Abstract

Chewing khat leaves has been associated with several adverse health effects, and there are very few case reports of cardiotoxicity, stroke and death resulting from this. In addition, postmortem distribution of cathine and cathinone, active components of khat, are not yet fully clear. This postmortem case report aimed to identify and determine the concentration of cathine and cathinone in different body organs and green chewed plants found in the mouth of the deceased. Immunoassay and non-targeted GC-MS analysis showed that samples were only positive for amphetamine type stimulants. LC-MS/MS quantitative analysis confirmed that samples were positive for cathinone and cathine. The results showed that cathinone concentration was 0.03, 0.03, 0.06, 0.07, 1.85 and 31 μg/ml in brain, liver, blood, vitreous humor, stomach and chewed green plant, respectively. Whereas, the concentration of cathine was 0.31, 3.28, and 141 μg/ml in kidney, stomach and chewed green plant, respectively. Cathine and cathinone concentrations were found to be changed with respect to site of sampling. The results suggest that stomach and chewed green plants are considered as good samples to show the concentration for both cathine and cathinone at the time of death of the khat chewer.

Keywords: Forensic Sciences, Khat, Cathine, Cathinone, Postmortem Distribution.
1. Introduction

Khat (Catha edulis) is a plant containing alkaloid compounds (cathine and cathinone) that are structurally related to amphetamine with similar effects. In the past, khat was used in the form of a tea obtained by boiling 5-15 g of the dried leaves in one liter of water [1]. Nowadays, the most common method of khat intake is by chewing fresh small young leaves of the plant. Khat leaves are chewed continuously and kept in the cheek for several hours, known as khat storage. The juice produced from khat chewing is swallowed while the khat residue is later spat out [2, 3]. Chewing khat leaves is becoming a habit in the Arabian Peninsula [4]. It is commonly used for its effects on mental alertness, as a physical stimulant, and to induce euphoria [5]. However, the khat plant is prohibited according to the list of psychotropic substances issued by the Saudi Food and Drugs Authority (SFDA) [6].

There is evidence that the habit of khat chewing in the Jazan region of Saudi Arabia is increasing among young people [7]. Reports show that 37.7% of college and high school student males in Jazan chewed khat [8]. Another study examined the reasons for Khat chewing, showing that the main reasons for chewing khat were to improve mental function, increase physical activity, euphoria and enhance orgasms [9]. Several studies found that khat chewing is associated with several toxic effects. These include anorexia, hyperthermia, tremors, hypertension, increased heart rate and forced heart contraction, mydriasis, and urinary retention [5, 10-13]. In addition, there are a few case reports showing that khat chewing was associated with cardiotoxicity, stroke and death [14-15]. Furthermore, the continuous intake of khat predisposes users to acute myocardial infarction, arrhythmias, convulsions, schizophrenia and mania [11, 16-20]. These toxic effects are mainly attributed to cathine and cathinone in khat leaves [21].

Regarding dependence, khat chewing results in development of psychic dependence, whereas physical dependence does not occur [22, 23]. In addition, the continuous use of khat results in development of tolerance and often leads to an increase in the usual consumption of khat [24]. Khat chewers usually chew 50-200 g per day of fresh khat leaves [25]. The extracted cathinone and cathine from khat leaves by chewing are absorbed through oral and gastrointestinal mucosa [26]. The peak plasma levels of cathinone and cathine are reached after 2 and 3 hours, respectively, after starting chewing, [27, 28]. The elimination half-life of cathinone and cathine after khat chewing were found to be 1.5 ± 0.8 and 5.2 ± 3.4 hours, respectively [26]. The distribution of cathinone and cathine are not yet fully clear in ante-mortem and postmortem.

Pathologists and toxicologists are requested to present the concentration of the substances in the postmortem samples and they found that the concentration of these substances found in the postmortem samples were similar to that found at the time of death [29]. Generally, the concentration of substances varies between antemortem and postmortem [29]. This variation in the concentration of these substances between the time of death and the time of autopsy is affected by a major phenomenon called postmortem redistribution [29, 30]. This phenomenon is important in order to avoid the wrong interpretation of misleading toxicological results [29].

Postmortem redistribution is a process that happens to substances leading to an alteration in their concentration after death [31, 32]. This process is believed to be affected by two important factors. The first factor is the site of sampling, and the other is the time gap between the collection of samples and time of death [33, 34]. For confirmation and quantification, postmortem analysis of khat and its constituents needs more focus in order to show their redistribution after death. There is still not enough research regarding this issue. Therefore, in this postmortem case we analyse khat in order to investigate the redistribution of its constituents in different body organs and compare the results with that of the blood.

This paper reports a postmortem case whose death was suspected to be khat overdose. The deceased, a young
adult male in the fourth decade of age (30 to 39 years old), was discovered by the police within 24 hours of death and brought for autopsy. External examination showed no signs of violence, and the suspected cause of death was cardiac arrest. There was a green substance in his mouth. The post-mortem toxicological analysis was carried out 72 hours after the autopsy. The concentration of cathine and cathinone in various biological specimens were determined.

2. Materials and Methods

2.1 Sample Preparation

Three grams of each tissue sample (brain, liver, kidney and stomach) and the green chewed sample found in the mouth of the deceased were homogenized with 12 ml of deionized water. For immunoassay analysis, aliquots of blood, vitreous humor and homogenates were screened for drugs of abuse using Randox Evidence analyzer. In immunoassay analysis, all samples were given false-positive results for amphetamines as cathine and cathinone are known interferences of this test [35]. Test results for other drugs of abuse were negative for all samples.

For the extraction procedure for GCMS (non-targeted) analysis, samples were extracted by solid phase extraction (SPE) and analyzed by GC-MS as described before [36]. The combined elutes were then evaporated under nitrogen stream to dryness. Finally, the residues were reconstituted by 100 µl methanol, vortexed and placed in GC-MS vials for chromatographic analysis.

For confirmation by LCMS-MS, control and calibration samples were prepared from 1 mg/ml cathine, cathinone and amphetamine authentic standards spiked in negative kidney homogenate and urine samples to eliminate matrix effects [37]. Lipomed reference solutions were used for d-Cathine HCl (1mg free base/1ml methanol), d,l-Cathinone HCl (1mg free base/1ml(ACN/H2O: 1/1), d,l-Amphetamine H2SO4 (1mg free base/1ml methanol) and Amphetamine-D5 HCl (1mg free base/1ml methanol). Calibration levels were 50, 100, 250, 500, 750 and 1000 ng/ml. 1ml of blood, vitreous humor, homogenate samples, calibrators and control samples were extracted by solid phase extraction method after adding 200 µl of amphetamine-D5 as internal standard. The extracts were evaporated to dryness under nitrogen stream at < 40 °C and reconstituted in 150 µl of mobile phase [38].

2.2 Instrumental Analysis (GC-MS; LC-MS-MS)

For GC/MS analysis, all samples were conducted using single quadrupole Agilent Technologies GC-MS instrument model number 5977B. 2 µl of each sample was injected using a fully automated liquid sampler (ALS) into the injection port at 260 °C at splitless mode, and analysis was done according to the previously described method [36].

For LC/MS analysis, cathine, cathinone, amphetamine and amphetamine-D5 were detected, identified and quantified by the use of a LCQ fleet ion trap mass spectrometer (MS-MS) (Thermo Scientific) equipped with Surveyor LC pump and autosampler (37). Instruments were linked and controlled by Thermo Xcalibur® software. Liquid chromatography of compounds was carried out on Hypersil Gold C18 column (150 × 3 mm I.D; particle size, 5 µm by Thermo Scientific) at ambient temperature. Mobile phase consists of 0.1% formic acid in acetonitrile and 10M ammonium formate buffer with 0.1% formic acid (20:80 by volume). Mobile phase was delivered in isocratic mode at a flow rate of 0.3 ml/min. MS detector parameters were optimized by directly injecting the compounds to the MSD and an autotuning was performed for amphetamine and the tune file was saved to be used in the acquisition method. All compounds were positively charged [M+H]+ at LCMS interface using electro-spray ionization (ESI). Compounds were detected by LCMS-MS in full scan mode for m/z range 85 – 200. Collision induced dissociation of precursor m/z 152, 150, 136 & 141 to produce fragment ions of m/z 134, 132, 119 & 124 was used to identify and quantitate cathine, cathinone, amphetamine and the internal standard amphetamine-D5, respectively.
3. Results and Discussion

The habit of khat chewing has increased among young people in the Jazan region of Saudi Arabia [7, 8]. The wrong belief that khat chewing has positive effects without any negative effects on health has contributed to the high rate of khat intake [9]. Khat leaves are reported to contain many constituents. The most important active components are cathine and cathinone, which produce the main actions of khat. In a recent cohort study, the prevalence of khat chewing among patients with acute coronary syndrome was shown to have increased and has been associated with higher risk of cardiac stroke and death [14]. Although some cases of fatalities have been reported in khat users [14, 39, 40], the distribution of cathine and cathinone are not yet fully clear. Therefore, this study report aimed to contribute to the clarification of the determination of cathine and cathinone in biological matrices and homogenate of green chewed plants found in the mouth of the deceased body.

Figure-1 shows the non-targeted analysis that indicates samples tested positive for cathine and cathinone, and immunoassay showed that samples tested positive for amphetamine type stimulants. Figure-2 shows the LC-MS-MS quantitative analysis that indicates that samples tested positive for cathinone and cathine. The results showed that cathinone concentration was 0.03, 0.03, 0.06, 0.07, 1.85 and 31 μg/mL in brain, liver, blood, vitreous humor, stomach and chewed green plant, respectively. Table-1 indicates the concentration of cathine as 0.31, 3.28, and 141 μg/mL in kidney, stomach and chewed green plant, respectively.

The results of immunoassay showed that samples were positive for amphetamine type stimulants. These results may be due to cross-reaction of cathinone and cathine with related compounds such as amphetamine or phenylpropanolamine [35]. Therefore, confirmatory analysis by LCMS/MS was done and showed that samples were positive for khat active components, cathinone and cathine, as indicated in Figure-2. Table-1 shows that the highest concentration of cathine and cathinone were found in the stomach, and this was expected as the deceased died while chewing khat and a green chewed plant was found in his mouth. The analysis of this green chewed plant showed the highest concentration of cathine and cathinone, which are responsible for the main effects of khat.

Previous studies determined that khat contains cathine and cathinone ranging from 0.005 to 0.75% and 0.01 to 0.32%, respectively. In addition, fresh khat samples contain up to 3.3% cathinone [41-42]. It should be noted that cathinone is largely converted to cathine within about 24 to 48 hours upon exposure to air or heat, and is therefore difficult to detect. In this regard, proper sampling procedures during handling and extraction are needed to avoid converting cathinone, Schedule I drug, to cathine, Schedule IV drug, which leads to misinterpretation [25].

On the other hand, because the mass spectra of the different isomers are similar and can possess different actions, potency and one isomer has different legal regulation than another; therefore, isomer detection procedures must be used in forensic analysis to avoid inaccurate interpretation [43]. For example, the presence of d-norpseudoephedrine in Ephedra plants is demonstrated to have the same chemical structure as cathine (1S,2S-(+)-norpseudoephedrine) which is present in the khat plant [44-47], suggesting that isomer identification is essential to determine the source of d-norpseudoephedrine.

Another important issue is that cathine is converted to cathinone in the body by dopamine B-hydroxylase enzyme, and this may explain our results showed that cathinone was detected in all organs except kidney and stomach [48]. This may due to the lack of dopamine B-hydroxylase enzyme in these organs, as previously demonstrated in experimental animals [49-50]. The other explanation is that cathine detected in the stomach during the absorption phase and about 85% of cathine is excreted through the kidneys within 24 hours [51]. Further studies are needed to determine the level and to explore the factors that can affect the post-mortem redistribution of cathine and cathinone.
Table 1 - Quantitative analysis of cathine and cathinone.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cathinone µg/ml</th>
<th>Cathine µg/ml</th>
<th>Amphetamine µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.03</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>0.03</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Kidney</td>
<td>ND</td>
<td>0.31</td>
<td>ND</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.85</td>
<td>3.28</td>
<td>ND</td>
</tr>
<tr>
<td>Blood</td>
<td>0.06</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>0.07</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chewed green plant</td>
<td>31</td>
<td>141</td>
<td>ND</td>
</tr>
</tbody>
</table>

Note: ND; Not-Detected

Figure 1 - GC-MS Screening results. (A, B, C, D, E & F) are total ion chromatograms (TIC) of brain, liver, kidney, stomach, blood and vitreous humor, respectively. (G) is a zoom in of the TIC of green chewed plant showing cathinone and cathine peaks at RT 6.74 and 6.86, respectively. (H) shows identification spectrum of cathine while (I) shows the identification spectrum of cathinone which were detected in green chewed plant.
4. Conclusion

This case showed that the concentration of cathinone in the brain and liver was similar. In addition, blood and vitreous humor concentration of cathinone were almost comparable. Cathine and cathinone concentrations were found to be different with respect to site of sampling. The results suggest that stomach and chewed green plants are considered as a good sample to show the concentration for both cathine and cathinone at the time of death of the khat chewer.
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Conflict of interest
None.

References


