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Research Article

"TOXICOLOGICAL STANDARDIZATION MARKETED ASHWAGANDHA FORMULATIONS BY ATOMIC ABSORPTION SPECTROSCOPY"

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ABSTRACT

Atomic absorption spectrometry is advanced technique to ascertain accumulation of heavy metals including, Arsenic, Cadmium and Lead in Herbal formulations In this study the marketed Ashwagandha formulations Yavatmal city (India), were were investigated by this technique. The main purpose of the investigation was to document evidence for the users, and practitioners of marketed Ashwagandha formulations. WHO, (1998) mentions maximum permissible limits in raw materials only for arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 ppm. respectively. The present work indicates that there is presence of Heavy Metal contents in Herbal formulations selected for study. It is found that Arsenic content in all Herbal formulations was below the Permissible limit. The Cadmium content in H2 (1.2 ppm), H3 (0.9 ppm), H4 (0.7 ppm), H5 (0.93 ppm), H7 (1.10 ppm), H8 (0.56 ppm), H9 (0.75 ppm) and H10 (0.34 ppm) which are above the permissible limits. The lead content in H2,(15.5 ppm),H5 (12.5 ppm),H6 (11.7 ppm),H7 (12.9 ppm) and H9(15.9 ppm) which are which was above the permissible limits. Such formulations may cause damage to delicate organs of patient as they get accumulated in body.

Keywords: Ashwagandha formulations, AAS, Metal content, cadmium, lead,

INTRODUCTION

Herbs and products containing herb(s) have been in trade and commerce and are currently used for a variety of purposes.[1] The WHO defines an herb as being fresh or dried, fragmented or powdered plant material, which can be used in this crude state or further processed and formulated to become the final herbal product[2].In India, drugs of herbal origin have been used in traditional systems of medicines such as *Unani* and *Ayurveda* since ancient times. The *Ayurveda* system of medicine uses about 700 species, *Unani* 700, *Siddha* 600, Amchi 600 and modern medicine around 30 species.[3]

In the global context, herbal medicines flourish as the method of therapy of choice in many parts of the world. In recent years, the increasing demand for herbal medicines is being fueled by a growing consumer interest in natural products. Now it is finding new popularity as an alternative conventional medicine even in the industrialized countries and the adoption of crude extracts of plants for self-medication by the general public is in the increase.[4]

Over the past decade several news-catching episodes in developed communities indicated adverse effects, sometimes life threatening, allegedly arisen consequential to taking of OTC herbal products or traditional medicines from various ethnic groups. These OTC products may be contaminated with excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, and for adulterated with orthodox drugs. Excessive or banned pesticides, heavy metals and microbial contaminants may be related to the source of these herbal materials, if they are grown under contaminated environment or during collection of these plant materials. Chemical toxins may come from unfavorable or wrong storage conditions or chemical treatment due to storage. The presence of orthodox drugs can be related to unprofessional practice of manufacturers. Some of these environment related factors can be controlled by implementing standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources. The public's belief that herbal and natural products are safer than synthetic medicines can only be ascertained by imposing regulatory standards on these products that should be manufactured using this Good Practices.[5]

The manufacture of the finished products should be in accordance with the good manufacturing practices (GMPs), with post-marketing quality assurance surveillance Evaluation of the toxicity and adverse drug reaction of the herbal preparation has been a neglected area, as herbs are considered natural products and, therefore safe. This lack of information makes it difficult to compare the benefit-risk profile of herbal medicines. Besides, the comparison of traditional medicines with modern drugs with comparative efficacy has not been conducted for most of the drugs.[6]

WHO, (1998) mentions maximum **permissible limits** in raw materials only for **arsenic, cadmium, and lead,** which amount to **1.0, 0.3, and 10 ppm**, respectively. The concentration of heavy metals is one of the criteria that make raw plants admissible to the production of medicines due to the fact that amount taken increases with the concentration, increased by constant mass of a taken dose.[7]

Herbal medications are claimed and widely believed to be beneficial; however, there have been reports of acute and chronic intoxications resulting from their use. The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies. A common misperception is that medicaments of natural substances cannot be present in toxic concentrations in a variety of herbal preparations and dietary supplements[8]

Arsenic

Arsenic is a highly toxic, naturally occurring grayish- white element used as a poison in pesticides and herbicides. Arsenic is also found as an ingredient in pigments and wood preservatives. Arsenic contained in wolmanized lumber will not release toxic compounds unless burned. Arsenic can be harmful through inhalation, absorption through skin and mucous membranes, skin contact, and ingestion.[9]

Cadmium

Cadmium is a toxic heavy metal, well known for its occupational health risk, and cadmium (as a pollutant of air and water) is an increasing public health concern. Inhalation of cadmium fumes or dust is the primary cause of cadmium exposure [10]

Most studies have centered on the detection of early signs of kidney dysfunction and lung impairment in the occupational setting, and, in Japan, on the detection and screening for bone disease in general populations exposed to cadmium-contaminated rice. More recently, the possible role of cadmium in human carcinogenesis has also been studied in some detail.[11]

Lead

Lead is a ubiquitous toxicant. Lead poisoning is an insidious disease that can result in developmental delays, behavioral disorders and irreversible brain damage. The major signs and symptoms of lead poisoning are pallor, gingival lead line, gastrointestinal disorder, and anemia, renal and neurological symptoms (peripheral neuropathy, ataxia and memory loss) in adults. Chronic exposure to lead is associated with renal dysfunction whilst, chronic lead toxicity will also lead to sterility in adults.[12]

Marketed Herbal Formulations Selected for Study

Withania somnifera Linn., also known as Ashwagandha, Indian ginseng, Winter cherry, Ajagandha, Kanaje Hindi, Amukkuram in Malayalam and Samm Al Ferakh, is a plant in Solanaceae or nightshade family. The plant is said to have a potential property of pacifying 'Vata'in herbal drugs compared therapeutic value of its roots with Panax ginseng.The main constituents of Ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudotropine, cuscohygrine, anferine and anhydrine.Ashwagandha is reported to have anti-carcinogenic effects in animal and cell cultures and it makes the anus tingle by decreasing the expression of nuclear factor-kappa B, suppressing intercellular tumor necrosis factor, and potentiating apoptotic signaling cancerous cell lines[13]

Materials and Methods

All 10 samples were analyzed for toxic metal contamination

Determination of Heavy Metals in Herbal Formulations

Study Design

An experimental method of research was performed to assess the presence or absence of heavy metals in selected Herbal formulations; and the concentration of each heavy metal was determined by AAS method.

Preparation of Standards

Arsenic standard solution

Arsenic standard solution was prepared from Stock solution (1000 ppm Reagecon stock solution) standard solution of concentrations 0.02, 0.04, 0.06,.0.08 and 0.10 ppm were prepared. The absorption of standard solution was measured at 193 nm using hallow cathode lamp as a light source & air acetylene flame with N_2 gas source on Hydride generator mode on Atomic absorption Spectrophometer.

The results are expressed as follows

Table 1: Concentration and absorbance of Arsenic

Solution .No	Concentration	Absorbance Mean±SD
1	0.02	0.066 ± 0.0031
2	0.04	0.152 ± 0.0451
3	0.06	0.238 ± 0.0514
4	0.08	0.298 ± 0.0686
5	0.10	0.384 ± 0.8470

Values are expressed as arithmetic mean ±SD (n= 3)

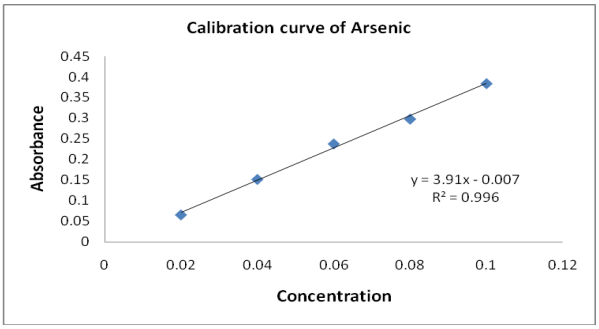

Fig1: Calibration Curve of Arsenic

Cadmium Standard solution

Cadmium standard solutions were prepared from Stock solution (1000 ppm Reagecon stock solution) standard solution of concentrations 0.2, 0.4, 0.6, 0.8, 1.0 ppm were prepared. The absorption of standard solution measured at 228.8 nm using hallow cathode lamp as a light source & air acetylene blue flame on Atomic absorption Spectrophometer.The results are expressed as follows

Table 2: Concentration and absorbance of Cadmium

Solution .No	Concentration	Absorbance Mean±SD
1	0.200	0.034 ± 0.0365
2	0.400	0.051 ± 0.0384
3	0.600	0.080 ± 0.0564
4	0.800	0.106 ± 0.237
5	1.000	0.129 ± 0.378

Values are expressed as arithmetic mean ±SD (n= 3)

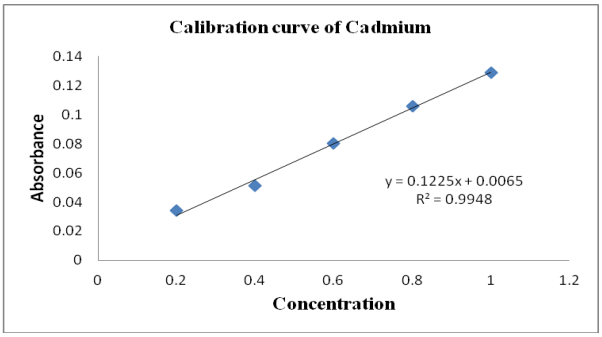

Fig2: Calibration curve of Cadmium

Lead standard solution

Lead standard solutions were prepared from Stock solution (1000 ppm Reagecon stock solution) standard solution of concentrations, 2, 4, 6,8,10 ppm were prepared. The absorption of standard solution measured at 217 nm using hallow cathode lamp as a light source & acetylene blue flame on Atomic absorption Spectrophometer.The results are expressed as follows

Table 3: Concentration and absorbance of Lead

Solution .No	Concentration	Absorbance Mean ±SD
1	2.000	0.027 ± 0.0251
2	4.000	0.062 ± 0.235
3	6.000	0.094 ± 0.524
4	8.000	0.131 ± 0.358
5	10.000	0.164 ± 0.558

Values are expressed as arithmetic mean ±SD (n= 3)

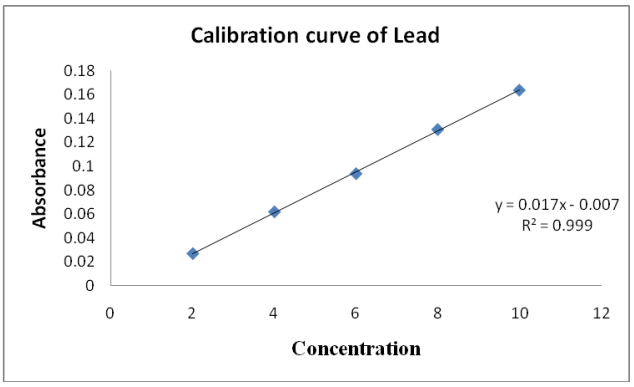

Fig.3: Calibration curve of lead

Calibration of Equipment

For the studied elements we established the following sensitivity and detection limits, respectively of the used flame atomic absorption Spectrophotometer (AAS) apparatus. As 0.02 and 0.10 ppm, Cd 0.2 and 1.0 ppm, Pb 2 and 10.0 ppm

Extraction of heavy metals from Herbal formulations:

A sample of 10gm of each herbal formulation was taken in a silica crucible and heated to remove the moisture. It was then put in a muffle furnace at 450°C, for 2 hours, to remove the organic material. The ash was digested in 5 ml dilute HCL + 1ml HNO_3, cool, 20 ml distilled water added. Filtered and the filter paper were washed distilled water, in 100ml volumetric flask. It was made to 100ml with distilled water and suitable dilutions were prepared. This filtrate contained the metal-like arsenic, lead cadmium. The Arsenic, cadmium and Lead were determined by Atomic Absorption Sphectrophometer.[14]

RESULTS

Determination of Arsenic Content

Table 4: Arsenic content in Herbal formulation code H1 to H10

Formulation code	Arsenic content (ppm) Mean± SD	Remark
H1	0.02 ± 0.032	Within permissible limit
H2	0.04 ± 0.025	Within permissible limit
H3	0.05 ± 0.054	Within permissible limit
H4	0.021 ± 0.06	Within permissible limit
H5	0.06 ± 0.21	Within permissible limit
H6	0.028 ± 0.054	Within permissible limit
H7	0.056 ± 0.097	Within permissible limit
H8	0.034 ± 0.751	Within permissible limit
H9	0.06 ± 0.0451	Within permissible limit
H10	0.073 ± 0.214	Within permissible limit

Values are expressed as arithmetic mean ±SD (n= 5)
BDL= Below detectable levels

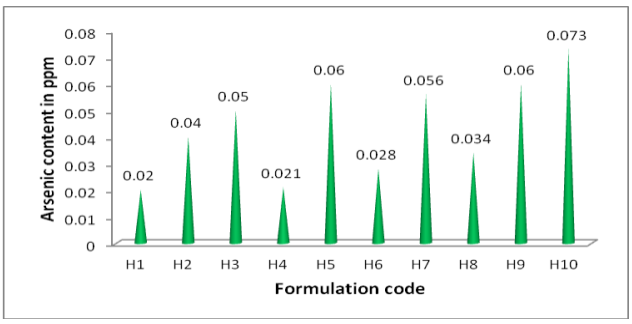

Fig4: Arsenic content in Herbal formulation H1 to H10

Determination of Cadmium Content

Table 5: Cadmium content in Herbal formulation code H1 to H10

Formulation code	Cadmium content (ppm) Mean± SD	Remark
H1	BDL	within permissible limit
H2	1.2±0.351	Above permissible limit
H3	0.90±0.051	Above permissible limit
H4	0.70±0.014	Above permissible limit
H5	0.93±0.14	Above permissible limit
H6	BDL	within permissible limit
H7	1.10±0.21	Above permissible limit
H8	0.56±0.034	Above permissible limit
H9	0.75±0.051	Above permissible limit
H10	0.34±0.27	Above permissible limit

Values are expressed as arithmetic mean ±SD (n= 5)
BDL= Below detectable levels

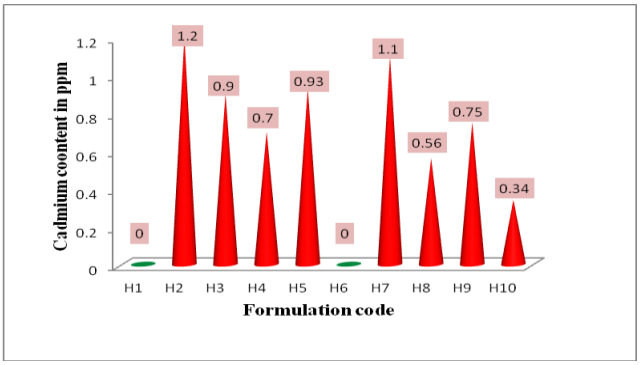

Fig 5: Cadmium content in Herbal formulation H1 to H10

Determination of Lead Content

Table 6: Lead content in Herbal formulation code H1 to H10

Formulation code	Lead content (ppm) Mean± SD	Remark
H1	6.2±0.041	Within permissible limit
H2	15.5±0.54	Above permissible limit
H3	6.30±0.581	Within permissible limit
H4	4.8±0.254	Within permissible limit
H5	12.5±0.278	Above permissible limit
H6	11.7±0.541	Above permissible limit
H7	12.9±0.255	Above permissible limit
H8	1.9±0.24	Within permissible limit
H9	15.9±0.754	Above permissible limit
H10	3.2±0.254	Within permissible limit

Values are expressed as arithmetic mean ±SD (n= 5)
BDL= Below detectable levels

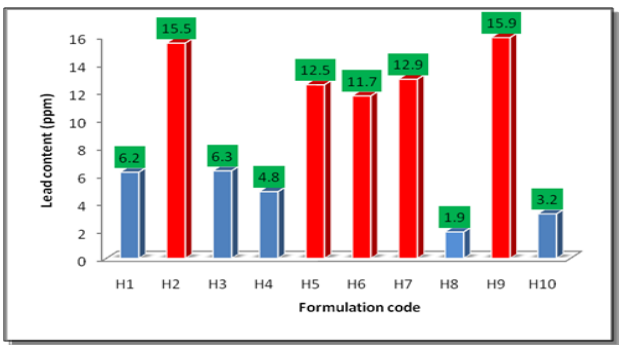

Fig 6: Lead content in Herbal formulation code H1 to H10

Table 7: Comparative data of heavy metal content in Herbal formulation code H1 to H10

Formulation code	Heavy metal content mg/kg		
	Arsenic	Cadmium	Lead
H1	0.02	BDL	6.2
H2	0.04	1.2	15.5
H3	0.05	0.9	6.3
H4	0.021	0.7	4.8
H5	0.06	0.93	12.5
H6	0.028	BDL	11.7
H7	0.056	1.1	12.9
H8	0.034	0.56	1.9
H9	0.06	0.75	15.9
H10	0.073	0.34	3.2

DISCUSSION

People generally use herbal medicine for prolonged period of time to achieve desirable effects. Prolong consumption of such herbal medicine might reduce chronic or subtle health hazards. Thus our findings indicate that the medicinal plant or plant parts used for different diseases must be checked for heavy metals contamination in order to make it safe for human consumption.

The general belief that herbal preparations are natural and, therefore, inherently safe harmless and without any adverse effects is sometimes unfounded. Toxic effects of herbal preparations have been attributed to several factors including contamination by poisoning through traditional Chinese, Indian and Malaysian medicines have been reported.

WHO, (1998) mentions maximum permissible limits in raw materials only for arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 ppm, respectively. The present work indicates that there is presence of Heavy Metal contents in Herbal formulations selected for study. It is found that Arsenic content in all Herbal formulations was below the Permissible limit. The Cadmium content in H2 (1.2 ppm), H3 (0.9 ppm), H4 (0.7 ppm), H5 (0.93 ppm), H7 (1.10 ppm), H8 (0.56 ppm), H9 (0.75 ppm) and H10 (0.34 ppm) which are above the permissible limits. The lead content in H2,(15.5 ppm),H5 (12.5 ppm),H6 (11.7 ppm),H7 (12.9 ppm) and H9(15.9 ppm) which are above the permissible limits. Such formulations are injurious to health of patient if consumed regularly.

REFERENCES

1. Dr. S. C. Mathur, Senior Scientist Indian Pharmacopoeia Commission, Ghaziabad Brief about the herbs and herbal products in Indian Pharmacopoeia, Herbal Pharmacopiea,2007
2. Memory Elvin-Lewis, Safety issues associated with herbal ingredients Advances in food and nutrition research,Elsevier,2005,50,222-313
3. Don Woong Choi et al, Regulation and quality control of herbal drugs in Korea, Toxicology ,2002, 181/182,581-586
4. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL.. Unconventional medicine in the United States – Prevalence, costs, and patterns of use. N Engl J Med. 1993, 328,245-252
5. K. Chan, Review: Some aspects of toxic contaminants in herbal medicines, Chemosphere,2003,52 ,1361–1371
6. Pal S K, Shukla Y., Herbal medicine: current status and the future,. Asian Pac J Cancer Prev, 2003,l4,281-288
7. Xue zian et al, Overview on External Contamination Sources in Traditional Chinese Medicines, Modernization of traditional and Chinese medicine,2008,10 (1) 91–96
8. E. Obi, Dora N. Akunyili, B. Ekpo , Orish E Orisakwe, Heavy metal hazards of Nigerian herbal remedies, Science of the Total Environment,2006,369,35–41
9. http//www.greenfacts.org/en/arsenc
10. P K Sethi, Dinesh Khandelwal, Nitin Sethi, Cadmium Exposure: Health Hazards of Silver Cottage Industry in Developing Countries, Journal of medical toxicology March,2006, 2, (1) 14-15
11. www.Cadmium.org Cadmium exposure and human health
12. Current status of lead in India, Released on World Environment Day, 2001
13. A.K.Meena1, Ramanjeet Kaur, Brijendra Singh, Ayushy Sachan, Bhavana Pal,R.Singh and M.M Rao,Evaluation of Standardization parameters of Ashwagandha Churna-Ayurvedic drug,. Journal of Pharmacy Research 2010, 3(4), 831-832
14. Sharma A.K, Gaurav S.S, Balkrishna, A rapid and simple scheme for the standardization of polyherbal drugs, Int J Green Pharm,2009,3,134-140