IDENTIFICATION OF SILDENAFIL CITRATE AS AN ADULTERANT IN HERBAL PRODUCTS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTODIODE ARRAY DETECTOR

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ABSTRACT

Objective: Investigation of sildenafil citrate as an adulterant in the traditional liquid herbal products of the local market in Bangladesh.

Methods: A reversed-phase high-performance liquid chromatographic (HPLC) method with photodiode array (PDA) detector system has been developed and validated for investigating the presence of synthetic phosphodiesterase 5 (PDE-5) enzyme inhibitor as an adulterant in the traditional herbal products. Nine of the liquid preparations (syrup), Balarista (A), Jinsant (B), Jernide (C), Bolarist (D), See Gopal Oil (E), Menostroge (F), Eneron (G), Ginseng (H) and I syrups were found to be 17, 22, 26, 25, 10, 24, 29, 22 and 17 mg/100 ml, respectively.

Results: All the products (A–I) were found to contain sildenafil citrate as an adulterant. HPLC peak of the adulterant was confirmed by comparing retention time, UV spectra generated by PDA detector and peak spiking with the authentic sample of sildenafil citrate. The quantity of sildenafil citrate in A, B, C, D, E, F, G, H and I syrups were found to be 17, 22, 26, 25, 10, 24, 29, 22 and 17 mg/100 ml, respectively.

Conclusion: The study indicated that all tested liquid herbal products contain sildenafil citrate as an adulterant. As PDE-5 inhibitors have severe side effects, possess drug-drug interaction and highly recommended to prescribe by registered physicians, the regulatory agency of Bangladesh should take necessary action to minimize the risk of patients.

Keywords: PDE-5 inhibitor, Herbal, Adulterant, Male erectile dysfunction (ED), HPLC

INTRODUCTION

PDE is a ubiquitous enzyme in the human cell that is the essential regulators of cyclic nucleotide signalling with diverse physiological functions. So far 11 subtypes of PDE (1–11) were identified. These enzymes are involved in various regulatory processes like ion-channel functions, cell differentiation, apoptosis, muscle contraction etc. [1]. Nowadays, PDE is considered as an important molecular target in drug discovery. Pfizer initiated the medicinal chemistry research program to develop selective PDE-5 inhibitors for the treatment of hypertension and other cardiovascular diseases in 1985. Sildenafil was synthesized in that project as an active drug [2]. During the clinical trial, sildenafil serendipitously found to have an effect in penile engorgement. This drug was eventually approved by Food and Drug Administration (FDA) of USA in 1998 for the treatment of ED. Sildenafil is selective to PDE–5 compared to PDE1–4, but it is less selective to PDE–6. As PDE–6 is found in the renal cell, use of sildenafil is associated with visual side effects [2]. Moreover, sildenafil also showed several drug-drug interactions and contraindicated to organic nitrates. Concomitant use of sildenafil and organic nitrates might cause severe and fatal hypotension [3].

Though sildenafil is approved as the prescription drug, it has great abuse potential for recreational purpose. Recently in the literature, it was reported that sildenafil is used as an adulterant in several traditional Chinese medicines that were marketed in Singapore and Denmark [4]. In the USA, the presence of sildenafil was reported in dietary supplements and bulk herbal products [5]. In Singapore, cases of 22 fatalities were reported during 1998–2009 due to the adverse reactions of adulterated herbal drugs and sildenafil was detected as one of the adulterants in those herbal products [6]. In India, one out of tested 85 Ayurvedic preparations from the local market was found to be adulterated with sildenafil [7]. Recently, in Bangladesh, a study was conducted using 35 traditional medicines and dietary supplements. The result indicated that 20 % traditional medicines and 70 % of the dietary supplements were found to contain sildenafil citrate as an adulterant. They also analysed two liquid dosages form among 35 products and found that one liquid dose of traditional medicines contains the high concentration of sildenafil citrate as an adulterant. They concluded that more study is essential for the falsified products available in Bangladesh to observe the trends and find out the risk [8]. As traditional liquid medicines contain the high concentration of sildenafil citrate and there are many popular liquid herbal products available in the market as the sole remedies for physical and sexual weakness, it’s important to conduct the study in those products to protect the people from deadly effect.

Many people of Bangladesh prefer to use liquid herbal products from their common believes that these products might have fewer side effects with maximum therapeutic benefit. However, if a patient with diabetes or ischemic heart diseases or related diseases uses sildenafil adulterated herbal products, it might possess a great health threat [3, 6, 9]. Therefore, monitoring for synthetic adulterants in marketed liquid herbal products which are most commonly used as strength and energy booster throughout Bangladesh is highly recommended for the safety of local people who solely depend on herbal remedies. In this study, we investigated the presence of specific PDE-5 inhibitor in the most popular marketed liquid herbal products in Bangladesh by HPLC system to confirm their safe use.

MATERIALS AND METHODS

Reagents and materials
Sildenafil citrate INN as a standard PDE-5 inhibitor was purchased from Pol Pharma (Poland, Batch No. 10112128, Potency 99.99%). Potassium dihydrogen phosphate and phosphoric acid (85%) were purchased from Merck (Germany), water (HPLC grade) and acetonitrile (HPLC grade) from Active Fine Chemicals Ltd. (Dhaka, Bangladesh).

Reagents and materials used in the study with the purchase details are given below:

- Sildenafil citrate INN as a standard PDE-5 inhibitor was purchased from Pol Pharma (Poland, Batch No. 10112128, Potency 99.99%).
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Keywords: PDE-5 inhibitor, Herbal, Adulterant, Male erectile dysfunction (ED), HPLC
The recovery study of the standard was determined using four concentration ranges. It was determined by different spiked concentrations of standard sildenafil citrate in the blank solvent. The calculation for the recovery studies was done using standard calibration lines. The result (table 2) indicated that the mean recoveries of sildenafil citrate were ranging from 92.4–105% and ensured that the method was suitable for analysis. From the recovery studies, accuracy and precision were also evaluated and shown in table 2. The limits of accuracy were between (-5.0)–7.60% and the limits of precision were in a range of 0.94–1.95%. The results of recovery studies, accuracy and precision indicated that the method was suitable for analysis. As all the results for validation of methods were suitable for analysis, therefore the analysis of herbal products was conducted in accordance with this method.

The Bronchocin used for the treatment of respiratory tract diseases was purchased from Unani Herbal Ltd (Bangladesh) as the blank herbal product. The herbal products (A) Balarista syrup 450 ml (Batch No. 071), (B) Jinsant syrup 450 ml (Batch No. 036) and (C) Jermade Syrup 100 ml (Batch No. 005) of Hamadard Laboratories (Bangladesh), (D) Bolarist Syrup 400 ml (Batch No. 11), and (E) Sree Gopal Oil 50 ml (Batch No. 02) of Modern Herbal Research Garden (Bangladesh), (F) Menostroge syrup 400 ml (Batch No. 12) of MNX Homoeo Laboratory (Bangladesh), (G) Enerton syrup 200 ml (Batch No. 307003) of Square Herbal and Nutracuticals Ltd. (Bangladesh), (H) Ginseng syrup 100 ml (Batch No. 03/12) of MaxFair and Company Ltd. (Bangladesh) and (I) Ginsin Plus Syrup 450 ml (Batch No. 001) of Muslim Pharmaceuticals (Unani, Rajshahi, Bangladesh) were purchased from local market of Bangladesh.

**Standard preparation**

The stock solution of standard sildenafil citrate was prepared by dissolving 1.5 mg/ml in methanol based on the solubility of the synthetic drug and stored in a refrigerator and brought to room temperature prior to use. The working solution was prepared by making a serial dilution of the stock solution with methanol to about 0.0467 mg/ml. The injection volume was 20 μl for each working solution and the procedures were done for triplicate.

**Samples preparation**

One ml of each liquid herbal formulation was put into a 50 ml volumetric flask and taken to the volume with methanol. The flask was shaken vigorously and sonicated for 20 min at 40°C temperature. The solutions were filtered by filter paper to remove the unwanted materials, and the supernatant was again filtered by a membrane filter to make a clear liquid solution of each herbal product. Then 1 ml of the clear solution of each formulation was taken in the HPLC vial and 20 μl of solution was injected.

**HPLC method development**

A Shimadzu SIL-20AHT prominence HPLC system controlled by LCsolution LC-Assist Software, version 2.1, with a Shimadzu SPD-M20A prominence PDA detector (Shimadzu Corporation, Kyoto, Japan) was used. Phenomenex® Luna Analytical column (particle size: 5μ, stationary phase: C18 (ODS), pore size: 100 Å) was used under the isothermal condition at 40°C. The system was pumped at a flow rate of 1.0 ml/min and full UV spectra were recorded on-line during the 30 min chromatographic run. The selection of the mobile phase was carried out in accordance with published literature [11] and consisted of (A) acetonitrile: 50 mmol potassium dihydrogen phosphate (adjust to pH 2.5 with phosphoric acid) (70:30, v/v) and (B) acetonitrile: 50 mmol potassium dihydrogen phosphate (adjust to pH 2.5 with phosphoric acid) (70:30, v/v) which were used in a gradient mode at a flow rate of 1.0 ml/min. The gradient system size: 5 µ, stationary phase: C18 (ODS), pore size: 100 Å) was used for standard sildenafil citrate. The working solutions were injected into HPLC system for the confirmation of standard sildenafil citrate in nine herbal products (A–I).

**Quantification of sildenafil citrate in herbal products (A–I)**

For the quantification of the amount of sildenafil citrate in herbal products (A–I), all products were injected in triplicate under the developed operating condition and the mean amount of the three injections was recorded as the total amount of standard sildenafil citrate in herbal products as an adulterant.

**RESULTS**

For standard sildenafil citrate, three calibration standards were prepared to evaluate the relationship between the area under the curve and the concentration. The linearity of the relationship was determined for standard sildenafil citrate in a concentration range of 0.75–0.047 mg/ml. The calibration curves were obtained using linear regression and were confirmed with the R² values. Table 1 showed the mean R²-values and demonstrated that the standard calibration curves for sildenafil citrate are linear within the selected concentration ranges. To evaluate the LOD and LOQ, the different concentrations of the standard were mixed in blank herbal products (herbal product without PDE–5 inhibitors). Table 1 showed the values of LOD and LOQ. The results indicated that the LOD was 0.02 μg and LOQ 0.07 μg/ml.

**Table 1: Linearity, LOD, LOQ**

| Compound       | R²      | LOD (μg) | LOQ (μg) |
|----------------|---------|----------|----------|
| Sildenafil citrate | 0.9997±0.0005 | 0.02     | 0.07     |

The results are mean±standard deviation for n= 3 determinations with detection at 293 nm
Table 2: Recovery, accuracy and precision studies

| Sample       | Spiked concentration (μg/ml) | Calculated spiked concentration (μg/ml) | Recovery (%) | Precision (%RSD) | Accuracy  |
|--------------|-----------------------------|----------------------------------------|--------------|------------------|-----------|
| Sildenafil citrate | 40                          | 42.0±0.82                              | 105.0        | 1.95             | -5.0      |
|               | 250                         | 231±2.16                                | 92.4         | 0.94             | 7.6       |
|               | 800                         | 748.25±8.14                             | 93.5         | 1.09             | 6.47      |

The results are mean±standard deviation for n=4 determinations with detection at 293 nm.

Fig. 1 showed the HPLC chromatogram of standard sildenafil citrate and herbal products (A–I). The chromatogram indicated the RT of standard sildenafil citrate at 13.6 min. The HPLC chromatogram also indicated that all herbal products (A–I) showed a large peak at 13.6 min. The results revealed that all herbal products might contain sildenafil citrate. Although the sildenafil citrate and herbal product showed the similar peak at 13.6 min, sometimes different compounds can show the same retention time in HPLC analysis. Therefore, the ultraviolet spectrums (UV max) of identified peaks at 13.6 min were also evaluated.
Fig. 1: HPLC chromatograms of standard sildenafil citrate and herbal products (A-I)

Fig. 2 showed the HPLC chromatograms of A (1)–I (1) of herbal products and A (2)–I (2) of sildenafil citrate spiked in herbal products (A–I). The results indicated that the retention time of peaks of herbal products and the retention time of sildenafil citrate spiked in herbal products were similar. Moreover, the UV spectrums of these peaks were also similar in both herbal product and spiked herbal products. These results indicated that the retention time of peaks of herbal products at 13.6 min was due to the presence of sildenafil citrate. Based on the above analytical parameters, it was confirmed that all herbal products contained PDE-5 inhibitor such as sildenafil citrate. Therefore, the quantity of sildenafil citrate in each herbal product was also evaluated.

Fig. 2: HPLC chromatograms of (1) herbal products (A-I), and (2) sildenafil citrate spiked in the herbal products (A-I)
As the PDE-5 inhibitors cause serious health problems, the use of these herbal products by patients may also cause fatal effect, as the patients received these products without proper examination and prescription by the registered physician [2,13]. In Bangladesh, patients are buying these liquid herbal products without physical and prescription by the registered physician [2,13]. In Bangladesh, most of the well reputed herbal companies are using synthetic sildenafil citrate as an adulterant in their liquid formulations. As the PDE-5 inhibitors cause the serious health problem, the use of these herbal products by patients may also cause fatal effect as the patients received these products without proper guidelines. Therefore, the government and the people of Bangladesh should be aware of these herbal products because the use of these products might cause patient death.

**CONCLUSION**

The study presents that all herbal products (A-I) were found to contain a synthetic PDE-5 inhibitors "sildenafil citrate". The results suggested that as the synthetic PDE-5 inhibitors have several side effects, possesses severe drug-drug interactions and highly recommended to prescribe with caution for patients with various health problems, the use of synthetic agent in herbal products for the purposes of ED, physical and sexual weakness, and fatigue and as tonic might cause fatal effects. Therefore, the drug regulatory agency of Bangladesh should take necessary action urgently to minimize the future risk in patients.

**AUTHORS CONTRIBUTIONS**

All authors equally contributed to drafting the paper. All authors have read and approved the final manuscript.

**CONFLICTS OF INTERESTS**

All authors declare no conflicts of interest.

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