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Research Article

Determination of the presence of sildenafil and tadalafil in herbal products collected in Bamako and surrounding area by HPLC at the National Health Laboratory (LNS)

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Abstract

Objectives: Adulteration of herbal products can have a negative impact on the health of populations, as adulterants can lead to developmental defects, chronic diseases or death. Recently, in the literature, it has been reported that these products are used as adulterants in several traditional medicines. Therefore, it is strongly recommended to monitor synthetic adulterants in marketed liquid herbal products, which are most commonly used as strength and energy stimulants in Mali, for the safety of populations.

Methods: In this study, we investigated the presence of sildenafil and tadalafil in the most popular marketed herbal products in Mali by HPLC for regulatory measurements.

Results: A total of 16 samples were collected and analyzed, of which 14 revealed the presence of adulteration corresponding to 87%. Among these samples, 8 revealed the presence of Sildenafil and 6, the presence of Tadalafil at varying concentrations. These samples consist mostly of liquid, powder or paste preparations and are used as traditional medicines and food supplements for the treatment of physical and sexual weakness.

Conclusion: These results clearly raise the issue of adulteration of traditional medicines and food supplements used for the treatment of physical and sexual weakness in Mali by chemicals and the need to strengthen regulatory controls to prevent the falsification of these products.

Keywords: sildenafil, tadalafil, herbal products

INTRODUCTION

Adulteration of herbal products can have a detrimental impact on the health of a population as adulterants can lead to developmental defects, chronic diseases or death[1].

PDE is a ubiquitous enzyme in the human cell that is the essential regulator of cyclic nucleotide signaling with various physiological functions. So far, 11 subtypes of PDE (1-11) have been identified. These enzymes are involved in various regulatory processes such as ion channel functions, cell differentiation, apoptosis, muscle contraction, etc.[2]. Nowadays, PDE is considered as an important molecular target in drug discovery. Sildenafil was synthesized in a large drug discovery project as an active drug[3]. During the clinical trial, sildenafil was serendipitously found to have an effect on penile engorgement. This drug was finally approved by the United States Food and Drug Administration (FDA) in 1998 for the treatment of erectile dysfunction. Sildenafil is selective for PDE-5 over PDE1-4, but it is less selective for PDE-6. Since PDE-6 is found in the retinal cell, the use of sildenafil is associated with visual side effects[4]. In addition, sildenafil has also shown several drug interactions and is contraindicated with organic nitrates. Concomitant use of sildenafil and organic nitrates can cause severe and fatal hypotension[5]. Although sildenafil is approved as a prescription drug, it has a high potential for abuse for recreational purposes. Recently, in the literature, it has been reported that sildenafil is used as an adulterant in several traditional Chinese medicines marketed in Singapore and Denmark[5]. In the United

States, the presence of sildenafil has been reported in dietary supplements and bulk herbal products[6]. In Singapore, 22 deaths were reported between 1998 and 2009 due to adverse reactions to adulterated herbal medicines, and sildenafil was detected as one of the adulterants in these herbal products[7]. In India, one out of 85 Ayurvedic preparations tested in the local market was found to be adulterated with sildenafil[8]. Recently, in Bangladesh, a study was conducted on 35 traditional medicines and dietary supplements. The results showed that 20% of traditional medicines and 70% of dietary supplements contained sildenafil citrate as an adulterant. They also analyzed two liquid dosage forms among 35 products and found that one liquid dose of traditional medicines contained high concentration of sildenafil citrate as an adulterant[9].

As traditional liquid medicines contain high concentration of sildenafil citrate and there are many popular herbal liquid products available in the market as remedies for physical and sexual weakness, it is important to conduct the study on these products to protect the populations from the deadly effects.

Many Malians prefer to use liquid herbal products because they generally believe that these products could have fewer side effects with maximum therapeutic benefit. However, if a patient with diabetes or ischemic heart disease or related diseases uses herbal products adulterated with sildenafil, it can pose a significant threat to his health[4,7,10].

Therefore, it is strongly recommended to monitor synthetic adulterants in marketed liquid herbal products, which are most commonly used as strength and energy boosters in Mali, for the safety of populations. In this study, we investigated the presence of a specific PDE-5 inhibitor in the most popular marketed liquid herbal products in Mali by HPLC system to confirm their safe use.

METHODOLOGY

Samples

All samples used in this study were sampled in Bamako district. The collected samples were stored in the sample library of the drug quality control department of the National Health Laboratory. A total of 16 samples were collected and analysed by HPLC.

Tests

Reagents, chemicals and solvents

Analytical reagents were used throughout the experiment. The reagents used were ultrapure water, potassium dihydrogen, phosphoric acid (85%), methanol and acetonitrile HPLC grade.

Instruments

HPLC INFINITY 1260, OHAUS analytical balance, Elmasonic ultrasonic water bath, Velp scientifica magnetic stirrer, Mettler Toledo pH meter, Arium confort distiller.

Analytical procedure

- **Preparation of the Sildenafil reference:** weigh 10 mg of sildenafil RS in 10 mL of Methanol, then pipette 1.7 mL of this solution into 25 mL of methanol, which corresponds to a concentration of 0.068 mg/mL.
- **Preparation of the Tadalafil reference:** 1 tablet of 20 mg tadalafil was crushed, then dissolved in 20 mL of methanol. Then 1.7 mL of this solution is taken in 25 mL of methanol.

- **Preparation of 50 mmol of Potassium dihydrogen phosphate:** weigh 6.8382 g of potassium dihydrogen phosphate and dilute in a 1000 mL flask with water. Shake until all products are completely dissolved and adjust the pH with phosphoric acid to pH.

Sample preparation

Liquid Solutions: 1 mL of each herbal liquid formulation was placed in a 50 mL volumetric flask and made up to volume with methanol. The flask was shaken vigorously and sonicated for 30 min at 40°C.

Powders and Pastes: 2 g of each herbal powder or paste formulation was placed in a 50 mL volumetric flask and made up to volume with methanol. The flask was shaken vigorously and sonicated for 30 min at 40°C.

The solutions were filtered through filter paper to remove unwanted materials, and the supernatant was filtered again through a membrane filter to obtain a clear liquid solution of each herbal product. Then, 1 mL of the clear solution of each formulation was taken into the HPLC vial and 20 µL of the solution was injected.

HPLC method

An Agilent INFINITY 1260 HPLC system with a DAD detector was used. The Avantor analytical column (C18; 150mm x 4.6 mm; 5µm) was used under isothermal conditions at 40 °C. The system was pumped at a flow rate of 1.0 mL/min and the full UV spectra were recorded online during the 30-minute chromatographic run. Mobile phase selection was performed according to published literature[11] and consisted of (A) acetonitrile:50 mmol potassium dihydrogen phosphate (adjusted to pH 2.5 with phosphoric acid) (10:90, v/v) and (B) acetonitrile:50 mmol potassium dihydrogen phosphate (adjusted to pH 2.5 with phosphoric acid) (70:30, v/v); which were used in gradient mode at a flow rate of 1.0 mL/min. The gradient system was: Tmin/A:B (V/V); T 0.01-5/100:0.00, T15/40:60, T20/0.00:100, T25/100:0.00, T30/100:0.00.

Chromatographic conditions

Parameter	Conditions
Column	Extension C-18 (150 mm x 3.9mm, 5 µm)
Mobile phase	Acetonitrile : Water (60:40)
Detector	DAD
Detection wavelength	365 nm
Flow	1 mL
Column temperature	40ºC
Sample volume	20µL

RESULTS

A total of 14 samples were analyzed, of which 6 revealed the presence of Sildenafil and 6 revealed the presence of Tadalafil at varying concentrations (Tables II and III). These samples consist mostly of liquid, powder or paste preparations and are used as traditional medicines and food supplements for the treatment of physical and sexual weakness.

Table I: Situation of samples according to commercial name

N°	Sample code	Commercial name
1	24-0379	KTM (MORINGA THE MENTHE) + Gingembre
2	24-0380 (E1)	VITAMAX (Jaune brun)
3	24-0381 (E2)	VITAMAX (mélange de cristaux blanchâtre et brun)
4	24-0382	7ème ciel
5	24-0385 (E3)	VITAMAX (cristaux marron)
6	24-0386	BIO HERBS Royal King Honey
7	24-0387	Shooting in the Enratp fire condition (5minutes)
8	24-0388 (E4)	VITAMAX (jaune brun)
9	24-0389	ATTOTE Tisane
10	24-0390	Le ROI
11	24-0391	LA PAIX CONGNON-MOUSSO YAKO
12	24-0392	ATTOTE Original
13	24-0393	4 AIR COLA EXCELENT
14	24-0394	CYCLONE
15	24-0395	WARABA KOLE SERE
16	24-0396	TCHEYA DAGA

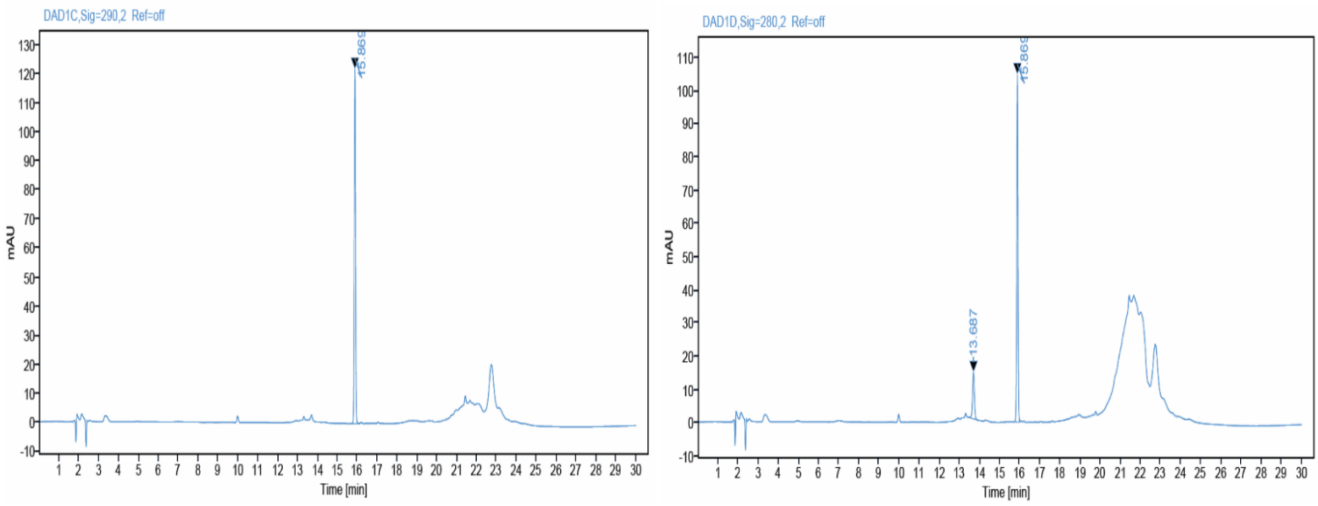

Figure 1: (A) Chromatogram of the Sildenafil standard identified at a retention time of 15.86 min with an area of 2472.927. **(B)** Chromatogram of the CYCLONE sample identified at a retention time of 15.86 min with an area of 807.621.

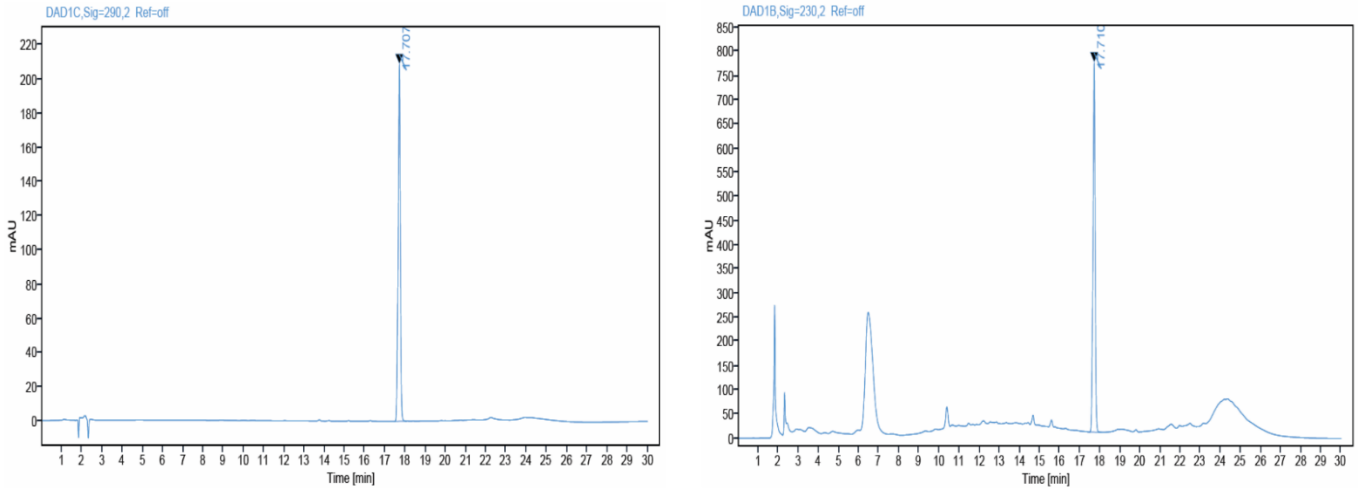

Figure 2: (A) Chromatogram of the Tadalafil standard identified at a retention time of 17.70 min with an area of 1342.769. **(B)** Chromatogram of the VITAMAX sample (E3) identified at a retention time of 17.71 min with an area of 2135.948.

Distribution of samples according to the presence or absence of adulteration

Of the 16 samples, 8 revealed the presence of Sildenafil and 6, the presence of Tadalafil. Sildenafil and Tadalafil were not found in 2 samples.

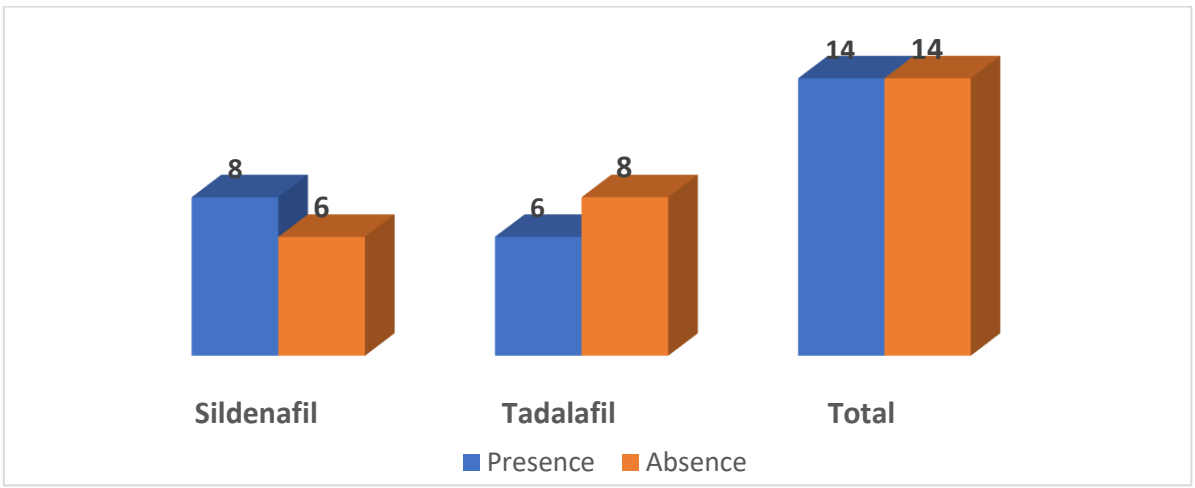

Figure 3: Situation of samples according to the presence and absence of Sildenafil or Tadalafil.

Distribution of samples according to the active ingredient found

Table II: Distribution of samples containing Sildenafil

Designation	Sildenafil	Concentration
Septième (7eme) ciel	Yes	1 mg/2g
Le ROI	Yes	28,79 mg/mL
LA PAIX CONGNON-MOUSSO YAKO	Yes	**135,42** mg/mL
ATTOTE Original	Yes	75,40 mg/mL
4 AIR COLA EXCELENT	Yes	20,35 mg/mL
CYCLONE	Yes	34,15 mg/mL
WARABA KOLE SERE	Yes	55,20 mg/mL
TCHEYA DAGA	Yes	6,20 mg/mL

PAIX CONGNON-MOUSSO YAKO had the highest concentration of Sildenafil at 135 mg/mL.

Table III : Distribution of samples containing Tadalafil

Designation	Tadalafil	Concentration
KTM (MORINGA THE MENTHE) + Gingembre	Yes	0,3 mg/sachet
VITAMAX (E1) (yellow brown)	Yes	15,52 mg/2g
VITAMAX (E2) (mixture of whitish and brown crystals)	Yes	19,81 mg/2g
VITAMAX (E3) (brown crystals)	Yes	**29,87** mg/2g
BIO HERBS Royal King Honey	Yes	20,30 mg/2g
VITAMAX (E4) (yellow brown)	Yes	14,17 mg/2g

The VITAMAX (E3) product had the highest concentration of Tadalafil at 29 mg/2g.

Overall results of the tests carried out

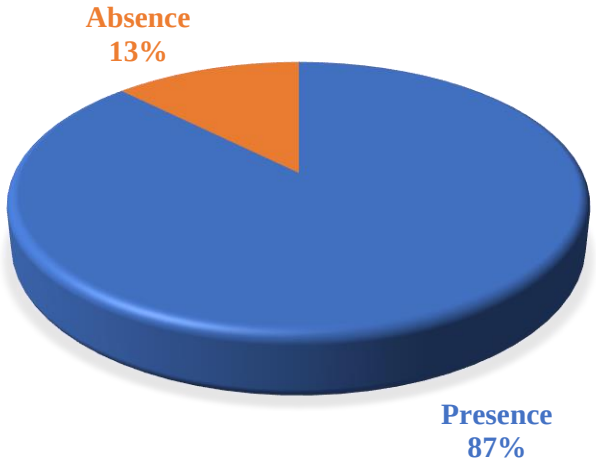

Figure 4: Overall result of the samples analyzed

DISCUSSION

Our results reveal an adulteration of traditional medicines and dietary supplements used for the treatment of physical and sexual weakness by Sildenafil and Tadalafil with 86% (14/16) of the samples analyzed. Some products even contain a high amount of sildenafil citrate compared to the recommended daily doses. Indeed, according to Pfizer laboratories, the recommended dose is 50 mg per day with a maximum dose not to exceed 100 mg[12]. This is all the more dangerous since the populations who buy and use these products are not aware of their dosages and are also unaware of the presence of synthetic sildenafil citrate and Tadalafil which have a fatal effect on human health at high doses. Oral PDE-5 inhibitors are the treatment for patients suffering from erectile dysfunction. The mechanism involves the release of nitric oxide in the corpora cavernosa during physical intercourse. Then, nitric oxide activates the enzyme guanylate cyclase, which increases the levels of cyclic guanosine monophosphate (cGMP), which causes relaxation of smooth muscles in the blood vessels and allows blood flow into the corpora cavernosa. The FDA has recommended sildenafil citrate for patients with erectile dysfunction. The use of these herbal products may have a serious adverse effect on the patient's health because patients have been given these products without proper precautions. In addition, oral PDE-5 inhibitors (sildenafil citrate and tadalafil) also increase the risk of many cardiovascular diseases, including heart attack, myocardial infarction, and sudden death. It is also possible that these drugs may interact with other drugs and cause a fatal effect, for example, sildenafil citrate causes the synergistic effect with alpha-blockers that could cause a dangerous health problem. It is also known that the use of Sildenafil in combination with organic

nitrates significantly increases the systemic hypotensive effects.

In this study, we found that all liquid herbal products mention that they use traditional medicines in their formulations. They also do not have any labeling regarding the use of synthetic sildenafil or tadalafil in their formulation. The use of synthetic products in herbal product formulations is prohibited because the herbal formulation must contain traditional medicines and the use of synthetic medicines may cause an inverse interaction with natural medicines[13].

CONCLUSION

In this study, we determined the presence of Sildenafil and Tadalafil in 14 products described as ginseng, ginger, lemon, Garcinia kola (little kola), herbal blends and herbal supplements, Moringa, caffeine on the product packaging. For others, the composition is undefined and may contain other synthetic products used as sexual stimulants. These samples were in the form of pills, capsules, powders, syrups, liquids and pastes. The powder products were generally brown and gray in color and had a strong spicy odor. The liquid products were light green. It is believed that these processes are intended to convince users that the products are natural.

These results clearly raise the issue of adulteration of traditional medicines and food supplements used for the treatment of physical and sexual weakness in Mali by chemicals and the need to strengthen regulatory controls to prevent the falsification of these products.

To minimize the risk of these herbal products adulterated by medicinal chemicals, consumers must be informed that "natural" does not necessarily mean "risk-free" and that the adverse effects due to these products are an undeniable reality.

Conflicts of interest: None, it was within the framework of public health and for the well-being of the Malian population in accordance with the mission of the LNS.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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