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Metabolism of Cathinone to *d*-Norpseudoephedrine in Humans

A. N. GUANTAI and C. K. MAITAI ˣ

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Abstract □ Cathinone, a potent psychostimulant isolated from young shoots of *Catha edulis* was given to four human volunteers. Examination of urine collected from the volunteers at predetermined intervals showed the presence of unchanged cathinone, *d*-norpseudoephedrine, and two unidentified basic substances. The observed biotransformation of cathinone to the less potent psychostimulant, *d*-norpseudoephedrine involves reduction of a ketone group to alcohol, a common metabolic pathway in humans.

Keyphrases □ Cathinone—metabolism to *d*-norpseudoephedrine in humans □ *Catha edulis*—metabolism in humans

Catha edulis Forsk grows well in several countries of Eastern, Central, and Southern Africa. Because of the psychostimulant and mental dependence associated with habitual chewing of young shoots of this plant, it has been investigated extensively. Although chemical investigation of *C. edulis* goes back to the 19th century, the most significant result was obtained by Wolfes in 1930 (1) when he isolated and identified *d*-norpseudoephedrine, a CNS stimulant with amphetamine-like properties. There was some controversy as to whether the amount of *d*-norpseudoephedrine present in *Catha* material could account wholly for the observed psychostimulant effect, thus providing impetus for further chemical investigation (2). Several years later, a second phenylalkylamine closely related to *d*-norpseudoephedrine was isolated and named "cathinone" (3). This compound is a much more potent psychostimulant than *d*-norpseudoephedrine, and has attracted much attention as a potential drug of abuse (4).

The excretion of *d*-norpseudoephedrine in human urine has been investigated (5). In preliminary investigations involving known habitual chewers of *C. edulis*, *d*-norpseudoephedrine was shown to be the major compound present in urine when it was collected several hours after chewing *C. edulis*. Since cathinone is a major constituent of the young shoots of *C. edulis*, it seemed odd that it was absent or present only in trace amounts in several urine samples examined. A literature survey showed that the fate of cathinone in humans has not been investigated and accordingly this work was undertaken.

EXPERIMENTAL

Collection of Urine Samples—Four volunteers who had never chewed the material before participated in the experiment. Two volunteers were given 16 mg of cathinone extracted from young shoots of *C. edula* by preparatory TLC (3, 6), while the other two were given 16 mg of synthetic cathinone. All four volunteers had only milk for breakfast. Urine samples were collected at the following intervals: 0 (control), 0–4, 4–8, 8–12, 12–15, and 15–24 hr. Each urine sample was examined for the presence of cathinone and related substances as described below.

Examination of Urine for Cathinone, *d*-Norpseudoephedrine, and Related Basic Substances— Approximately 40 ml of urine was taken, 2–3 ml of strong lead acetate solution (7) was added, and after thorough mixing the precipitated proteins were removed by centrifugation. The supernatant was acidified to pH 5–6 with 0.1 *N* sulfuric acid and any precipitate removed by centrifugation. The acidified urine sample was extracted with chloroform, four times to remove acids and neutrals. The aqueous urine portion was then alkalinized (pH 9) using dilute ammonia solution and extracted with 4 × 100 ml of chloroform. The combined chloroform was distilled off at 40° using a rotary evaporator and the residue examined by TLC.

Examination of residue for *d*-norpseudoephedrine, cathinone, and related basic substances using TLC was carried out as follows. The residues were taken up in 2 ml of chloroform and ~10 μl was spotted on TLC plates coated with silica gel[1], and activated at 100° for 1 hr. Cathinone and *d*-norpseudoephedrine were spotted on the same TLC plates as the basic residues from urine. The plates were developed in either of the following solvent systems: (*A*) ethyl acetate–methanol–ammonia (17:2:1) or (*B*) cyclohexane–chloroform–diethylamine (5:4:1). Usually the plates were developed for 35–45 min. After development the plates were examined under UV light, then sprayed with 0.3% ninhydrin solution (8) after which they were heated at 105° for 10 min. The intensity of fluorescence under UV light and that of color after spraying with ninhydrin solution were judged on a 3-point scale as shown in the results. Where cathinone or *d*-norpseudoephedrine (or both) was not detected, the chloroform basic residue was concentrated almost to dryness and the experiment repeated.

The presence of *d*-norpseudoephedrine and cathinone in the basic residue extracted from urine was also investigated with a gas–liquid chromatograph[2] equipped with a flame ionization detector. The GLC column used was glass, 4-mm i.d. × 1.5 m long, packed with nonacid washed Chromosorb W (100–200 mesh)[3] coated with 1% polyethylene glycol 20 *M* (carbowax 20 *M*)[4]. The column was conditioned for 8–10 hr at 230°. The experimental operating conditions were as follows: hydrogen pressure, 1.4 kg/cm^2; condensed air pressure, 0.65 kg/cm^2; nitrogen flow rate 30 ml/min; column temperature programmed from 80–200° at 5°/min and finally left at 200° for 10 min; chart speed, 1 cm/2 min. The retention times and peaks were recorded[5].

For quantitative analysis, the areas under the curves were measured by the method of triangulation (9) and compared with areas obtained with standard solutions of cathinone and *d*-norpseudoephedrine. The standard solutions were prepared by dissolving 5 mg of either cathinone or *d*-norpseudoephedrine in 40 ml of control urine and subjecting this to the same extraction procedure as the experimental urine. It was then assumed that the percentage recovery of both compounds from experi-

[1] GF254, Merck & Co.
[2] Pye Unicam Series 104.
[3] Sigma Chemical Co.
[4] British Drug House.
[5] Pye Unicam linear recorder, type AR55.

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Table I—TLC Examination of Basic Residue from Urine of Volunteers who had Ingested Cathinone[a]

Urine Sample	Collection Time, hr[b]	Substance Detected Cathinone	Substance Detected *d*-norpseudoephedrine
1	0 (control)	none	none
2	0–4	++[c]	++
3	4–8	++	++
4	8–12	+[d]	++
5	12–15	0[e]	+
6	15–24	0	+

[a] Two unidentified substances in trace amount, associated with cathinone were observed in samples 2, 3, 4 and 5. The R_f values of unidentified substances in solvent system *A* were 0.71 and 0.85. [b] After ingestion of cathinone. [c] ++ = Easily detected. [d] + = Trace amount. [e] 0 = not detected.

mental and control urine was approximately the same. Control experiments had shown recovery of >90% under the standardized conditions.

RESULTS

Both cathinone and *d*-norpseudoephedrine separated well in solvent systems *A* and *B*, the R_f values being as follows: *A*-cathinone 0.55, *d*-norpseudoephedrine 0.42; *B*-cathinone 0.38, *d*-norpseudoephedrine 0.47. Results of TLC are summarized in Table I. Results of GLC were in agreement with those obtained with TLC. With GLC only one *d*-norpseudoephedrine peak was observed at 165° (retention time 7.5 min) while the major cathinone peak was observed at 151° (retention time 5.1 min). Another peak associated with cathinone appeared at 145° (retention time 3.7 min). The results were fairly reproducible, and it was possible to calculate the amount of cathinone and *d*-norpseudoephedrine excreted in the urine. There was no significant variation in the excretion pattern of either cathinone or *d*-norpseudoephedrine in the four volunteers even when the experiment was repeated after 4 weeks. The amount of cathinone and *d*-norpseudoephedrine recovered from the urine of each of the volunteers agreed to within 20% and the results are summarized in Table II.

DISCUSSION

Results obtained in the present study show that some of the cathinone ingested by humans is metabolized to *d*-norpseudoephedrine and possibly two other unidentified metabolites. Metabolism of cathinone to *d*-norpseudoephedrine involves reduction of a ketone group to alcohol, a fairly common metabolic pathway in humans, catalyzed by liver microsomal enzymes. In several drugs (*e.g.*, cortisone and warfarin) reduction of a ketone or aldehyde to an alcohol is often associated with significant change in potency. Usually both the parent drug and the metabolite will contribute to the biological activity, the net result often being determined by such pharmacokinetic parameters as the rate of elimination. The finding that little, if any cathinone is excreted in human urine after 15 hr, may be important in forensic toxicology since *d*-norpseudoephedrine, also present in *C. edulis*, is known to be excreted over a much longer period.

Table II—Amount of Cathinone and *d*-Norpseudoephedrine Excreted in Human Urine (Result of GLC)

Urine Sample(s)	Cathinone Recovered, mg	*d*-norpseudoephedrine Recovered, mg
2 (0–4 hr after ingestion of cathinone)	0.72 ± 0.12 (4.4% of amount ingested)	3.2 ± 0.3 (equivalent to 20% ingested cathinone)
3, 4, 5, 6 (4–24 hr after ingestion of cathinone)	1.04 ± 0.08 (6.5% of amount ingested)	9.9 ± 0.6 (equivalent to 61.9% ingested cathinone)
2, 3, 4, 5, 6 (0–24 hr)	1.765 (11% of amount ingested)	13.12 (equivalent to 81.9% ingested cathinone)

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Nonparametric Pharmacokinetic Calculations: One-Compartment Open Model

WILLIAM H. SHELVER ˣ and FRED F. FARRIS

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Abstract □ A nonparametric method suitable for estimation of parameters in nonlinear problems was developed for one-compartment pharmacokinetic data. The method was tested by running 500 simulations with various types of error and comparing the results with a standard nonlinear regression computation. The nonparametric method was superior to nonlinear regression techniques if the assumptions for the error structure of the regression were not true.

Keyphrases □ One-compartment pharmacokinetic model—nonparametric method, comparison with standard nonlinear regression procedure, estimation of parameter

Calculating the parameters of a pharmacokinetic model containing more than one exponential term requires the use of either the method of residuals (sometimes referred to as stripping or feathering) or one of various nonlinear regression techniques. As they have been applied classically, both general techniques have proven somewhat

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