European Journal of Integrative Medicine 25 (2019) 55–59

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/locate/eujim

Research paper

Herbal medicines in Iran advertised as opioid withdrawal drugs - analysis by gas chromatography-mass spectrometry

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ARTICLE INFO

Keywords:
Opioid addiction
Herbal medicines
Illegal drugs
Safety
Substance misuse
Gas chromatography mass - spectography

ABSTRACT

Introduction: Herbal medicines are used to address some of the problems experienced by individuals addicted to drugs, but there are also many reports about the adulteration of products sold as medicinal herbs for addiction treatment or opioid withdrawal. The aim of the present study was to explore the products sold as herbal medicines by herbal remedy shops in Iran.

Method: Fifty samples of medicinal herbs were collected from herb shops located in different parts of Iran. Samples were coded according to their brand name and included: Dragon tablets, Deta capsules, compound capsules and Vincamin DF. Samples were transferred to the toxicology laboratory of the Medical Jurisprudence Organization of Mashhad and the samples were analyzed for their physical and chemical properties.

Results: Out of the samples, 74% contained illegal or chemical drugs. In most of the counterfeit samples, we found a high dose of tramadol, methadone, and diphenoxylate. In some samples, small amounts of nicotine, barbiturate, acetaminophen, diclofenac, lysergic acid diethylamide, diphenhydramine, codeine were detected. An investigation of the physical properties (smell) indicated the presence of fragrant compounds in some samples such as ginger, Peganum harmala, and pepper.

Conclusions: Nearly all of the handmade and illegally distributed herbal medicines for the treatment of drug withdrawal contained at least one opioid or illegal drug, indicating that there was evidence of adulteration and highlighting the dangers to users. It is important to raise awareness of people, particularly warning them against the use of such products sold by herbal shops for the treatment of addiction.

1. Introduction

Generally, more than 230 million people worldwide are suffering from substance abuse with a mortality rate of nearly two hundred thousand annually [1]. Recently, the rate of substance abuse and addiction (especially opioid addiction) has increased in Iran [2]. So, the need to find solutions regarding the treatment of addiction has increased. Since chemical drugs have not succeeded in reducing withdrawal symptoms, alternative therapies (especially herbal remedies) have been considered [3]. Lately, the use of medicinal herbs has increased in the treatment of many diseases including addiction [4]. It is possible that these herbs could be used to solve many of the problems experienced by addicts, including mental, emotional and behavioral control, and as a complementary treatment alongside other psychological and pharmacological interventions [4].

One of the herbs which have long been used in South East Asia, particularly in Thailand, as a withdrawal remedy is Kratom [5]. Kratom leaves contain several alkaloids which impact the alpha adrenergic and opioid receptors, These tablets contain the active substance of Mitragynine, which is obtained from Kratom tree (Scientific name: *Mitragyna Speciosa Kortth*) [6,7]. This herbal medicine is sold under the name of Dragon and Indian Deta in Iran.

Unfortunately, many reports of legal medicine in Iran indicate non-scientific practices in addiction treatment. Generally, the impurity in herbal medicines and the counterfeiting of drugs are a growing problem and a concern for many countries today. It is estimated that counterfeit

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https://doi.org/10.1016/j.eujim.2018.12.001
Received 22 June 2018; Received in revised form 29 November 2018; Accepted 1 December 2018

drugs form 10% of the developing countries' pharmaceutical markets that Africa, Southeast Asia and many countries in Latin America are the most affected areas [8,9]. There are many reports of deaths from counterfeit opioids, between late 2013 and 2014, there were more than 700 counterfeit fentanyl-related deaths reported in the United States. During this same period, the Centers for Disease Control and Prevention (CDC) reported that deaths from synthetic opioids (including fentanyl) increased by 79% from 3097 to 5544. [10]. Since nobody deserves to be killed with counterfeit opioids, it is essential that we examine the counterfeit drugs distributed in the black markets. Also, there are many reports of counterfeit traditional herbals that are sold in other countries. In Japan in 2002, a designer added fenfluramine to an herbal tea and caused over 800 cases of serious health problems and even some deaths too. Similar clinical examples were reported on a smaller scale in Singapore, Belgium and the United Kingdom [11].

The World Health Organization (WHO) stated that counterfeit drug is "a medicine that is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging" [12].

Universally, counterfeit herbal medicines with low prices are substandard medicines with no therapeutic benefit [9]. Due to the existence of harmful ingredients in counterfeit drugs, they may cause serious harm to health or exacerbate the condition of the person under treatment. Since there are not enough analytical or quantitative reports on the use of adulterated medicinal herbs, the present study is designed to analyze and investigate the quality and quantity of various pills, capsules, and handmade herbs that are sold as remedies for drug dependence or opioid withdrawal by some herbal remedy shops in Iran.

2. Methods

2.1. Sample collection

In the present study, the five largest and most populous Iranian cities (Tehran, Mashhad, Esfahan, Shiraz, and Tabriz) were selected for the investigation of herbal medicines, where opioid withdrawal drugs were advertised and sold by the herbal shops from June to December 2016. Five herb shops were randomly selected in different parts of each city and two samples were collected from each herb shop. According to the brand name of samples, they were coded, including Dragon tablets, Deta capsules, Compound capsules (hand-made capsules that named by sellers), and Vincamine DF. Vincamine is a monoterpenoid indole alkaloid found in the leaves of Vinca minor. Some pharmaceutical companies produce Vinca brand capsules as accredited and licensed herbal medicines for the treatment of drug dependency. Due to the high prices of its packets, a number of herb shops divide tablets into small parts and sell them unpacked. Therefore, Vincamine DF as legal of herbal medicine was sampled and investigated. After coding, all of the samples were transferred to the toxicology laboratory of the Medical Jurisprudence Organization of Mashhad.

2.2. Method

Firstly, the physical and chemical properties of the samples such as weight, color, smell, melting point, water solubility, and pH were recorded. In order to detect all the drug types or chemicals existing in the samples, we set up a general method of using gas chromatography with the mass spectrometry detector GC/MS. After the sample preparation and purification, the samples were qualitatively analyzed for the determination of drug type by GC/MS. In the next step, for the quantification of identified compounds, relevant standard preparations were performed.

2.3. Materials

Tramadol hydrochloride and methadone were supplied by Modava Company (Tehran, Iran). Diphenoxilate hydrochloride was supplied by TAMAD Pharmaceutical Products Factory Co (a branch of Darou-Pakhsh Company) and HPLC-grade methanol was purchased from Merck (Darmstadt, Germany).

2.4. GC/MS conditions

The GC/MS was provided by the Agilent Company (6890 N-5978B). Helium carrier gas was supplied by helium cylinders with a purity of 99.990. The scan mode was used for the GC/MS analysis. Data analysis was performed using MSD chemstation G1701EA E.02.01.1177. To detect compounds, Agilent Library software was used. A capillary column with a length of 30 m and an internal diameter of 250 µm was used. The temperature of the injector was 280 °C and the initial temperature of the column and the rate of temperature rise were 65 °C and 10 °C/min respectively. The final temperature of the column was 300 °C. Total run time was 32 min with a flow rate of 1 ml/min. To minimize the manual injection errors, auto-injector was used and the volume of sample injected into the GC was 1 µL with 1/20 split ratio.

2.5. Calibration curve

Quantitative analysis was only performed for tramadol, methadone, and diphenoxilate. Because in the qualitative analysis step, we observed a high concentration of tramadol, methadone, and diphenoxilate in the chromatogram peak of most samples, the unknown samples were analyzed using calibration curve prepared by adding tramadol, diphenoxilate, and methadone to blank methanol. Each calibration curve consisted of six calibration points and was prepared in concentration ranges of 40–400 µg/mL, 10–100 µg/mL, and 5–50 µg/mL for tramadol, methadone, and diphenoxilate respectively. The calibration curves were determined by least-square linear regression analysis. Since we did not start with extraction before injection, the internal standard for each analyte was not used. For all the analytes in the sample, the limit of detection (LOD) was 0.35 µg/ml and the limit of quantification (LOQ) was 1.2 µg/mL. Three chromatograms of blank methanol were spiked with diphenoxilate, tramadol, and methadone.

2.6. Sample preparation

To prepare the samples, each tablet or capsule was weighed and crushed. Then, 0.1 g of mixed powder samples was dissolved into 10 ml of methanol as solvent.

2.7. Statistical analysis

The statistical analysis is presented as values ± SD. All statistical analyses were performed using the SPSS software, version 20. Statistical significance was determined using a one-way ANOVA test, followed by the post-hoc Tukey test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. General properties

The Mean ± SD of weights for Dragon, Deta, Compound capsules and Vincamine DF were respectively 1.79 ± 0.23, 0.96 ± 0.14, 0.81 ± 0.20 and 0.58 ± 0.004 g. The colors of these compounds were various, including brown and orange.The number of samples collected for each product were as follows; Dragon n = 9, Deta n = 12, Compound n = 16 and Vincamine DF n = 13. Also, investigation of physical properties (smell) was indicated the presence of fragrant compounds such as ginger, Peganum harmala, and pepper in some

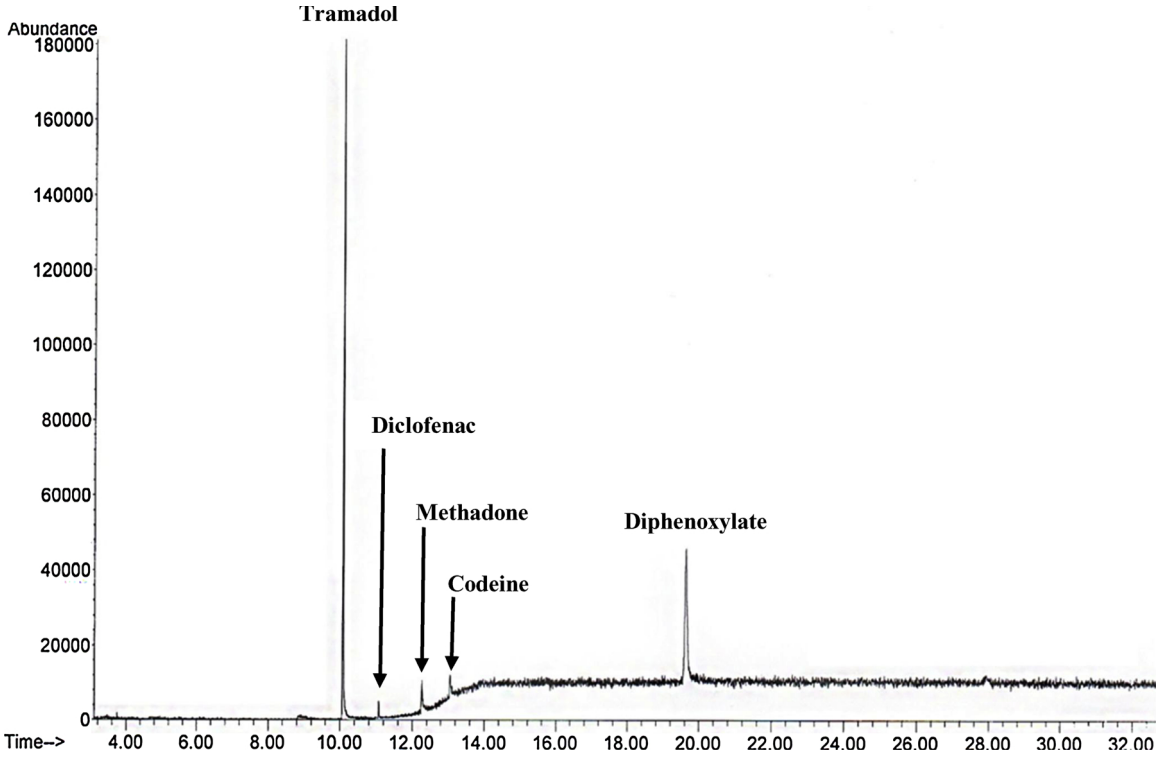

Fig. 1. The chromatogram of one counterfeit sample with illegal drugs and high concentration of tramadol and diphenoxylate.

samples. The brochure of Vincamine DF capsules shows that this product contained a mixture of Atropine, Duboisine, Tigloidine, Noix-Vomique, Vincamine, and vitamin B2. The average cost of each tablet or capsule was 10000–25000 Rial (25–60 cents).

3.2. Qualitative analysis

Out of a total of 50 samples, 74% were sold as herbal compounds containing illegal or chemical drugs. In most of the counterfeit samples, we found high doses of tramadol, diphenoxylate, and methadone. The chromatogram of one counterfeit sample that contained some illegal drugs is shown in Fig. 1. In some of the collected samples, GC/MS identified small amounts of nicotine, barbiturate, acetaminophen, diclofenac, Lysergic acid diethylamide, diphenhydramine, and codeine. In all of the illegal samples, at least one drug or chemical compound was identified in the qualitative analysis step. Tramadol, methadone and diphenoxilate were not detected in Vincamine DF capsules (Table 1). No other chemical substance or illegal drugs were detected.

3.3. Quantitative analysis

a) **Tramadol:** The highest concentrations of tramadol were detected in Dragon tablets. No statistically significant differences were observed between tramadol concentrations in Deta, Dragon, and Compound capsules ($p = 0.61$) (Table1).

b) **Diphenoxylate:** The highest concentration of diphenoxylate was in Deta samples. Statistically significant differences were found between diphenoxylate concentrations in Deta, Dragon and Compound capsules ($p = 0.03$) (Table 1).

c) **Methadone:** The highest concentration of methadone was found in Compound capsules, but there was no significant difference of methadone concentration between Dragon, Deta and Compound capsules (Table1).

4. Discussion

In this study, opiate withdrawal herbal remedies which are supplied in forms of tablets and capsules in herb shops in Iran were analyzed and evaluated both qualitatively and quantitatively. The presence of illegal drugs in these samples is sufficient to recommend that these desired drugs (herbal medicine) should not be sold. Results showed that most of the herbal medicines supplied in herb shops are adulterated.

Counterfeit drugs represent an enormous public health challenge worldwide. There are a number of studies on counterfeit herbal medicines worldwide. In 2015, the New York attorney general, along with the FDA, targeted supplement companies selling counterfeit products. Authorities tested top-selling herbal supplements at GNC (General Nutrition Center), Target, Walgreens (As the second-largest pharmacy store chain in the United States), and Walmart (American multinational retail corporation) and they understood that 80% of the tested products

Table 1
Concentration (Mean ± SD) of Tramadol, Diphenoxilate and Methadone in various brand samples. Statistical significance was set at $P < 0.05$.

Analytes	Dragon tablets (N = 9)	Deta capsules (N = 12)	Compound capsules (N = 16)	Vincamine DF (N = 13)	P Value
Diphenoxilate (ppm)	3.64 ± 7.29	26.94 ± 9.08	22.12 ± 17.76	N.D*	0.03
Tramadol (ppm)	214.06 ± 212.57	137.79 ± 123.0	132.02 ± 131.71	N.D*	0.61
Methadone (ppm)	21.9 ± 43.26	35.81 ± 12.75	48.67 ± 23.98	N.D*	0.07

N = Number.
* N.D: None Detected.

did not contain the medicinal herbs stated in their labels [10].

Another study found that one out of five Europeans has bought a prescription-only medicine from an illegal source. While most of these provided their medicines on the internet [13–16]. According to a WHO report, more than 50% of the medicines supplied from websites are counterfeited [13–16]. In rich countries, people buy herbal remedies for obesity or erectile dysfunction disorders via the internet, and they believe that there is no risk for their health. However, some studies detected synthetic drugs as adulterants in the herbal compounds that caused high risks for public health [17,18]. In the study by Azizi et al. products sold as Chinese Herbal medicines for weight loss were assayed by using immuno chromatographic and radioimmunoassay methods and they found thyroid hormones and Phencyclidine in Chinese herbal slimming products, while they were not mentioned as contents in the labels [11].

In a study by Akhgari and his colleagues, the chemical profile of counterfeit buprenorphine vials seized in Iran was investigated; the qualitative analysis of counterfeit buprenorphine by gas chromatography-mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) showed the presence of diacetylmorphine, acetylcodeine, and pheniramine, in the most samples [19].

Gas chromatography was used to confirm the identity of essential oils, the presence of residual solvents, volatile compounds (especially in the quality control of herbal medicines) and anonymous compounds or analogues [20]. Therefore, in the present study, GC/MS was selected as an efficient technique.

The quantitative results of our testing show a high dose of the opioid compound such as tramadol, methadone, and diphenoxylate in the collected samples. Abdollahi et al. in 2009 reported that the presence of CNS-depressant compounds caused exacerbation of tramadol toxic effects [21].

While our results show that some samples consisted of high concentrations of tramadol and were accompanied with CNS-depressant compounds such as barbiturates, this raises concern about tramadol toxicity.

Tramadol is a chemical with pain-relieving effects, which belongs to opioid analgesics and has been in widespread use in recent years [22–24]. Due to its opioid effects on pain relief and its euphoric effects, dude serotonin inhibitor reuptake can be abused in addiction treatment. Reports show dependence and severe withdrawal symptoms among people who use it as an addiction treatment. Moreover, such medicines can bring about several complications and side effects like seizures, liver and kidney diseases, cardiovascular diseases, and even death in the case of overdose [23–25].

Furthermore, this study has also shown the presence of diphenoxylate and methadone with a high concentration in the counterfeit drugs which are the synthetic derivative of analgesic and opioid for the supportive treatment of acute and chronic diarrhea and alternative addiction treatment. However, it has features similar to those of opiates and is often abused [12].

In a study by Movagghar et al., the treatment of addiction by the herbalists in Tehran was examined by using thin layer chromatography (TLC), and the results show that all samples contained at least one illegal drug, and qualitative results and interviews with addicts indicate that these herbal medicines were addictive [26]. Also, Foroughi and colleagues showed that opiate withdrawal herbal remedies often contain diphenoxylate, tramadol, codeine, sertraline, fluoxetine, and acetaminophen to improve their effectiveness [27]. Our quantitative and qualitative analytical results are consistent with the findings of Movaghar and Foroughi and furthermore, in our qualitative analysis with GC/MS, we found a small amount of nicotine, barbiturate, acetaminophen, diclofenac, Lysergic acid diethylamide, diphenhydramine in some of the collected samples. Moreover, our results and previous studies show that in some instances they contain high doses of drugs that are dangerous for users [12]. Generally, in the previous studies, sampling was performed from one city, while, in this study, we tried to sample from several cities to provide more comprehensive data.

On the one hand, the lack of reliable service centers and sale of mixtures of anonymous compounds as herbal medicines by profiteers have caused serious problems in this regard. On the other hand, Iran has long shared common borders with Afghanistan and Pakistan; some of these substances are illegally imported from neighboring countries [3,28]. Drug counterfeit is a major public health problem that can lead to death, especially in poor countries with some extreme cases.

The WHO reports that about 10% of the global drug market is counterfeit. This is estimated to be up to 25–50% in developing countries; in some African countries, counterfeiting rate was reported to be up to 80% [12,29]. Overall, people are satisfied with herbal medicines and prefer to receive these herb drugs compared to chemical drugs because they often felt that these drugs are cheaper and more easily available, therefore, profiteers supply the market with unknown counterfeit drugs, which are sometimes even more dangerous than the substances consumed by addicts. Unfortunately, these counterfeit herbal medicines are largely supplied by traditional herb shops and by the black market, so that fake herbal pills have morphed into an important branch of addiction. Generally, the concerns about addiction and substance abuse are heightened with counterfeit drugs because the use of these compounds—in addition to imposing a heavy economic burden on the public health system—can inflict families with more substantial damage. There are few applied studies on addiction treatment with counterfeit herbal medicines in Iran. The findings of this study indicate the necessity of raising the awareness of people and warning them against the use of such compounds.

One of the main limitations of the present study was collecting samples. Black market dealers have their own particular customers, they rarely confide in strangers and the products obtained for analysis in this study possible only represents a fraction of the wider problem in the market place. Another limitation in this study was that the GC/MS device in toxicology laboratory of the Medical Jurisprudence Organization of Mashhad did not have the equipement for evaluating plant materials. If this method had been available to examine the materials in our study, better and more comprehensive results from all the compounds found in the samples would have been obtained.

5. Conclusion

A safe therapeutic source is essential for public health. According to the present qualitative analysis, all handmade and illegal distributed herbal medicine for treatment of drug withdrawal contained at least one opioid or illegal drug that indicate an adulteration which is dangerous for users. Therefore, there is a need to raise the awareness of people and warn them against the use of such compounds sold by herb shops for the treatment of addiction. Additionally, it is important to analyze such medicines and governmental healthcare authorities need to enact regulations to control the production of counterfeit herbal medicines that may cause serious health problems for users.

Conflict of interest

The authors declare that there are no conflicts of interests.

Acknowledgment

This study was supported by a grant from the Medical Jurisprudence organization of Mashhad, Iran (95021, 2016).

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