International Journal of
Medical Toxicology & Forensic Medicine

April 2024, Volume 14, Number 2

Research Paper

Investigating the Detection of Undeclared Cyproheptadine in Weight Gain Herbal Supplements, Creajensing

Sajjad Sadeghi[1, 2*]

1. Department of Forensic Toxicology, Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran.
2. Food & Drug Laboratory, Semnan University of Medical Sciences, Semnan, Iran.

Use your device to scan and read the article online

Citation: Sadeghi, S. Investigating the Detection of Undeclared Cyproheptadine in Weight Gain Herbal Supplements, Creajensing. International Journal of Medical Toxicology and Forensic Medicine. 2024; 14(2):E43922. https://doi.org/10.32598/ijmtfm.v14i2.43922

https://doi.org/10.32598/ijmtfm.v14i2.43922

Article info:
Received: 19 Nov 2023
First Revision: 20 Jan 2024
Accepted: 26 Feb 2024
Published: 19 May 2024

Keywords:
Weight gain supplement, Serotonin antagonist, Histamine antagonist, Adulteration, Cyproheptadine

ABSTRACT

Background: Nowadays many people depend on herbal medicine for their healthcare needs; however, handmade herbal drugs are not screened for efficacy and safety. Undeclared active pharmaceutical ingredients have been detected in herbal medicine, even if there are claims to be natural. This study determines the undeclared active pharmaceutical ingredients in a weight gain herbal supplement collected from an Iranian online herbal shop.

Methods: One packet of herbal supplement (containing 45 tablets), advised as a weight gain product, was gathered from an online herbal shop in Iran. The sample was analyzed to detect undeclared active pharmaceutical ingredients using gas chromatography/mass spectrometry instrument, based on Iranian forensic standard operating procedures 920118-2655.

Results: The cyproheptadine was detected in this herbal supplement at a concentration higher than the therapeutic dose.

Conclusion: Although synthetic drugs cannot be produced except by permission of the licensing authorities, there is no regulation for herbal supplement production in Iran. Therefore, herbal supplements' serious quality and safety concerns must be assured for patients' health.

*** Corresponding Author:**
Sajjad Sadeghi, PhD.
Address: *Department of Forensic Toxicology, Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran.*
Tel: *+98 (21) 55983326*
E-mail: *s.sadeghi@mazums.ac.ir*

Introduction

According to the World Health Organization (WHO), nearly 80% of the world's population depends on traditional medicine (herbal) for their primary healthcare needs [1]. People tend to self-prescribe herbal medicines without consulting a health professional [2]. In contrast to conventional pharmaceuticals, herbal medicines are presumed harmless, safe, and without side effects, because of their natural origin [3]. Concerns have been raised repeatedly regarding the adulteration and contamination of herbal medicinal products [4]. Herbal medicines may occasionally cause idiosyncratic or dose-related toxicity [5]. Handmade herbal medicines are not screened from efficacy and safety perspectives and this issue does not have priority for manufacturers due to high cost [6]. Several departments in the ministry of health, treatment and medical education of Iran are involved in implementing good agricultural practices for herbal drugs [7]. Meanwhile, developing and developed countries are facing the threat and health consequences caused by counterfeit medicines [8]. The presence of undocumented pharmaceuticals in herbal medicines is widespread, especially in formulations of Asian origin [9]. There are some reports about counterfeit medicines in the pharmaceutical market of Iran [7].

A body mass index of 18.5 kg/m^2 or less is considered underweight [10]. Underweighting is classified into three groups as follows: Mild (>17.5 kg/m^2), moderate and severe (<16.5 kg/m^2) [11]. According to several studies, mortality is higher in underweight people compared to individuals with normal weight [10]. Underweight is increasingly reported among children in the West and South Asia [12]. In Iran, underweight has been reported in men and children, and there are also reports of an increase in underweight in rural areas compared to urban areas [13, 14] The prevalence of underweight among 6-14-year-old children in Iran is 19% [15]. The use of herbal medications and natural dietary supplements has also increased greatly in Iran [7].

There are many protocols for underweight treatment. Medications, nutrition therapy, and psychotherapy are used in underweight treatment. Medication-assisted therapy includes the administration of some medications, such as cyproheptadine, benzodiazepine, and atypical antipsychotic drugs for weight gain [16, 17]. Accordingly, this study determines undeclared active pharmaceutical ingredients (API) in a weight-gain herbal supplement.

Materials and Methods

Study chemicals

Methanol was purchased for the study (Merck Co.; Darmstadt Germany). The standard for cyproheptadine hydrochloride sesquihydrate was prepared by Sigma Chemical Co. Meanwhile, helium gas (99.999% purity) was purchased from Faransanat Co. (Tehran, Iran). All the employed chemicals and solvents were of analytical reagent grade.

Sampling and sample preparation

One packet of herbal products (containing 45 tablets), advertised as a weight gain supplement, was gathered from an online herbal shop in Iran. The herbal shop was not registered with the Iran Food and Drug Administration (FDA). Some tablets were analyzed to detect undeclared API in the forensic toxicology laboratory, based on Iranian forensic standard operating procedures number 920118-2655. Based on the hypergeometric distribution, 20 tablets were analyzed. Initially, for each tablet, organoleptic characteristics (sample weight, odor, and color) were reported. The samples were crushed and uniformed with mortar and pestle. The extraction of drugs was done using simple liquid-liquid extraction as follows: 1 mg of sample and 3 mL methanol mixed for 20 min in a test tube, using a rotator then the mixture was centrifuged (3 min at 3000 rounds per min) and finally the supernatants were filtered with PTFE syringe filter (Macherey-Nagel, Germany) and collected. For qualitative analysis, extracted samples were injected into gas chromatography/mass spectrometry (GC-MS) [18]. The injection volume was equal to 0.2 µL in the split-less mode.

Method validation procedures

All of the samples were analyzed with the validated GC-MS techniques, as the mainstays of pharmaceutical analysis for systematic toxicological analysis [19]. A GC (7890B model, Agilent, USA) fitted with a split/splitless injector was applied and an HP5- MS capillary column (5% phenyl silicone, and 95% dimethyl polysiloxane, 30 m length×0.25 mm ID×0.25 µm film thickness) was used. The GC parameters were as follows: Injector temperature at 280°C, transfer line temperature at 310°C, initial column oven set to 80°C and held constant for 1 min. The oven temperature program rate was 10°C/min and the final temperature was set to 300°C and the final hold was 22 min (total time=45 min). Helium carrier gas

Figure 1. Packaging of counterfeit herbal weight gain supplement labeled "Creajensing"

(99.99% purity) was maintained at a constant flow rate of 1.2 mL/min.

The mass analyzer (5977B model, Quadrupole, Agilent, USA) was connected to the column and operated by electron impact (70 eV) in positive full scan mode (50-550 m/z). National Institute of Standards and Technology (NIST-2014), Maurer/ Pfleger Weber (MPW) (MPW; 2011) and Wiley (2011) libraries were used to identify undeclared APIs.

The GC-MS method for the detection of many drugs was pre-validated in the forensic laboratory. Sample preparation steps and instrumental conditions were set as a general method for the detection of drugs with natural, acidic and basic structures. The linearity of the proposed method was examined from a calibration graph for cyproheptadine. The graphs were plots of the analyte peak area for five concentrations. Three replicates per concentration level were used. The data obtained were fitted to the Equation 1:

1. $y=ax+b$

Using regression analysis. The limit of detection and the limit of quantitation of this method were obtained by the standard deviation of results for ten injections of a low concentration of cyproheptadine. Cyproheptadine was the reference compound for the complete recovery of 30 µL methanol solution (1 µg/mL) cyproheptadine was used as an internal standard for quantitative analysis. The linearity of cyproheptadine was evaluated using five cyproheptadine concentrations of 200, 400, 800, 1600, and 3200 ng/mL after triplicate analysis. The regression line was plotted and expressed as a correlation coefficient (R^2=0.9990) using the least squares method to evaluate the correlation between the area under the curve (AUC) and cyproheptadine concentration.

Results

The current study employed the liquid-liquid extraction technique with methanol and GC-MS to detect undeclared APIs in herbal supplements. The results showed that the pocket of herbal supplements has a product number (2941), batch number (57488K), manufacture date (25/05/2022), expiration date (25/05/2023), manu-

Figure 2. Counterfeit herbal weight gain tablet labeled "Creajensing" containing cyproheptadine

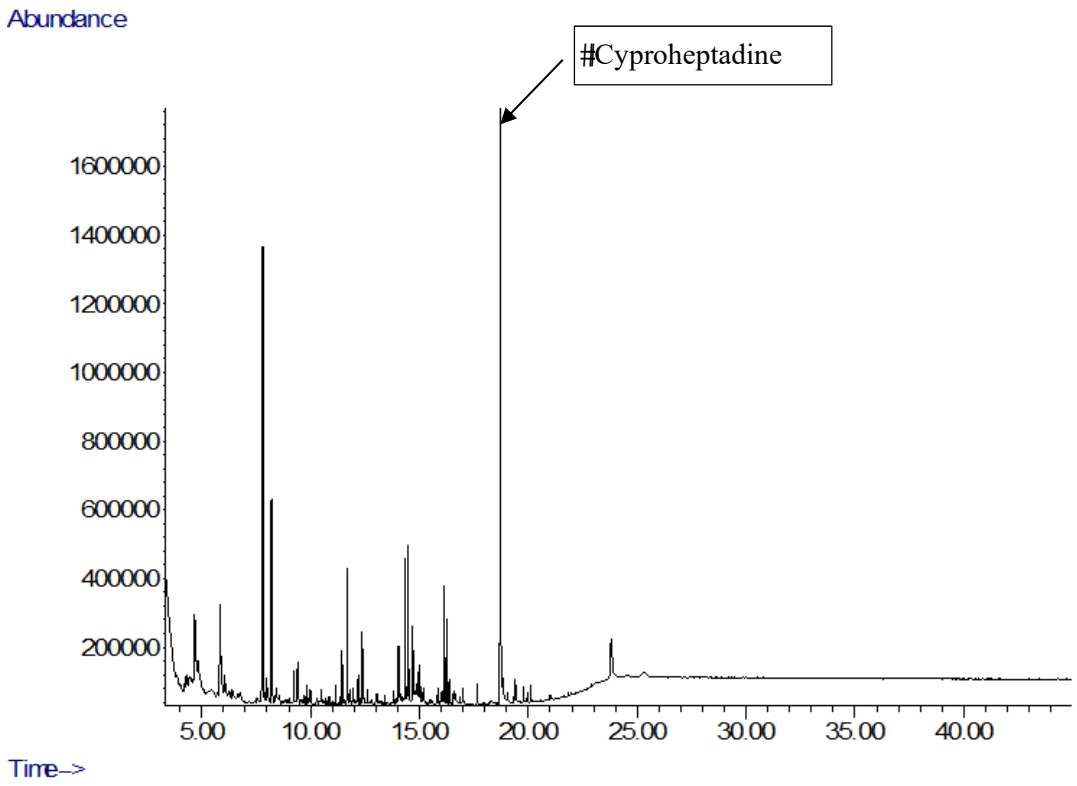

Figure 3. GC-MS chromatogram of cyproheptadine separated from adulterated herbal weight gain drug

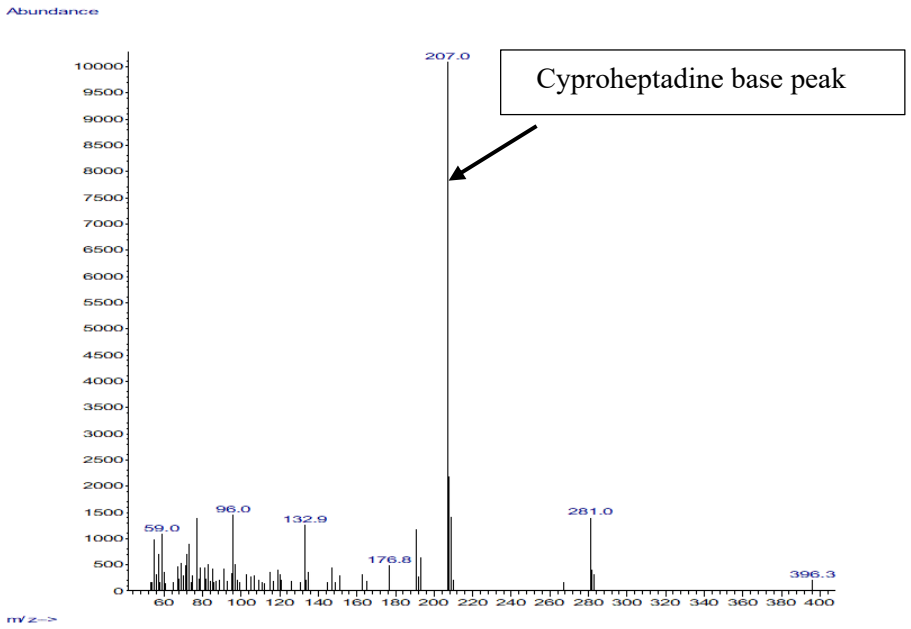

Figure 4. Mass spectrum of cyproheptadine separated from adulterated herbal weight gain drug

Table 1. Sample numbers and active pharmaceutical ingredients detected in adulterated herbal weight gain drugs

Sample Number	Dosage Form	Detected Active Pharmaceutical Ingredients	Quantity of Drugs in Formulations
1	White hard tablet	Cyproheptadine	6.53 mg/tablet
2	White hard tablet	Cyproheptadine	6.62 mg/tablet
3	White hard tablet	Cyproheptadine	6.45 mg/tablet
4	White hard tablet	Cyproheptadine	6.58 mg/tablet
5	White hard tablet	Cyproheptadine	6.63 mg/tablet
6	White hard tablet	Cyproheptadine	6.79 mg/tablet
7	White hard tablet	Cyproheptadine	6.49 mg/tablet
8	White hard tablet	Cyproheptadine	6.44 mg/tablet
9	White hard tablet	Cyproheptadine	6.55 mg/tablet
10	White hard tablet	Cyproheptadine	6.59 mg/tablet
11	White hard tablet	Cyproheptadine	6.64 mg/tablet
12	White hard tablet	Cyproheptadine	6.31 mg/tablet
13	White hard tablet	Cyproheptadine	6.57 mg/tablet
14	White hard tablet	Cyproheptadine	6.57 mg/tablet
15	White hard tablet	Cyproheptadine	6.54 mg/tablet
16	White hard tablet	Cyproheptadine	6.73 mg/tablet
17	White hard tablet	Cyproheptadine	6.53 mg/tablet
18	White hard tablet	Cyproheptadine	6.46 mg/tablet
19	White hard tablet	Cyproheptadine	6.55 mg/tablet
20	White hard tablet	Cyproheptadine	6.22 mg/tablet

Note: This table indicates Mean±SD of cyproheptadine tablets that is 6.5± 0.12.

International Journal of
Medical Toxicology & Forensic Medicine

facturers' name (KWEILIN drug manufactory) and list of ingredients. There is no standard logo on the pocket. Other than herbal constituents, the drug labels did not disclose the presence of pharmaceutical ingredients. All of the samples had an herbal smell, were white in color, CG tablet labels, and the mean weight of tablets was 1.001±0.138 g (Mean±SD) (Figures 1 and 2).

Table 2. The results of method validation

Parameters	Equations	Results
Recovery percentage	100 (spiked matrix/actual concentration)	109.92%
Coefficient of variation (CV%) or relative standard deviation	100 (standard deviation/mean)	5.5%
Limit of detection	3 (standard deviation of low concentration/slope of calibration curve)	0.02 ng/mL
Limit of quantification	10 (standard deviation of low concentration/slope of calibration curve)	0.3 ng/mL

International Journal of
Medical Toxicology & Forensic Medicine

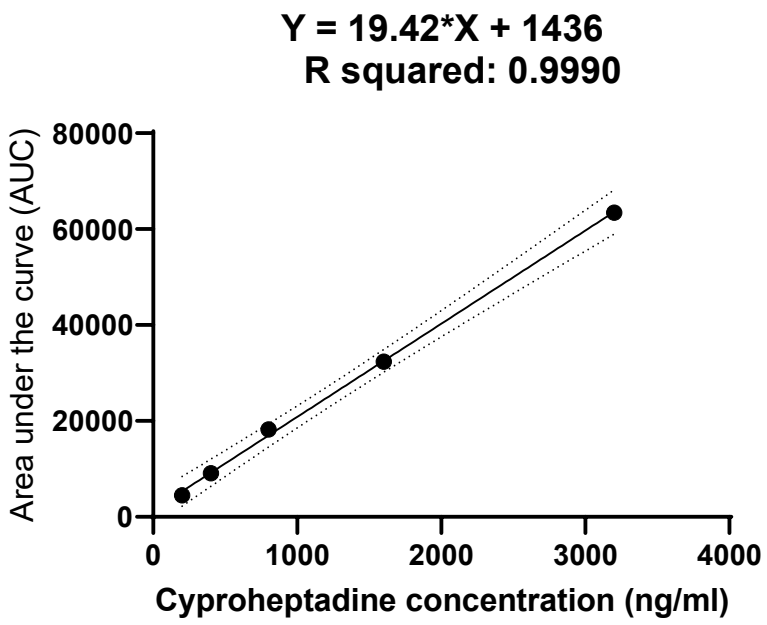

Figure 5. Linearity plot for five different concentrations of cyproheptadine using GC-MS instrument

All samples contained cyproheptadine based on the quantitative analysis results. The identification of cyproheptadine was confirmed with retention time (18.71 min) and spectrums (base peak is 207 m/z) of standards. Therefore, to identify, the analyte was confirmed by comparing the ion ratios of the unknowns to those of the standards. Quantitative analysis of tablets showed that cyproheptadine concentration was 6.5±0.12 mg/tablet. It means that Mean±SD=6.5±0.12 mg/tablet (Figures 3 and 4 and Table 1). Meanwhile, the method validation results are presented in Table 2 and Figure 5.

Discussion

Herbal medicines can be bought over the counter from pharmacies, supermarkets, markets, or the Internet without any consultation with a health professional [2]. Detecting and proving adulteration of drugs is one of the important tasks for forensic toxicologists and pharmaceutical analysts [20]. One explanation for adding undeclared API to herbal supplements is to make them more effective for consumers [21]. Various guidelines for the proper and safe use of herbal medicines have been published by the WHO, the European Medicines Agency, and the US-FDA [22-25].

Side effects caused by adulteration and contamination of herbal products can be extremely serious [4]. Mortazavi et al. demonstrated avascular necrosis due to an unapproved weight gain supplement [26]. The results of the present study demonstrated that an herbal weight gain supplement was counterfeited with cyproheptadine. Some studies declared cyproheptadine in herbal weight gain products [27-29]. Cyproheptadine the main use in allergy and hay fever, is one of the drugs that is considered obesogenic [30]. Cyproheptadine is a safe, well-tolerated medication that has utility in helping facilitate weight gain in underweight populations and children with cancer/treatment-related cachexia [31, 32]. Also, the use of cyproheptadine in infants and children with poor appetite and poor growth is a safe and effective treatment [33]. Cyproheptadine is approved by the US FDA for use in the prevention of allergic reactions as an antihistamine. Other, non-FDA-approved uses were appetite stimulation in anorexia nervosa, the control of symptoms of carcinoid syndrome, adjunctive therapy with growth hormone in growth hormone-deficient children, and management of anorgasmia during cyclic antidepressant therapy [34]. The therapeutic concentration during a steady state of cyproheptadine is about 0.05 mg/L [35]. One study reported that there were no adverse effects from the use of cyproheptadine [36]. On the contrary, one case of cyproheptadine poisoning was reported in a patient receiving approximately 22.5 mg of the drug [37]. A case of death with cyproheptadine has

been reported in a 28-year-old man with a high blood concentration of cyproheptadine (0.46 mg/L) [38].

In light of the findings, it is suggested that in addition to conducting more related research and study, new protocols for the regulate produce and marketing of herbal drugs and avoidance of unapproved herbal drugs.

Conclusion

This article mainly identified undeclared APIs in herbal supplements used for weight gain in Iran. The results of the present study showed that herbal weight gain products may not be natural. They contain API in higher doses than therapeutic amounts. Although synthetic drugs cannot be produced except by permission of the licensing authorities, there is no regulation for the production of herbal drugs. Therefore, the quality and safety of natural supplements must be assured for patients' health.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Acknowledgements

The author wish to thanks all technical staff of the Forensic Toxicology Laboratory of the legal Medicine Center, Bojnurd City, Iran, for their invaluable assistance.

References

[1] Kumar G, Jalaluddin M, Rout P, Mohanty R, Dileep CL. Emerging trends of herbal care in dentistry. Journal of Clinical and Diagnostic Research. 2013; 7(8):1827-9. [DOI:10.7860/JCDR/2013/6339.3282] [PMID] [PMCID]

[2] Skalli S, Jordan SA. Herbal and traditional medicines, now and future. In: Edwards IR, Lindquist M, editors. Pharmacovigilance. Berlin: Springer; 2017. [DOI:10.1007/978-3-319-40400-4_14]

[3] Jung J, Hermanns-Clausen M, Weinmann W. Anorectic sibutramine detected in a Chinese herbal drug for weight loss. Forensic Science International. 2006; 161(2-3):221-2. [DOI:10.1016/j.forsciint.2006.02.052] [PMID]

[4] Posadzki P, Watson L, Ernst E. Contamination and adulteration of herbal medicinal products (HMPs): An overview of systematic reviews. European Journal of Clinical Pharmacology. 2013; 69(3):295-307. [DOI:10.1007/s00228-012-1353-z] [PMID]

[5] Routledge PA. The European Herbal Medicines Directive: Could it have saved the lives of Romeo and Juliet? Drug Safety. 2008; 31(5):416-8. [DOI:10.2165/00002018-200831050-00006] [PMID]

[6] Brown AC. An overview of herb and dietary supplement efficacy, safety and government regulations in the United States with suggested improvements. Part 1 of 5 series. Food and Chemical Toxicology. 2017; 107(Pt A):449-71. [DOI:10.1016/j.fct.2016.11.001] [PMID]

[7] Foroughi MH, Akhgari M, Jokar F, Mousavi Z. Identification of undeclared active pharmaceutical ingredients in counterfeit herbal medicines used as opioid substitution therapy. Australian Journal of Forensic Sciences. 2017; 49(6):720-9. [DOI:10.1080/00450618.2016.1273387]

[8] Höllein L, Kaale E, Mwalwisi YH, Schulze MH, Holzgrabe U. Routine quality control of medicines in developing countries: Analytical challenges, regulatory infrastructures and the prevalence of counterfeit medicines in Tanzania. TrAC Trends in Analytical Chemistry, 2016; 76:60-70. [DOI:10.1016/j.trac.2015.11.009]

[9] Mackey TK, Liang BA, York P, Kubic T. Counterfeit drug penetration into global legitimate medicine supply chains: A global assessment. The American Journal of Tropical Medicine and Hygiene. 2015; 92(6 Suppl):59-67. [DOI:10.4269/ajtmh.14-0389] [PMID] [PMCID]

[10] Lee JY, Kim HC, Kim C, Park K, Ahn SV, Kang DR, et al. Underweight and mortality. Public Health Nutrition. 2016; 19(10):1751-6. [DOI:10.1017/S136898001500302X] [PMID] [PMCID]

[11] Park J, Han S, Jang HD, Shin G, Han K, Hong JY. Underweight as a risk factor for vertebral fractures in the South Korean population. The Spine Journal. 2023; 23(6):877-84. [DOI:10.1016/j.spinee.2023.02.015] [PMID]

[12] Mak KK, Tan SH. Underweight problems in Asian children and adolescents. European Journal of Pediatrics. 2012; 171(5):779-85. [DOI:10.1007/s00431-012-1685-9] [PMID]

[13] Pourabdian S, Golshiri P, Janghorbani M. Overweight, underweight, and obesity among male long-distance professional drivers in Iran. Journal of Occupational Health. 2020; 62(1):e12114. [DOI:10.1002/1348-9585.12114] [PMID] [PMCID]

[14] Mohseni M, Ahmadi S, Asadi H, Mohammadian ED, Asgarlou Z, Ghazanfari F, et al. A systematic review and meta-analysis of the prevalence of malnutrition among 6-14-year-old children in Iran. International Journal of Preventive Medicine. 2022; 13:138. [DOI:10.4103/ijpvm.IJPVM_564_20] [PMID] [PMCID]

[15] Jalali-Farahani S, Chin YS, Amiri P, Mohd Taib MN. Body mass index (BMI)-for-age and health-related quality of life (HRQOL) among high school students in Tehran. Child: Care, Health and Development. 2014; 40(5):731-9. [DOI:10.1111/cch.12103] [PMID]

[16] Fitzpatrick KK, Lock J. Anorexia nervosa. BMJ Clinical Evidence. 2011; 2011:1011. [PMID] [PMCID]

[17] Halmi KA, Eckert E, LaDu TJ, Cohen J. Anorexia nervosa. Treatment efficacy of cyproheptadine and amitriptyline. Archives of General Psychiatry. 1986; 43(2):177-81. [DOI:10.1001/archpsyc.1986.01800020087011] [PMID]

[18] Fakhri S, Mohammadi B, Jalili R, Hajialyani M, Bahrami G. Screening and confirmation of different synthetic adulterants in slimming products. Asian Journal of Pharmaceutical and Clinical Research. 2018. 11(2):260-4. [DOI:10.22159/ajpcr.2018.v11i2.22516]

[19] Dastjerdi AG, Akhgari M, Kamali A, Mousavi Z. Principal component analysis of synthetic adulterants in herbal supplements advertised as weight loss drugs. Complementary Therapies in Clinical Practice. 2018; 31:236-41. [DOI:10.1016/j.ctcp.2018.03.007] [PMID]

[20] Görög S. Identification in drug quality control and drug research. TrAC Trends in Analytical Chemistry. 2015; 69:114-22. [DOI:10.1016/j.trac.2014.11.020]

[21] Zhang J, Wider B, Shang H, Li X, Ernst E. Quality of herbal medicines: Challenges and solutions. Complementary Therapies in Medicine. 2012; 20(1-2):100-6. [DOI:10.1016/j.ctim.2011.09.004] [PMID]

[22] World Health Organization (WHO). Guidelines for the appropriate use of herbal medicines. Geneva: World Health Organization; 1998. [Link]

[23] Margine I, Martinez-Gil L, Chou YY, Krammer F. Residual baculovirus in insect cell-derived influenza virus-like particle preparations enhances immunogenicity. Plos One. 2012; 7(12):e51559. [DOI:10.1371/journal.pone.0051559] [PMID] [PMCID]

[24] World Health Organization (WHO). WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems [Internet]. 2004 [Updated 2004 June 5]. Available from: [Link]

[25] Calapai G. European legislation on herbal medicines: A look into the future. Drug Safety. 2008; 31(5):428-31. [DOI:10.2165/00002018-200831050-00009] [PMID]

[26] Mortazavi SMJ, Moharrami A, Shafiei H, Ebrahimzadeh MH, Karimi M. Unapproved weight gain supplement as a cause of avascular necrosis: A cautionary report. The Archives of Bone and Joint Surgery. 2019; 7(6):561-5. [PMID] [PMCID]

[27] Saberi N, Akhgari M, Bahmanabadi L, Bazmi E, Mousavi Z. Determination of synthetic pharmaceutical adulterants in herbal weight gain supplements sold in herb shops, Tehran, Iran. Daru. 2018; 26(2):117-27. [DOI:10.1007/s40199-018-0216-2] [PMID] [PMCID]

[28] Bogusz MJ, al Tufail M, Hassan H. How natural are 'natural herbal remedies'? A Saudi perspective. Adverse Drug Reactions and Toxicological Reviews. 2002; 21(4):219-29. [DOI:10.1007/BF03256198] [PMID]

[29] Coghlan ML, Maker G, Crighton E, Haile J, Murray DC, White NE, et al. Combined DNA, toxicological and heavy metal analyses provides an auditing toolkit to improve pharmacovigilance of traditional Chinese medicine (TCM). Scientific Reports. 2015; 5:17475. [DOI:10.1038/srep17475] [PMID] [PMCID]

[30] Leslie WS, Hankey CR, Lean ME. Weight gain as an adverse effect of some commonly prescribed drugs: A systematic review. QJM. 2007; 100(7):395-404. [DOI:10.1093/qjmed/hcm044] [PMID]

[31] Harrison ME, Norris ML, Robinson A, Spettigue W, Morrissey M, Isserlin L. Use of cyproheptadine to stimulate appetite and body weight gain: A systematic review. Appetite. 2019; 137:62-72. [DOI:10.1016/j.appet.2019.02.012] [PMID]

[32] Couluris M, Mayer JL, Freyer DR, Sandler E, Xu P, Krischer JP. The effect of cyproheptadine hydrochloride (periactin) and megestrol acetate (megace) on weight in children with cancer/treatment-related cachexia. Journal of Pediatric Hematology/Oncology. 2008; 30(11):791-7. [DOI:10.1097/MPH.0b013e3181864a5e] [PMID] [PMCID]

[33] Sant'Anna AM, Hammes PS, Porporino M, Martel C, Zygmuntowicz C, Ramsay M. Use of cyproheptadine in young children with feeding difficulties and poor growth in a pediatric feeding program. Journal of Pediatric Gastroenterology and Nutrition. 2014; 59(5):674-8. [DOI:10.1097/MPG.0000000000000467] [PMID]

[34] American Society of Health-System Pharmacists. AHFS drug information. Bethesda: American Society of Health-System Pharmacists; 2000. [Link]

[35] Moffat AC, Osselton MD, Widdop B, Watts J. Clarke's analysis of drugs and poisons. London: Pharmaceutical Press; 2011. [Link]

[36] Graudins A, Stearman A, Chan B. Treatment of the serotonin syndrome with cyproheptadine. The Journal of Emergency Medicine. 1998; 16(4):615-9. [DOI:10.1016/S0736-4679(98)00057-2] [PMID]

[37] Vikram Kumar V, Rama Devi K. Acute cyproheptadine poisoning. Indian Journal of Pediatrics. 1989; 56(4):521-3. [DOI:10.1007/BF02722431] [PMID]

[38] Levine B, Green-Johnson D, Hogan S, Smialek JE. A cyproheptadine fatality. Journal of Analytical Toxicology. 1998; 22(1):72-4. [DOI:10.1093/jat/22.1.72] [PMID]