Pak. J. Bot., 45(1): 141-144, 2013.

EVALUATION OF TRACE AND TOXIC HEAVY METALS IN SELECTED CRUDE DRUGS USED IN *KHYBER PUKHTONKHAWA*, PAKISTAN

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Abstract

The present study was conducted for the evaluation of essential trace and toxic heavy metals such as Pb, Mn, Zn, Co, Cr, Cu, Fe, Ni, K and Na in 31 crude drugs commonly used in Khyber Pukhtonkhawa. These crude drugs were purchased from local venders in Peshawar. Pb was present in excessive (more than 10 µg /g) concentration in four tested samples while Mn concentration exceeded only in two samples. Zn concentration exceeded the permissible limit (50 µg /g) in *Trigonella foenum gracum* (102 µg /g) and *Berberis lyceum* (59 µg /g). Except *Glycyrrhiza glabra* all these samples of crude drugs exceeded the standard limit for Co (0.2 µg /g). Twelve crude drugs were within permissible limits of C r(1.5 µg/g). Highest concentration of Cu was found in *Berberis lyceum* (57 µg/g) followed by *Trigonella foenum graecum* (16 µg/g), *Glycyrrhiza glabra* L. (11 µg/g) and *Silybum marianum* (11 µg/g). The concentrations of remaining metals were in safe range for all tested samples. Crude drugs are abundantly used for various ailments, but due to presence of heavy toxic metals, these must be subjected to various pharmacological and toxicological evaluation for their affective and safe use.

Introduction

Like other developing countries of the world, practice of herbal drugs is very common in Pakistan (Saeed *et al*., 2010). Acceptance of herbal drugs in the country can be judged from the fact that the government has allowed the practice of herbal medicines in the Public Hospitals, besides thousands of private herbal practitioners. These drugs have substantial share in drug market due to affordable prices and easy access. The most appealing aspect of herbal products is the belief that they are safe and harmless because of their natural origin. However, reports from different groups have ruled out the concept of inherit safety. In continuation of our efforts to validate Pakistani herbal products (Khan *et al*., 2009; Khan *et al*., 2010a; Khan *et al*., 2010; Sultan *et al*, 2009, 2010), the current study was designed to estimate the concentration of various trace and toxic heavy metals in thirty one crude drugs, available in markets all over Pakistan.

Materials and Methods

Plant materials: All the crude drugs having *Trigonella foenum graecum* were purchased from the local market of Peshawar. The crude drugs and plants were identified with the help of flora of Pakistan in the Herbarium Department of Botany, University of Peshawar, Peshawar. Voucher numbers were positively allotted to all of the identified samples (Table 1) and a sample of each was kept in the herbarium of the department of Botany University of Peshawar, Peshawar for ready references. The materials were garbled, washed and grounded to fine powder.

Instruments and chemical used: Atomic Absorption Spectrometer (Parkin Elmer AAS 700), HCl (37% Sigma Aldrich), HNO_3 (65% Sigma Aldrich), $HClO_4$ (70% Sigma Aldrich), aqua regia (mixture of concentrated Nitric acid and concentrated Hydrochloric acid in the ratio of 1:3 respectively), glass wares, Whatman filter paper # 42, standard solutions of Pb, Mn, Zn, Co, Cr, Cu, Fe, Ni, K and Na.

Sample preparation (wet digestion method): Two grams (2 g) of each grounded crude drug as listed in Table 1 was taken and placed in a small beaker. 10mL of concentrated Nitric acid was added and allowed to stand overnight. Each beaker was heated carefully on hot plate until the production of red NO_2 fumes ceased. They were then allowed to cool down and 2-4 mL perchloric acid was added to each beaker. All the beakers were again heated to evaporate to a small volume and cooled again. 10 mL of aqua regia was added to each sample and heated to evaporate to a small volume. The samples were transferred to a 50 mL volumetric flask and diluted with deionized water (Saeed *et al*., 2010; Saeed *et al*., 2011). The stock solutions were marked with code numbers and analyzed through atomic absorption spectrometer for quantitative detection of Pb, Mn, Zn, Co, Cr, Cu, Fe, Ni K and Na.

Results and Discussion

Concentration of various elements detected are given in Table 2.

Lead (Pb): Pb was present in the range of 1-68 µg/g. According to Word Health Organization the permissible limit of Pb in medicinal plants is 10 µg/g (Issac & Jonson, 1975). Highest concentration was present in *Carum copticum* (68 µg/g), followed by *Allium sativum* (52 µg/g), *Silybum marianum* (51 µg /g) and *Berberis lyceum* (29 µg /g). Out of 31 samples, 4 contained Pb higher than the WHO limits. Pb poisoning is serious at any age, but children are relatively more vulnerable (Asiri. 2006). Pb toxicity causes anemia due to inhibition of heam biosynthesis and affects multiple body systems (Meredith *et al*., 1978; Perlstein *et al*., 1966., Staessen *et al*., 1994; Ang & Lee, 2006).

Table 1. Botanical names, family, common name, part used and uses of the crude drugs.

S. No.	Voucher No.	Botanical name	Family	Uses
1.	Bot.7911	*Allium sativum* L.	Alliaceae	Antihypertensive, cholesterol lowering agent, antipyretic
2.	Bot.7912	*Aloe vera*	Lilliaceae	Tonic, purgative, wound healing, epilepsy, skin burns, anti depressant
3.	Bot.7913	*Asparagus officinalis* L.	Lilliacaea	Tonic & anthelmentic
4.	Bot.7914	*Berberis lyceum* Royle	Berberidaceae	Ophthalmic diseases, antiemetic
5.	Bot.7915	*Camellia sinensis* (L.)	Theaceae	CNS stimulant, diuretic, bronchodilator
6.	Bot.7916	*Carum carvi* Linn.	Apiacaea.	Carminative, flavors
7.	Bot.7917	*Carum copticum* benth	Apiaceace	Smooth muscles relaxant, demulcent, carminative
8.	Bot.7918	*Cassia angustifolia* M.Vahl	Caesalpiniaceae	Laxative/purgative
9.	Bot.7919	*Cinnamomum zeylanicum* Blume	Lauraceae	Astringent, carminative, antiseptic, sexual tonic
10.	Bot.7920	*Colchicum luteum* Baker.	Liliaceae	Rheumatism, Blood Purifier, for Gout
11.	Bot.7921	*Commiphora myrrha*	Burseraceae	Protective stimulant, stomachache, astringent
12.	Bot.7922	*Ephedra gerardiana* Wall.	Ephedraceae	Asthma & cough
13.	Bot.7923	*Fagonia indica* (Linn).	Zygophyllaceae	Blood purifier, astringent
14.	Bot.7924	*Foeniculum vulgare* Mill	Umbelliferae	Carminative, flavors, galactogouge
15.	Bot.7925	*Glycyrrhiza glabra* L.	Fabaceae	Demulcent, expectorant, as flavoring agent
16.	Bot.7926	*Mentha longifolia* L.	Labiateae	Carminative, in children for diarrhea
17.	Bot.7927	*Pistacia integerrima* Stew ex Brandis	Anacardiaceae	Jaundice & hepatitis
18.	Bot.7928	*Plantago ovata* Forssk	Plantaginaceae	Emollient, demulcent, laxative
19.	Bot.7929	*Rauvolfia serpentine* Bth	Apocyanaceae	Antihypertensive, sedative, tranquilizer
20.	Bot.7930	*Salvia moorroftiana* Wall	Lamiaceae	Applied to wounds as poultice, used in coughs, cold & dysentery
21.	Bot.7931	*Silybum marianum* Gaerth	Compositeae	Hepatitis & liver diseases
22.	Bot.7932	*Strychnos nux-vomica* L.	Loganiaceae	As spinal stimulant, potent cholinergic,
23.	Bot.7933	*Terminalia belerica* (Gaertn.) Roxb.	Combretaceae	Antibacterial, sore throat, diarrhea, piles
24.	Bot.7934	*Tribulus terristris* L.	Zygophyllaceae	Diuretic, urinary & kidney diseases, aphrodisiac
25.	Bot.7935	*Trigonella foenum* graecum	Papilionaceae	Chest infections, rheumatism
26.	Bot.7936	*Valeriana wallichii* DC.	Valerianaceae	Tonic, astringent, febrifuge
27.	Bot.7937	*Verbascum thapsus* Linn	Verbinaceae	Chest complaints such as cough & pulmonary diseases, rheumatism, diarrhea, analgesic.
28.	Bot.7938	*Withania coagulans* Dunal.	Solanaceae	Digestive disorders, blood purification, diabetes, Asthma
29.	Bot.7939	*Withania somnifera* (L.) Dunal	Solanaceae	Diuretic, used in bronchitis & ulcers
30.	Bot.7940	*Zea mays* L.	Poaceae	Diuretic in heart diseases, rheumatism, diabetes mellitus
31.	Bot.7941	*Zingiber officinale* Rose.	Zingiberaceae	Abdominal bloating, Stimulate digestion, as flavoring agent, anti-emetic

Table 2. The Concentrations of trace and heavy metals in drug samples (µg/g).

S. No	Botanical name	Pb	Mn	Zn	Co	Cr	Cu	Fe	Ni	K	Na
1.	*Allium sativum* L.	52±2.67	18±1.59	28±1.74	3±0.15	1±0.02	5±0.00	136±2.99	8±1.25	7354±3.85	4008±2.47
2.	*Aloe vera*	ND	19±0.00	21±1.21	1±0.71	6±0.21	5±0.00	637±2.44	8±1.94	1829±2.86	1017±0.00
3.	*Asparagus officinalis* L.	3±0.31	35±1.55	19±1.11	4±0.52	ND	7±1.00	727±2.19	11±2.00	2129±3.21	8935±2.78
4.	*Berberis lyceum* Royle	29±1.68	53±1.15	59±1.78	4±0.05	9±1.00	57±1.01	410±1.00	13±0.35	5281±2.48	1472±2.00
5.	*Camellia sinensis* (L.)	5±1.55	404±2.15	29±1.11	2±0.01	ND	8±0.02	317±2.10	8±0.01	4194±3.45	3632±0.00
6.	*Carum carvi* Linn.	ND	18±0.00	16±0.00	3±0.00	1±0.02	9±0.90	302±2.80	8±0.09	3348±4.00	1503±2.49
7.	*Carum copticum* benth	68±1.35	30±1.11	49±1.85	3±0.01	2±0.11	8±1.00	298±0.00	9±0.00	6580±0.00	3098±0.00
8.	*Cassia angustifolia* M. Vahl	ND	114±2.15	18±1.71	5±0.12	1±0.01	6±0.00	533±2.20	10±0.00	6408±4.81	2400±1.19
9.	*Cinnamomum zeylanicum* Blume	5±0.00	460±2.15	25±1.14	3±0.02	1±0.01	8±0.00	248±2.16	8±0.55	4730±0.00	1358±2.68
10.	*Colchicum luteum* Baker.	ND	12±0.00	26±1.70	2±0.09	2±0.00	5±1.00	73±2.97	7±0.06	5212±2.47	9712±2.46
11.	*Commiphora myrrha*	1±0.01	23±1.85	12±1.22	2±0.01	7±0.00	7±1.02	656±2.00	10±0.00	1161±2.79	1072±2.74
12.	*Ephedra gerardiana* Wall.	9±1.25	25±0.00	16±1.31	3±0.02	5±0.00	4±0.00	257±3.10	11±0.66	4645±2.79	5473±1.49
13.	*Fagonia indica* (Linn).	2±0.03	32±1.15	20±1.00	4±0.00	ND	6±1.05	350±2.91	11±0.00	1943±2.86	1505±2.00
14.	*Foeniculum vulgare* Mill	ND	47±1.23	28±1.11	4±0.44	1±0.00	10±1.19	368±2.18	9±1.11	17198±4.36	2333±2.63
15.	*Glycyrrhiza glabra* L.	1±0.00	76±1.15	31±0.00	ND	5±0.03	11±1.15	227±2.12	11±0.00	2528±1.86	2813±2.47
16.	*Mentha longifolia* L.	1±0.02	49±1.40	23±0.00	3±0.02	3±0.10	7±1.10	561±2.82	11±1.88	8179±3.79	1508±1.75
17.	*Pistacia integerrima* Stew ex Brandis	ND	9±0.00	14±1.18	2±1.00	1±0.00	5±0.03	57±2.20	8±0.00	11589±1.13	3729±2.11
18.	*Plantago ovata* Forssk	ND	13±1.55	44±1.75	1±0.02	3±0.00	6±0.00	54±1.10	7±0.22	3729±0.00	1931±2.46
19.	*Rauvolfia serpentine* Bth	ND	19±0.03	20±1.14	2±0.01	ND	6±0.01	642±2.10	9±1.23	4385±0.00	4037±2.81
20.	*Salvia moorroftiana* Wall	3±0.01	266±2.15	23±1.41	6±0.02	18±0.06	10±0.00	6059±4.10	12±0.00	3622±2.36	17516±0.00
21.	*Silybum marianum* Gaerth	51±1.24	75±1.50	32±1.52	3±1.01	7±0.55	11±1.00	1123±3.10	12±0.01	7007±0.00	1983±2.63
22.	*Strychnos nux-vomica* L.	ND	120±0.00	19±0.00	2±0.11	2±0.02	7±1.11	680±2.00	16±1.48	2715±5.12	1932±2.48
23.	*Terminalia belerica* (Gaertn.) Roxb.	ND	15±1.08	13±0.00	1±0.04	4±0.00	5±1.19	157±1.10	8±0.00	5636±1.00	1365±0.00
24.	*Tribulus terristris* L.	ND	24±1.55	15±1.71	2±0.01	1±0.02	4±1.00	237±2.68	8±0.00	3945±1.10	969±3.75
25.	*Trigonella foenum* Graecum	ND	29±0.00	102±0.00	2±0.00	5±0.02	16±1.16	429±0.11	9±1.01	10122±4.85	2806±3.86
26.	*Valeriana wallichii* DC.	ND	37±0.00	23±0.08	3±0.00	ND	10±1.11	523±0.00	8 ±1.92	7584±3.18	3821±2.00
27.	*Verbascum thapsus* Linn.	3±0.01	15±0.00	24±1.10	1±0.00	7±0.00	7±1.15	349±2.45	9±1.45	6725±1.15	1624±3.46
28.	*Withania coagulans* Dunal.	ND	78±2.15	32±1.61	2±0.11	4±0.00	10±1.16	197±2.10	8±0.11	22543±2.79	1312±1.45
29.	*Withania somnifera* (L.) Dunal	ND	37±1.00	26±0.00	3±0.11	2±0.00	9±1.14	640±2.67	8±0.02	13391±1.88	2754±2.99
30.	*Zea mays* L.	ND	18±1.04	28±1.46	2±0.01	7±1.01	5±1.16	179±2.78	9±1.02	6025±1.10	1224±2.47
31.	*Zingiber officinale* Rose.	ND	71±2.15	17±0.00	2±0.02	3±0.00	5±1.00	54±2.84	8±0.66	6700±2.36	1422±2.99

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

Manganese (Mn): Manganese is one of essential metal which is normally found in traces in human as well as animals bodies**.** Mn deficiency causes tissue damage and impairs CNS functions (Friedman *et al*., 1987). However, it's excessive amount can results in breathing disorders like pneumonia and affects reproductive system, which may lead to infertility (Chandra & Shukla., 1976). The present study reveals that Mn was present in the range of 9- 460 µg /g. The permissible limit of Mn in plants is 200 (Srivastava *et al*., 2006). The highest concentration of Mn was found in *Cinnamomum zeylanicum* (460 µg /g) followed by *Camellia sinensis* (404 µg /g) and *Salvia moorcroftiana* (266 µg /g). These three samples have excessive amounts of Mn, while the remaining 28 samples showed limited concentrations.

Zinc (Zn): Zn is one of the essential trace metals for all living organisms. About 100-300 enzymes in human body are Zn dependant (Saeed *et al*., 2010). The permissible limit of Zn in medicinal plants is 50 µg /g (Markert, 1994). In the present study, the concentration range of Zn was 12-102 µg /g. The results showed that over permissible concentration was present in only 2 drugs, *Trigonella foenum gracum* (102 µg /g) and *Berberis lyceum* (59 µg /g). Taking Zn in a dose of 225- 450 mg induce nausea and vomiting. Zn deficiency also causes Copper deficiency due to its displacement character and weakness, rapid breathing, profuse sweating (Anon., 2001).

Cobalt (Co): Co is a natural metals found in plants, animals, rocks, and water. The permissible limit of Co in plants is 0.2 µg/g (Markert, 1994). The concentration range of Co in the present study was 1-6 µg/g (Table 2). It means that except *Glycyrrhiza glabra* all the samples of crude drugs exceeded the standard limits (0.2 µg /g). Animal study has shown cobalt toxicity (Karovic *et al*., 2007). Excess of Co causes neurotoxicity, like memory loss, cardiomyopathy, hyperglycemia and allergic dermatitis (Elbetieha *et al*., 2008).

Chromium (Cr): Cr is one of the abundant metals on the earth (Emsley & John., 2001). The food source of Cr is milk, eggs, cheese and mushrooms. It plays an important role in the synthesis of fatty acids and cholesterols (Food and Nutrition Board, 2001). The permissible limit of Cr in plants is 1.5 µg/g (Markert, 1994). The results obtained in the present study showed a concentration range of 1-18 µg/g in various samples as shown in Table 2. It is apparent that out of thirty one samples, only 12 were within the permissible range the rest 19 exceeded to limits. *Salvia moocroftiana* has the highest (18 µg/g) concentration, followed by acute oral toxicity of chromium (III) occurs in dose of 1500- 3300 µg/kg body weight while chromium (VI) is toxic at the dose of 50-150 µg/kg (Katz & Salem, 1993). Inhaling high level of chromium (VI) may lead to irritation of the nose, asthma, shortness of breath and wheezing. It is also responsible for allergic reactions (Anon., 2008).

Copper (Cu): Cu is an essential trace metals found in human and animal bodies. It is involved in oxidation-reduction reactions, energy production, connective tissues formation, iron metabolism, neurotransmitter synthesis, metabolism of neurotransmitters and formation of myelin (Amin, *et al.*, 2003). The permissible limit of Cu is 10 µg/g in plants (Markert, 1994). The range of Cu in our study was 4-57 µg/g. As given in Table 2, the highest concentration of Cu was found in *Berberis lyceum* Royle (57 µg/g) followed by *Trigonella foenum graecum* (16 µg/g), *Glycyrrhiza glabra* L. (11 µg/g) and *Silybum marianum* (11 µg/g).

Iron (Fe): The range of iron in our tested samples was 54-6059 µg/g as presented in Table 2. Fe is the key micronutrient and very essential component of hundreds of proteins and enzymes in human body. It is a well know hematinic. The highest concentration was observed in *Salvia moorcroftiana* (6059 µg/g) followed by *Silybum marianum* (1123 µg/g). The recommended dietary allowance (RDA) of Iron in Adult male is 8 mg per day, while it is 7-10 mg per day in children (Anon., 2004). The most common toxicity caused by iron daily intake in over dose is being observed in children under the age of 6 years.

Nickel (Ni): Ni is available in abundance in nature. It is found in atmosphere due to release from industrial activities. Primary sources of Ni are vegetables or plants that contain Ni. It is also present in cigarette and detergents. Taking in larger quantities, Ni can cause cancer of different organs such as nose, lungs, prostate and contact allergic condition (Anon., 2008). The concentration of Ni was in the ranges of 7-16 µg/g as shows in Table 2 and all the samples exceeded the permissible limit i.e., 1.5 µg/g (Markert, 1994).

Potassium (K): Like sodium K is an electrolyte of prime importance. The normal body functions of living organisms are mostly dependent on the concentrations of K ions inside and outside of the cell thus maintaining action potential (Peterson, 1997). There is no international limit for K which reflects the content of Potassium in plants; however the average intake of Potassium is 2300 mg/day for adult women and 3100 mg/day for adult men (Hajjar *et al*., 2001). The present analysis showed that K was present in the range of 116-22543 µg/g to tested drugs. The highest concentration (22543 kg/g) was in *Withania coagulance*. Most of the other drugs also have highest concentration (Table 2).

Sodium (Na): Na is the most important element of common table salt. It has got prime role in the maintenance of normal physiology in all living organisms. The concentration of Na ranged from 969-17516 µg/g (Table 2). Fifteen samples contained 17.516-2.333 gm of Na. Frequent use of these crude drugs either alone or in combinations, may cause hypernatremia as it exceeded the RDA in all ages. It is now established through research that low Sodium salt intake is recommended in hypertension and pre-eclampsia (Harper *et al*., 1997).

The current study shows that the selected samples of crude drugs contained high concentrations of heavy metals. In light of this study it can be said with confidence that most of the crude drugs used in the Unani or Ayurvedic systems of medicines in Pakistan may produce severe long term toxic effects in human beings,

who blindly take these drugs due to a common perception that they are very safe. The severity of the situation is neither felt by the people nor by the State Agencies. The significance of this trends becomes manifold when there is no control of the government of on quality control, trade, manufacturing, storage and sales of herbal drugs due to the absence of legislation as observed for allopathic drugs. The results of this research on one hand will create awareness in the general masses regarding the miss concept of safe use of herbal drugs and at the same time it will provide guidelines to Government to make legislation and tools of Standardization for Manufacturing, trade and use of herbal preparations.

As most of the tested crude drugs contained large concentrations of both micro and macro nutrients which could be a potential natural sources of these nutrients. However, considerable number of samples exceeded the permissible limits for human use and pose a potential threat to human health and life.

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(Received for publication 13 January 2011)