard was dissolved in 10 ml of methanol to get a concentration of 1000 µg/ml stock solution of sildenafil.

Preparation of stock solution of carbamazepine internal standard

An equivalent weight of 10 mg of carbamazepine working standard was dissolved

in 100 ml of Acetonitrile to get a concentration of 100 µg/ml stock solution of carbamazepine.

Preparation of working solution of carbamazepine I.S

20 µl were taken from carbamazepine stock solution (100µg/ml) and diluted to 100 ml using ACN which was considered to be an I.S working solution that contains 20 ng/ml of carbamazepine.

Method of extraction

The described procedures were applied for subject samples, calibrator, and quality control samples.

In order to perform the sample extraction, disposable Eppendorf tubes were labeled and placed on a rack, then 100.0 µl aliquots of each test sample (blank, zero, standards, quality control low, quality control medium, quality control high and rat samples) were drawn into the tubes by pipette and mixed with 150.0 µl of internal standard (20 ng/ml carbamazepine in ACN), then vortex of each sample vigorously for 1.0 min, and finally Centrifugation at 14000 rpm for 15 minutes.

Chromatographic conditions and detection conditions were as follow in table I:

Analytical method validation

Accuracy and Precision

The intra-day precision and accuracy were evaluated by analyzing six replicates of the QC samples (low, mid, high) and lower limit of quantification (LLOQ) samples on a single day. The inter-day precision and accuracy were determined by analyzing three runs of QC samples and LLOQ samples on three different days. The accuracy (%) was calculated by dividing a measured mean concentration over the nominal analyte concentration. Precision was presented as CV%. The acceptable limits of accuracy and precision should be below 15% except at the LLOQ, for which accuracy and precision should be below 20%²⁸.

Linearity

Linearity was determined by a series of six injections to a seven calibration concentration levels for the analyte. Peak areas of the calibration standards were plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve was evaluated through the value of the correlation coefficient (R²) which should be more than 0.98²⁸.

Stability

Stability of the analyte in the rat serum was evaluated using both low and high QC samples, which were analyzed immediately after preparation and after the applied storage conditions. Evaluation included: autosampler stability, freeze-thaw stability “after 3 cycles”, and Short-term stability at room temperature “24 h”.

The mean concentration should be within $\pm 15\%$ of the nominal concentration²⁸.

RESULTS AND DISCUSSION

Validation

The validation of sildenafil was conducted according to EMA guideline²⁸. Selected chromatograms for sildenafil validation (blank, zero, LLOQ) are shown in figures (4, 5 and 6).

Accuracy and precision

Table II represents inter-day precision and accuracy for quality control samples of sildenafil in three days of validation. All of the

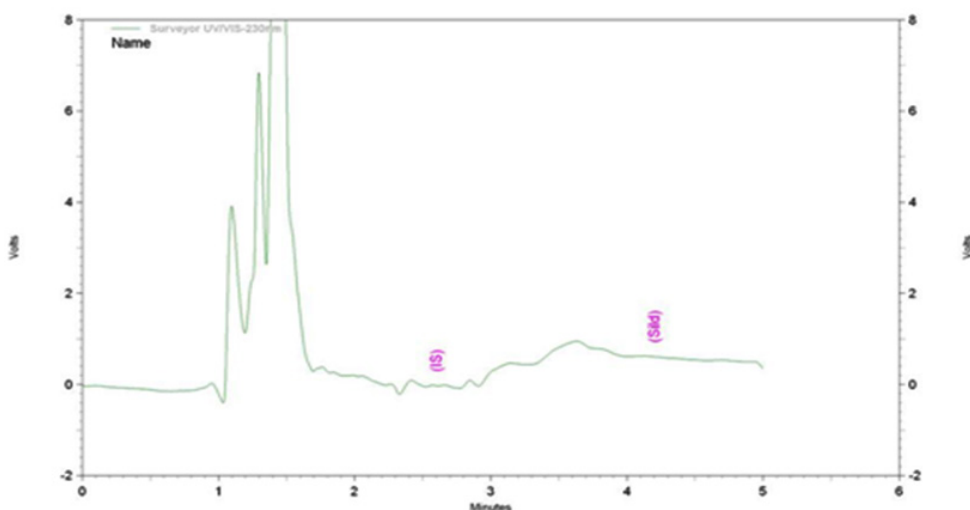


Fig. 4. Sildenafil blank chromatogram

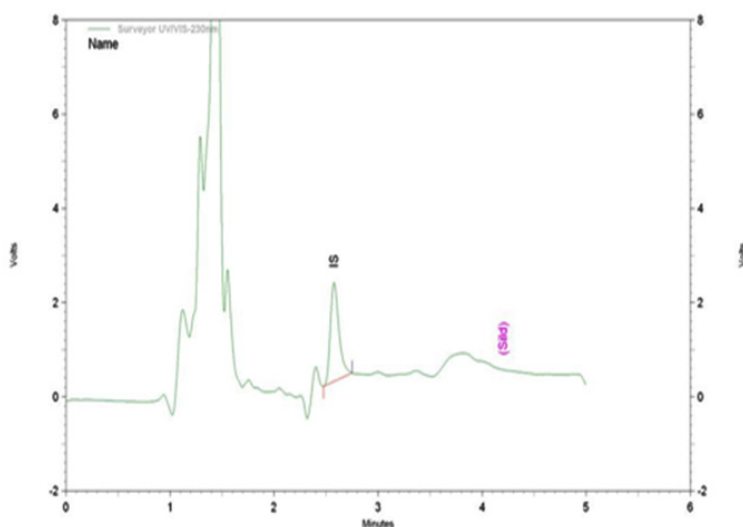


Fig. 5. Sildenafil zero chromatogram

obtained accuracy and precision data were within the required range which is (85-115 % for all concentration except for LLOQ, which is 80-120 %) for the accuracy, and (20 % for LLOQ and 15 % for other concentrations) for the precision.

Linearity

R^2 which represents the strength of correlation coefficient for standard calibration curve was greater than 0.99 during validation course. Data of standard curve with regards to correlation, slope, R^2 , and intercept are shown in tables III.

Therefore, validation results passed the required criteria in term of linearity and accuracy.

Stability

Tables IV shows data for short-term stability indicated by two QC concentrations (low, high) for sildenafil after preparation procedure (auto-sampler stability), $T=10^\circ\text{C}$ and for room temperature or processing temperature. Regarding to the stability of plasma samples during freezing and thawing processes; two QC samples were stored and frozen in a freezer at the intended temperature -60°C and there after thawed and processed at room temperature twice for 72 hours. All results were within the accepted range of 85%-115%.

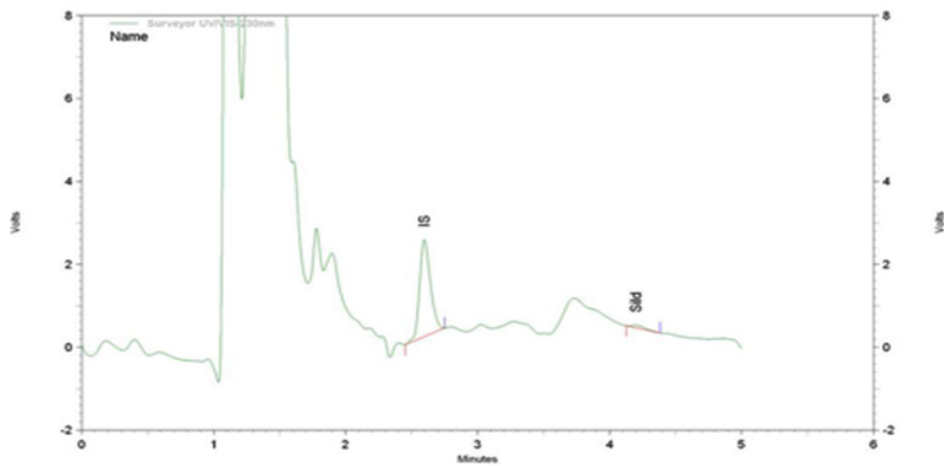


Fig. 6. Sildenafil 20 ng/ml chromatogram

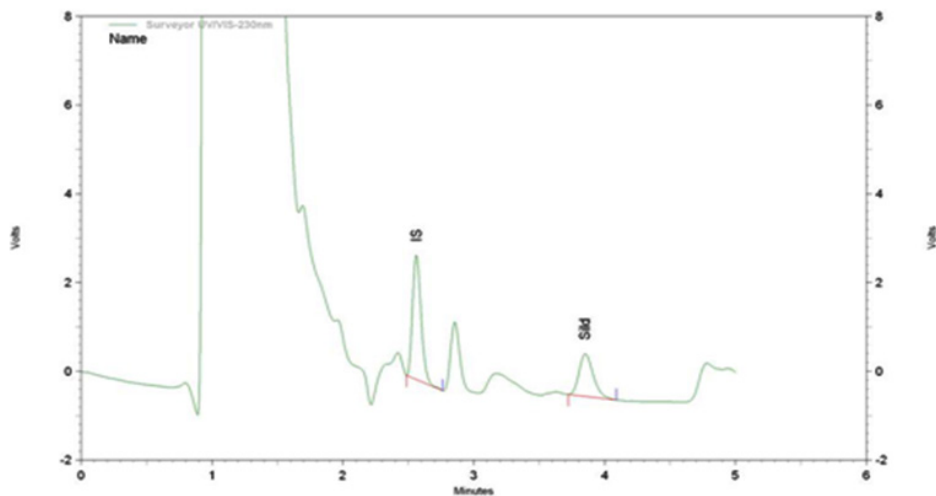


Fig. 7. Sildenafil sample chromatogram after 0.5 hours of administration for one group

Pharmacokinetic data of sildenafil in presence of RED BULL®

The plasma concentrations of sildenafil with or without RED BULL® were measured in the population rats using a sample size of 10 rats for the drug alone and then repeated for the combination of the drug with RED BULL® in a crossover study with washout period of 2 weeks.

7 discrete samples were obtained from rats following drug administration to the last time interval of 7.5 hours.

This study was conducted on female rats since the sildenafil bioavailability is more comparable to humans (regardless the gender)

and the plasma levels of sildenafil male rats are much lower when compared to its bioavailability in female rats (16) (17).

The plasma levels of sildenafil fed rats has reached its maximum level at the first 0.5 hours (C_{max}) (162.05 ng/ml) (T_{max}) when administered alone as depicted in figures (3, 7).

Whereas, sildenafil plasma levels were intimately decreased when administered with RED BULL® as C_{max} was only 44.68 ng/ml at the first 0.5 hours, as shown in figures (3, 8). The sharp decrease in sildenafil levels as a consequent of administration with RED BULL® reached a proportion of 72% decrease ($P < 0.001$) compared with sildenafil fed rats alone.

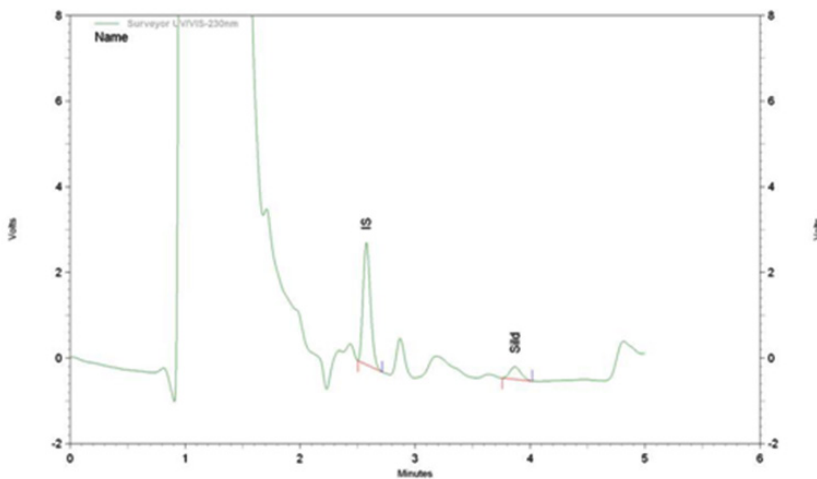


Fig. 8. Sildenafil with RED BULL® sample chromatogram after 0.5 hours of administration for one group

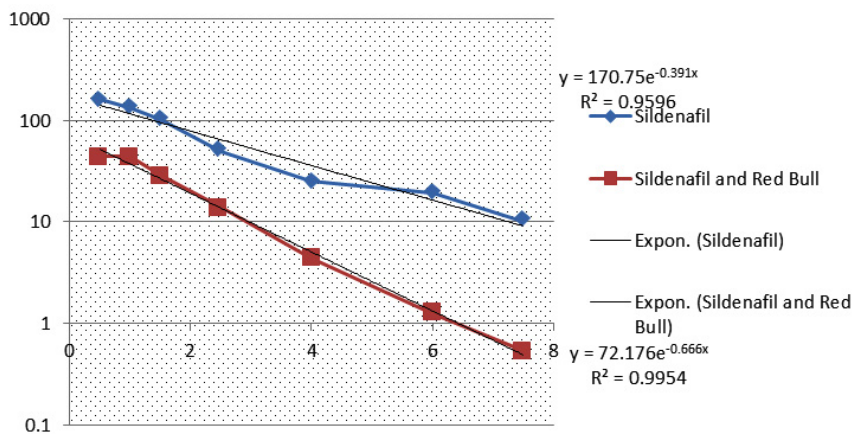


Fig. 9. Mean plasma sildenafil concentration in female rats with time after drug administration, compared to its combination with RED BULL® on a semilog scale. Each data point represents the mean (n=10)

Figure 3 shows Mean plasma sildenafil concentration of female rats in a crossover study, sildenafil was administered alone and in combination with RED BULL®. Each data point represents the mean (n=10).

The pharmacokinetic interaction of Sildenafil and RED BULL® could happen at any stage of ADME: "Absorption, Distribution, Metabolism, and Excretion."

However, the absorption of sildenafil from the intestine happens through a passive mechanism, no carriers are needed so we rule out this is the cause of the difference²⁹. The pH of RED BULL® solutions (with and without Sildenafil) is 3.28, which indicates the minimum effect on stomach pH. In addition, we noted that complete dissolution occurred for Sildenafil in RED BULL®.

However, this cannot rule out the presence of interaction at the absorption level in the stomach

as the presence of REDBULL will effect of the gastric emptying time²⁶.

By plotting the data on the semilog scale as shown in figure (9), the slope (which represents elimination rate constant) of Sildenafil when administered with RED BULL beverage was steeper, this indicates a higher elimination rate of the drug when combined with RED BULL.

Figure 9: Mean plasma sildenafil concentration in female rats with time after drug administration, compared to its combination with RED BULL® on a semilog scale. Each data point represents the mean (n=10).

The lower AUC, Cmax is accompanied by an increase in the clearance of the drug and shortening of the half-life. These changes elimination parameters indicate an enhancement in elimination processes of the drug which mainly eliminated by metabolism in the liver.

Table 1. Chromatographic conditions and detection conditions.

Column Oven Temp	Auto-sampler Temp 30°C	Auto-sampler Injection Volume 10°C	Pump Flow Rate 25 µl	HPLC Conditions 1.0 ml / min
Mixture of (57.5 % ACN, 42.5 Water), 675 µl Triethylamine /1L of mixture, pH=7.0 adjust pH with phosphoric acid Sepax GP-C18, (150 x 4.6 mm, 5µm)			Mobile phase	Chromatography
Sildenafil 4.2 min 230 nm	Carbamazepine(I.S) 2.5 min		Column type Expected Retention times(minutes) Wavelength	Detection Conditions

Table 2. Inter-day precision and accuracy data for sildenafil samples in the three days of validation

Conc./Days	20 ng/ml	60 ng/ml	250 ng/ml	425 ng/ml
Mean (n=6)	20.398	60.535	257.891	426.620
STD	1.324	2.436	6.425	7.376
CV%	6.492	4.023	2.491	1.729
Accuracy%	101.99	100.89	103.16	100.38

Table 3. Raw data of the calibration curve for sildenafil

Correlation	Slope	Factor	R ²	Intercept
0.999963	0.002158	463.45431	0.999927	-0.002794

Table 4. Short and long term stability data for Sildenafil

Time	Sildenafil QC low samples stability auto-sampler procedure at 10°C.			Sildenafil QC high samples stability auto-sampler procedure at 10°C.				
	Measured Conc.	Mean Measured	Accuracy %	Stability	Time	Mean Measured Conc.	Accuracy %	Stability
0.00 Hour	61.959	61.195	103.27	100.00	0.00 Hour	427.436	100.57	100.00
	60.624		101.04			423.162	99.57	
24.00 Hours	61.002	59.902	101.67	97.89	24.00 Hours	428.190	100.75	99.25
	59.474	60.462	99.12			426.317	100.31	
	60.462	59.771	100.77			424.062	99.78	
			99.62			418.864	98.56	
Time	Sildenafil QC low samples stability at room temperature (bench stability).			Sildenafil QC high samples stability at room temperature (bench stability).				
	Measured Conc.	Mean Measured	Accuracy %	Stability	Time	Mean Measured Conc.	Accuracy %	Stability
0.00 Hour	61.959	61.195	103.27	100.00	0.00 Hour	427.436	100.57	100.00
	60.624		101.04			423.162	99.57	
24.00 Hours	61.002	58.031	101.67	94.83	24.00 Hours	428.190	100.75	99.21
	59.693	57.071	99.49			426.246	100.29	
	57.071	57.329	95.12			420.336	98.90	
			95.55			422.109	99.32	
Time	Sildenafil QC low samples stability at freeze and thaw.			Sildenafil QC high samples stability at freeze and thaw.				
	Measured Conc.	Mean Measured	Accuracy %	Stability	Time	Mean Measured Conc.	Accuracy %	Stability
0.00 Hour	61.959	61.195	103.27	100.00	0.00 Hour	427.436	100.57	100.00
	60.624		101.04			423.162	99.57	
72.00 Hour	61.002	58.871	101.67	96.20	72.00 Hour	428.190	100.75	99.68
	58.712	58.713	97.85			423.093	99.55	
	58.713	59.187	97.86			426.925	100.45	
			98.65			424.678	99.92	

Table 5. Pharmacokinetic data of sildenafil

	Sildenafil	Sildenafil and Red bull
T-max(hr)	0.5	0.5
C-max(ng/ml)*	162.05	44.68
AUC(ng/ml*hr)*	370.53	87.74
AUMC-t (ng.hr ² /ml)	810.90	155.73*
MRT-t (hr)	2.18	1.66*
Kel (hr ⁻¹)	0.45	0.56*
T1/2 (hr)	1.51	1.15*
Systemic Cl (L/hr/kg)	135	535.6*

All parameters are within a CI of 5%.

By reviewing the literatures, it has been demonstrated that taurine has an activating effect on the expression level of CYP 3A4 in the presence of CYP3A4 inducer. Taurine enhances the induction of CYP3A4 mRNA in a concentration-dependent manner³⁰. Taurine is a potent inducer of CYP3A4 mRNA via pregnane X receptor (PXR), an orphan member of the steroid/retinoid/thyroid hormone receptor superfamily of ligand-activated transcription factors^{31,32}. It was recently reported that PXR serves as a functional bile acids receptor, and lithocholic acid, which is a kind of bile acid, induces CYP3A mRNA³³⁻³⁵. Attention should be paid to drug interactions when taurine-containing medicinal agents, foods, and drinks are administered together with both sildenafil and drugs metabolized mainly by CYP3A4³⁵.

Moreover, in a similar manner of drug interaction, high levels of taurine have lowered the plasma concentrations of acetaminophen and rifampicine by increasing the enzyme activity of CYP3A4^{36,37}. Moreover, we tried to find a single clinical report about overdose abuse of sildenafil and REDBULL but up to the date there is no case report about the adverse effect of Sildenafil intake with energy drink. Our findings could explain the reason of why there are no reports on sildenafil interaction with such drinks but even though we don't know anything about the sildenafil clinical effect since it has reached to a sub therapeutic dose.

In addition, we propose that the change in sildenafil plasma levels in female rates is an enzyme induction mechanism due to a significant change in elimination parameters. Whereas Kel has been changed significantly from 0.45 hr⁻¹ for sildenafil

alone to 0.56 hr⁻¹ when given in combination with RED BULL beverage. The terminal elimination t_{1/2} was shortened significantly from 1.51 hr to 1.15 hr in combination, and the clearance increased also highly significantly from to 135 L/hr/kg to 535.6 L/hr/kg in combination.

Also, the MRT-t was shortened. All these changes suggest an enhancement in elimination processes by metabolism of sildenafil by hepatic enzymes. However, the interaction still could have done at any stage of ADME.

Moreover, caffeine has a great impact on the cardiovascular system by causing increased heart rate and blood pressure which results in increased cardiac output³⁸. So the blood flow to the liver will increase, and the metabolism of sildenafil in presence of caffeine may increase because of this increase in blood flow.

This interaction between sildenafil and RED BULL[®] could be of clinical significance, because the decrease of sildenafil concentrations to subtherapeutic levels may make the effect of sildenafil clinically insignificant. Table V shows Pharmacokinetic data of sildenafil.

The difference between both C_{max} and AUC of sildenafil alone and sildenafil with RED BULL[®] was statistically significant (P<0.001).

CONCLUSION

There is a pharmacokinetic interaction between sildenafil and RED BULL[®] when administrated concomitantly. Such interaction effects significantly (P<0.001) the C_{max} and AUC of sildenafil. The interaction between sildenafil and RED BULL[®] could happen in all stages with high probability to be executed in metabolism stage.

This *in vivo* trial on rats suggests further investigations, such as assessing the possible interaction between sildenafil and RED BULL[®] in humans, checking the pharmacodynamic effect of this combination, testing sildenafil levels in plasma in presence of certain concentrations of caffeine and explaining the exact mechanism of this interaction and the responsible compounds.

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