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DETERMINATION OF CADMIUM, LEAD, COPPER AND ZINC IN THE HAIR OF HEAVY KHAT USERS

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ABSTRACT

The concentrations of Cd, Pb, Cu and Zn in scalp hair of 100 khat chewers and 100 normal subjects were determined by anodic stripping voltammetry. Hair samples were brought into solution by using a mixture of nitric and perchloric acids. The efficiency of the decomposition, and the accuracy and precision of the proposed procedure was studied using a human hair standard. The results indicated that the hair of Yemeni khat users had significantly higher concentrations of cadmium, lead and copper than did normal controls but slightly less amount of zinc. The possible connection of these elements with the etiology of disease is discussed. The results confirm the need for reducing the amount of khat currently digested and the immediate improvements of "khat sessions" ventilation.

Keywords: Hair analysis; khat chewing; Anodic stripping voltammetry Corresponding author.

INTRODUCTION

The chewing of the stimulant leaf khat (Catha edulis Forsk. Celastraceae) is a habit that is widespread in certain countries of East Africa and the Arabian Peninsula. The central nervous system action of this drug is due to the presence of the alkaloid cathinone and the results of various experiments indicate that this substance must be considered a natural amphetamine [1]. It has been recommended by the World Health Organization that cathinone should be a Schedule I drug of the United Nations and be put under international control [2].

During the last two decades, important *progress* has been made in understanding the pharmacological and social effects of khat [3-8]. Much less attention has been paid to the concentration of trace elements in khat and the role of cathinone in disturbing the trace element levels in human tissues and body fluids. Recently, we have reported that khat has significantly higher concentrations of cadmium, lead, copper and zinc than did leafy vegetables [9]. Continuous exposure to these toxic heavy elements results in their gradual accumulation in human body which may cause profound biochemical and neurological changes. The aim of the present paper was to use scalp hair as a possible indicator of trace element abnormality and to determine whether differences in their levels might occur due to khat chewing. The biological monitoring of toxic metals in hair has become a matter of wide interest since (i) the concentrations of most trace elements are higher in hair than in other human materials, (ii) the specimens can be collected quickly, easily and without stress and (iii) the material is relatively inert as well as homogeneous.

Trace analytical determinations, subsequent to sampling and sample preparation, are often carried out using instrumental methods with multielemental capabilities. Among the most suitable are atomic absorption spectrometry, neutron activation analysis and inductively coupled plasma atomic emission and mass spectrometry. Unfortunately, most of these techniques are very expensive and sometimes do not offer sufficient sensitivity for accurate determination of elements at the ultratrace concentrations usually encountered in environmental samples. Electrochemical techniques, specially polarography and voltammetry are also applicable to trace determinations. During the past two decades the use of stripping voltammetry has grown rapidly. Such interest is attributed to the remarkable sensitivity of stripping methods, and also to its speciation capabilities and low coast [10]. The present study was designed to determine Cd, Pb, Cu and Zn in human hair of heavy khat users compared with non-users using anodic stripping voltammetry.

Apparatus

Stripping voltammetric experiments were carried out with a Metrohm (Herisau, Switzerland) 746 VA Trace Analyzer connected to a Metrohm 747 VA multimode electrode used in the hanging mercury drop electrode (HMDE) regime. A platinum rod and a saturated Ag/AgCl electrode were used as auxiliary and reference electrodes, respectively [11]. pH was measured with a digital pH-meter JENWAY, Model 3310. Dissolved oxygen was removed from the samples by purging with purified nitrogen (99.99%) through the measuring vessel for 5 min. During the experiments, nitrogen was passed over the solution to prevent oxygen interference.

Software sampling enabled complete recording of the entire course of the analysis. Voltammetric curves could be smoothed, added, subtracted and copied. Parameters in all measuring procedure were as follows: preconcentration potential –1.2V, preconcentration time 60 s for Cd, Pb and Cu and 20 s for Zn, scan rate 6 mV/s, pulse amplitude 50 mV and final potential 0.2 V.

Chemicals:

All chemicals were of Suprapure grade (Merck, Darmstadt, Germany). Deionized water was used to prepare all solutions. The standard metal solutions were prepared as follows: cadmium, copper and lead stock solutions were prepared by dissolving the corresponding nitrates and a zinc stock solution by dissolving the sulphate in deaerated 2% (v/v) HNO_3. The working standard solutions were prepared daily by suitable dilution of this stock solution in the matrix required. Ammonium acetate (1.5 M) solution was purified for heavy metals using a controlled potential electrolysis assembly with – 1.5 V as initial potential for 24 h and then diluted with deionized water. All glassware were stored in 8 M nitric acid for 1 week and rinsed thoroughly with deionized water.

Sampling:

The study covered 100 heavy khat chewers and 100 normal subjects aged from 20 to 40 years. Hair samples were collected from the vertex of the scalp by cutting approximately 2 mm from the scalp. The collected samples were stored in polyethylene containers. Sample weight was about 0.5 g.

Wash procedure

Prior to analysis, all hair samples were cut into 2 cm segments with a stainless steel blade and cleaned to remove surface contamination. To achieve this, hair samples were extracted with acetone for 1 h, washed three times with deionized water and finally dried at 70 °C for 1 h.

Digestion procedure:

Samples of hair were digested as follows: 100 mg of samples was wet ashed with 3 ml of 70% nitric acid and 2 ml of 70% perchloric acid, using a reflux condenser. When the solution became colorless, it was evaporated to nearly dryness. The residue was taken in 10 ml of 0.25% nitric acid (pH = 2), which was directly transferred to the voltammetric cell for lead, cadmium and copper estimation. Zinc was determined after adjusting the pH to about 4 with ammonium acetate. Optimum quantities of acids were used so that the wet oxidation of the sample was complete and at the same time the reagent blank values for Cd, Pb, Cu and Zn were sufficiently low.

Dietary intake:

Ten volunteers were requested to collect weekly (for one month) duplicate samples of all foods (mainly bread, egg, potatoes, rice, beans and vegetables and occasionally fish or meat) that they consumed during a 24-h period. The samples were weighed to 1% accuracy, homogenized, dried, powdered and burned to ash at 500° C. The ash was dissolved in 0.5 ml of concentrated nitric acid, then diluted appropriately and analyzed in duplicates for Cu and Zn using the same analytical technique.

Statistical analysis:

Quantification of metal concentrations in the samples was carried out by the standard addition method. This is the preferred method as the sensitivity of the stripping voltammetric analysis may vary between samples of different ionic strength. The best fitting line through the data pairs was calculated by linear least-squares regression analysis. The concentration of each element in the sample is equal to the quotient of the intercept and the regression coefficient. The metal concentrations in the hair of khat users are compared with the corresponding non-users values by Students t-test ($P < 0.05$) [12].

Results and Discussion

Voltammetric measurements

An anodic stripping voltammogram of a digested hair sample is shown in Fig. 1; well defined peaks for cadmium, lead, copper and zinc were observed, indicating that the digestion of the sample was relatively complete.

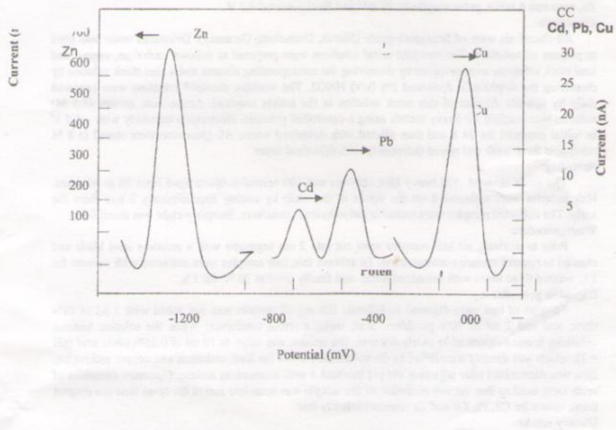

Fig. 1 Typical voltammogram of wet digested hair (100 mg) after dilution to 20 ml. The peaks represent 1 $\mu g\ g^{-1}$ Cd, 4 $\mu g\ g^{-1}$ Pb, 16 $\mu g\ g^{-1}$ Cu and 180 $\mu g\ g^{-1}$ Zn. Deposition potential –1200 mV; deposition time 60 s for Cd, Pb, and Cu and 20 s for Zn; scan rate 6 $mV\ s^{-1}$; pulse amplitude 50 mV.

The calibration curves were linear in the concentration range of 0-300 μg g –1 with a correlation coefficient lies between 0.9950 and 0.9957 for the four elements. Based on the calibration curve, the limits of detection were also determined. The limit of detection is the analyte concentration giving a signal equal to the blank signal, plus three standard deviations [12,13]. The limits of detection were 0.15 μg g –1 Cd, 0.30 μg g –1 Pb, 2.80 μg g –1 Cu and 1.20 μg g –1 Zn.

The precision of the proposed method was estimated by calculating the relative standard deviation (RSD) for ten replicate analyses of three hair samples. For all metals, the relative precision was better than 8%.

The accuracy of the proposed method was checked with "Human Hair" standard No 397-064 EC Bureau of Reference, Brussels, after digestion to provide aqueous solutions whose final concentrations were within the range of the metal contents expected in hair samples. Our mean results agree to 94% with the certified values. Recoveries were done by five replicate voltammetric determinations of the metals under consideration in five hair samples. The average recoveries were 99% (range 90 – 108%).

The concentrations of examined metals in tested Yemeni hair samples

The concentrations of Cd, Pb, Cu and Zn in the studied samples are given in Table 1. Values for these elements in fresh khat [9,14] are reproduced in Table 2. The estimated daily intakes of th four elements during Yemeni khat chewing are collected in Table 3.

Table 1: Comparison of cadmium, lead, copper and zinc concentrations* in Yemeni hair of khat users and non-users.

Group**	Cd	Pb	Cu	Zn
Normal controls	$\mu g\ g^{-1}$	2.1 ± 1.0	12.6 ± 3.1	129 ± 25
	0.38 ± 0.12	(0.8 – 7.3)	(5.7 – 21.6)	(58– 178)
	(0.16 – 1.18)	3.2 ± 1.2	15.9 ± 4.1	114 ± 26
Khat users	0.51 ± 0.18	(1.1 – 9.3)	(7.7 – 28.5)	(52 – 179)
	(0.25 – 1.80)			

*Each value is the mean ± SD with the range shown in parentheses.

**Number of specimens analyzed = 100 for each group.

Table 2 : Concentrations of cadmium, lead, copper and zinc elements of khat and selected Yemeni vegetables (fresh weight basis) [9,14].

Sample**	Cd / $\mu g\ kg^{-1}$	Pb / $\mu g\ kg^{-1}$	Cu / $\mu g\ kg^{-1}$	Zn / $\mu g\ kg^{-1}$	Plant Part** Examined
Khat	20.3 ± 10.3	333.6 ± 141.5	5308 ± 1888	6622 ± 1822	Leaf
Cabbage	3.6 ± 1.4	84.8 ± 58.6	521 ± 182	1023 ± 280	Leaf
Parsley	9.8 ± 4.2	212.0 ± 70.1	3218 ± 1634	3741 ± 1596	Leaf
Leek	8.5 ± 3.9	169.5 ± 99.4	1968 ± 1233	1559 ± 658	Leaf
Radish	14.0 ± 9.3	269.3 ± 175.7	1406 ± 1092	2903 ± 1120	Leaf
Mint	9.6 ± 7.6	292.8 ± 123.3	3948 ± 2044	4852 ± 1460	Leaf
Coriander	11.8 ± 7.0	175.3 ±74.1	3207 ± 1188	4546 ± 2067	Leaf
Lettuce	19.9 ± 11.4	222.3 ± 148.3	1793 ± 859	2116 ± 1126	Leaf
Carrot	3.0 ± 1.2	44.5 ± 22.3	873 ± 504	1215 ± 541	Root
Egg - Plant	8.1 ± 3.9	36.9 ± 20.9	1311 ± 346	1277 ± 360	Immature fruit
Potato	7.5 ± 5.0	34.0 ± 21.1	1504 ± 554	1957 ± 768	Tuber
Zucchini	3.5 ± 2.6	39.9 ± 34.5	928 ± 218	1969 ± 546	Young fruit
Onion	5.8 ± 3.8	57.9 ± 48.8	1057 ± 420	1276 ± 783	Bulb
Okra	9.0 ± 4.4	58.6 ± 24.2	2145 ± 770	3791 ± 1636	Immature fruit
Tomato	4.4 ± 2.2	30.2 ± 15.6	1410 ± 704	559 ± 305	Ripe fruit
Pepper, sweet	6.3 ± 3.6	63.5 ± 26.2	1279 ± 365	1350 ± 718	Mature fruit
Cucumber	2.6 ± 1.7	30.4 ± 19.4	554 ± 296	859 ± 363	Immature fruit

Table 3: Mean cadmium, lead, copper and zinc contents of Yemeni khat and estimated daily intake of these elements during regular khat chewing (100-500 gm fresh khat).

Element	Mean* ± SD $\mu g\ kg^{-1}$ Fresh Khat	Estimated Daily Intake $\mu g\ day^{-1}$
Cd	20.3 ± 10.3	2.0 – 10.2
Pb	333.6 ± 141.5	33.4 – 166.8
Cu	5308 ± 1888	530.8 - 2654
Zn	6622 ± 1822	662.2 - 3311

* Number of samples analyzed = 100

Table 1 indicates that khat has the highest concentrations of cadmium and lead compared with other vegetables. The average quantity of khat chewed by ordinary Yemenis is ranging from 100 to 500 gram daily. These quantities contribute 2.0 to 10.2 µg of cadmium and 33.4 to 166.8 µg of lead daily. The FAO/WHO Joint Expert Committee on Food Additives recommended a provisional maximum tolerable daily intake of Cd and Pb from all sources (food, air and water) of 60 – 72 and 210 – 240 µg (assuming an average Yemeni weight of 60 kg) [15]. According to these directives the daily intake of Cd and Pb by Yemeni consumers from khat alone is below the FAO/WHO Provisional Tolerable Daily Intakes. However, if other Pb and Cd sources are included and if khat is digested without washing (as most Yemenis do), the daily intake may exceed the recommended levels. The analysis of hair is a convenient first step to confirm such exposure. The mean (± SD) of cadmium and lead levels in the hair of normal controls were 0.38 ± 0.12 and 2.1 ± 1.0 $\mu g\ g^{-1}$, respectively (Table 1). These values matched well those recently reported by other workers [16,17] and with the recommended levels quoted by Chatt and Katz [18] (Table 4).

able 4 Recommended concentrations for zinc, cadmium, lead and copper

in adult hair [18].

Element	Reference value ($\mu g\ g^{-1}$)	Lower limit range ($\mu g\ g^{-1}$)	Upper limit range ($\mu g\ g^{-1}$)
			Zinc
15-30 yr	180-190	140-160	210-220
30-40 yr	180-155	140-115	190-210
>45 yr	155-130	115-130	190-195
Cadmium	0.23-0.27	0.1-0.15	0.45-0.55
Lead	3-4	1.7-2.5	5-6
Copper	8.5-10	7-8	11-12

The concentration of Cd and Pb in the hair of khat users were 0.51 ± 0.18 and $3.2 \pm 1.2\ \mu g\ g^{-1}$, respectively. Both values are significantly higher than that of the normal controls ($p<0.02$) but still remain at the highest range of the reference levels. In a similar work Wasiak et al. [16] observed high lead and cadmium concentrations in the hair of employees in factories manufacturing lead-acid batteries and nickel-cadmium batteries. These observations suggest that the intake of cadmium and lead by Yemeni khat chewers, whether through khat digestion or by absorption from the lungs after inhalation (during smoky khat sessions), may result in their gradual accumulation in human vital organs, which may cause profound biochemical and neurological changes in the body.

In addition to Cd and Pb, Table 1 demonstrates that khat has the highest concentrations of copper and zinc compared with the selected vegetables. Because khat is chewed heavily and daily in Yemen, it is important to assess the total daily Cu and Zn intakes by khat consumers.

The mean estimated dietary Cu intake of the Yemeni subjects included in this study was 1.4 ± 0.3 mg/d. This level is below the current estimated safe and adequate daily intake (ESADDI) range of 2 to 3 mg/d [19]. The average quantity of khat chewed by Yemeni consumers contributes 0.530 to 2.654 mg of copper daily (Table 3). The overall Cu intake by heavy khat chewers is therefore 1.93 to 4.05 mg/d. These figures are at the higher end of the ESADDI level and much higher than the usual intake of copper in the United States population (<2 mg/d).

The influence of high copper intake is expected to increase the concentration of Cu in scalp hair of khat users. Indeed, the mean value of hair copper levels in normal controls was $12.6 \pm 3.1 \mu g\ g^{-1}$ (Table 1). This figure is slightly higher than the reference level [18] and those recently published by Naidu et al. [17], lower than that quoted by Linder and Azam [20] and much lower than that reported by Raie [21]. The hair copper level of Yemeni khat users $15.9 \pm 4.1\ \mu g\ g^{-1}$ was significantly higher ($P < 0.01$) than that of the normal controls. Since none of the volunteers had Wilson's disease, haematuria, azotaemia or hepatic necrosis, the result provides unambiguous evidence that khat chewing is responsible for the high copper level. Negative consequences of excess of copper are extensively documented [20]. Liver cirrhosis typically develops from toxic intakes, and abnormalities in red blood cell formation also occur.

The present results reinforce the impression that hair analysis potentially useful in experimental medicine but its use in clinical medicine for diagnosis, prognosis, and therapy will remain limited until validation by the standard methods of clinical investigation is achieved [22,23].

The mean estimated di etary zinc intake of the low-income Yemenis (from all sources except khat) was 9.8 ± 4.1 mg/d. None of the volunteers consumed the Recommended Daily Allowance (RDA) [19] of 15 mg Zn/day. These low levels are expected in undernourished populations, such as those in Yemen, since their intake of meat products, which are good sources of zinc, is limited [24]. The potential contribution of Zn from khat to the Yemeni diet is 0.662 to 3.311 mg (Table 3). The total zinc intake by heavy khat chewers is thus, about 10.46 to 13.11 mg/d which is still below the RDA levels.

The concentration of zinc in the hair of khat non-users was $129 \pm 25\ \mu g\ g^{-1}$. Although, this value is at the lower limit range of the recommended levels (Table 4), it is higher than those reported for other populations (for Indians ~ $91.21 \pm 20.37\ \mu g\ g^{-1}$) [17]. These results may confirm that the Yemeni daily Zn intakes seemed to be nutritionally adequate. More surprising is the fact that although khat chewing supplied the body with an extra daily intake of 0.662 – 3.311 mg zinc daily, the hair zinc level of Yemeni khat users ($114 \pm 26\ \mu g\ g^{-1}$) was slightly lower than that of the normal controls (Table 1). These observations suggest the existence of a competition between the absorption of Cu and Zn by humans, which provide strong support to the general attitude that the interaction between zinc and copper is mutually antagonistic. The exact mechanism of the reciprocal relationship between zinc and copper in the above conditions is not known.

Conclusions

The results showed that:

I-The levels of cadmium, lead and copper in the hair of khat users were all significantly higher than those in the normal controls.

II-The concentration of toxic elements in the hair of Yemeni khat chewers is generally not considered critical, although the continuous exposure to these heavy metals do require attention.

III -It is possible to decrease the daily metals intake by washing khat properly and reducing the amount of khat currently digested.

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