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Metals and Organochlorine Pesticide Residues in Some Herbal Ayurvedic Formulations

V. Rai · P. Kakkar · C. Misra · S. K. Ojha · N. Srivastava · S. Mehrotra

Herbal drugs are gaining popularity worldwide, but the cases of adverse health consequences caused by the use of herbal medicines have also been increasing (WHO, 2003; Chan, 2003; Stewart et al., 1999). There may be various reasons for it, but one of the major causes of reported adverse effects is the poor quality of herbal drugs. Therefore, it has been recognized that insufficient attention has been paid to herbal drug quality. The safety and quality of herbal drugs depends on various factors, and contamination by metals and pesticides during any stage of production can lead to the deterioration of quality and safety. In recent years, some initiatives have been taken toward improvement of quality, and few reports are available on the metal content in medicinal plants (Naithani and Kakkar, 2004, 2005, 2006a,b; Haider et al., 2004; Dwivedi and Dey, 2002; Rai et al., 2001; Abou-Arab and Abou-Donia, 2000; Samudralwar and Garg, 1996), but it is also important to see the concentration of heavy metals and pesticides in the finished products (Ayurvedic formulations), which are used more widely in the developed societies as compared to crude drugs. A study conducted on ayurvedic medicines by Harvard Medical School (Saper et al., 2004) reported that some of these drugs had potentially harmful levels of lead, mercury, and arsenic. Thus, it is imperative to measure the concentration of metals and pesticide residues in frequently used ayurvedic formulations (Naithani and Kakkar, 2006b). In this context, an attempt has been made to estimate the concentration of metals (Pb, Cd, Cr, Ni) and persistent pesticide residues (isomers of HCH, DDT, DDE, DDD, α- endosulfan) in samples of some popular ayurvedic formulations. These herbal preparations are extensively prescribed by Ayurvedic practitioners for the management of diseases.

Materials and methods

A total of 24 samples representing three herbal formulations of different brands were purchased from a local market in Lucknow, India. All these three preparations are sold over the counter and consumed orally, and in this article these preparations are arbitrarily coded as A, B, and C, respectively. Although ‘‘A’’ is in powder form, ‘‘B’’ and ‘‘C’’ are liquid. The major constituents of these products are listed in Table 1. Metals and organochlorine pesticides analysis was carried out according to AOAC (1995) . For metal analysis, 1 g powder of each sample of ‘‘A’’ and 5 mL of each sample of ‘‘B’’ and ‘‘C’’ were taken. All the samples were digested in HNO_3: $HClO_4$, (6:1), using a wet digestion method, by heating slowly on a hot plate in the fume hood chamber until a white residue was obtained. The residue was dissolved in 0.1 N HNO_3, and the volume yielded up to 10 mL. All the chemicals used were of analytical grade (AR, E-Merck, Germany). The digested samples were analyzed on Inductively Cou-

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V. Rai
Society of Ethnobotanists, National Botanical Research Institute,
Lucknow 226 001, India

P. Kakkar · C. Misra
Herbal Research Section, Industrial Toxicology Research
Center, MG Marg, Lucknow 226 001, India

S. K. Ojha · N. Srivastava · S. Mehrotra (✉)
National Botanical Research Institute, Rana Pratap Marg,
Lucknow 226 001, India
e-mail: nivaran_lko@rediffmail.com or
vartikarai@rediffmail.com

Table 1. List of plant ingredients of analyzed herbal preparations

Product	Ingredients
A	*Areca catechu*, *Asparagus racemosus, Phyllanthus emblica*, *Mesua ferrea, Cyperus rotundus*, *Santalum album, Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Buchanania lanzan*, *Cinnamomum zeylanicum*, *Elettaria cardamomum, Cinnamomum tamala*, *Cuminum cyminum*, *Carum carvi*, *Trapa natans*, *Myristica fragrans*, *Syzygium aromaticum*, *Coriandrum sativum*, *Piper cubeba*, *Aristolochia indica*, *Valeriana wallichii*, *Coleus vettiveroides*, *Eclipta alba*, *Vetiveria zizanioides*, *Withania somnifera*
B	*Saraca asoca*, *Woodfordia fruticosa, Cuminum cyminum*, *Cyperus rotundus, Zingiber officinale*, *Berberis aristata, Nymphaea stellata*, *Terminalia chebula, Terminalia bellirica*, *Phyllanthus emblica*, *Mangifera indica*, *Cuminum cyminum*, *Adhatoda zeylanica*, *Santalum album*
C	*Aloe barbadensis*, *Terminalia chebula,Woodfordia fruticosa*, *Myristica fragrans, Piper cubeba*, *Nardostachys jatamansi*, *Piper chaba*, *Ricinus communis, Myristica fragrans*, *Pistacia integerrima,Terminalia bellerica*, *Inula racemosa*

pled Plasma Emission Spectrophotometer (ICP 8440 Plasma Labtam). The standard reference material of Pb, Cd, Cr, and Ni (E-Merck, Germany) were used to provide calibration and quality assurance for each analytical batch. The efficiency of digestion of samples was determined by adding standard reference material (E- Merck, Germany) to the different samples. After addition of standards, samples were digested, and metals were estimated as described previously. Mean recoveries obtained were Cr: 85 ± 8%, Pb: 83 ± 7%, Cd: 90 ± 9%, and Ni: 86 ± 8%. The detection limits of the instrument for each metal was Cr 0.0061 μg/g, Pb 0.042 μg/g, Cd 0.0025 μg/g, and Ni 0.010 μg/g. Replicate (n = 3) analyses were conducted to assess the precision of the analytical techniques. Each sample of herbal formulation was processed in triplicate for pesticide analysis (50 mL liquid or 5 g powder). For solid samples, n-hexane Soxhlet extractive was taken, and for liquid, sample 100 mL n-hexane (HPLC grade) was mixed and kept overnight. Organic phase was separated from aqueous phase and was collected together. Aqueous phase was further extracted 3 times with 50 mL n-hexane in a separation funnel. Three x 30 mL of acetonitrile (HPLC grade), saturated with n-hexane, was added in the total pool of hexane extract in separation funnel, and after vigorous shaking the lower layer was collected. This extract was further passed through anhydrous sodium sulfate and activated florisil column. The clean hexane extract was concentrated and was made to 5 mL in a clean volumetric flask. Aliquots of the concentrate described were injected into a precalibrated GC machine equipped with ^{63}Ni electron capture detector. Operation temperature were programmed at 195°C, 200°C, 220°C for column, injector, and detector, respectively. Purified nitrogen gas was used as carrier gas at flow rate of 60 mL/min. Limit of detection was 0.1 to 0.5 ppb for organochlorine pesticides analyzed. Procedural blanks were periodically used to check cross contamination. Recovery studies with purified samples indicated that overall recovery value exceeded 80%. Identification and quantification were accomplished using a known amount of external standard received from Sigma-Aldrich.

Table 2. Metal concentration (ppm) in herbal preparations

Preparations	Pb	Cd	Cr	Ni
A 1	0.38 ± 0.02	0.03 ± 0.01	1.37 ± 0.25	9.5 ± 0.72
A 2	1.43 ± 0.45	0.12 ± 0.04	2.72 ± 0.50	6.30 ± 0.53
A 3	2.79 ± 0.12	0.26 ± 0.03	8.35 ± 0.65	12.15 ± 1.23
A 4	2.25 ± 0.68	0.03 ± 0.00	4.05 ± 0.47	12.00 ± 0.98
B 1	3.98 ± 0.76	0.18 ± 0.01	0.95 ± 0.12	2.68 ± 0.38
B 2	4.24 ± 0.13	0.14 ± 0.01	1.20 ± 0.05	2.91 ± 0.34
B 3	3.47 ± 0.85	0.21 ± 0.01	0.72 ± 0.47	2.84 ± 0.56
B 4	3.37 ± 0.65	0.22 ± 0.04	1.25 ± 0.34	2.71 ± 0.32
C 1	3.33 ± 0.82	0.16 ± 0.03	1.00 ± 0.556	2.42 ± 0.36
C 2	3.76 ± 0.29	0.09 ± 0.02	0.92 ± 0.12	2.43 ± 0.76
C 3	4.94 ± 0.54	0.14 ± 0.00	1.00 ± 0.37	2.43 ± 0.23
C 4	3.30 ± 0.34	0.11 ± 0.04	1.17 ± 0.15	2.46 ± 0.33

Average of four samples in triplicate; ± standard deviation

Results and discussion

The results of the analysis for metals are shown in Table 2. It is evident from the results (Table 2) that Pb, Cd, Cr, and Ni were present in all the samples of A, B, and C. Formulation A, which is used in pain, hyperacidity, and infertility, was found to have Pb and Cd within the permissible limit of WHO. Maximum lead detected, that is, 2.79 ppm, was present in A 3 samples, which is far below the 10 ppm that is the WHO permissible limit. Sample A 3 also has maximum Cd, that is, 0.26 ppm, which is close to the WHO permissible limit of 0.3 ppm. Maximum Cr (8.35 ppm) and Ni (12.15 ppm) were also found in the same sample. Cr present in this sample is two times higher than sample A 4 and is approximately four times higher than sample A 2. Sample A 3 also had more than two times the amount of Ni as did sample A 2.

Table 3. Isoforms of HCH (mg/kg) detected in herbal preparations

Preparations	α- HCH	β- HCH	γ- HCH	δ – HCH	Total HCH
A 1	0.0716	BDL	0.3012	0.0098	0.3826
A 2	0.0656	BDL	0.3023	0.0250	0.3929
A 3	0.1363	0.0942	0.4653	BDL	0.6958
A 4	0.1943	BDL	0.9205	0.0032	1.1180
B 1	0.0782	BDL	0.3694	0.0015	0.4491
B 2	0.0600	BDL	0.2528	0.0014	0.3142
B 3	0.1146	BDL	0.6780	BDL	0.7926
B 4	0.0757	BDL	0.4892	BDL	0.1649
C 1	0.0814	BDL	0.3655	BDL	0.4469
C 2	0.2035	BDL	0.3900	0.0562	0.2597
C 3	0.0771	BDL	0.4821	BDL	0.5592
C 4	0.0011	BDL	0.0109	BDL	0.0120

BDL, Below detection limit

Table 4. DDT and its metabolites (mg/kg) in herbal preparations

Preparations	pp′-DDT	pp′-DDD	O′p′-DDT	pp′-DDE
A 1	BDL	BDL	BDL	BDL
A 2	BDL	BDL	BDL	0.0159
A 3	BDL	BDL	BDL	0.0057
A 4	BDL	BDL	BDL	BDL
B 1	BDL	BDL	BDL	0.0048
B 2	BDL	BDL	BDL	0.0049
B 3	BDL	BDL	BDL	BDL
B 4	BDL	BDL	BDL	BDL
C 1	BDL	BDL	BDL	0.0368
C 2	0.0213	BDL	BDL	BDL
C 3	BDL	BDL	BDL	BDL
C 4	BDL	BDL	BDL	BDL

BDL, Below detection limit

Formulation B is used in leucorrhoea, fever, and piles, whereas formulation C is used in abdominal lump, cough, and asthma. Both these formulations had Pb and Cd within the permissible limit of WHO, and the level of Cr and Ni were also less than formulation A.

Table 3 and Table 4 show the mean concentration values of residues of different isomers of HCH and metabolites of DDT, respectively. From Tables 3 and 4 it was revealed that presence of residues of HCH was more prominent than the residues of DDT in all the preparations analyzed. α-Endosulfan was not detected in any sample. The residue level pattern in all the three formulations was HCH > DDT. As evident from the data shown in Table 3, the level of γ – HCH was much greater than other isomers in all the samples, which is understandable, as this is one of the more persistent isomers among all HCH isomers. Although use of DDT is restricted in India, and its use is only allowed for vector control, but because of its persistent nature, some of its metabolites are detected even until date in various food, vegetable, and milk products (Dogheim et al., 2004; Raizada, 1996).

Thus, from ongoing studies it can be inferred that the presence of more than the permissible limit of DDT, metals and pesticide residues may cause hazards to human life, and these residues would also affect the export potential of herbal drugs. Therefore, it should be mandatory for the pharmaceutical industries to estimate the heavy metals and pesticide residues in every batch of ayurvedic formulations, so that the consumer is assured of its quality.

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