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Excretion of the Active Principle of *Catha edulis* (Miraa) in Human Urine

C. K. MAITAI *x and G. M. MUGERA ‡

Abstract □ *d*-Norpseudoephedrine, a central stimulant present in *Catha edulis*, is excreted unchanged in human urine. The alkaloid was detected in urine 30–50 min after ingestion of synthetic *d*-norpseudoephedrine, and trace amounts of the drug could still be detected 24 hr later. Approximately 40% of ingested *d*-norpseudoephedrine was recovered in urine in the first 6 hr.

Keyphrases □ *Catha edulis*—urinary excretion of *d*-norpseudoephedrine after mastication, humans □ *d*-Norpseudoephedrine—urinary excretion after mastication of *Catha edulis*, humans □ CNS stimulants—urinary excretion of *d*-norpseudoephedrine after mastication of *Catha edulis*, humans

Catha edulis Forsk. (Celastraceae) is an evergreen shrub, which usually is about 3–7 m tall but can grow to a height of 18 m under favorable climatic and soil conditions. It is found in the Eastern part of Africa, from Ethiopia to South Africa, and in Arabia. In Kenya, the plant is cultivated on a commercial scale on the slopes of Mount Kenya in the Nyambeni Division of the Meru District. The shoots of this plant are masticated while fresh to combat mental fatigue, allay hunger, and generally induce euphoria (1). The earliest recorded direct reference to the use of *Catha* was in a 1237 (2) prescription for relief of depression. Alles *et al.* (3) showed that the central stimulant activity of *Catha* can be attributed wholly to its *d*-norpseudoephedrine content.

Information gathered from those who masticate *Catha* indicated that the effect on the central nervous system is biphasic, *i.e.* initial stimulation followed by a compensatory phase of depression (4). It was reported that it is necessary to chew the material continuously for sustained mental stimulation, an effect that could be interpreted to mean that the material is rapidly eliminated from the body. A literature survey has not revealed any study of the elimination of *d*-norpseudoephedrine from the human body and, accordingly, this aspect was investigated. Since most phenylalkylamine derivatives are eliminated through the renal route (5), the present study was restricted to investigation of the presence of *d*-norpseudoephedrine and metabolites in urine.

EXPERIMENTAL

Each of four human volunteers was requested to masticate three bundles of *Catha* material at a rate of about two bundles per hour and to submit samples of urine at predetermined intervals. The average weight of a fresh bundle of *Catha* material was 80.6 g, of which 70–80% was ingested; the rest, a fibrous residue, was discarded. The volunteers had only drunk milk in the morning and were requested to give samples of urine just before the experiment and thereafter at the following intervals: 0.5–1, 1–2, 2–4, 6–8, 8–12, 12–15, and 15–24 hr. No food or beverage was consumed in the first 6 hr of the experiment, but the volunteers were encouraged to drink water freely so as to promote frequent voiding of urine.

Qualitative examination of urine for *d*-norpseudoephedrine and metabolites was carried out as follows. To each urine sample (usually 20–50 ml) was added 1–2 ml of saturated lead acetate solution and, after thorough mixing, centrifugation was performed to remove any precipitate. The urine was acidified with 0.1 *N* sulfuric acid, and any precipitate of lead sulfate was removed by centrifugation. The acidic supernatant liquid was extracted with an equal volume of ether three times to remove organic acids and neutrals.

The aqueous phase (urine) was made alkaline with 2 *N* sodium hydroxide and then saturated with sodium chloride and extracted with an equal volume of ether for 8 hr. Preliminary work had shown that the "salting-out" process with sodium chloride improves the percentage recovery of *d*-norpseudoephedrine from urine considerably. This extraction was repeated three times, and the combined ether extract was washed twice with 2 ml of 2.5% sodium bicarbonate solution and dried with anhydrous sodium sulfate. The ether was then distilled off, and the basic residue was examined by TLC and GLC techniques.

This experiment was repeated using synthetic *d*-norpseudoephedrine. Approximately 30 mg was accurately weighed, dissolved in a small volume of water, and given to each of four human volunteers with urine samples collected as described previously.

Examination of residues for *d*-norpseudoephedrine and metabolites using TLC was carried out as follows. Residues were taken up in 2 ml of ether, and approximately 2–5 μl was spotted on a TLC plate coated with silica gel. Approximately 5 μl of the following reference compounds was also spotted on the same plate as the residue: *l*-ephedrine, *d*-pseudoephedrine, and *d*-norpseudoephedrine. Plates were developed in one of the following solvent systems: A, butanol–acetic acid–water (60:15:25); B, butanol saturated with water (upper phase); C, methanol–ammonia (100:1.5); or D, isopropanol–water–ammonia (80:15:5). The spots were revealed by ex-

Table I—R_f Value of Basic Residue Isolated from Urine of Persons Who Had Consumed *C. edulis* Compared to that of Closely Related Compounds

Compound	Solvent			
	A	B	C	D
d-Norpseudoephedrine	0.61	0.31	0.47	0.80
Basic residue from urine	0.61	0.31	0.47	0.80
l-Ephedrine	0.53	0.26	0.39	0.75
d-Pseudoephedrine	0.53	0.26	0.39	0.75

posing the plates to iodine vapor and, after warming to remove iodine, the plates were sprayed with either ninhydrin or iodoplatinate reagents (6).

The basic residue from urine was also examined with a gas chromatograph[1] equipped with a flame-ionization detector. The GLC column used was stainless steel, 0.3 cm (0.125 in.) o.d, 1.5 m in length, and packed with Celite CQ (100–120 mesh) coated with 3% methyl silicone gum (OV-1[2]). The column was conditioned for 12 hr under the following operating conditions: oven temperature, 150°; detector temperature, 150°; hydrogen pressure, 17 psi; air pressure, 12 psi; and nitrogen flow rate, 50 ml/min.

In the quantitative estimation of the alkaloid recovered from urine, the residue was dissolved in ether and diluted to 2 ml in a standard flask. A fixed volume, usually 5 μl, was injected into the column using a 10-μl syringe[3]. The amount of compound present was calculated from the area under the curve by comparison with the area of a standard amount of *d*-norpseudoephedrine subjected to a similar extraction procedure. Preliminary experiments were done to establish the percentage recovery of *d*-norpseudoephedrine from urine under specified conditions.

RESULTS

Only one compound was detected in urine from humans who had consumed *Catha* material or synthetic *d*-norpseudoephedrine, and this compound had the same R_f value (TLC) as reference *d*-norpseudoephedrine in all solvent systems used (Table I). Similarly, the retention time (GLC) for the basic compound isolated from urine was the same as that for *d*-norpseudoephedrine (Fig. 1). This basic compound was first detected in urine 30–50 min after ingestion of *d*-norpseudoephedrine, and trace amounts could still be detected 24 hr later when the experiment was discontinued.

The percentage recovery of *d*-norpseudoephedrine from control urine was 84.6 (6.2 *SD*). Since the amount of drug excreted in the urine within any specified interval was very small, it was necessary to combine two or three residue fractions for the purpose of quantitative estimation of the drug in urine with GLC. The peaks obtained when *d*-norpseudoephedrine was injected into the column were symmetrical and reproducible to within 10% for each urine sample. Approximately 40% of *d*-norpseudoephedrine was recovered in urine within the first 6 hr. The percentage of *d*-norpseudoephedrine excreted in urine varied considerably between the four individuals and even in the same individual at different times, but the values were always within the range of 30–40%.

Hydrolysis of urine (acidified with 2 *N* hydrochloric acid) by heating under reflux in a boiling water bath for 1 hr before extraction did not alter the results, an indication that conjugation is not an important metabolic pathway for *d*-norpseudoephedrine.

DISCUSSION

Since the basic residue recovered from urine of humans who consumed *Catha* material had the same R_f value as *d*-norpseudoephedrine in four different solvent systems and had the same retention time (GLC), it is logical to assume that the two compounds are identical.

d-Norpseudoephedrine is a polar basic compound; consequently, a large percentage of the free base would not be reabsorbed from the glomerular filtrate. This expectation is consistent with results obtained in the present work in which the drug was detected in urine 30–50 min after oral administration. Elimination of many drugs from the body follows a first-order kinetic rate; therefore, even though the drug might appear in urine soon after administration, the elimination process often continues for a long period. Research work has shown that amines of the ephedrine series are excreted unchanged (7). Excretion of drugs in urine after oral administration depends on the time course of absorption and distribution. In addition, urinary pH and output might influence the quantitative excretion of the drug. Since both urinary pH and output fluctuate considerably throughout the day, the amount of drug excreted also can be expected to fluctuate, even in the same individual.

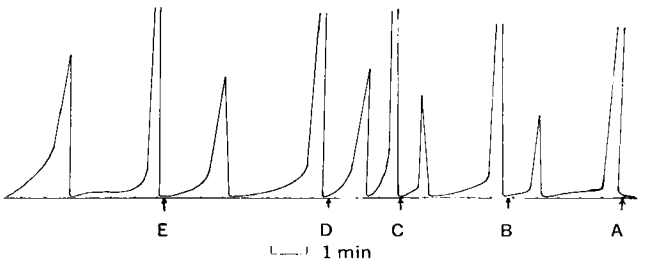

Figure 1—*Retention time for: A, basic residue from urine (4.1); B,* d-*norpseudoephedrine (4.1); C, amphetamine (1.6); D,* l-*ephedrine (4.7); and E,* d-*pseudoephedrine (4.7).*

Central stimulation by amphetamine-like substances is invariably accompanied by a compensatory phase of depression, but the exact mechanism by which central stimulation gives way to depression is not understood (8). Information received from people who chew *Catha* material indicates that the onset of mental fatigue sometimes occurs very suddenly. Continuous ingestion of the drug should forestall the onset of central depression, and this is apparently what happens in people who masticate *C. edulis* continuously, sometimes for more than 12 hr.

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‡ To whom inquiries should be directed.

[1] Pye Unicam gas chromatograph P.S. 104.
[2] Supplied by Pye Unicam Ltd., Cambridge CBI 2PX, England.
[3] Hamilton.