Detection of a Tadalafil Analogue as an Adulterant in a Dietary Supplement for Erectile Dysfunction

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ABSTRACT

Introduction. Several cases of adulteration of dietary supplements with tadalafil, sildenafil, and vardenafil, or their unapproved analogues have been reported worldwide. Mainly, the presence of the latter represents a serious health risk to consumers as their efficacy and toxic effects have not been assessed and may result in unpredictable adverse effects.

Aim. To investigate the suspected adulteration with synthetic phosphodiesterase type 5 (PDE-5) inhibitors in a dietary supplement marketed in Argentina for the treatment of erectile dysfunction (ED).

Methods. The content of the capsules of the dietary supplement (sample A) was analyzed by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) diode-array detection. From the organic extract of sample A, a major compound was purified by column chromatography (CC). The isolated compound was identified by proton nuclear magnetic resonance (1H NMR) and carbon NMR (13C NMR), heteronuclear single quantum coherence, distortionless enhancement by polarization transfer (DEPT 135), electrospray ionization mass spectrometry, and ultraviolet, and infrared (Fourier transform infrared spectroscopy) spectroscopy.

Main Outcome Measure. Proof of adulteration of herbal products with synthetic PDE-5 inhibitors.

Results. By TLC and HPLC analysis, a major compound was detected in sample A organic extract. The purification of this extract by CC led to the isolation of a pure compound which was identified according to its spectral data as (6R,12aR)-2-amino-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6] pyrido [3,4-b] indole-1,4-dione or aminotadalafil.

Conclusions. An unapproved PDE-5 inhibitor analogue, which was identified as aminotadalafil, has been detected in a dietary supplement. This study represents the first report in Latin America and one of the few independent studies of an adulteration with an unapproved PDE-5 inhibitor of an herbal product for ED treatment. **Ulloa J, Sambrotta L, Redko F, Mazza ON, Garrido G, Becher EF, and Muschietti L. Detection of a tadalafil analogue as an adulterant in a dietary supplement for erectile dysfunction. J Sex Med 2015;12:152–157.**

Key Words. Erectile Dysfunction; Dietary Supplements; PDE-5 Inhibitor Analogues; Adulteration; Aminotadalafil

Introduction

Erectile dysfunction (ED) is a common and widespread health problem. Its prevalence is 1–10% in men younger than 40 years, 2–9% among men between 40 and 49 years, and it increases to 20–40% among men between 60–69 years, reaching the highest rate in men older than 70 years (50–100%) [1]. In addition, it has been estimated that the worldwide prevalence of ED will rise to 322 million cases by the year 2025 [2].

Tadalafil (Cialis™, Eli-Lilly, Indianapolis, IN, USA) is a selective inhibitor of the cGMP-specific phosphodiesterase type 5 (PDE-5) enzyme, which has been approved for the treatment of ED by the European Union in 2002 and by the U.S. Food and Drug Administration in 2003 [3]. It is capable of enhancing the relaxation of the cavernosal smooth muscle, similar to sildenafil citrate (Viagra™, Pfizer, New York, NY, USA) and vardenafil hydrochloride (Levitra™, Bayer, Leverkusen, Germany) and thus has the ability to enhance erection [4]. Compared with sildenafil, tadalafil has an improved PDE-5/PDE-6 selectivity [5].

PDE-5 inhibitors are massively used around the world with a good safety profile. However, these drugs are contraindicated in men taking nitrates and need to be administered carefully in subjects under multiple antihypertensive medications.

In Argentina, dietary supplements (DSs) are incorporated into the Argentina Food Code (CAA) since 1998 and are defined as "products to increase regular dietary intake, supplementing the incorporation of nutrients in the diet of healthy people who, in the absence of pathological conditions, present an unmet dietary need . . ." DSs are sold in pharmacies, natural product stores, health food stores, etc, and do not require a medical prescription. According to their labels, certain DSs are promoted as enhancers of sexual performance and are supposed to contain only natural plant extracts. These kinds of DS are widely consumed around the world, but since they are frequently sold informally and even online, it is difficult to assess the exact market penetration.

In recent years, several cases of adulteration of herbal products with tadalafil, sildenafil, or vardenafil and at least 46 synthetic analogues of these drugs have been reported [6–10]. In particular, new tadalafil analogues such as aminotadalafil, N-octylnortadalafil, 2-hydroxypropylnortadalafil, N-butylnortadalafil, chloropretadalafil, and other analogues are continuously being synthesized [11]. The side effects of these analogues are not fully known, and their safety profiles have not yet been established.

Aim

The aim of this study was to investigate the suspected adulteration with synthetic PDE-5 inhibitors in a DS marketed in Argentina, which is claimed to be natural and to enhance sexual performance.

Methods

The DS (sample A) was submitted to our laboratory by professionals from the Urology Department from "Hospital de Clínicas José de San Martín," University of Buenos Aires. Apart from this sample, three different lots of sample A were purchased from the local market. The product is labeled to have the following: *Panax ginseng*, *Astragalus membranaceus*, *Schizandra chinensis*, *Ginkgo biloba* and vitamins. The content (brown powder) of four capsules was ground and extracted with dichloromethane (50 mL/g sample) by ultrasonic shaking at 25°C for 1 hour. The extract was filtered, and the solvent was evaporated under vacuum at 40°C yielding the dried extract from sample A (DESA). Tablets of Cialis™ (tadalafil, 20 mg), Viagra™ (sildenafil, 50 mg), and Levitra™ (vardenafil, 20 mg) were obtained from pharmacies and used as standards and treated in the same way as sample A. Standard solutions were prepared at about 0.1 mg/mL. Thin layer chromatography (TLC) analysis of DESA and standard solutions was performed on silicagel 60 F_{254} using ethylacetate : water : n-buthanol (25:50:100) (upper layer) (system I) and dichloromethane : ammonia : methanol (15:3:2) (lower layer) (system II) as mobile phases. The detection was done with UV light (254 and 366 nm) and by derivatization with anisaldehyde/sulfuric acid reagent. High-performance liquid chromatography-diode-array detection (HPLC-DAD) was performed on a Waters™ 600 liquid chromatographer (Waters, Milford, MA, USA) equipped with a Waters 2996 DAD under the following chromatographic conditions: C18 analytical column (Phenomenex Luna, 250.0 × 4.6 mm, 5 μm), the gradient elution performed with 0.010 M KH_2PO_4, pH 3.0 (A)/methanol (B), at a flow rate at 1.0 mL/min, and an injection volume of 20 μL at room temperature. The working solutions were prepared by diluting stock solutions of DESA with MeOH to about 0.4 mg/mL. The elution was initially performed with 43% A and 57% B for 20 minutes. The mobile phase reached 40% A at 24 minutes and 20% at 26 minutes. The initial conditions were restored at 30 minutes and maintained for 5 minutes. Detection was set at 220 nm, and the UV spectrum for each peak was recorded between 190 and 400 nm. The purification of DESA was done by open column chromatography (CC) over silica gel 60 and eluted successively with n-hexane, CH_2Cl_2, and MeOH in a gradient of increasing polarity. A total of 70 fractions (F_1-F_{70}, 20 mL each) were eluted. Accord-

ing to the TLC profile, fractions F_{53} and F_{54} contained one pure compound (**1**). These fractions were pooled and taken into dryness, and **1** was studied by spectroscopic techniques. Fourier transform infrared spectroscopy (FT-IR) was performed on solid phase (KBr, Sigma, Sigma, St. Louis, MO, USA) on a NICOLET 380 FT-IR Smart Multi Bounce H ATR ZnSe 45° spectrometer (Madison, WI, USA) and recorded over the spectral range 4,000–400 cm^{-1} with an optical resolution of 4 cm^{-1}. Electrospray ionization mass spectrometry (ESI-MS) was performed on a LCQ Duo spectrometer coupled to a Surveyor HPLC pump (Thermo Scientific, Waltham, MA, USA). The nuclear magnetic resonance (NMR) spectroscopy spectra were determined on a 300-MHz Avance III 300 spectrometer (Bruker, Billerica, MA, USA). Compound **1** was dissolved in dimethylsulfoxide-*d6* (DMSO-*d6*) 99.8% (Sigma), and 1D and 2D NMR spectroscopy (^{1}H, ^{13}C, distortionless enhancement by polarization transfer—DEPT 135- and heteronuclear single quantum coherence [HSQC-NMR]) were performed. Chemical shift values (δ) are expressed in parts per million (ppm).

Main Outcome Measures

A herbal product marketed in Argentina suspected of adulteration was investigated. The sample was analyzed by TLC and HPLC-DAD, and the main compound was isolated by CC. Its identification was achieved by its spectral data that included UV, FT-IR, ESI-MS, ^{1}H-, ^{13}C-, DEPT-135, and HSQC-NMR.

Results

The results of the TLC analysis are shown in Figure 1. DESA showed one major compound (**1**) in both chromatographic systems tested with retention factor (Rf) values of 0.81 and 0.74 in systems I and II respectively.

As shown in Figure 2, the HPLC analysis of DESA showed the presence of one main peak with a retention time (11.79 minutes) that eluted before tadalafil (16.31 minutes) and a UV spectrum pattern (λ max 291.4, 284.3, and 220.6 nm) similar to that of tadalafil standard under the same chromatographic conditions.

The purification of DESA, performed by CC, led to the isolation of a pure compound (**1)** that was obtained as an amorphous yellowish powder (97.5 mg). The IR spectrum of **1** showed an absorption band at 1672.1 cm^{-1} indicating the existence of a carbonyl group and characteristic bands of amine (3296.6 and 1654.3 cm^{-1}), aromatic rings (1480.7 cm^{-1}) and other bands at 2905.0, 1427.3, 1031.2, and 746.4 cm^{-1}.

ESI-MS data indicated that the molecular formula of compound **1** was $C_{21}H_{18}N_4O_4$ which showed a molecular ion peak at *m/z* 391.1 $[M+H]^+$, indicating the presence of one more nitrogen atom but one less carbon and hydrogen atom than tadalafil.

A comparison of the ^{1}H and ^{13}C-NMR spectra of compound **1** with that of tadalafil [12] shows that in **1**, the N-methyl group of tadalafil is replaced, as evidenced by the two-proton signal at δH 5.11 (2H, *s*) and the disappearance of the signal at δH 2.93 (3H, *s*) that corresponds to the methyl group (Table 1).

The absence of a primary carbon is corroborated by the disappearance of the signal at δ32.8 in the ^{13}C-NMR spectrum of **1** and the presence of 21 carbons, including three secondary carbons, and nine tertiary and nine quaternary carbons in the DEPT spectrum [3]. The 2D HSQC spectrum shows correlations consistent with

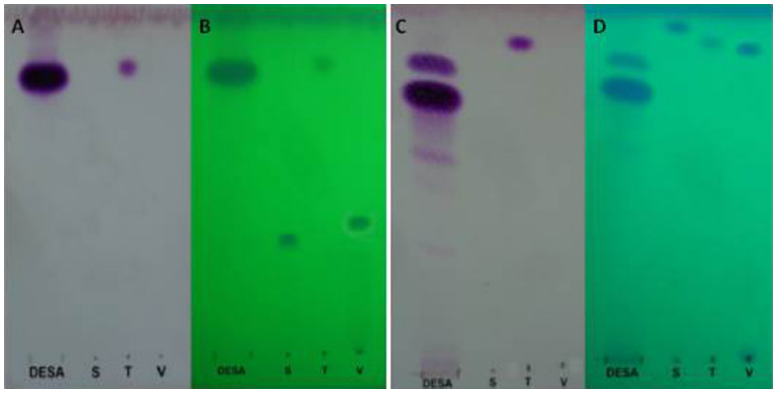

Figure 1 TLC analysis of DESA, sildenafil (S), tadalafil (T), and vardenafil (V) standards in system I (A and B) and system II (C and D). (A/C) Detection with anisaldehyde/sulphuric acid reagent; (B/D) detection with UV light 254 nm.

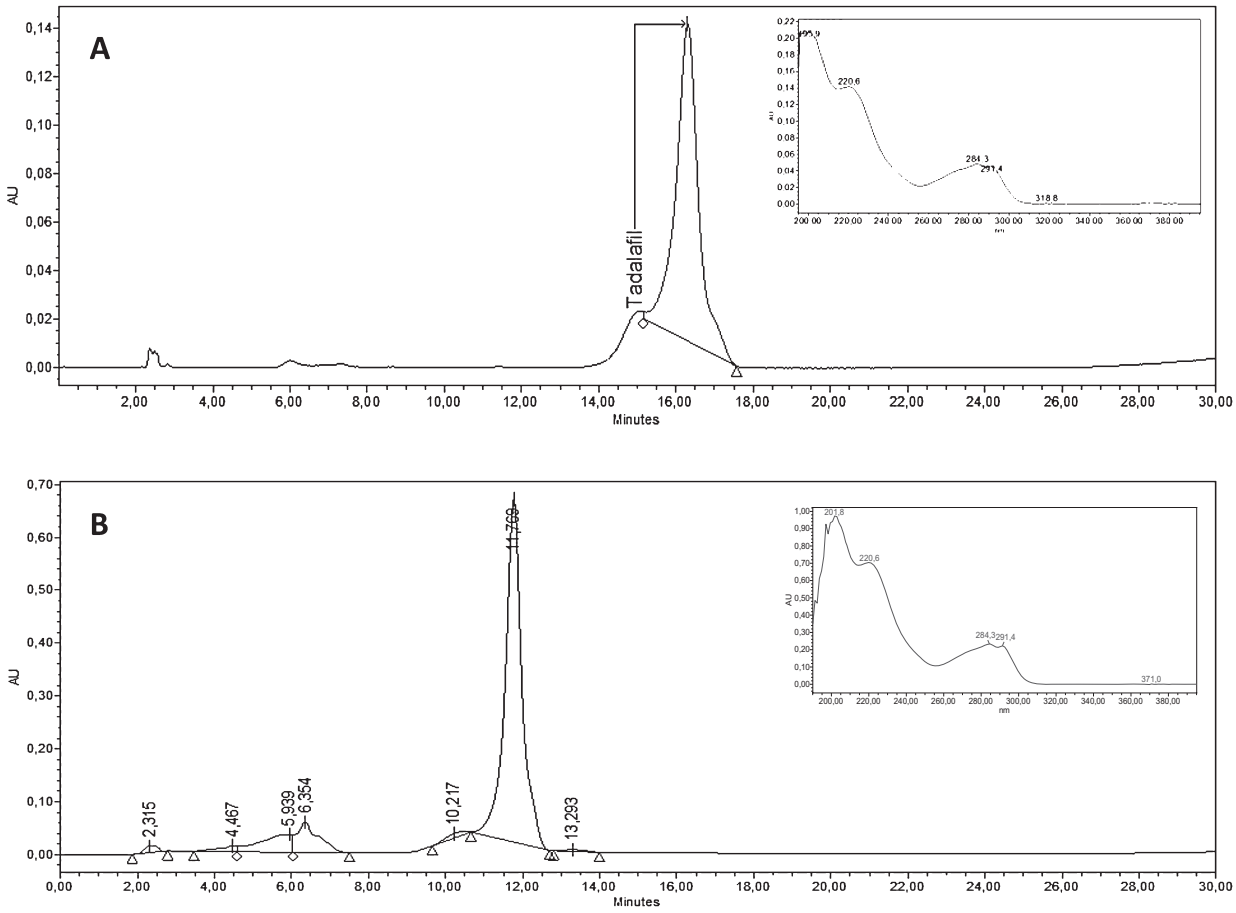

Figure 2 (A) HPLC chromatogram and UV spectrum corresponding to the tadalafil standard. (B) HPLC chromatogram of DESA and UV spectrum of the major compound (**1**) with a retention time of 11.79 minutes.

the chemical structure of the compound aminotadalafil (Figure 3).

By comparing the results obtained with published data [3,10], it was concluded that the structure of compound **1** was (6R,12aR)-2-amino-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydropyrazino [1′,2′:1,6] pyrido [3,4-b] indole-1,4-dione or aminotadalafil (Figure 4).

Discussion

In this study, we have analyzed a DS, marketed in Argentina for ED, that was submitted by professionals from the Urology Department at "Hospital de Clínicas José de San Martín," University of Buenos Aires, on suspicion that it contained not only natural extracts but also synthetic PDE-5 inhibitors. This suspicion for this research and the reason for selecting this DS was based upon the observed therapeutic effect, its duration, and the presence of adverse effects similar to PDE-5 inhibitors such as flushing, headache, and nasal congestion.

We first approached the analysis of the organic extract of sample A (DESA) using chromatographic techniques. Among them, TLC represents a very useful tool for a preliminary analysis of the chemical composition of herbal products and for the detection of adulterations [13]. The analysis of DESA, by this technique, detected a major compound (**1**). This was neither vardenafil, sildenafil, nor tadalafil since its Rf values did not coincide in the chromatographic systems tested. Notwithstanding, compound **1** showed a similar behavior to tadalafil as assessed by its Rf values and detection. Tadalafil and compound **1** were detected with anisaldehyde/sulphuric acid reagent, but vardenafil and sildenafil were not. Compound

Table 1 ^{1}H- and ^{13}C- NMR data for compound **1** in DMSO d_6

Position	^{13}C (ppm)	^{1}H (ppm)
1	164.5	—
2	—	—
3	53.3	3.96 (1H, d)
		4.26 (1H, d)
4	166.3	—
5	—	—
6	55.5	6.09 (1H, s)
6a	134.0	—
7	—	11.00 (1H, s)
7a	136.2	—
8	111.3	7.29 (1H, d)
9	121.2	7.05 (1H, m)
10	118.9	7.00 (1H, m)
11	118.1	7.55 (1H, d)
11a	125.7	—
11b	104.8	—
12	23.5	2.98 (1H, dd)
		3.56 (1H, dd)
12a	55.4	4.44 (1H, dd)
13	—	5.11 (2H, s)
1′	137.1	—
2′	106.9	6.87 (1H, s)
3′	145.0	—
4′	147.0	—
5′	107.9	6.77 (1H, d)
6′	119.3	6.79 (1H, d)
7′	100.9	5.92 (2H, s)

δ in ppm from TMS
d = doublet; dd = double doublet; m = multiplet; s = singlet

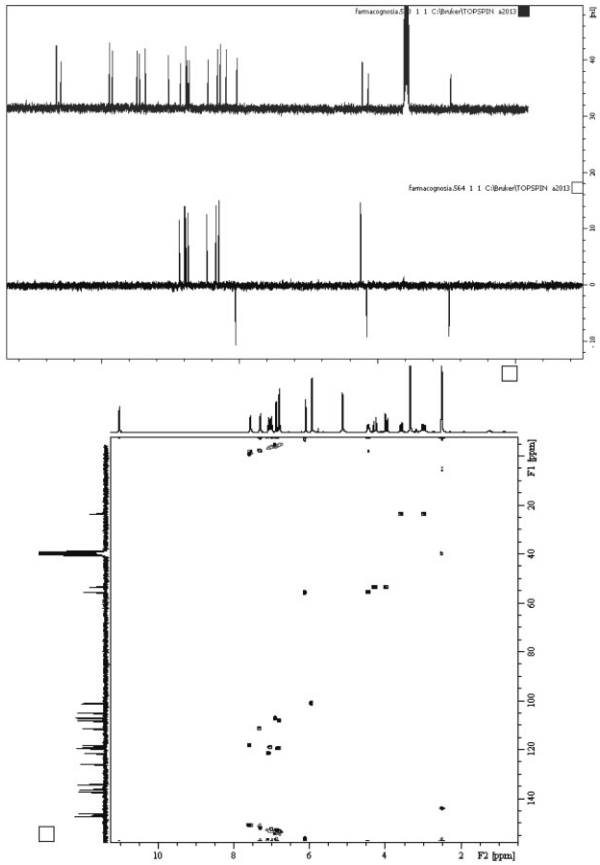

Figure 3 DEPT 135 and HSQC NMR spectra of compound **1**.

1 proved to be a more polar molecule than tadalafil, a fact that was also confirmed by the analysis by HPLC. The comparison of the UV spectra revealed a UV spectrum pattern similar to that of tadalafil. We presumed that **1** might have a similar structure to tadalafil and therefore proceeded to its isolation and purification by CC. The structural elucidation of **1** was based on the analysis of its spectral data and identified as aminotadalafil, which has been previously reported as an adulterant in DS [3,10].

The presence of analogues of PDE-5 inhibitors as illicit adulterants has been detected in herbal products marketed for ED. The occurrence of this sort of adulterations has increased significantly over the last years due to the widespread belief that natural products are safer and healthier than synthetic ingredients. One of the major concerns of these compounds is that they are available without prescription; therefore, they are sold informally and even online, not only in Argentina but also in many Latin American countries and even in the

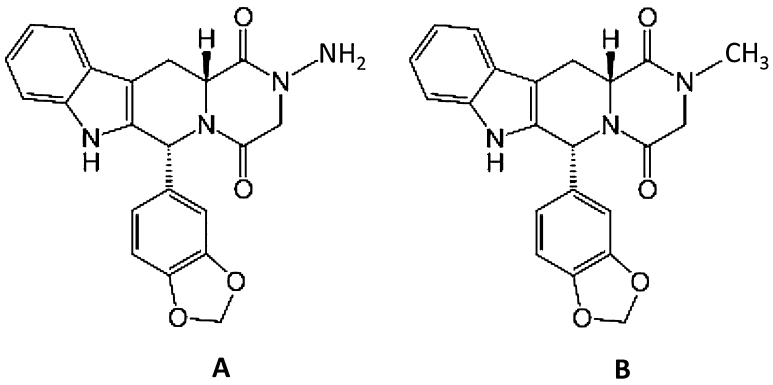

Figure 4 Chemical structures of aminotadalafil (A) and tadalafil (B).

United States. This represents a serious health risk to consumers as their efficacy and toxic effects have not been clinically assessed and may result in unpredictable adverse effects. Therefore, better regulation of these natural health products is necessary [14]. Moreover, patients with a contraindication to take PDE-5 inhibitors may consume herbal products or DS with its inherent risk in case of adulteration. To our knowledge, this study is the first report in Latin America and one of the few independent studies of an adulteration with an unapproved PDE-5 inhibitor of a herbal product for ED treatment.

Conclusions

An unapproved PDE-5 inhibitor analogue, which was identified as aminotadalafil, has been detected as an adulterant in a DS marketed in Argentina for the treatment of ED. The presence of untested drugs in herbal products, which are not declared on the packaging/labelling, constitutes a high risk to public health as the effects and side effects are unknown. These products require a better regulation given that although marketed as natural and devoid of adverse effects, they might have potentially fatal drug interactions.

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Statement of Authorship

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