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Identification and quantitation of alkaloids in coca tea

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Abstract

The consumption of coca tea is a common occurrence in many South American countries. The tea is often packaged in individual servings as tea bags which contain approximately 1 g of plant material. The consumption of coca tea leads to ingestion of cocaine and other alkaloids: however, there is little information available regarding the pharmacological or toxicological effects that result from consumption of coca tea. We performed a series of studies with coca tea bags from two South American countries, Peru and Bolivia. The alkaloidal content of the 'coca leaf' in coca tea bags was determined by two different extraction methods: Soxhlet extraction with methanol (exhaustive extraction), and mechanical agitation with methanol. Extracts were purified by solid-phase extraction (SPE) followed by analysis by gas chromatography/mass spectrometry (GC/MS). Coca tea prepared from Peruvian and Bolivian coca tea bags was also analyzed by SPE-GC/MS assay. In addition, urine specimens were analyzed from an individual who consumed one cup of Peruvian coca tea and one cup of Bolivian coca tea on separate occasions. Urine samples were analyzed by immunoassay (TDx^R) and SPE-GC/MS. Analysis of coca tea bags and coca tea indicated that cocaine, benzoylecgonine, ecgonine methyl ester and *trans*-cinnamoylcocaine were present in varying quantities. With exhaustive extraction, an average of 5.11 mg, and 4.86 mg of cocaine per tea bag were found in coca leaf from Peru and Bolivia, respectively. The average amounts of benzoylecgonine and ecgonine methyl ester in Peruvian coca leaf were 0.11 and 1.15 mg, and in Bolivian coca leaf were 0.12 and 2.93 mg per tea bag, respectively. *trans*-Cinnamoylcocaine was found in trace amounts in Peruvian tea bags and 0.16 mg/tea bag of Bolivian tea. When tea was prepared, an average of 4.14 mg of cocaine was present in a cup of Peruvian coca tea and 4.29 mg of cocaine was present in Bolivian tea. Following the consumption of a cup of Peruvian tea by one individual, a peak urine benzoylecgonine concentration of 3940 ng/ml occurred 10 h after ingestion. Consumption of Bolivian coca tea resulted in a peak benzoylecgonine concentration of 4979 ng/ml at 3.5 h. The cumulative urinary excretion of benzoylecgonine after approximately 48 h, determined by GC/MS, was 3.11 mg and 2.69 mg after consumption of Peruvian and Bolivian coca tea, respectively. This study demonstrated that coca tea bags and coca tea contain a significant amount of cocaine and cocaine-related alkaloids and the consumption of a single cup of Peruvian or Bolivian coca tea produces positive drug test results for cocaine metabolites.

Keywords

Coca tea bags; Cocaine; Benzoylecgonine

1. Introduction

The ingestion of herbal teas is a common occurrence in many countries. In South America, herbal teas frequently consist of pure coca leaf or coca leaf mixed with herbs. The plant material may be loose or bagged for individual servings. Typically, one or two 'tea bags', consisting of

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approximately 1 g of plant material per bag, is steeped in hot water for a few minutes. Sugar and lemon or milk may be added and the mixture is ingested. Although coca leaf is known to contain cocaine, only limited information is available on the alkaloidal content of these 'tea bags', the amount of cocaine that is extracted during the 'tea-making' process, or the amount of cocaine metabolite subsequently excreted as a result of drinking coca tea.

Siegel et al. [1] indicated that there was an average amount of 4.8 mg of cocaine per bag in Health Inca Tea (in which the ingredients were listed as 'decocainized coca leaves') and 5.7 mg of cocaine in a regular coca tea bag (Mate de Coca). In addition, benzoylecgonine was detected in the urine of some coca tea drinkers by gas chromatography with nitrogen phosphorus detection and gas chromatography/mass spectrometry (GC/MS). In a second study, benzoylecgonine was measured by GC/MS in urine specimens for 29 h following consumption of one cup of Health Inca tea [2]. Peak benzoylecgonine concentrations occurred 2 h after ingestion and decreased to 274 ng/ml by 22 h. The total amount of benzoylecgonine excreted after 29 h was 0.82 mg. Analysis of the tea indicated that 2.15 mg of cocaine was present and no benzoylecgonine was detected. Jackson et al. [3] measured the urinary excretion of benzoylecgonine in four volunteers following ingestion of 180 ml of Health Inca Tea and concluded that the tea contained 1.87 mg of cocaine. Urine samples collected for 36 h after tea consumption were analyzed by GC/MS for benzoylecgonine and by immunoassay for cocaine metabolites. Peak benzoylecgonine concentrations ranged from 1.4–2.8 mg/1 and occurred 4–11 h post ingestion. Positive immunoassay results (300 ng/ml cutoff) were obtained for 21–26 h. Floren and Small [4] reported a peak urine concentration of 2608 ng/ml for cocaine metabolite in a 100 kg subject 4 h after drinking one cup (240 ml) of 'Mate de Coca' purchased in Bolivia. Urine drug screens for two individuals were negative (no cutoff concentration reported) 24 h after drinking two cups of coca tea. The two subjects reported 'mild, generalized stimulant effects, indistinguishable from the effects of two cups of coffee...' [4]. These previous studies have several shortcomings including: no indication of the specific origin of the coca tea utilized or determination of alkaloidal content [3]; no description of how the coca tea was prepared [2]; and assaying a limited number of alkaloids (cocaine and/or benzoylecgonine only) following coca tea consumption [2,3].

We performed a comprehensive study utilizing coca tea bags from Peru and Bolivia, with the following three objectives: (1) to identify and measure the major coca alkaloids in coca tea bags; (2) to determine the amounts of cocaine and cocaine analogs that are transferred to the aqueous phase during the tea making process; and (3) to determine the urinary excretion profile of cocaine and benzoylecgonine following the consumption of coca tea.

2. Materials and methods

2. 1. Chemicals

Coca tea bags were obtained from commercial sources in Peru (Mate de Coca produced by the National Enterprise of Coca, Inc. (ENACO), Cuzco, Peru) and Bolivia (Mate de Coca, 'Lupi', Bolivia). Methanol was HPLC grade (J.T. Baker, Inc.). All other chemicals were reagent grade.

2.2. Methanolic extraction (Soxhlet)

The contents of four Peruvian coca tea bags were weighed, then placed in a Soxhlet extraction apparatus. The material was refluxed with 500 ml of methanol for 24 h. After 24 h, the methanolic extract was removed and fresh solvent was added. A second extraction was performed for an additional 24 h. The extracts were stored at 2°C until analyzed by SPE-GC/MS assay. Similarly, the contents of Bolivian tea bags also were extracted, but due to a limited supply, only two bags were utilized in the extraction.

2.3. Methanolic extraction (Agitation)

The contents of two Peruvian coca tea bags were weighed and placed in sealed containers with 250 ml of methanol. The material was mechanically shaken for 24 h. After 24 h the methanolic extract was removed and 250 ml of fresh solvent added. A second extraction was performed for an additional 24 h. The extracts were stored at 2°C until analyzed by SPE-GC/MS. The contents of one Bolivian tea bag was extracted in a similar manner.

2.4. Preparation of Peruvian and Bolivian coca tea

Coca tea bags were randomly selected from each source, weighed, and then tea prepared by the addition of a single bag to 180 ml of deionized water at 94°C. The tea bag was maintained in the hot water for 3 min, removed and the pH of the infusion determined. To study the effects of infusion time, tea bags were also immersed in hot water for 6, 9, 12, and 15 min. Aliquots of tea were assayed for cocaine and cocaine-related compounds by SPE-GC/MS.

2.5. Consumption of Peruvian and Bolivian coca tea

An individual consumed one cup of coca tea prepared with one Peruvian coca tea bag. On a separate occasion, the same individual ingested one cup of tea prepared with one Bolivian tea bag. All urine samples were collected for a minimum of 48 h. Urine samples were analyzed by fluorescence polarization immunoassay (FPIA) (TDx®, Abbott Laboratories) for the presence of cocaine metabolites and by SPE-GC/MS for cocaine-related alkaloids [5].

2.6. Sample extraction

Aliquots of the Soxhlet and mechanical extracts, coca tea and urine samples were treated with deuterated internal standards and extracted by SPE (Clean Screen DAU, 130-1 ml, United Chemical Technologies, Inc.) according to a previously published procedure [5]. After extraction, the samples were reconstituted with acetonitrile, transferred to auto sampler vials and derivatized with 0.02 ml of BSTFA (with l% TMCS). Sample vials were heated at 60°C for 30 min and then analyzed by GC/MS.

2.7. GC/MS assay

Derivatized extracts were analyzed on an HP-1 cross-linked fused silica capillary column (12 m, 0.20 mm I.D., 0.33 μm film thickness) with a Hewlett-Packard 5890A gas chromatograph, equipped with a 7673A automatic liquid sampler and interfaced with a 5970B mass selective detector (MSD). Alkaloids were identified by comparison of full scan electron impact spectra with that of reference standards. Quantitation was achieved by operating the MSD in the selected ion monitoring mode (SIM). The following ions were monitored for each compound: nicotine, *m/z* 84, 133, 162 (qualitative only); anhydroecgonine methyl ester, *m/z* (152), 166, 181; ecgonine methyl ester, *m/z* 82,(96), 271: [$^2H_3$]-ecgonine methyl ester, *mlz* 85. (99); ecgonine ethyl ester, *mlz* 83, (96), 285; cocaine, *m/z* 82. (182), 303; [$^2H_3$]-cocaine, *m/z* 85, (185); cocaethylene, *m/z* 82, (196), 317; benzoylecgonine, *m/z* 82, (240), 361; [$^2H_3$]-benzoylecgonine, *m/z* 85, (243); norcocaine, *m/z* (140), 240, 346; norcocaethylene, *m/z* 140, (254), 360; benzoylnorecgonine, *m/z* 140, 298, (404); and *trans*-cinnamoylcocaine, *m/z* 82, (182), 329. Ions used for quantitation are shown in parenthesis. Standard curves (6.25–1000 ng) were constructed based on ion peak area ratios of analyte to their respective deuterated analogs.

2.8. Caffeine determination

Coca tea was analyzed for caffeine by a liquid-liquid extraction and gas chromatographic (GC) assay for basic drugs according to a previously published procedure [6].

3. Results

3. 1. Identification and measurement of alkaloids in coca tea bags

Methanolic extracts of coca tea bags were analyzed by GC/MS in the full scan mode. Alkaloids were identified by comparison of electron impact spectra with those of authentic standards. Quantitation was performed in the SIM mode by the internal standardization method. The average Peruvian coca tea bag ($N = 21$) contained 1.09 g of coca leaf. The average Bolivian coca tea bag ($N = 7$) contained 0.82 g of coca leaf. Table 1 lists the quantities of alkaloids isolated by two different extraction methods in coca tea bags from Peru and Bolivia. Cocaine was present in the highest amount followed by ecgonine methyl ester by both methods. Traces of benzoylecgonine and *trans*-cinnamoylcocaine also were present. Anhydroecgonine methyl ester was present in trace amounts in extracts prepared by the agitation method and in larger amounts in the Soxhlet extract. It is likely that the presence of anhydroecgonine methyl ester was due to artifactual production from cocaine as a result of heat exposure during extraction.

Generally, the Soxhlet extraction method recovered greater amounts of alkaloids than the agitation method. The one exception occurred when cocaine was recovered from Bolivian tea bags in slightly higher amounts by the agitation method. With Soxhlet extraction, all alkaloids in Bolivian coca tea bags were removed in the first 24-h extraction. For Peruvian coca tea bags, 100% of ecgonine methyl ester, 98% of cocaine, and 54% of benzoylecgonine was removed in the first extraction. Extraction by agitation was less efficient and recovered 88% and 95% of available cocaine in Peruvian and Bolivian coca tea bags, respectively.

3.2. Alkaloids in coca tea

Cocaine, ecgonine methyl ester, benzoylecgonine and *trans*-cinnamoylcocaine were present in both Peruvian and Bolivian tea. Anhydroecgonine methyl ester was identified, but was most likely produced as an artifact. Other alkaloids such as caffeine and nicotine were not detected. An average concentration of 4.14 mg of cocaine was transferred to coca tea from one Peruvian coca tea bag (Table 2). This indicated that 81% of the available cocaine was extracted from the tea bag during coca tea preparation. Coca tea also contained an average of 1.15 mg of ecgonine methyl ester. This amount was similar to that found in coca tea bags. In addition, 0.50 mg of benzoylecgonine was found in the coca tea infusion. This was approximately ten times the amount of benzoylecgonine measured in one coca tea bag. Traces of *trans*-cinnamoylcocaine were also found in coca tea.

To determine whether ecgonine methyl ester and benzoylecgonine were present naturally in coca leaf or were being produced as an artifact during the tea making process, 3.4 mg of d_3-cocaine was added at the beginning of coca tea preparation. Assay by SPE-GC/MS without the addition of deuterated internal standard indicated that only trace amounts of d_3-cocaine were hydrolyzed to d_3-ecgonine methyl ester and d_3-benzoylecgonine during tea preparation. This finding indicated that ecgonine methyl ester and benzoylecgonine were present in coca tea bags and were not produced by hydrolysis during the preparation of tea.

Table 2 also shows the alkaloids measured in Bolivian coca tea. The amount of cocaine in the coca tea infusion was similar to that measured in Peruvian coca tea. However, less benzoylecgonine, an average of 0.26 mg/180 ml tea, and more ecgonine methyl ester, an average of 1.81 mg/ 180 ml tea, were present in the Bolivian coca tea.

Increasing the steeping time during tea preparation produced an increase in the amount of cocaine in the coca tea (Table 3). For example, 3.94 mg of cocaine was present in Peruvian coca tea at 3 min; this amount increased to 5.88 mg of cocaine when the coca tea bag was steeped for 15 min. An increase in the amount of cocaine present in Bolivian coca tea was also observed as the steeping time was increased from 3 to 12 min. The amount of benzoylecgonine

extracted remained essentially unchanged, but there was a slight increase in the amount of ecgonine methyl ester extracted.

3.3. Excretion of cocaine metabolites after consumption of coca tea

Urine samples were periodically collected from a single individual following consumption of one cup of Peruvian and one cup of Bolivian coca tea on separate occasions. The samples were analyzed by TDx for cocaine metabolites and GC/MS for cocaine, benzoylecgonine and ecgonine methyl ester. Urine samples collected prior to ingestion of tea were negative for cocaine and metabolites. The first sample collected after ingestion of coca tea from Peru and Bolivia was highly positive for cocaine metabolites by TDx. The concentration of benzoylecgonine equivalents remained above 1000 ng/ml for approximately 17 h. Thereafter, the concentration of cocaine metabolites decreased to below 100 ng/ml by approximately 45 h after coca tea consumption. The excretion profile for cocaine and metabolites over time is shown in Fig. 1 for Peruvian coca tea and Fig. 2 for Bolivian coca tea. The figures illustrate the high correlation ($r = 0.999$) between immunoassay results for cocaine metabolites and GC/MS benzoylecgonine results. GC/MS analysis indicated that the first sample obtained after Peruvian coca tea consumption contained 794 ng/ml of benzoylecgonine, 91 ng/ml of cocaine and 1093 ng/ml of ecgonine methyl ester. Concentrations of all analytes continued to increase and peaked at 3368 ng/ml of benzoylecgonine at 10 h, 2520 ng/ml of ecgonine methyl ester at 10 h, and 196 ng/ml of cocaine at 5 h. Thereafter, concentrations declined, but benzoylecgonine consistently remained above 300 ng/ml for 20 h. At 48 h, the benzoylecgonine concentration in urine was 23 ng/ ml, cocaine was negative and ecgonine methyl ester was 22 ng/ml. The cumulative urinary excretion of benzoylecgonine after 47.75 h was 3.11 mg. After consumption of one cup of Bolivian coca tea, the first urine sample obtained after 2 h contained 719 ng/ml of benzoylecgonine, 97 ng/ml of cocaine and 345 ng/ml of ecgonine methyl ester. The concentrations of each analyte peaked at 3.5 h at 4155 ng/ml of benzoylecgonine, 587 ng/ml of cocaine and 2314 ng/ml of ecgonine methyl ester. Concentrations of benzoylecgonine remained consistently above 300 ng/ml for approximately 19 h. After 52.5 h, the last collection time, the urine benzoylecgonine concentration was 21 ng/ml, cocaine was negative and ecgonine methyl ester was 12 ng/ml. The cumulative urinary excretion of benzoylecgonine after 47 h was 2.69 mg.

4. Discussion

The consumption of coca tea results in ingestion of varying amounts of cocaine and cocaine-related alkaloids. Although the contents of the coca tea bags from Peru and Bolivia were not identified botanically, this study demonstrated that approximately 5 mg of cocaine was present in the tea bags and approximately 80% of the available cocaine was transferred to the aqueous phase during the preparation of tea. Lesser amounts of benzoylecgonine and ecgonine methyl ester also were transferred to the tea during preparation. In addition, trace amounts of the *trans*-isomer of cinnamoylcocaine were identified in the contents of the coca tea bags and were transferred to the infusion during the tea making process.

Other studies have identified various alkaloids in coca leaves. Engelke and Gentner [7] quantitated the amount of cocaine in coca tea by gas chromatography. They determined that when the coca tea was prepared according to the labeling instructions, the average amount of cocaine present was 0.8 mg per g dry weight of coca leaf tissue [7]. This quantity was less than that determined in the present study. Differences in results are most likely due to differences in coca leaf, coca tea preparation and in the analytical procedures. In 1889, Liebermann [8] reported the isolation of cinnamoylcocaine from coca leaves and, later, Moore [9] identified the *cis*- and *trans*-isomers in illicit cocaine samples, illicit coca paste and authentic coca leaves. Identification was made by comparison of relative retention times of samples with standards

on a GC/FID. The compounds were also characterized by UV, IR, NMR and GC/MS. In these samples, the isomers were present in approximately equal quantities. Turner et al. [10], identified cocaine and the *cis*- and *trans*-isomers of cinnamoylcocaine in *Erythroxylum coca* leaves obtained from three different regions of Peru. However, in this study, they found that the relative proportions of the isomers of cinnamoylcocaine and the ratio of total cinnamoylcocaine to cocaine differed among samples from different regions. This suggested that ratios of different cocaine constituents could be used to identify samples from different geographic regions.

Benzoylecgonine has been found in Peruvian and Colombian coca leaves but not Javan [11, 12]. It has been suggested that the presence of benzoylecgonine may be an artifact, produced by hydrolysis of cocaine during the extraction process utilized in the analysis [13]. Our studies with deuterated cocaine indicated that cocaine was stable during the tea-making process and benzoylecgonine and ecgonine methyl ester were already present in the coca leaf. Ecgonine methyl ester [11] and tropacocaine [14,15] have also been identified in coca leaves from a variety of regions. The former in Javan, and the latter in both Javan and Peruvian coca leaves.

In the present study, nicotine was not identified. However, the alkaloid was identified by Fikenscher [16] in young plants and adult roots and stems of commercial Javan coca (*E. novogranatense* var. *novogranatense*) utilizing thin layer chromtography and color reactions. Rivier [17] repeated the work of Fikenscher, using *Erythroxylum coca* leaves, but did not identify nicotine when the leaves were analyzed by GC/MS in the selective ion monitoring mode.

Coca tea is regularly consumed by the people of some countries in South America. In addition, travellers to these countries may purchase the tea and return with it to their native countries. The National Enterprise Institute of Peru (ENACO), which sells coca tea, estimated that between 1984 and 1989, over 22 million coca tea bags were sold in Peru [18]. In a single year, 1990, according to estimates, ENACO sold approximately 5.7 million bags of coca tea, and over one-half million of these bags were purchased by American tourists [18]. These figures illustrate that a large number of American residents may be consuming coca tea. This study has shown that consumption of one cup of coca tea results in detectable concentrations of cocaine metabolites in the urine for at least 20 h. Therefore, coca tea drinkers may test positive in a urine drug test for cocaine. At least one case has been documented [19] in which a South American woman failed a pre-employment drug test in the United States due to use of coca tea following an operation. Therefore, it is important that health officials and the general public be aware that consumption of coca tea may result in production of a positive urine drug test for cocaine metabolites.

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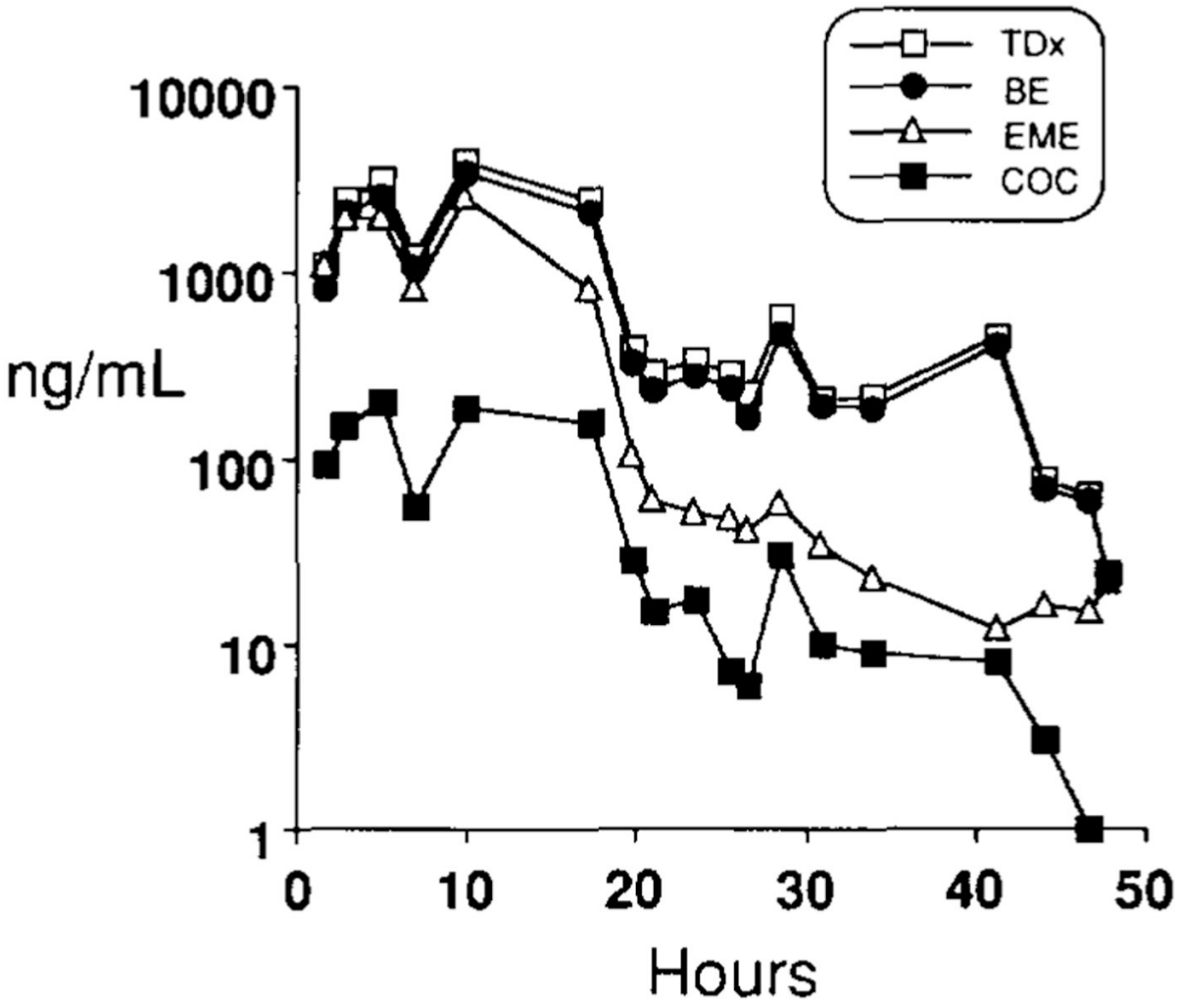

Fig. 1.
Urinary excretion of cocaine and metabolites by an individual after ingestion of one cup of Peruvian coca tea. TDx, benzoylecgonine (BE) equivalents by TDx assay for cocaine metabolite. BE, ecgonine methyl ester (EME) and cocaine (COC) were measured by GC/MS.

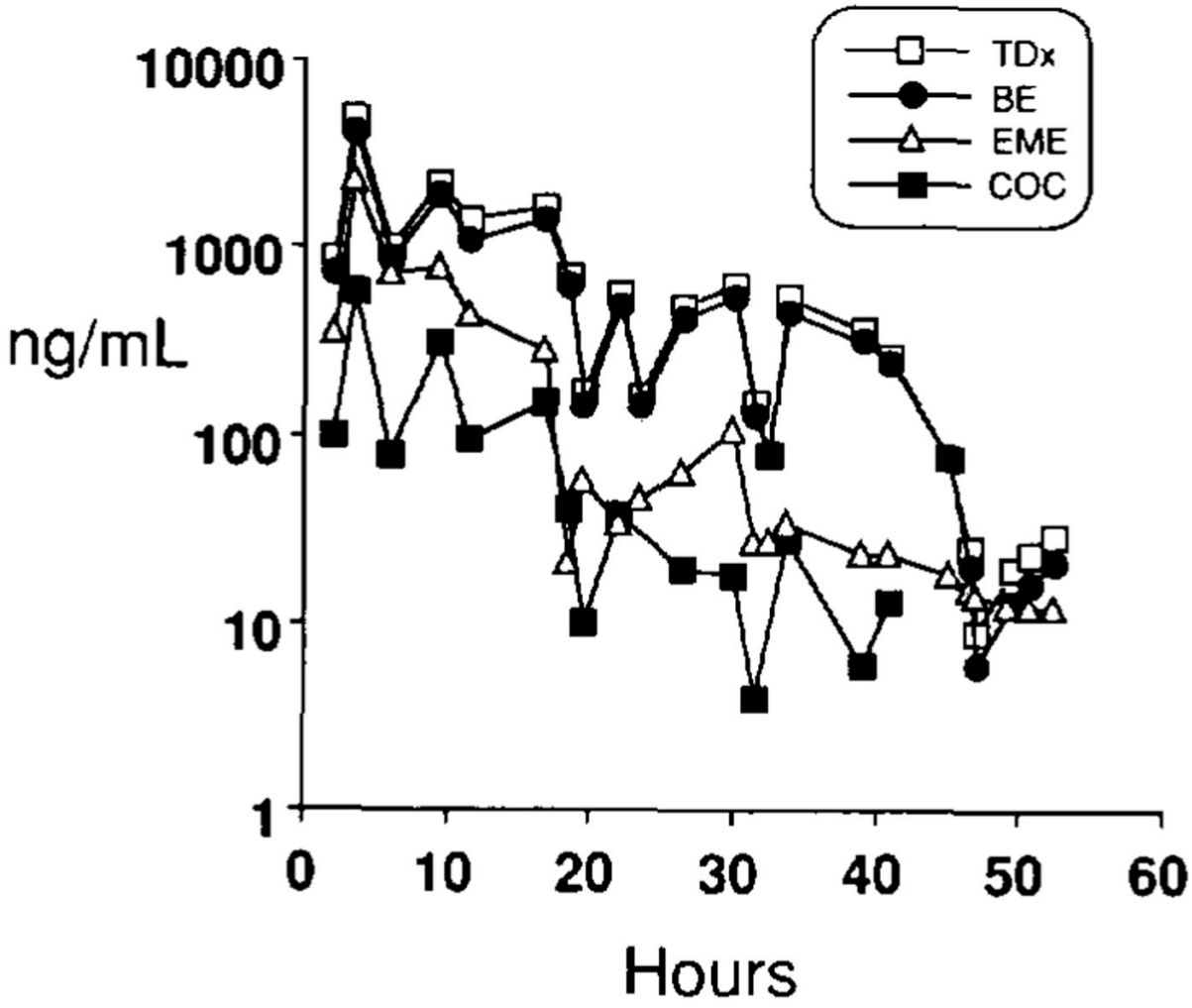

Fig. 2.
Urinary excretion of cocaine and metabolites by an individual after ingestion of one cup of Bolivian coca tea. TDx, benzoylecgonine (BE) equivalents by TDx assay for cocaine metabolite. BE, ecgonine methyl ester (EME) and cocaine (COC) were measured by GC/MS.

Table 1

Cocaine-related alkaloids in coca tea bags by Soxhlet and agitation extraction

Alkaloid	Extraction method (mg/tea bag)							
	Soxhlet				Agitation			
	Peru		Bolivia		Peru		Bolivia	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
COC	5.02	0.09	4.86	0	3.94	0.56	5.15	0.26
BE	0.06	0.05	0.12	0	0.05	0.05	0.11	0
EME	1.15	0	2.93	0	0.88	0.11	2.12	0.07
t-CINNCOC	Trace	—	0.16	0	Trace	—	0.15	0.02
AEME[a]	0.36	0.01	1.26	0	0.02	0	0.12	0

1st = first extraction (0–24 h); 2nd = second extraction (24–48 h).

COC, cocaine: BE, benzoylecgonine; EME, ecgonine methyl ester; t-CINNCOC, *trans*-cinnamoylcocaine; AEME, anhydroecgonine methyl ester.

[a]The presence of this material appears to be due primarily to thermal decomposition of cocaine during the extraction process.

Table 2

Alkaloids in coca tea prepared with a single tea bag and measured by GC/MS

Alkaloid	Amount (mg)[a]		N
	Mean	Range	
Peruvian coca tea			
Cocaine	4.14	3.40–4.76	13
Benzoylecgonine	0.50	0.41–0.63	11
Ecgonine methyl ester	1.15	0.94–1.33	13
trans-Cinnamoylcocaine	0.07	0.05–0.08	6
Anhydroecgonine methyl ester	0.01	0.00–0.02	13
Bolivian coca tea			
Cocaine	4.29	4.09–4.49	2
Benzoylecgonine	0.26	0.17–0.35	2
Ecgonine methyl ester	1.81	1.29–2.33	2
trans-Cinnamoylcocaine	0.12	—	1
Anhydroecgonine methyl ester	0.07	0.06–0.08	2

[a] mg per 180 ml coca tea utilizing one tea bag.

N = number of tea preparations analyzed.

Table 3

Effect of steeping time on amount of coca alkaloids extracted from coca tea bags (mg/tea bag)

Alkaloid	Steeping time (min)				
	3	**6**	**9**	**12**	**15**
Peruvian coca tea					
Cocaine	3.94	4.89	5.29	5.71	5.88
Benzoylecgonine	0.68	0.79	0.87	0.90	0.89
Ecgonine methyl ester	1.81	2.18	2.20	2.26	2.34
Bolivian coca tea					
Cocaine	4.46	5.24	5.34	5.51	5.02
Benzoylecgonine	0.17	0.17	0.19	0.19	0.19
Ecgonine methyl ester	2.32	2.58	2.53	2.55	2.51

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