

Original Research Article

Determination of the heavy metal contents of frequently used herbal products in Pakistan

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Sent for review: 23 September 2020

Revised accepted: 24 January 2021

Abstract

Purpose: To determine the heavy metal content of selected local and international herbal medicines sold for the treatment of various diseases in Pakistan.

Methods: The different dosage forms of herbal medicines assessed were crude forms of syrups, gel, capsule, powder and tonic. Wet digestion method was used to prepare the herbal samples using nitric acid, and then analyzed for arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg), using MHS-15 mercury/hydride system and flame atomic absorption spectrometry (FAAS).

Results: The investigated results displayed the Arsenic level (0.00 ppm to 0.580 ppm); Cadmium (0.001 ppm to 0.006 ppm); Lead (0.00 ppm to 1.078 ppm) and Mercury (0.001 ppm to 0.012 ppm). All results were found below the permissible limit of acceptability intake of the World Health Organization (WHO) and American Herbal Products Association (AHPA). The pH of the samples were in the range of 1.52 to 6.99.

Conclusion: The findings reveal that the investigated herbal products available in Pakistan are safe with reference to heavy metals, and considered non-toxic for human consumption.

Keywords: Heavy metals, Branded herbal products, Atomic Absorption Spectroscopy, Toxicity

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INTRODUCTION

Over the past few decades, the consumption of herbal medicines has constantly been on the rise all over the world. They have been playing an active part in the prevention as well as the management of various ailments. Approximately two-thirds of the global population rely on herbal based traditional systems for basic health care.

Herbal medicines are gaining popularity around the globe because of their lesser side effects, enhanced effectiveness, and cost friendliness. Different traditional systems have been utilizing herbal drugs to cure different ailments by numerous methods [1,2].

Climatic conditions have profound effects on plants. Heavy metal accumulation in different

parts of plants from the soil is the biggest cause of changes in plant composition. Heavy metal can be defined as an element with a relatively higher density and atomic weight than sodium, and could have lethal effects at low concentrations [3]. For instance, lead, mercury, cadmium, arsenic, chromium and thallium are well known heavy metals. There is a tendency in plants to accumulate both lethal and non-lethal metals at diverse concentrations. Plants take in water with minerals and heavy metals from soil and in this way, these toxic or non-toxic heavy metals are deposited in different parts of the plants [2].

Heavy metal pollution is a great health and also an ecological issue as well. Very minute amounts of some of these non-toxic heavy metals are useful for health, but if they exceed safe levels, may have deleterious effects on organs. Plants possess a greater affinity to store heavy metals compared to the environment. Lead, mercury, cadmium and arsenic are considered as the most toxic heavy metals [4]. They are also categorized as non-essential elements, and regarded as human carcinogens. Non-essential elements have the capability to produce reactive oxygen and nitrogen species. Lead poisoning leads to high blood pressure, and damage to the brain and kidneys. Cadmium is harmful to the respiratory system, and leads to cardiac as well as kidney problems [5].

Pakistan has an abundant tradition of growing medicinal plants, utilized for the cure of several diseases. It is estimated that 70 % of Pakistani residents depend on traditional and alternative system of medicine specifically in rural areas, which greatly demands the incorporation of modern complementary and alternative medicine (CAM) practices for the sake of evidence-based data [6]. Hence, the present study was conducted to determine the concentration of toxic heavy metals i.e., lead, mercury, arsenic and cadmium levels in frequently used herbal products available in Karachi at various medicine outlets and herbal shops.

EXPERIMENTAL

Collection of herbal samples

Different dosage forms of herbal medicines i.e., crude forms of syrups, gel, capsule, powder and tonic were collected from various renowned drug outlets and herbal shops in Karachi. Arq-e-mehzal, Digas syrup, Tut siah, Mehzileen, Shahi herbal tonic, *Aloe vera* 100% pure gel, *Cichorium intybus* seeds, *Lepidium sativum* seeds, Xest capsules, *Glycyrrhiza glabra* roots, Banafsha

shehtoot and Cranmax Pro were included in the study. Selected herbal samples were of local and foreign origin, and sold frequently for the treatment of different diseases. Sample numbers, origin/source, brand names/scientific names, constituents, dosage form and usage of herbal samples have been presented in Table 1 and Table 2.

Processing of crude drug samples into powdered form

Crude forms of herbal samples were milled with an electrical grinder (Moulinex DPA) to convert them into powder form. The powdered form is then kept in moisture free, air tight amber-colored bottles in a refrigerator.

Atomic absorption spectrophotometer with hollow cathode/EDL lamps, model A Analyst 700 by Perkin Elmer USA was used for the assessment of heavy metals. Lead and cadmium were analyzed on an acetylene flame, while mercury and arsenic were analyzed using Perkin Elmer MHS-15, using argon as the pressure gas.

Chemicals

Nitric acid was used for the digestion of herbal samples, and was purchased from Sigma Aldrich.

Preparation of standard solutions

Prepared standard stock solutions (1000 ppm) of lead, mercury, arsenic and cadmium were purchased from Fisher Scientific UK, and were further diluted by means of serial dilution to 100 ppm, 6 ppm, 4 ppm and 2 ppm consecutively to attain calibration curve.

Preparation of herbal sample solution

Wet digestion method was used to prepare herbal samples. 2 g of sample was accurately weighed and transferred to a 100 mL conical flask, then 10 mL of concentrated nitric acid was added and it was kept overnight for better digestion and maximum recovery of heavy metals. Afterwards, it was heated on an electric hot plate at 250 °C for a duration of 2 h, until the solution became totally colorless. After the digest had been cooled, 10-15 mL of distilled water was added to the conical flask, shaken and then filtered through Whatman grade 42 filter paper into a 25 mL volumetric flask, and then diluted up to mark by distilled water. Later on, the under examined heavy metals were determined using atomic absorption spectrophotometer (AAS) [7].

Table 1: Basic profile of understudied herbal products

Sample #	Origin/Source	Brand/generic name	Dosage form	Usage
1	Local	Arq-e-Mehzal by Lasani	Syrup	Weight loss
2	Local	Digas by Medics Laboratories	Syrup	Heartburn, acidity, bloating, abdominal pain, flatulence, nausea & vomiting
3	Local	Tut Siah + Mulathi by Lasani	Syrup	Diphtheria, hoarseness of voice, pharyngitis, sore throat
4	Local	Mehzileen by Azeemi Laboratories	Tablet	Weight reduction, remove stomach worm, swelling and inflammation of liver, abdominal discomfort
5	Local	Shahi Herbal Health Tonic by Tayyebi Pakistan	Tonic	Immunity booster and energizer
6	Foreign	<i>Aloe vera</i> 100% Pure Gel by Fruit of the Earth USA	Gel	Cooling, soothing, forms a protective layer, retain moisture and promote healing
7	Local	<i>Cichorium intybus</i> seeds	Crude Powder	Hepatoprotective, blood purifier, fever, diarrhea, spleen enlargement, jaundice, liver enlargement, gout, and rheumatism
8	Local	<i>Lepidium sativum</i> seeds	Crude Powder	Anti-inflammatory, dysentery, diarrhea, rheumatic pain, healing of bone fracture
9	Foreign	Xest by The Vitamin Company USA	Capsule	Aphrodisiac
10	Local	<i>Glycyrrhiza glabra</i> roots	Crude Powder	Anti-inflammatory, gastrointestinal ulcers, cough, arthritis, liver diseases
11	Local	Banafsha Shehtoot by Farzana Dawakhana	Syrup	Catarrh, cough, fever, headache, throat irritation
12	Local	Cranmax Pro by Hinucon	Powder Sachet	Urogenital and urinary tract infections

Table 2: Profile of herbal products ingredients/ constituents

Sample #	Constituents of herbal products/crude samples
1	<i>Illicium verum</i> , <i>Hyoscyamus niger</i> , <i>Ocimum sanctum</i> , <i>Rosa damascena</i> , <i>Tribulus terrestris</i> , <i>Cichorium intybus</i>
2	<i>Foeniculum vulgare</i> , <i>Cuminum cyminum</i> , <i>Mentha piperita</i> , <i>Phyllanthus emblica</i> , <i>Terminalia bellerica</i> , <i>Zingiber officinale</i> , <i>Terminalia chebula</i> , Sodium bicarbonate
3	<i>Morus nigra</i> , <i>Glycyrrhiza glabra</i> , Sucrose Base Q.S
4	<i>Ptychotis ajowan</i> , <i>Artemisia absinthium</i> , <i>Foeniculum vulgare</i> , <i>Artemesia vulgaris</i> , <i>Ruta graveolans</i> , <i>Carum carvi</i> , <i>Mentha pulegium</i> , <i>Nigella sativa</i>
5	<i>Laccifer lacca</i> , <i>Rosa damascena</i> , <i>Onosma bracteatum</i> , <i>Centaurea behen</i> , <i>Salvia hematodes</i> , <i>Doronicum hookeri</i> , <i>Cinnamomum zeylanicum</i> , <i>Santalum album</i> , <i>Nepeta hindostana</i> , <i>Asarum europaeum</i> , <i>Rheum emodi</i> , <i>Crocus sativus</i> , Pearls, <i>Corallium rubrum</i> , Bezoar, Agate, Silver foil, <i>Emblica officinalis</i> , <i>Cucumis melo</i> , <i>Citrullus vulgaris</i> , <i>Vitis vinifera</i> , <i>Punica granatum</i> , <i>Pyrus malus</i> , <i>Vitis vinifera</i> , <i>Pyrus pyrifolia</i>
6	<i>Aloe barbadensis</i> leaf juice, Triethanolamine, Tocopheryl acetate, Carbomer
7	Anthocyanins, coumarins, fructans (inulin), sesquiterpene lactones, coffee acid derivatives, flavonoids, polyphenols, Beta-sitosterol
8	Alkaloids, flavonoids, cyanogenic glycosides (traces), tannins, glucosinolates, calcium, iron, carotene, riboflavin, uric acid, cellulose, phosphorus, thiamine, niacin, stearic acid, palmitic acid, linoleic acid, sterols
9	<i>Epimedium alpinum</i> , <i>Urtica dioica</i> , L-Arginine, <i>Tribulus terrestris</i> extract, <i>Eurycoma longifolia</i> , <i>Lepidium meyenii</i> , Niacin
10	Glycyrrhizin, Liquirtin, Isoliquertin, liquiritigenin, rhamnoliquiritin, glucoliquiritin apioside, C liquocoumarin, coumarin
11	<i>Viola odorata</i> , <i>Morus nigra</i>
12	<i>Vaccinium macrocarpon</i> , <i>Bacillus coagulans</i> , <i>Curcuma longa</i>

Determination of pH

The pH was determined by HANNA HI2210 pH meter. The pH of tonic, gel, crude powders, capsule and powder sachet were measured after dissolving the herbal samples in distilled water in the ratio of 1:1, while pH of samples in syrup form was measured directly [8].

RESULTS

The present research investigated different dosage forms of herbal medicines, including crude forms, syrups, gel, capsule, powder and tonic for As, Cd, Pb and Hg levels, by using MHS-15 mercury/hydride system and flame atomic absorption spectrometer (FAAS). Results have been displayed in table-3 and expressed as mean, which revealed the concentration of arsenic in herbal samples (0.00 ppm to 0.580 ppm), cadmium (0.001 ppm to 0.006 ppm), lead (0.00 ppm to 1.078 ppm) and mercury (0.001 ppm to 0.012 ppm). Maximum level of arsenic was observed in sample 6, cadmium in sample 8, lead in sample 6, and mercury in sample 5. Results were compared with the allowable limit acceptability intake of the World Health Organization (WHO) and American Herbal Products Association (AHPA). The pH of the detected samples was in the range of 1.52 for sample 11, to 6.99 for sample 6.

Table 3: Concentrations of heavy metals in ppm/mean and pH of respective herbal samples

Sample #	As (ppm)	Cd (ppm)	Pb (ppm)	Hg (ppm)	pH
1	0.022	0.004	0.066	0.001	6.53
2	BDL*	0.004	0.144	0.007	6.30
3	BDL*	0.001	0.281	0.006	2.96
4	0.032	0.003	0.160	0.001	5.54
5	0.021	0.003	0.098	0.012	6.00
6	0.580	0.002	1.078	0.007	6.99
7	0.130	0.005	0.227	0.002	4.74
8	0.071	0.006	0.773	0.001	5.40
9	0.035	0.003	0.034	0.009	5.06
10	0.121	0.003	0.161	0.010	5.89
11	0.236	0.002	0.128	0.001	1.52
12	0.316	0.003	BDL*	0.003	3.38

BDL*= Below Detectable Level; ppm = parts per million

Permissible limit	As (ppm)	Cd (ppm)	Pb (ppm)	Hg (ppm)
	10	0.3	10	0.1

DISCUSSION

The current study displayed the lead concentration in herbal samples ranged from 0 (below detectable limit) to 1.078 ppm. The

maximum concentration was found in sample 6, while the lowest concentration was found in sample 12. As stated by the World Health Organization (WHO), the provisional weekly consumption limit ranges from 25 – 50 µg/g or ppm, whereas acceptable daily intake (ADI) is 10 µg/g or ppm [9]. It was observed that lead concentration in sample 1 to 12 was in the recommended range. The results are mentioned in table-3. The positive feature was that the herbal supplements had minute amount of lead because these supplements are consumed frequently, so the safety aspect is highly essential. Another study was conducted in Libya to determine the heavy metal levels in different teas imported from different parts of the world in Libya, and it was found that Pakistani products had a negligible amount of lead [10]. Studies conducted on different herbal preparations utilized in Ayurvedic, Chinese and Tibetan medicine, show that traditional herbal preparations surpass the safety limit for heavy metals [11,12].

The WHO permitted limit for mercury in herbal products is 0.1 µg/g or 0.1 ppm [9]. The results revealed that all the herbal products selected for the study had negligible amounts of mercury. Mercury concentration in herbal samples ranged from 0.001 ppm to 0.012 ppm, which were under the WHO permissible limit. Maximum value was seen in sample 5, i.e., 0.012 ppm, while the minimum value was seen in samples 1, 4, 8 and 11, i.e., 0.001 ppm. Research has shown that exposure to mercury vapors for an extended period, leads to neurological ailments. Minimum exposure is liable to cause symptoms such as anorexia, lethargy and abdominal disorders. Excessive exposure to mercury may cause tremor, irritability, abnormal excitement, memory loss, insomnia, lethargy, delirium, hallucinations and gingivitis [13]. The study conducted on herbal products to assess the safety of herbal preparations revealed that the products containing mercury ranged from 0.00-1.0 ppm [14].

WHO allowable limit of cadmium in herbal products is 0.3µg/g or 0.3 ppm [9]. In the present study, cadmium concentration in herbal products ranged from 0.001 ppm to 0.006 ppm. Minimum level, i.e., 0.001 ppm, was seen in sample 3, while maximum level was observed in sample 8 i.e., 0.006 ppm. All were far below the recommended limit for cadmium. Results revealed that these herbal products contain very minute amount of cadmium. A study was conducted on different brands of kohl and henna to determine the concentration of heavy metals in Tunisia. It was found that Pakistani brands

contain 1.0 ppm of cadmium in kohl, while in henna, the concentration was 0.8 ppm, which was a small amount compared to other brands. Although concentration was slightly elevated, these products were usually intended for topical use [15].

WHO acceptable level of arsenic is 10 µg/g or ppm in herbal medicines [9]. The present study revealed that the arsenic concentration in selected herbal samples ranged from 0.00 pm or BDL (Below detectable limit) to 0.580 ppm. Maximum concentration was observed in sample 6, while minimum was seen in sample 2 and 3. All samples were found below the recommended range. Minor exposure to arsenic may lead to nausea and vomiting, reduced number of red blood and white blood cells, disturbance in cardiac rhythm, injury to blood vessels and tingling effect in the hands and feet.

Extended exposure to arsenic may produce blackening of the skin, and formation of tiny corns on the soles, palms and torso. Arsenic is one of the major causes of hypertension. Severe consequences related to the cardiovascular system and hepatic impairment were also observed at higher doses [16]. Studies have been reported where herbal products may have produced arsenic toxicities because of the toxic level of arsenic. It was also found that the herbal medicines sold via the internet contained toxic levels of heavy metals [17,18].

CONCLUSION

The investigated herbal products were analyzed for Arsenic (As), Cadmium (Cd), Lead (Pb) and Mercury (Hg) levels, and all samples were found below to the WHO-set recommended limits for the respective metals. Hence, they may have no adverse effect with reference to heavy metals associated toxicity on communities who patronize these products for their healthcare requirements.

DECLARATIONS

Acknowledgement

The author/research supervisor, Iqbal Azhar, is thankful for the Dean's Research Grant 2018, University of Karachi.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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