SIMULTANEOUS DETERMINATION OF CADMIUM, LEAD, COPPER AND ZINC IN URINE OF HEAVY KHAT-CHEWERS USING ANODIC STRIPPING VOLTAMMETRY

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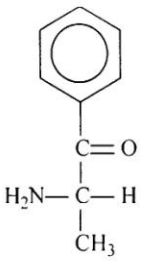
يقدم هذا البحث النتائج العملية لتحديد عناصر الكادميوم، الرصاص، النحاس والزنك الموجودة في مستويات منخفضة جدا في بول المتعاطين للقات بشدة باستخدام طريقة الخلع المصعدي الجهدي. وقد بين التحليل أن المتوسط الهندسي للعناصر الأربعة في بول المتعاطين للقات تفوق بشكل ملحوظ مقدار تلك العناصر في بول غير المتعاطين له. وأظهرت متابعة تركيز النحاس والزنك خلال ٢٤ ساعة بان تركيز النحاس يزداد بشكل كبير خلال فترة تعاطي القات ثم ينخفض إلى المستوى الاعتيادي بعد انتهاء تأثير القات وعلى العكس ينخفض مستوى الزنك خلال فترة المضغ ثم يزداد بعدها.
فسرت النتائج على أساس تكون ارتباط كلابي مرجح بين الكاثينون والنحاس (المرتبط بالبلازما) لا تستطيع الكلية إعادة امتصاصه فيفرز بمقدار كبير في بول المتعاطين للقات. أما نقصان مستوى الزنك في البول أثناء المضغ فيرجع إلى تأثير التضاد بين العنصرين.

The concentration of cadmium, lead, copper and zinc in urine of heavy khat- chewers with matched normal controls was investigated by differential pulsed anodic stripping voltammetry (DPASV). The analysis showed that urinary cadmium, lead, copper and zinc of regular khat users were significantly higher than those of the controls. The peak for urinary copper levels was attained after about 2-3 h following khat chewing. The height of the peak was found to depend on the type and amount of khat digested. In contrast, the circadian rhythm of urinary zinc demonstrated a remarkable and progressive decline of urinary zinc during khat chewing that is independent of zinc nutritional status. Cathinone, the active principle of khat may resemble penicillamine and other chelators in their strong capability to form copper complexes and that the interaction between zinc and copper is mutually antagonistic.

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].



Cathinone ((S)-2-amino-1-phenyl-1-propanone)

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 the concentrations of Cd, Pb, Cu and Zn in fresh khat have been determined [9]. It was found that khat had significantly higher concentrations of copper and zinc than did leafy vegetables, but almost the same amounts of cadmium and lead [9]. The aim of the present study is to investigate the effect of khat chewing on the urinary Cd, Pb, Cu and Zn excretion of heavy khat users compared to the non-users. The biological monitoring of toxic metals in urine has become a matter of wide interest owing to the toxicity of these metals and their influence in controlling the course of biological processes.

In urine, trace metals often occur at very low concentrations ($<10^{-8}$ M). Several techniques have been used in trace metal analysis with varying degree of success and convenience. Among the various techniques, anodic stripping voltammetry (ASV) have demonstrated great sensitivity for Cd, Pb, Cu and Zn and can easily determine these metals quantitatively in the low parts per billion ranges in biological matrices such as blood and urine [10]. This remarkable sensitivity of stripping analysis is attributed to the preconcentration step that takes place before the actual measurement (stripping) step. When compared with spectrometric methods, only electrothermal atomic absorption spectrometry has nearly the same sensitivity but is more expensive [11].

Materials and Methods:

Sampling and Sample Pretreatment:

In urine, the concentration of elements varies with the urinary flow. One-way of handling this problem is to relate the excretion to time, e.g., 24 h, which is adopted in the present work. The subjects for this study were hundred Yemeni male students aged 19-26 y and weighing 45-65 kg. Fifty of them were regular heavy khat users chewing about 300 grams of freshly khat daily and the other fifty are normal controls (khat non-users). All subjects were non-smokers and in good health as evidenced by medical history, physical examination, and routine blood chemistry studies.

Usually urine has a pH values in the range of 5.0 – 6.0. The urine sample was pretreated by adjusting the pH to 4.0 with 1:50 doubly distilled nitric acid to prevent the precipitation of calcium phosphate and the consequent loss of heavy metals due to coprecipitation or adsorption on the precipitate.

Different wet digestion procedures given in the literature were examined. In addition, a high temperature dry ashing was also examined. Based on the experience with these methods the following recipe was adopted. Five ml of urine was wet ashed with 3 ml of 70% nitric acid, 1 ml of sulphuric acid and 1 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.

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 [12]. 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 −1200 mV, preconcentration time 120 s for Cd, Pb and Cu and 60 s for Zn, scan rate 6 mV s^{-1}, pulse amplitude 50 mV and final potential 200 mV.

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 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.

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 urines of khat users are compared with the corresponding non-users values by Students *t*-test ($P < 0.05$) [13].

RESULTS AND DISCUSSION

An anodic stripping voltammogram of a digested khat 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.

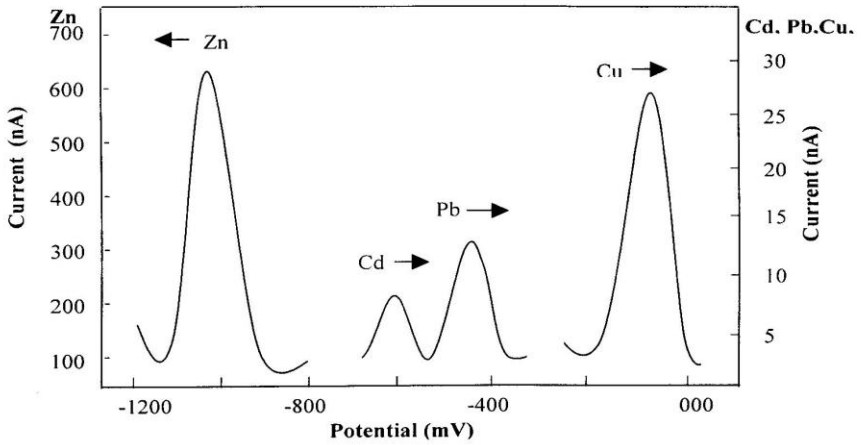


Fig. 1: Typical voltammogram of wet digested urine (5 ml) after dilution to 20 ml. The peaks represent 3 μg l^{-1} Cd, 10 μg l^{-1} Pb, 16 μg l^{-1} Cu and 380 μg l^{-1} Zn. Deposition potential –1200 mV; deposition time 120 s for Cd, Pb, and Cu and 60 s for Zn; scan rate 6 mV s^{-1}; pulse amplitude 50 mV.

The calibration curves were linear in the concentration range of 0-300 μg l^{-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 [13,14]. The limits of detection were 0.15 μg l^{-1} Cd, 0.30 μg l^{-1} Pb, 2.80 μg l^{-1} Cu and 1.20 μg l^{-1} Zn.

The precision of the proposed method was estimated by calculating the relative standard deviation (RSD) for ten replicate analyses of three urine samples. For all metals, precision was better than 5%.

The accuracy of the proposed method was checked with a standard reference urine issued by the United States National Bureau of Standards (SRM 2670) after digestion to provide aqueous solutions whose final concentrations were within the range of the metal contents expected in urines. 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 urine samples. The average recoveries were 99% (range 90 – 108%).

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] are reproduced in Table 2 together with the estimated daily intakes during heavy khat chewing (300 gm).

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

Group**	Cd	Pb	Cu	Zn
	µg / d			
Normal controls	1.21 ± 0.58 (0.25 – 2.23)	6.9 ± 3.8 (1.4 – 12.3)	42.1 ± 27.9 (7.5 – 93.9)	222.2 ± 126.6 (83.3 – 585.2)
Khat users	2.48 ± 2.12 (0.32 – 9.47)	16.1 ± 13.4 (1.6 – 48.9)	346.7 ± 281.7 (62.4 – 779.7)	427.1 ± 200.8 (88.2 – 1345)

*Each value is the mean ± SD with the range shown in parentheses.
**Number of specimens analyzed = 50 for each group.

Table 2: Mean cadmium, lead, copper and zinc contents of Yemeni khat [9] and estimated daily intake of these elements during heavy khat chewing (300 gm).

Element	Mean* ± SD µg kg^{-1} Fresh Khat	Estimated Daily Intake µg / d
Cd	20.3 ± 10.3	6.1
Pb	233.6 ± 141.5	70.1
Cu	5308 ± 1888	1592
Zn	6622 ± 1822	1987

* Number of samples analyzed = 100

As shown in Table 1 the mean values (± SD) of urinary cadmium and lead levels in normal controls were 1.21 ± 0.58 and 6.9 ± 3.8 µg/d, respectively. These values agree well with those reported by other workers [15,16]. The urinary Cd and Pb values of heavy khat users, who received 6.1 µg Cd and 70.1 µg Pb daily (Table 2), were 2.48 ± 2.12 and 16.1 ± 13.4 µg/d, respectively. Both values are higher than that of the normal controls but do not present any health risk to khat consumers according to the reference levels [17]. If khat is digested without washing (as most Yemeni do), the daily intake may exceed the recommended levels. Continuous exposure to Cd and Pb results in their gradual accumulation in human vital organs, which may cause profound biochemical and neurological changes in the body.

The mean value of urinary copper levels in normal controls was 42.1 ± 27.9 µg/d (Table 1). This figure is lower than that reported (69 ± 24.9

μg/d) [18], very close to the reference level (36.9 ± 1.47 μg l^{-1}) [19] and slightly higher than those accepted by Tankanow (< 40μg/d) [18]. The urinary copper levels in heavy khat users 346.7 ± 281.7 μg/d are significantly higher ($P < 0.05$) 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 "unusual" high copper urinary levels.

To decide whether the high urinary level reflects the physiological effect of cathinone, or is merely due to the intensive copper uptake from khat it is necessary to assess the Cu status of khat consumers. The average daily intake of copper by heavy khat chewers (from khat only) is 1.59 mg/d (Table 2). Based on the metabolic research [20]; neither urinary nor salivary Cu is affected by the amount of Cu in the diet when young men fed: (1) with an adequate Cu diet (1.68 mg/d for 24 days), (2) with a low Cu diet (0.79 mg/d for 42 days) and (3) with a high Cu diet (7.53 mg/d for 24 days). These data suggest that cathinone; the active principle of khat was a major factor in inducing substantial alterations of the renal tubular function.

The minimal loss of copper through the urine of normal control is consistent with the evidence that there is little or no free copper in blood plasma, and suggests that any low-molecular-weight copper complexes that might be filtered by the glomeruli are specifically reabsorbed [21]. Treatment with specific chelators like penicillamine and trientine greatly increases the size of the plasma copper compartment associated with low-molecular-weight ligands and leads to much greater urinary excretion of the element. Such chelators are applied in Wilson disease. Cathinone may do the same, but definitive conclusions about its role await additional investigation.

The mean value of urinary zinc levels in normal controls was 222.2 ± 126.6 μg/d (Table 1). This value is expected in undernourished populations, such as those in Yemen, since their intake of meat products, which are good sources of zinc, is limited. Zn levels in the present work agree well with those reported (260 μg/d) [22]for men consuming low-meat diets and with those mentioned by Johnson et al [23], who observed a significant decrease in urinary zinc when dietary intake decreased from 10.3 to 4.5 mg Zn/d and further decreases as dietary zinc intake decreased to 1.4 mg/d. The urinary zinc levels of khat users 427.1 ± 200.8 μg/d were significantly higher ($P < 0.05$) than those for normal controls. Based on our calculations (Table 2) the mean daily intake of Zn by heavy khat consumer, from khat only, is 1.99 mg. This additional zinc may be the factor that affects the magnitude of the urinary zinc in khat users compared with non-users.

The 24-urine copper excretion (in μg l^{-1}) levels of heavy khat users compared with non-users are illustrated in Fig. 2. The peak was attained 2-3 h subsequent to khat chewing and remains high as long as khat chewing continues. The height of the peak was found to depend on the type and amount of khat digested. These interesting results plus the evidence already presented [24], that the peak plasma cathinone levels were attained after about 1.5-3.5 h following khat-chewing, support our assumption that cathinone is the constituent mainly responsible for the unusual loss of copper.

In contrast, zinc urinary concentration decline rapidly during the khat chewing session, thereafter rises steadily (Fig. 3). These observations provide strong support to the general attitude that the interaction between zinc and copper is mutually antagonistic and justify the use of zinc therapy to reduce the copper burden in patients with Wilson's disease. The exact mechanism of the reciprocal relationship between zinc and copper in the above conditions is not known.

The important conclusion from the above experimental data and calculations is that cathinone; the active principle of khat may resemble penicillamine and other chelators in their strong capability to form copper complexes and that the interaction between zinc and copper is mutually antagonistic.

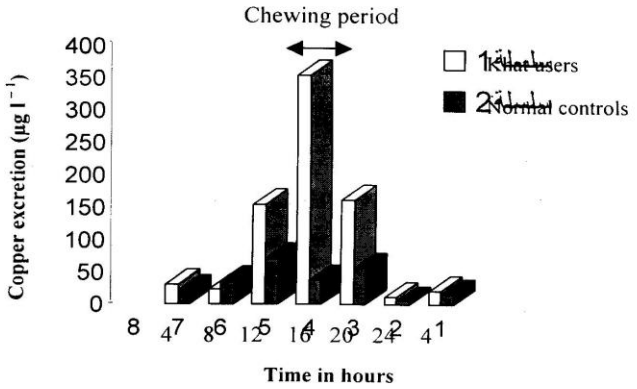


Fig. 2: Influence of khat chewing on the urinary copper excretion over 24 h.

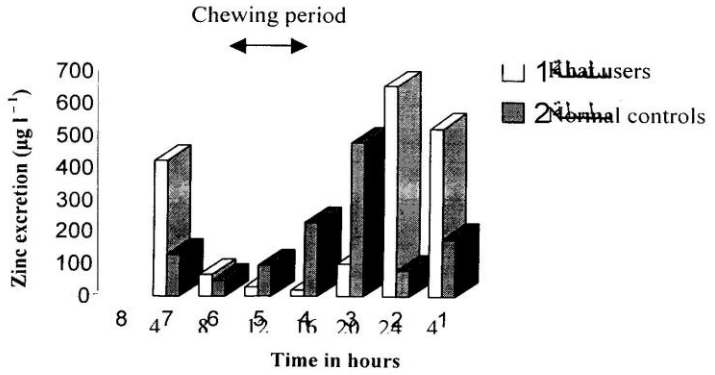


Fig. 3: Influence of khat chewing on the urinary zinc excretion over 24 h.

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