Analysis of Toxic Heavy Metals in Branded Pakistani Herbal Products

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(Received on 19th October 2009, accepted in revised form 13th March 2010)

Summary: The present study was designed to estimate the concentration of heavy toxic metals in Pakistani herbal products frequently used for the treatment of various ailments. For this purpose, twenty five herbal products of well reputed herbal manufacturers were selected. The results of our investigation revealed that the concentrations of lead, cadmium, nickel and chromium were far beyond the permissible limits proposed by the International Regulatory Authorities for herbal drugs. Therefore, this study conveys a strong message to the ministry of health to establish proper rules and regulations for the validation of herbal products on scientific grounds in order to protect the general public from the harmful effects of these heavy metals in herbal products.

Introduction

Industrially prepared herbal drugs are commonly used in various communities of the world. They are available in different shapes such as Ayurvedic, Traditional Chinese Medicines (TCMs) and Homeopathic. The wide spread usage of herbal formulations are not limited to the third world countries but are also extensively use in all the Member States of the European Union [1]. The popularity of the herbals notably increased in the past decade, probably due to rapid increased in Allopathic drugs prices and media reports on their safety [2]. In Pakistan, peoples are often using herbal products especially in the rural areas because of availability, affordability and safety [3].

There is a general public perception that the herbal products are safer and harmless but most of these products are not validated according to the recommended pharmaceutical guidelines, often herbal products contained toxic and lethal concentrations of toxic heavy metals [4]. Even in the United States, the herbal products are formulated and marketed without the prior approval of Food and Drug Administration [5]. It is well documented in literature that medicinal plants and plant based herbal products contained toxic concentrations of heavy metals [6-9]. Similarly, a large scale screening test was carried out on more than 500 Chinese patent medicines and 134 drugs for the level of heavy metals, approximately 10 percent were found to contain undeclared drugs or toxic levels of metals [2].

Recent scientific advancement in terms of chemical, pharmacological and biological technologies facilitated healthcare professionals to explore the potential healthcare benefits of herbal products. The heavy metals contamination in traditional medicines may occur due to polluted environment in which the medicinal plants grow, the polluted conditions in which the plants are dried and processed, the storage conditions or during manufacturing of the final dosage form [10, 11]. High level of toxic metals can be found due to the use of metals containing agricultural expedient and contaminated irrigation water [12]. The purpose of carrying out this research project was to determine the levels of toxic heavy metals in order to ensure the safety of the locally manufactured traditional medicines.

Results and Discussion

Like most other developing countries of the world, the practice of herbal treatment is well established in Pakistan. Despite of poor marketing, these products have substantial share in drug market. Apart from affordability and availability, the advocates of herbal products believe that they are safe and harmless because of their natural origin without any scientific evaluation. Similarly, the potential serious side effects of synthetic drugs also diverted the peoples towards natural products. But unfortunately, most of the herbal products contain undisclosed components or toxic concentration of heavy metals. It has been noticed that the exposure to heavy metals like lead, cadmium etc, are toxic to human health even in traces [2].

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The concentration of metals like lead, nickel, manganese, cadmium, zinc, chromium, copper and iron were investigated in herbal products collected from the local market of known manufacturers using atomic absorption spectrophotometer. Statistical analyses were performed using Graphpad Instate. The results are shown in Table-1.

Lead

As shown in Table-1, the estimated lead concentration was in the range of 2.6-70.1 µg/g. The high concentration of lead was found in *Zubex* (70 µg/g), *Arq-gaozaban* (52.5 µg/mL), *Kushta Qali* (45.2 µg/g), and Safi (44.3 µg/mL). According to FDA/WHO, the tolerable weekly intake of lead per Kg body weight is 25 µg [13]. Based on the manufacturer's recommended daily dose of these products as shown in Table-2, sixteen out of twenty five products exceeded the permissible limit. For instance, the expected daily lead consumption of *Arq-gaozaban* for adults (weigh approximately 70 kg) would be 4200 µg per day, and for *Biomint* 1089-2178 µg per day. Taking such a high concentration of lead per day is dangerous for all ages because study has shown a broad range of adverse effects in humans and animals [14,15]. According to a study, a 10 month old infant was reported to be a victim of lead poisoning because of using lead (37.5 µg/g) containing herbal medicines [10]. Lead has an extensive history as a reproductive toxin and lead toxicity due to herbal products has been associated with status epilepticus, infant fatal encephalopathy, congenital paralysis and sensorineural deafness, and developmental delay [10, 16].

Cadmium

Cadmium is one of the most toxic natural elements. Chronic exposure to cadmium through environment or using contaminated food causes kidneys and lungs failure, affect bones, and stomach. The toxic effects of cadmium on humans are same in both adults and children. However, the experimental data showed that younger animals absorb more cadmium than adults [17]. According to Food and Drug Administration (FDA)/ World Health Organization (WHO), the tolerable weekly intake of chromium per kg body weight is 7 µg (*i.e.* 70 µg/day for normal adults) [13]. The concentration range of cadmium in tested samples was 1.9-45.2 µg/g as shown in Table-1. According to manufacturer's recommended dose, the daily consumption of cadmium was calculated as shown in Table-2. Majority of the tested products contained high concentration of cadmium as the daily consumption is above the recommended limit *e.g.* the daily consumption of cadmium were 464 µg, 189-378 µg and 141-282 µg for *Arq- gaozaban*, *Gastoplex* and *Gasgon*, respectively. Out of twenty five products fourteen products exceeded the permissible limit.

Table-1: Concentration (ppm) of various toxic heavy metals in Pakistani branded herbal products.

S. No	Product Name	Manufacturer	Fe	Cd	Cr	Cu	Mn	Pb	Zn	Ni
1	Aller- Z cap (S)	Qurshi	206.5±0.31	45.2±0.02	82.4±0.12	14±0.03	29.4±0.05	2.6±0.01	1071.1±0.87	26±0.02
2	Arq- gaozaban (L)	Qurshi	22.4±0.11	5.6±0.15	113.9±0.42	1.7±0.00	0.7±0.00	16.2±0.15	6.70±0.11	0.2±0.01
3	Arq-e-gulab (L)	Qurshi	20.7±0.02	5.9±0.01	105.5±0.52	2.3±0.03	ND	22.2±0.31	10.30±0.05	1±0.00
4	Biomint (L)	Falcon lab	19.4±0.25	4.7±0.10	105.8±0.32	2.7±0.10	ND	16.4±0.12	7.40±0.20	2.4±0.00
5	Carminative (L)	Sharex	16.2±0.20	7.6±0.01	112.8±0.31	3±0.00	3.8±0.01	44.3±0.17	10.90±0.02	4.7±0.00
6	Ferroglobin (L)	Falcon lab	446.2±0.45	23.8±0.04	86±0.01	7.7±0.02	23.4±0.11	23.1±0.13	37.80±0.20	5.3±0.01
7	Fever-x (L)	Qurshi	18.5±0.02	6.3±0.06	106.8±0.05	2.9±0.01	ND	30.8±0.01	8.50±0.01	3±0.11
8	Gasgon (L)	New pak lab	9.2±0.00	3.8±0.39	92.9±0.06	2.5±0.09	ND	19.2±0.03	7.70±0.01	3.7±0.11
9	Gastoplex (L)	Taj lab	18.3±0.00	4.9±0.15	101.9±0.30	3.6±0.02	ND	12.5±0.14	10.1±0.02	1±0.00
10	Gastoran (L)	Kamran lab	697.8±0.03	5.9±0.02	94.5±0.70	18±0.20	26.5±0.03	35.2±0.01	87.9±0.08	9.3±0.50
11	Gestofill (L)	Qurshi	2731.8±0.26	4±0.01	87.2±0..48	10.5±0.00	346.4±0.75	4.6±0.05	253.8±0.02	7.4±0.02
12	Gestrosin (L)	Taj lab	2514.3±0.59	6.5±0.05	131.5±0.70	14.6±0.01	158.2±0.42	70.1±0.01	86.6±0.02	25.4±0.09
13	Hayatheen (L)	Qurshi	101±0.33	3.7±0.05	68.4±0.36	4.1±0.01	5±0.30	ND	6.5±0.33	5.3±0.01
14	Humdardguty (L)	Hamdard	634.3±0.84	4.2±0.01	144.6±0.52	12.1±0.00	38.8±0.40	22.7±0.01	31.4±0.19	56.3±0.32
15	Iksir-e-jigar (L)	Qurshi	21.5±0.01	6.3±0.02	104.6±0.11	4.5±0.00	0.3±0.00	35.3±0.02	13.2±0.07	3±0.01
16	Kushta Qali (S)	Qurshi	50.5±0.06	3.9±0.02	56.7±0.31	9.8±0.06	ND	45.2±0.02	11.7±0.09	9.7±0.11
17	Lungsol (L)	Ashraf dawakhana	10.8±0.08	4.4±0.00	99.1±0.00	3±0.25	ND	15.2±0.03	8±0.02	3.3±0.10
18	Peppermint (L)	Aqsa lab	59.3±0.33	03.8±0.03	93.2±0.02	04.8±0.55	04±0.02	16.8±0.06	24.7±0.02	2.8±0.11
19	Rahat. Tab (S)	Qurshi	190.6±0.54	05.8±0.27	55.8±0.08	12.5±0.49	25.6±0.02	13.8±0.11	16.3±0.29	2.3±0.10
20	Reminol (L)	Mumtaz lab	18.4±0.04	5.5±0.21	98.1±0.10	4.2±0.00	ND	13.7±0.42	14±0.43	8.1±0.04
21	Safi (L)	Hamdard	10.1±0.01	6.1±0.02	103.1±0.66	2.4±0.11	ND	20.5±0.56	12.5±0.25	11±0.14
22	Surficol (L)	Qurshi	16.90±0.01	5.1±0.04	94.2±0.04	6±0.06	ND	17.7±0.32	16.5±0.22	5.1±0.02
23	Typhex (L)	Qurshi	9.2±0.01	5.5±0.09	117.9±0.00	3.5±0.01	ND	24.2±0.36	08.70±0.11	2.4±0.00
24	Zerjam cap (S)	Qurshi	667.1±0.01	5.8±0.00	68.9±0.07	26.9±0.01	42.5±0.01	28.2±0.00	09±0.09	8.4±0.03
25	Zubex (S)	Qurshi	2514.3±0.90	6.5±0.01	131.5±0.09	14.6±0.01	158.2±0.02	70.1±0.00	86.6±0.08	25.4±0.09

ND= Not detected. Data are expressed as the Mean ± SD (n = 3)

Table-2: Daily intake of metals (µg/day) according to manufacturer's dose.

S. No	Products	Pb	Zn	Ni	Mn	Cu	Fe	Cd	Cr
1	Aller- Z cap	2.6	1071.1	26	29.4	14	206.5	45.2	82.4
2	Arq- gaozaban	4200	1392	552	ND	456	1512	464	9552
3	Arq-e-gulab	208	670	10	ND	20	99	46	1145
4	Biomint	1089-2178	391.5-783	67.5-135	66	157.5-315	414-828	ND	5305.5-10611
5	Carminative	214.5-429	115-231	91.5-105	ND	61.5-123	192-384	87-174	1818-3636
6	Ferroglobin	192-864	77-346.35	371-166.5	ND	25-112.5	92-414	57-171	929-4180
7	Fever-x	549	204	45	ND	66	480	nd	4455
8	Gasgon	492-984	222-444	72-144	ND	18-162	582-1164	141-282	3174-6348
9	Gastoplex	924-1848	255-510	90-180	ND	87-174	555-1110	189-378	3204-6408
10	Gastoran	205.5-411	210-420	121.5-243	ND	63-126	276-552	82.5-165	1471.5-2943
11	Gestofill	102-204	370.5-741	42-84	60-120	72-144	889.5-1779	57-114	1398-2796
12	Gestrosin	265.5-531	247.5-495	76.5-153	ND	90-180	253.5-507	76.5-153	1413-2826
13	Hayatheen	ND	11.7	9.5	9	7.3	181.8	7	132.1
14	Humdardguty	62.5	50.5	5	ND	18	91.5	24.5	509.5
15	Iksir-e-jigar	243-486	100.5-201	6	10.5-21	25.5-51	310.5-621	84-168	1708.5-3417
16	Kushta Qali	22.6	5.8	4.8	ND	4.9	25.2	1.9	28.35
17	Lungsol	999	463.5	45	ND	103.5	832.5	265.5	4747.5
18	Peppermint	228-456	120-240	49.5-99	ND	45-90	162-324	66-132	1486.5-2973
19	Rahat. Tab	51.3	60.6	8.5	95.2	46.2	709	21.5	207.5
20	Reminol	529.5-1059	198-396	45-90	4.5-9	67.5-135	322.5-645	94.5-189	1569-3138
21	Safi	443	109	24	38	30	162	76	1128
22	Surficol	307.5-615	187.5-375	165-330	ND	36-72	151.5-303	91.5-183	1546.5-3093
23	Typhex	507-1014	8334-16668	27-54	ND	36-72	447-894	120-240	3735-7470
24	Zerjam cap	28.2	9	8.4	42.5	26.9	667.1	5.8	68.9
25	Zubex	210	259.8	76.2	474.6	43.8	7542.9	19.5	394.5

ND= Not-detected.

Nickel

According to the Agency for Toxic Substances and Disease Registry (ATSDR), chronic exposure to nickel is commonly associated with allergic reactions in humans, especially skin rashes appear at the site of contact. Animal data showed that intake of nickel in large amount causes blood, stomach, liver, and kidney disorders [18]. The daily dietary intake of nickel estimated by various authors is 35 µg [19] or 25–35 µg [20]. Variable concentrations of nickel were found in tested products ranging from 0.2-56.3 µg/g. The high nickel concentration was found in *Aller- Z cap* (26 µg/g).There is no significant data about the toxic level of nickel. The daily consumption of nickel was calculated for all the samples as shown in Table-2. Majority of the tested samples exceeded the recommended daily intake, therefore, there is possibility of toxicity.

Chromium

Chromium is available in two ionic forms *i.e.* trivalent and hexavalent. The trivalent chromium is present in food and is utilized by humans because of its safety. Chromium participates in glucose metabolism. The information to set the recommended dietary allowance for chromium is not enough. However, the Food and Nutrition Board set out an adequate intake level based on chromium contents in normal diet *i.e.* for children 11-25 µg/day and for adult 30-35 µg/day. Exposure to high level of chromium causes lungs cancer and dermatitis [17, 21]. The presence of chromium in our study was ranging from 55.8-154.2 µg/g as shown in Table-1.

Table-2 shows the daily consumption of all the tested products calculated on the bases of the recommended daily dose given on the product label. The daily intake of chromium as per recommended dose for all the tested herbal products exceeded the limit set by Food and Nutrition Board and therefore could lead to chromium toxicity.

Copper

Copper is an essential element for the human's metabolic system. It regulates various biological processes inside the body like oxidation-reduction (redox) reactions, energy production, connective tissues formation, iron metabolism, synthesis of neurotransmitters etc, [20, 22]. However, chronic exposure to high concentration of copper causes irritation of nasal mucosa, vomiting, nausea, diarrhea, damaging kidney and liver [23]. The recommended dietary allowance for children is 340-440µg/ day and for adult 890- 900 µg/day [21]. As shown in Table-2, the daily consumption of copper was 456 µg in *Arq-geozaban*, exceeding the permissible limit for children.

Zinc

Zinc is one of the essential trace elements for human and animals. The catalytic activity of approximately 100 enzymes are zinc dependent in our body. It also plays an important role in the structural stability of proteins and cell membranes. In addition, zinc is involved in cell signaling, release of hormones and in apoptosis [24]. Acute zinc toxicity (oral dose of 225-450 mg) causes abdominal pain, nausea, vomiting and diarrhea. Chronic exposure of zinc causes copper deficiency [21]. The recommended dietary allowance for children is 4-5 mg/day, for men 13-19 mg/day, and for women 9-13 mg/day [25]. The concentration of zinc in tested samples was 5.1-1071 µg/g. The daily consumption of zinc was calculated according to the manufacturer's recommended dose as shown in Table-2. Out of twenty five samples only one product, *Typhex* (8334-16668 µg) exceeded the recommended permissible limit for women and children.

Manganese

Manganese is also an essential element for health. In the tested samples, manganese was present in the concentration of 0.3-346.4 µg/g. The highest concentration was found in *Gestofill* (346.4 µg/mL) followed by both *Gestrosin* and *Zubex* (158.2 µg/g). However, manganese was not detected in most of the tested samples. The recommended dietary allowance for children is 1.2-1.5 mg/day, for men 2.3 mg/day, and for women 1.8-2 mg/day [21].

Iron

The estimated concentration of Iron in our samples was 8.6-2731.8 µg/g. The maximum concentration among the tested samples was present in *Zubex* (2514.3 µg/g) followed by *Aller Z cap* (206 µg/g). The recommended dietary allowance is 7-10 mg/ day for children, 8 mg/day for adults and 27 mg/ day during pregnancy [21]. Therefore, the iron concentration in the tested samples was within the permissible limits.

Experimental

In the present work, twenty five commercial products of various brands were analyzed. These herbal products were available in both solid and liquid dosage forms. Briefly, 1g of material in powder form was taken in a flask and 10 mL of concentrated Nitric acid (67%) added and kept over night at room temperature. After 24 h, 4 mL of perchloric acid was added to the sample and concentrated on hotplate at 60 °C so that approximately 1 mL of suspension remained in the flask. Later on, the suspension was cooled and diluted with deionized water. The resulting solution was filtered through Whatman (# 42) filter paper. After filtration, sufficient amount of deionized water was added to make the final volume up to 100 mL [6]. The liquid samples were prepared according to the standard method of Gomez *et al.,* [26]. The prepared samples (stock solution) were kept in transparent bottles and then analyzed by Flame Atomic Absorption Spectrophotometer (A. Analyt 700, Perkin Elmer) for the quantitative determination of described heavy metals. The working conditions of Atomic Absorption Spectrophotometer (AAS) for each metal are presented in Table-3. The readings were replicate of three different experiments in ppm.

Conclusion

The current investigations were carried out on twenty five selected herbal products from the local market of known manufactures contained heavy metals concentrations beyond permissible limits. The accumulation of toxic levels in these herbal products could be due to various plants sources used or in different process / solvents / excipents used in the

Table-3: Working conditions of Atomic Absorption Spectrophotometer for determining toxic heavy metals.

Metal name	Flame type	Wavelength (nm)	Slit width (nm)	Acetylene flow (L/min)	Air oxide flow (L/min)	Cathode lame current (mA)	Energy
Pb	Air-Acetylene	283.3	0.7H	2.0	17	10	46
Zn	Air-Acetylene	213.9	0.7H	2.0	17	15	45
Cr	Air-Acetylene	357.9	0.7H	2.5	17	25	75
Cd	Air-Acetylene	324.8	0.7H	2.0	17	15	50
Fe	Air-Acetylene	248.3	0.7H	2.0	17	30	25
Ni	Air-Acetylene	232	0.2H	2.0	17	25	46
Mn	Air-Acetylene	279.5	0.2H	2.0	17	20	38
Cu	Air-Acetylene	324.5	0.7H	2.0	17	15	68

manufacturing. However, this level can be reduced or eliminated by strictly following the guidelines of Current Good Manufacturing Practice (cGMP) or other quality control parameters for herbal products. Similarly, it is strongly recommended to the ministry of health and related regulatory authorities that they should make well defined rules / regulations for pre-marketing safety studies on herbal products in order to protect the public health. Moreover like allopathic drugs, herbal products should be subjected to all pharmaceutical and pharmacological tests to ensure the efficacy, potency and safety of these products and protect the ultimate users from unwanted effects.

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