

Analysis of Cathinone and Cathine in Urine Sample of Khat Chewer Presenting with Hemorrhagic Stroke

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Abstract

Khat contains cathinone and cathine, which are known to be associated with myocardial infarction and cerebral hemorrhage. There is limited data showing a relationship between khat chewing and hemorrhagic stroke. In Jazan region, the most of stimulant toxicity cases were toxicated by both khat and amphetamine. This report represents a khat chewer whose toxicity was only suspected to be from khat chewing. The levels of cathine and cathinone in the blood and urine samples of a khat chewer were determined. The method of extraction and analysis were discussed. The results of initial analysis were negative for amphetamine, cocaine, opiate, barbiturate and tricyclic antidepressant. The urine initial analysis results were positive for amphetamine like substance. On further investigation, the confirmatory analysis by liquid chromatography-tandem mass spectrometry LC-MS/MS in urine were detected and quantified cathine and cathinone.

Introduction

Khat chewing is high prevalent habit in Jazan region^[1,2]. Khat contains cathinone and cathine, which are known to be associated with myocardial infarction and cerebral hemorrhage^[3,4]. It's also reported to cause vasoconstriction and thrombogenicity^[4-6]. Generally, khat is stimulating the release of dopamine and adrenaline, which are mediated its sympathomimetic effects^[7]. These effects proportional to cathinone blood levels, which rise within 60 minutes and peak at 90 to 210 minutes after khat chewing, which is increase the cardiac oxygen demand and platelet aggregation^[5,8,9]. In addition, khat extract is increased oxidative stress and apoptosis in cardiomyocytes, which peaks at 48 hours post khat extract exposure^[8]. However, there is limited data showing a relationship between khat chewing and hemorrhagic stroke. In Jazan region, the rate of khat toxicity were the most frequent cases among stimulant toxicities according a report from Poison Control and Medical Forensic Chemistry Center in Jazan, and most of these cases were toxicated by both khat and amphetamine. This report represents a khat chewer whose toxicity was only suspected to be from khat chewing. Therefore, the levels of cathine and cathinone in urine sample of a khat chewer were determined. The method of extraction and analysis were discussed.

Case Report

A 35-year-old male was admitted to hospital with initial symptoms of left limb weakness, loss of consciousness. He was diagnosed as hemorrhagic stroke with hypertension and started on symptomatic and supportive treatment. Lab analysis of serum biochemistry showed a normal renal and liver function tests. His symptoms were gradually improved and upon the review of previous history, this patient had never been diagnosed for chronic diseases and he was chewing Khat on a daily basis. The toxicological sample was sent to poison control center after 2 days of admission. His general toxicological screening

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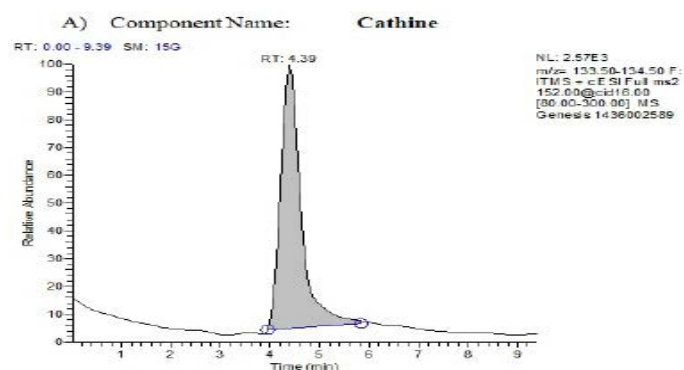
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results were negative for amphetamine, cocaine, opiate, barbiturate and tricyclic antidepressant in blood sample and positive for amphetamine like substance in urine sample (Table 1). On further investigation, the confirmatory analysis by liquid chromatography-tandem mass spectrometry LC-MS/MS in urine were detected and quantified cathine and cathinone (Figure 1).

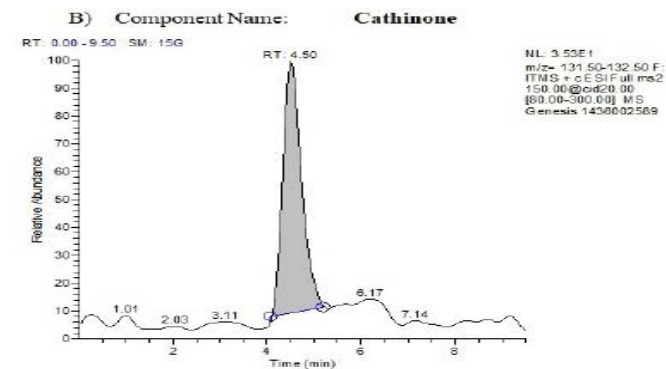
Table 1: Immunoassay results for patient blood and urine samples.

Assay	Results ng/ml
Amphetamines	< 100
Barbiturates	< 25
Cocaine	< 40
Opiates	1.3
Tricyclic antidepressants	< 0.0



Details

Component Name: Cathine
 Actual RT(min): 4.4
 Calculated Amount: 2346.18237



Details

Component Name: Cathinone
 Actual RT(min): 4.5
 Calculated Amount: 14.31188

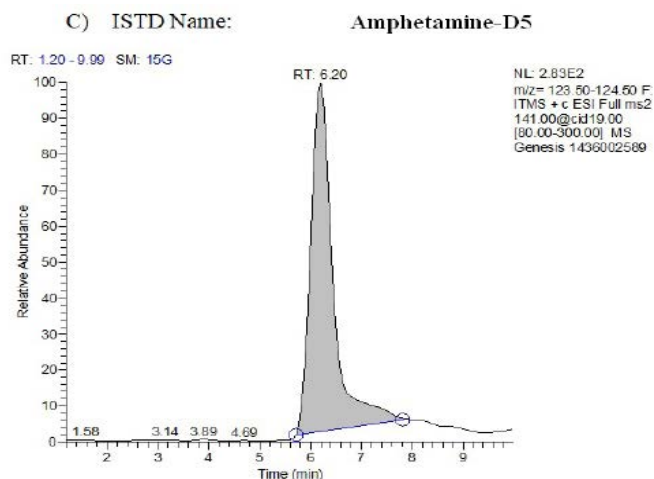


Figure 1: LCMS chromatograms (A) Cathine, (B) Cathinone, (C) Amphetamine-D5

Materials and Methods

Chemicals and Reagents

Dichloromethane, Isopropyl alcohol, ammonium hydroxide, methanol, acetic acid and acetonitrile were purchased from Sigma Aldrich (Germany). All chemicals were HPLC grade. De-ionized (DI) water prepared from Millipore purification system. SPE cartridges (CSDAU203) were purchased from united chemicals technologies (Philadelphia, USA).

Samples Preparation

Samples were extracted by solid phase extraction method using SPE cartridge^[10]. Briefly, the SPE cartridge was conditioned with 3 ml methanol, 3 ml DI water and 1 ml phosphate buffer (pH 6). Then, 1 ml of urine sample mixed with 1 ml phosphate buffer was loaded and allowed to elute on gravity. The SPE cartridge was cleaned with 3 ml DI, 1 ml 0.1M acetic acid and 3 ml methanol, allowed to dry under air stream for 5 min and finally the compounds were eluted into clean 16 ml glass tube using 3 ml dichloromethane, isopropyl alcohol and ammonium hydroxide mixture (87:20:2). The elution solvent was evaporated under nitrogen stream at < 40 °C and the residues were reconstituted by 150 µl of mobile phase (80% ammonium formate buffer 0.1 M with 1% formic acid and 20% acetonitrile with 1% formic acid).

Liquid Chromatography-mass spectrometry (LC-MS/MS) analysis

The analysis was carried out using LC-MS/MS system consisted of a LCQ Fleet Single quadrupole Ion Trap Mass spectrometer (Thermo Scientific) equipped with Thermo Finnigan Surveyor MS Pump and Thermo Finnigan Surveyor Autosampler.

The samples were then analyzed by LC-MS/MS (LCQ Fleet, Thermo Scientific) using the method described by Syam Mohan, et al, 2016^[8]. Briefly, 10 µl of the sample was injected and the analytes were separated on a Hypersil GOLD column (150 × 3 mm i.d.: 5µm, Thermo Scientific, USA). The compounds were eluted by isocratic mobile phase made from 85% of 10 mmol ammonium formate buffer and 15% of 0.1% formic acid in acetonitrile (B). The run time was 7 minutes with a flow

rate of 0.3 ml/minute.

After chromatographic separation, cathine, cathinone and internal standard (amphetamine d5) reached the Electro-spray Ionization (ESI) interface they were positively charged. The ESI conditions were 5 kV spray voltage, 275 °C capillary temperature, 50 capillary voltage, 110 tube voltage and 30 arb flow rate of nitrogen sheath gas. The analysis was performed in the scanning mode, monitoring the following transitions: m/z 150 → 150 and m/z 150 → 132 for cathinone, m/z 152 → 152 and m/z 152 → 134 for cathine and m/z 141 → 124 for amphetamine d5. Helium gas was used as fragmentation gas in the Collision-induced decompositions (CID). The CID value was 17 for cathine, 19 for cathinone and 20 for amphetamine d5.

Discussion

The toxic effects of khat chewing usually occurs within 20 minutes after chewing and persist for several hours after chewing cessation and ranged from central stimulation, mild increases in blood pressure, heart rate, respirations, and temperature, to severely dysrhythmias, myocardial ischemia and pulmonary edema^[11,12]. Indeed, these toxic effects may occur with normal use, particularly in prolonged use, in predisposed persons, elderly, and during exercise^[11,13,14].

Admassie and Engidawork (2011) demonstrated that acute high dose of khat produced a significant increase in blood pressure at 2 and 3 h post-dosing, which is related to the duration of action of cathinone^[15]. While, chronic and heavy khat chewing can result in increased the risk of myocardial infarction^[16].

In this case report, the serum concentration of cathine was more than hundred times than cathinone, indicating that symptoms are most likely due to cathine. In this regard, khat has been shown to cause nervousness, emotional instability and irritability within 2 hours after khat chewing, followed by decreased the alertness and loss of consciousness among khat chewers^[17]. Benois et al, (2009) showed that 10 of 16 patients with hemorrhagic stroke were chewing khat on a daily basis and they suggest that khat chewing were of the major risk factors for hemorrhagic stroke^[18].

In addition, a case report of young man, who was chewing khat on a daily basis, presented with left limb weakness and diagnosed as stroke has also been reported^[19]. This patient was recovered after khat cessation with no further complications during the follow up, suggesting that khat chewing was the cause of stroke. Moreover, postmortem toxicological analysis of cases suspected to die due to drug overdose demonstrated that death was attributed to intracranial hemorrhage and cardiac arrest, which may due to khat intoxication^[20,21]. These effects may be due to the release of dopamine and adrenaline, which are result in vasoconstriction and thrombogenicity^[4-7]. In addition to stimulate release of dopamine and norepinephrine, cathinone is also reported to exhibit MAO inhibition, resulting additional accumulation of dopamine and norepinephrine in the brain and other organs^[22-25]. In this regard, the administration of dopamine receptor blockers, such as haloperidol and chlorpromazine, or dopamine or norepinephrine uptake inhibitors, such as bupropion and desipramine, or dopamine and norepinephrine synthesis inhibitors, such as alpha-methyltyrosine, antagonized the khat effects^[26,27].

The present case report has indicated that hemorrhagic stroke may be attributed to Khat chewing. Therefore, it is advisable to used confirmatory analysis such as LC-MS/MS in toxicological investigation for patient with hemorrhagic stroke to confirm the present of cathinone and cathine and to exclude other suspected amphetamines in khat chewers.

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