DETECTION OF SILDENAFIL AND TADALAFIL IN HERBAL HONEY MIXTURES IN LIBYAN MARKET

By

Abdulhakeem Alssamo[1], Abdulrahman Abuzoraida[1], Samia Ibrahim Msimier[2], Aisha Alhadi Eghliow[2], Hawa Ibrahim Elkawk[2], Nuha Ahmed Elmangoush[2]

1-Faculty of Pharmacy-Misurata University, Misrata. 2-Libyan Academy, Janzour

ABSTRACT

The study focuses on the analysis of herbal honey mixtures (HHMs) available in the Libyan market. Due to the illegal adulteration of HHMs, there have been concerns regarding their harmful pharmacological effects on consumers. The specific compounds of interest in this study are sildenafil (SLD) and tadalafil (TAD), which are commonly used as phosphodiesterase type 5 (PDE-5) inhibitors for the treatment of erectile dysfunction (ED).

To detect the presence of SLD and TAD in the HHMs, the researchers employed High Performance Liquid Chromatography-Ultraviolet (HPLC-UV) as the analytical method. The mobile phase used in the analysis consisted of a gradient elution system comprising 0.1% formic acid in water and 0.1% formic acid in acetonitrile (ACN). The detection wavelength was set at 290 nm.

Out of the seven samples analysed, two were found to contain TAD, confirming the presence of this compound in the tested HHMs. This finding highlights the issue of adulteration and the need for quality control measures to ensure the safety and efficacy of herbal products in the market.

KEY WORDS: Sildenafil, Tadalafil, Herbal Honey Mixtures, High Performance Liquid Chromatography-Ultraviolet

INTRODUCTION

The global use of herbal medicines and dietary supplements has significantly increased in recent years. This can be attributed to the rising awareness of the detrimental side effects of synthetic drugs, prompting individuals to seek out "all-natural" alternatives. Consequently, the industry for herbal medicinal products and dietary supplements is experiencing a surge, with annual sales exceeding several billion euros [1]. According to a report from the World Health Organization (WHO), at least 80% of the population in developing countries depends on traditional medicine as their primary healthcare source. This highlights the importance and relevance of traditional medicine in such regions and emphasizes the need for further research and investment in this field. The efficacy and safety of herbal supplements are being debated in many countries [2]. Since there are no regulations, these herbal supplements tend to be adulterated in order to produce immediate and intensified pharmacological effects by using synthetic drugs instead of natural products [3]. One commonly cited claim in the literature is that herbal

For correspondence: Abdulhakeem Alssamo
Email:a.aissamo@phar.misuratu.edu.ly
ORCID: 9433-8498-0003-0009

supplements may be adulterated with drugs to guarantee or enhance the effects of the final product. However, it is important to note that the majority of cases involving adulteration are intentional [2]. Counterfeit medications and adulteration pose a grave risk to the well-being and safety of the general public. According to the WHO, counterfeit medicines are intentionally and fraudulently mislabelled in terms of their identity and/or source. Adulteration, on the other hand, involves the deliberate addition of substandard or harmful substances to medications [4]. Instances of adulteration in herbal remedies and dietary supplements are most frequently observed in products that are advertised as aids for improving sexual performance and aiding in weight loss [1]. According to a report from the Food and Drug Administration (FDA), there were 572 cases of adulterated supplements reported in the United States between 2007 and 2014. Out of these, 41.6% were identified as sexual enhancer drugs.[2] The most frequently added medications are synthetic PDE-5 inhibitors and SSRIs, which are used to treat ED and penile dysfunction. [3]

The use of herbal remedies for the treatment of erectile dysfunction has gained popularity in recent times. It is important to note that counterfeit herbal drugs may contain higher levels of undeclared drugs compared to approved pharmaceutical dosage forms, which may be seriously harmful or even fatal [2]. The concept of erectile dysfunction (ED), or impotence, refers to the inability to achieve and/or maintain a strong erection enough to perform a pleasurable sexual act [5]. In the world, 150 million men suffer from ED. The number is expected to double by 2025 [6]. It affects many males over 40. Studies often reveal that with increasing age comes a sharp rise in the risk of getting ED. A study of men between the ages of 40 and 70 was conducted as part of the Massachusetts Male Aging Study [7]. Additionally, participants were observed to have a higher prevalence of ED if they had cardiovascular disease, diabetes, or inactivity [8]. Causes of erectile dysfunction (ED):

Psychogenic ED: An important psychogenic factor related to ED is performance anxiety [9].

Vasculogenic ED: Caused by diminished blood flow, arterial insufficiency, or arterial stenosis, which are all symptoms of vascular disease and endothelial dysfunction [10].

Neurogenic ED: Numerous neurological diseases, such as multiple sclerosis, spinal cord injury, and cavernous nerve damage, are caused by major pelvic cancer surgery [11].

Endocrinological ED:

Several endocrine diseases, including end-stage renal failure, hypogonadism, hyperprolactinemia, hypothyroidism, and diabetes mellites [12].

Drug-induced ED: Many drugs are able to cause ED, including: zoladex, ketoconazole, spironolactone, thiazide diuretics, B-blockers, calcium channel blockers, digoxin, amiodarone**,** tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and recreational substances such as marijuana, opiates, nicotine, and alcohol [9].

Treatment of ED:

The Food and Drug Administration (FDA) defines an aphrodisiac drug product as “any product that bears labelling claims that it will arouse or increase sexual desire, or that it will improve sexual performance” [13].

According to the American Urological Association (AUA) and the European Association of Urology (EAU), phosphodiesterase type 5 (PDE-5) inhibitor medications like SLD and TAD are the preferred course of treatment for ED patients who do not

have a specific contraindication prohibiting their use [5].

A general mechanism of action for PDE5 inhibitors in ED As a result of sexual stimulation, nitric oxide (NO) is released directly into the penis by nerves and endothelial cells. NO binds to guanylyl cyclase in smooth muscle cells. Through this interaction, guanosine 5′-triphosphate (GTP) is converted into 3′-5′-cyclic guanosine monophosphate (cGMP) [14].

PDE5 inhibitors have a structure that is similar to that of cGMP (Figure 1); they competitively bind to PDE5 and prevent cGMP hydrolysis [11]. As cGMP accumulates, cGMP-dependent protein kinase (PKG) is activated, resulting in a decrease in intracellular calcium levels. Relaxation of arterial and trabecular smooth muscles increased arterial inflow, increasing penile rigidity (Figure 2) [14].

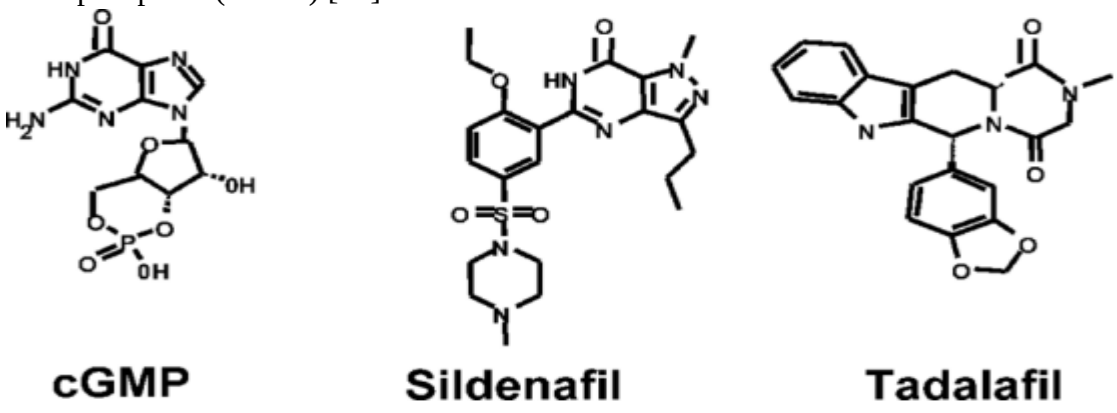

Figure 1. The chemical structures of cGMP, SLD, and TAD, respectively [15]

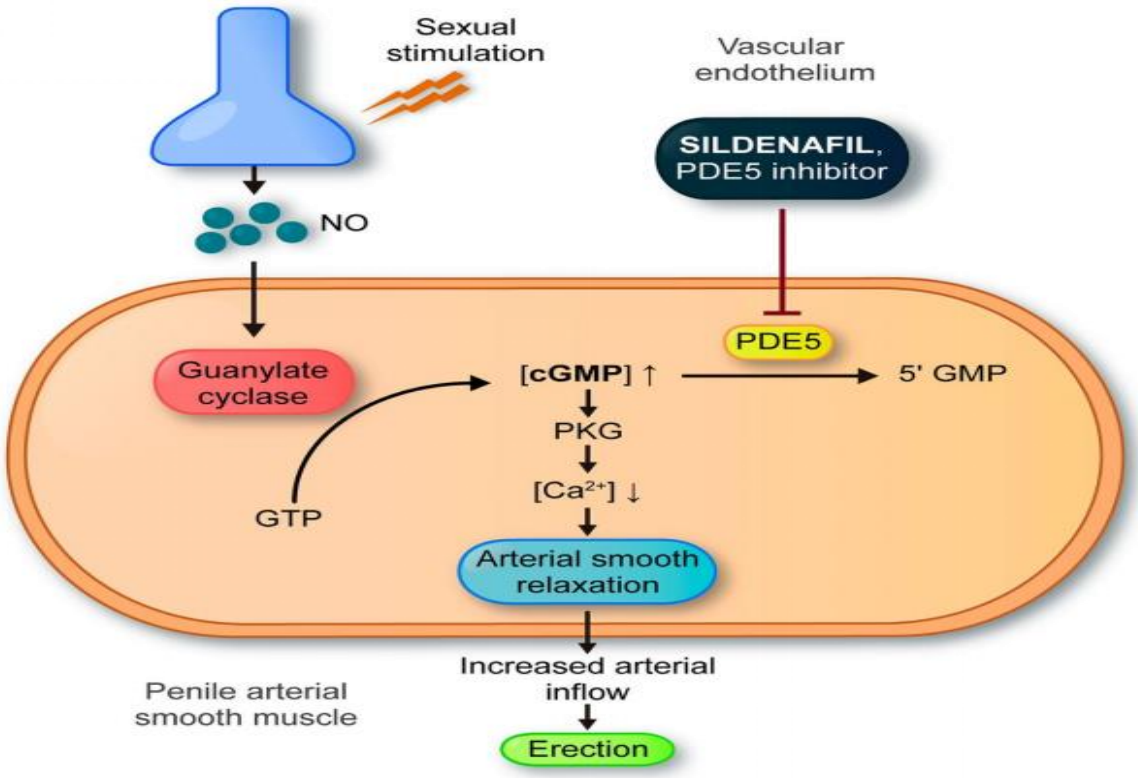

Figure 2. The mechanism of action for PDE5 inhibitors [14]

AIM OF STUDY

Detection of the presence of SLD and/or TAD in different types of mixed herbal honey that are used as OTC remedies for erectile dysfunction in Misurata, Libya. The analysis was conducted using HPLC technology.

MATERIALS

-Methanol HPLC-grade (MeOH) (CARLO ERBA Reagents, DASIT Group, Italy)
-Formic acid (0,1%)
-Acetonitrile (ACN) (AppliChem GmbH, Darmstadt, Germany).
-Distilled water
-Filter paper 0.45 µm Minisart® (Sartorius, Germany)
-Phosphate buffer (pH 3)
-Sildenafil 50mg (Sildenafil STADA®, Germany) and TAD 20mg (Victogon Normon®, Spain) tablets were used as reference standards dissolved in MeOH.
-HPLC-UV analysis: An HPLC (KNAUER, Berlin, Germany) instrument was equipped with a model series EA4300 smartline pump, SCL-10 AVP system controller, E4320V2 Smartline Manager 5000 Degasser, and an E4310 UV-VIS detector. An HPLC column measuring 150 x 4 mm with a precolumn was used. Data acquisition was performed using the Class-VP software.
-Vortex mixer: Vortex Mixer, (STUART) SA7 Fixed speed of 2500 p.m. for rapid mixing of samples contained in test tubes, small flasks, and bottles. It starts automatically when the rubber cup is depressed and stops once the vessel is removed.
-Centrifuge: This device is designed for separating substance mixtures with different densities, in particular for processing and analysing samples from the human body. It is manufactured by the Eppendorf company, Germany.
Sample collection: The samples used for analysis were seven different commonly used herbal honey blends provided by local suppliers in Misrata, Libya, which are used as OTC medications to treat ED.
Procedure of experiment:
-Standard solution preparation:
The preparation of standard solutions of SLD and TAD involved carefully weighing 5 mg of the drug, which was then dissolved in 20 mL of MeOH to provide a concentration of 0.25 mg/ml.
-Sample preparation:
To obtain the best extraction, two extraction methods were used to treat the sample using a different solvent each time, and then the results were compared.
In the first one, 5g of the HHM was dissolved in 15 mL of MeOH, and in the other, it was dissolved in 15 mL of ACN/water (50:50, v/v).
After 15 minutes of shaking with a vortex mixer, the samples were centrifuged at 4500 rpm for 30 minutes. The HPLC autosampler has been filled with two vials containing 2 mL of each supernatant solution after microfiltration [16].
Preparation of mobile phase
In the first method of extraction, the mobile phase consisted of phosphate buffer (pH 3)/ACN/MeOH (50:25:25, v/v), and in the second method, the mobile phase consisted of 0.1% formic acid in an aqueous solution and 0.1% formic acid in ACN (40:60, v/v).
A cellulose acetate membrane with a pore size of 0.2 µm was used to filter the two mobile phases, which were then degassed in an ultrasonic bath for five minutes. MilliQ® ultrapure water was used for mobile phase preparation**.** [10].
Chromatographic conditions
HPLC-UV analysis HPLC separation was performed using a Knauer® Eurospher 100-5 C18 column (150 * 4 mm with precolumn). The mobile phase was water containing 0.1% formic acid (aqueous phase A) and 0.1% formic acid in acetonitrile (organic phase B).

The flow rate was set to be 1 mL/min in 40:60 ratio between the A and B phases. Qualification and identification were performed with UV detection at 290 nm. All the determinations were carried out at 25 °C, and the volume injected was 10 µL.

RESULTS AND DISCUSSION

Using the specified chromatographic conditions, the standard and test analytes were separated. Various solvents were used to extract HHM samples, and these different solvents gave the same results. Depending on the mobile phase type used, different retention times for the SLD and TAD standards were observed during the chromatographic examination. Therefore, when the mobile phase (50% pH 3 phosphate buffer: 25% MeOH: 25% ACN) was used and the MeOH as solvent, we obtained a retention time of 6.15 and 6.35 min for SLD and TAD, respectively. But the problem that arose when using this mobile phase was the obvious overlap between the two standard peaks (the resolution value is poor), so we changed the mobile phase to obtain better resolution.

When the first mobile phase was replaced by another one consisting of 40:60% (0.1% formic acid in water/0.1% formic acid in ACN) and the MeOH was also used as a solvent, this change gave a retention time of 4.033 min for the SLD and 2.283 min for the TAD. This showed good separation between the peaks and no overlap between them. Accordingly, this mobile phase was adopted and used to analyse all the other samples.

The herbal honey sample containing SLD and/or TAD should have retention times similar to the reference standards. Then the results are summarised in Table 1.

Table 1: Results and components discovered for the studied herbal honey mixtures

No. of sample	Retention time (minutes)	Detection of SLD/TAD
SLD-Standard	4.033	—------
TAD-Standard	**2.283**	—------
Sample M1	(1.533), (3.300).	Not detected
Sample M2	(1.683), (**2.317**).	Tadalafil
Sample M3	(1.483), (1.650), (3.300).	Not detected
Sample M4	(**2.333**), (1.533).	Tadalafil
Sample M5	(1.500), (3.500).	Not detected
Sample M6	(1.467), (1.650), (3.650), (4.817).	Not detected
Sample M7	(1.533), (3.433).	Not detected

As mentioned earlier, this study was conducted to investigate the prevalence of the OTC sexual enhancement drugs adulterated with SLD and/or TAD in the Libyan market. Two of the seven products tested in this study were found to contain undeclared TAD, as shown in Table 1, Therefore, the percentage of positive results in our investigation represents approximately 28.6% of all

samples, as the results of the analysis of samples 2 and 4 showed that they have peaks with retention times similar to the TAD reference standard (+/- 0.1). While the rest of the sample results showed no similarity to either of the two reference standards, neither SLD nor TAD.

Likewise, several studies have also found adulteration in herbal supplements. For example, one study examined sildenafil and tadalafil in 30 herbal preparations marketed in Khartoum city, Sudan. Among these preparations, 40% were found to be adulterated with sildenafil and tadalafil. [17] and in another study, 27% of 80 sexual enhancer samples examined in Iran study were found to contain declared substance [18] Similar to this, sildenafil and other PDE-5 inhibitors were discovered in 61% of the items in French research looking at herbal supplements for sexual performance [19], While an investigation in the United States showed that 81% of 74 sexual enhancement products were found to contain PDE-5 inhibitors [20]. It should be noted that the absence of TAD and SLD in the other samples 1,3,5,6 and 7 does not guarantee that the products are safe for use as an OTC remedy for ED, as the possibility of the presence of other active pharmaceuticals for example, the rest of the PDE5 inhibitor family or SSRIs, still exists. This possibility is reinforced by the fact that the chromatographic results of these samples showed the presence of unknown suspicious substances within them. This hypothesis is also supported by a study conducted in Egypt in 2021, the results of which revealed the presence of other substances such as vardenafil, dapoxetine, citalopram, and tramadol in certain herbal supplements[5].

In doing so, it was necessary for everyone to know that the risks of using fake herbal honey mixture to enhance sexual ability outweigh any potential benefits. These mixtures often contain off-label medications that can have negative side effects and interact with other medications, especially in patients with existing conditions such as diabetes and heart disease.

CONCLUSION:

As noted, after analysing all the samples, two of the seven samples were found to be adulterated with the addition of TAD, as their retention times were found to be similar to the reference standard. In actuality, the presence of some positive results was not unexpected, because numerous earlier research studies had also established the existence of synthetic compounds added to these HHMs.

RECOMMENDATIONS

1-Further analysis should be conducted to determine the concentrations of sildenafil and tadalafil in the herbal honey mixture. Additionally, expanding the detection range by including other substances that may have been mixed with the herbal honey would be beneficial.

2-Educate consumers about the potentially lethal consequences of unauthorised products that are freely sold, marketed, and advertised in the market and sold without prescription.

3-Increasing consumer protection by passing and enforcing stricter laws and regulations, those laws must be active and deal with the registration, marketing, sales, and advertisement of nutraceuticals and cosmeceuticals, as well as holding herbal manufacturers responsible for their actions and ensuring that advertising standards are pure and truthful.

4-Researchers must verify the safety and effectiveness of illegally marketed products, such as male and female sexual enhancements, that are freely sold on the market.

REFERENCES

1-Skalicka-Woźniak K, Georgiev MI, Orhan IE. Adulteration of herbal sexual enhancers and slimmers: The wish for better sexual well-being and perfect body can be risky. Food and chemical toxicology. 2017 Oct 1;108:355-64.

2-Fard HH, Akhgari M. Analytical perspectives of chemical adulterants in herbal sexual enhancer drugs. Journal of Pharmacy & Pharmacognosy Research. 2018;6(1):45-53.

3-Maria J, Noordin MI. Fast detection of SLD in adulterated commercial products using differential scanning calorimetry. Journal of Thermal Analysis and Calorimetry. 2014 Feb; 115:1907-14.

4-World Health Organization. Substandard and Falsified Medical Products. Retrieved from https://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products.

5-Abdelshakour MA, Salam RA, Hadad GM, Abo-ElMatty DM, Hameed EA. HPLC-UV and UPLC-MS/MS methods for the simultaneous analysis of SLD, vardenafil, and tadalafil and their counterfeits dapoxetine, paroxetine, citalopram, tramadol, and yohimbine in aphrodisiac products. RSC advances. 2021;11(14):8055-64.

6-Azimi S, Mistry NS, Wood M. UPLC-MS/MS for the Screening, Confirmation, and Quantification of Drugs Illegally Added to Herbal/Dietary Supplements for the Enhancement of Male Sexual Performance. Waters the Science of what's possible. 2012:1-6.

7-Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. The Journal of urology. 1994 Jan 1;151(1):54-61.

8-Johannes CB, Araujo AB, Feldman HA, Derby CA, Kleinman KP, McKINLAY JB. Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. The Journal of urology. 2000 Feb;163(2):460-3.

9-Shamloul R, Ghanem H. Erectile dysfunction. The Lancet. 2013 Jan 12;381(9861):153-65.

10-Yafi FA, Jenkins L, Albersen M, Corona G, Isidori AM, Goldfarb S, Maggi M, Nelson CJ, Parish S, Salonia A, Tan R. Erectile dysfunction. Nature reviews Disease primers. 2016 Feb 4;2(1):1-20.

11-McMahon CG. Erectile dysfunction. Internal Medicine Journal. 2014 Jan;44(1):18-26.

12-McMahon CG. Current diagnosis and management of erectile dysfunction. Medical Journal of Australia. 2019 Jun;210(10):469-76

13-West E, Krychman M. Natural aphrodisiacs—a review of selected sexual enhancers. Sexual medicine reviews. 2015 Oct;3(4):279-88.

14-Huang SA, Lie JD. Phosphodiesterase-5 (PDE5) inhibitors in the management of erectile dysfunction. Pharmacy and therapeutics. 2013 Jul;38(7):407.

15-Weeks JL, Blount MA, Beasley A, Zoraghi R, Thomas MK, Sekhar KR, Corbin JD, Francis SH. Radiolabeled ligand binding to the catalytic or allosteric sites of PDE5 and PDE11. Phosphodiesterase Methods and Protocols. 2005:239-62.

16-Mustabasic N, Isik S. Determination of SLD mixed into herbal honey mixture by ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry. Croatian journal of food science and technology. 2017 Dec 20;9(2):168-72.

17-Saeed SM, Shantier SW, Gadkariem EA, Ismail EM. Determination of undeclared sildenafil citrate and tadalafil in aphrodisiac herbal preparations by TLC and HPLC.

18-Fard HH, Akhgari M. Analytical perspectives of chemical adulterants in herbal sexual enhancer drugs. Journal of Pharmacy & Pharmacognosy Research. 2018;6(1):45-53.
19-Gilard V, Balayssac S, Tinaugus A, Martins N, Martino R, Malet-Martino M. Detection, identification and quantification by 1H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. Journal of pharmaceutical and biomedical analysis. 2015 Jan 5;102:476-93.
20-Campbell N, Clark JP, Stecher VJ, Thomas JW, Callanan AC, Donnelly BF, Goldstein I, Kaminetsky JC. Adulteration of purported herbal and natural sexual performance enhancement dietary supplements with synthetic phosphodiesterase type 5 inhibitors. The journal of sexual medicine. 2013 Jul;10(7):1842-9.