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Cathine and cathinone disposition kinetics and neurotransmitter profile in several organs of rats exposed to a single dose of *Catha edulis (Vahl) Forssk. ex Endl.* extract

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Abstract

Objectives: *Catha edulis (Vahl) Forssk. ex Endl.* (Khat) is a stimulant plant that contains cathine and cathinone, which its abuses induce euphoria, alertness, and motor activity. Since the toxicokinetics of these substances remain unclear, this study was carried out to investigate the disposition kinetics of cathine and cathinone, the neurotransmitter profile, following a single dose of *C. edulis* extract in rats.

Methods: Twenty-four adult male Wistar albino rats (250–300 g) were randomly selected and divided into six groups of four rats each. All groups received a single oral dose of 2,000 mg/kg body weight, and blood and tissue samples from the brain, lung, heart, liver, and kidney were obtained at intervals of 0.5, 1, 2.5, 5, 12, and 24 h. The cathine and cathinone concentrations were identified and quantified using ion trap ultra-high performance liquid chromatography (HPLC-IT/MS). The neurotransmitter profile was detected using the quadrupole time of flight UPLC-QTOF/MS method.

Results: The lung, liver, and heart tissues attained the highest levels of cathine, while the highest level of cathinone was determined in the heart. Cathine and cathinone concentrations in the blood and heart peaked at 0.5 h. The concentrations peaked in the brain 2.5 h later, indicating that the heart had an immediate effect, whereas the brain had a longer-lasting one. They have longer half-lives (2.68 and 5.07 h, respectively) and may remain in the brain for longer durations (3.31 and 2.31 h, respectively). The neurotransmitters epinephrine, dopamine, norepinephrine, and serotonin were detected in a delayed, prolonged and organ-specific manner.

Conclusions: Cathine and cathinone were deposited in considerable concentrations in all tissues analyzed, with the highest C_{max} in the lung and T_{max} in the heart tissues but not in the brain. In addition, neurotransmitters such as adrenaline, dopamine, norepinephrine, and serotonin were differentially detected in all tested samples in a organ-specific fashion. More study is needed to identify cathine and cathinone's effects on neurotransmitter profiles. Nevertheless, these findings provided a further basis for experimental, clinical, and forensic investigations.

Keywords: *Catha edulis*; cathine; cathinone; disposition; HPLC-IT/MS; kinetics; UPLC-QTOF/MS.

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Introduction

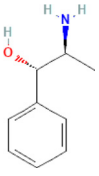
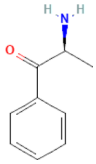
Catha edulis (Vahl) Forssk. ex Endl. (*C. edulis*) is commonly referred to as Khat. It is a stimulant plant where leaves are usually chewed and abused to induce euphoria, alertness, and motor activity. Its main stimulant components are cathine and cathinone, listed as mind-manifesting substances in Schedules IV and I of the Controlled Substances Act. Thus, the *C. edulis* plant has potential drug abuse and is therefore categorized as a controlled plant in Saudi Arabia [1]. Although the percentage range of cathine and cathinone in *C. edulis* leaves is relatively low, ranging from 0.1 to 0.3%, *C. edulis* toxicities occur by consuming a large quantity of

the leaves with long-time chewing [2, 3]. Other *C. edulis* components include cathedulin, tannins, flavonoids, triterpenes, sterols, and amino acids [4]. Chronic *C. edulis* consumption has been linked with various adverse effects, including anorexia, insomnia, hyperthermia, hypertension, arrhythmia, constipation, and urinary retention [5]. *C. edulis* alters the urinary inorganic profile, which can affect the metabolic pathway of medications, potentially increasing their toxicity or resulting in therapy failure [6]. In addition, cerebral hemorrhage, psychoactive disorders, and cancer have also been reported [4, 7].

Cathine (C9H13NO; MW: 151.21) and cathinone (C9H11NO; MW: 149.19) (Table 1) are highly lipophilic basic compounds with pKa of 8.9 and 5.5; respectively, thus both can undergo postmortem redistribution [8]. Cathinone is a keto analog of cathine, the most critical stimulant component of *C. edulis*, with a potency estimated to be 4–10 times higher than cathine. On the other hand, cathinone in fresh *C. edulis* leaves is unstable and can easily be reduced into cathine over time through exposure to air or heat [9, 10].

Cathine and cathinone were reported to be eliminated in human urine as the parent of *C. edulis*. Cathinone is chemically reduced rapidly to norephedrine and pseudoephedrine [11], whereas cathine is a minor metabolite of pseudoephedrine and cathinone [12, 13]. Therefore, positive cathine and cathinone detection in biological samples indicates the consumption of *C. edulis* [14]. However, the current disposition kinetics studies of cathine and cathinone after *C. edulis* administration are limited, and the neurotransmitter profile of cathine and cathinone is not yet fully explored. Therefore, this study aimed to determining the disposition kinetics indices of cathine and cathinone and exploring the neurotransmitter profile after a single oral dose of *C. edulis* in rats.

Table 1: Cathine and cathinone structures and chemical information.

Cathinone	Cathine	
		Chemical structure
149.19	151.21	Molecular weight
Norephedrone (S)-2-Aminopropiophenone (PubChem CID 62258)	(+)-norpseudoephedrine d-Phenylpropanolamine (PubChem CID 441457)	Synonyms
https://pubchem.ncbi.nlm.nih.gov/compound/cathinone.	https://pubchem.ncbi.nlm.nih.gov/compound/Cathine.	Reference

Materials and methods

Animal study design, *C. edulis* administration and sampling

Adult male Wistar albino rats (8–12 weeks old, 250–300 g weight) were obtained from the Experimental Animal Center of Medical Research Center, Jazan University, Jazan, Saudi Arabia. A total of 24 rats were randomly selected, group-caged (four rats per group) by time points (0.5, 1, 2.5, 5, 12, and 24 h), and kept five days of acclimatization before starting experiments. The rats were fed a standard laboratory diet consisting of 5% fat, 65% carbohydrate, 20.3% protein, 5% fiber, 3.7% salt mixture, and 1% vitamins with free access to water. *C. edulis* extracted powder was obtained using a methanolic extraction method as described previously [15]. The levels of cathine and cathinone in extracted khat powder were determined using an HPLC-IT/MS system. Every 100 g of *C. edulis* extract powder contains 0.3 mg cathine and 0.01 mg cathinone. According to previously published studies, *C. edulis* extract dose selection was based on the average daily amount of khat leaves (100–200 g) chewed by khat consumers [16, 17]. The single target dose of 2,000 mg/kg body weight was administered by oral gavage to the rats. The dose was prepared by dissolving 1 g of dried methanolic khat extract in 5 mL of distilled water (200 mg/mL). All rats were food fasted before dosing, and food was withheld for 3 h after dose administration. The animals were cared for and handled following international guidelines (e.g., NIH 1976). The study protocol was approved by Standing Committee for Scientific Research Ethics, Jazan University (HAPO-10-Z-001), reference number (REC-43/12/282).

Rats in each group were euthanized at specific time schedules as follows: 0.5 h for group I, 1 h for group II, 2.5 h for group III, 5 h for group IV, 12 h for group V, and 24 h for group VI. Immediately, 1 mL of blood samples were collected, and then the serum was separated by centrifugation. Organ tissues of the brain, lung, heart, liver, and kidney from each rat were collected, placed in a 0.9% sodium chloride solution and blotted on a filter paper to remove the blood; after that, they were weighed and stored in a specimen collection tube. All separated serum and tissue samples were stored at −20 °C for consequent experimental analysis.

Sample preparations

One gram of each organ tissue was homogenized in one mL of deionized water, then centrifuged for 15 min at 3,000×*g* using a Heraeus Labofuge 400 centrifuge (Thermo Fisher Scientific, USA). The supernatant was mixed with 1 mL phosphate buffer pH 6 and then vortexed for solid phase extraction. One milliliter of each serum and one gram of tissue homogenate were spiked with 1,000 ng/mL of 3,4-methylenedioxymethamphetamine (MDMA). Calibration samples were prepared by spiking 1 mL blank of the homogenate tissues with cathine, cathinone, and MDMA (Lipomed, Switzerland). Six calibration points were prepared at concentrations of 50, 100, 250, 500, 1,000 and 1,500 ng/mL of cathine and cathinone and 1,000 ng/mL of MDMA as internal standard. Three quality control samples were prepared by spiking 1 mL of the blank homogenate tissues with cathine and cathinone at the concentrations of 100, 250, 500, and 1,000 ng/mL of MDMA as internal standard. Blank samples were prepared by adding MDMA (1,000 ng/mL) into 1 mL of blank homogenate tissues. Cathine and cathinone were extracted from various sample matrices, along with calibrators, controls, and blanks, using the solid phase extraction method recommended by the

manufacturer of the SPE cartridges (UCT, Philadelphia, USA) for amphetamines type stimulants (ATS) extraction from biological samples.

After adding 1 mL of phosphate buffer with a pH of 6 to each sample and waiting for 10 min, 1 mL of acetonitrile was added to each sample, followed by vortexing. After 10 min, the mixture was centrifuged for 15 min at 4,000×*g*, and the supernatant was extracted by SPE. Consequently, SPE cartridges were preconditioned with 3 mL methanol and 3 mL deionized water and equilibrated with 1 mL phosphate buffer (pH 6). Upon sample load completion, SPE cartridges were washed with 3 mL of deionized water, 1 mL of 0.1 M acetic acid, and 3 mL of methanol and then dried for 10 min under a high nitrogen stream. Next, Cathine, cathinone, and MDMA were eluted into clean 12 mL glass tubes with 3 mL of dichloromethane, isopropanol, and ammonium hydroxide (78:20:2) mixture. Then one drop of 0.1 M HCL was added into each tube, and all elutions were evaporated to dryness under a nitrogen stream. Finally, all samples were reconstituted with 100 µL of the aqueous part of the mobile phase (10 mM ammonium formate with 0.11% formic acid) for HPLC-IT/MS analysis.

Cathine and cathinone disposition analysis

Tissue distribution of cathine and cathinone after a single oral dose of *C. edulis* extract was determined in the serum, lung, heart, brain, liver, and kidney tissues of each rat at the specified intervals using the HPLC-IT/MS system.

HPLC-IT/MS analysis

Cathine and cathinone levels were identified and quantified by HPLC-IT/MS system, using LCQ fleet mass analyzer coupled with Surveyor Auto-Sampler and Surveyor Quaternary Pump and controlled by X-Caliber Software (Thermo Scientific, USA). The analytical method was validated with some in-house modifications [18]. Briefly, 10 µL of each sample was injected by an autosampler. The chromatographic separation of cathine, cathinone, and MDMA was achieved by HPLC column (Hypersil GOLD, 5 µm, 150 × 4.6 mm, Thermo Scientific, USA), using mobile phase (A) [ammonium formate (10 Mm; 0.639 mg ammonium formate in 1 L HPLC water] and a mobile phase (B) [formic acid in acetonitrile (0.1%; 1 mL formic acid in 999 mL acetonitrile]. The mobile phase follows gradient mode as follows: 0–1 min, 100% A; and 1–7.5 min, 80% A; 7.5–8.5 min, 50% A, 8.5–9.5 min, 0% A; 9.5–10.5 min, 50% A; and 10.5–11.5 min 100% A. Flow rate was 300 µL/min, and the injection volume was 5 µL. Cathine, cathinone, and MDMA retention times were 5.9, 5.9, and 7.0 min, respectively.

As an optimized tuning profile of ATS, the electrospray ion source (ESI) runs in positive ionization mode with 5 kV spraying voltage, 275 °C capillary temperature and sheath gas value of 30. The mass analyzer runs scan mode, scanning for *m/z* 152 for Cathine, *m/z* 150 for cathinone, and *m/z* 194 for MDMA. Cathine, cathinone, and MDMA were furtherly fragmented in the collision cell with helium gas by Pulsed Q collision-induced dissociation (PQD) mode into *m/z* 134 and 117 for Cathine, *m/z* 132 and 105 for cathinone and *m/z* 163 and 135 for MDMA. The PQD value for cathine and cathinone was 19 and 22 for MDMA. Qualitative and quantitative analyses were performed by X-Caliber Software. This analytical method was optimized, validated, and verified by the technical staff of the Poison Control and Forensic Medical Chemistry Center of Jazan.

Cathine and cathinone kinetics indices analysis

Kinetics indices of cathine and cathinone in serum and organ tissues were calculated by non-compartmental pharmacokinetic analysis using WinNonlin 2.1. The goodness of fit statistic was determined by Rsq (Rsq>0.9). The average maximal concentrations (C_{max}), time to reach C_{max} (T_{max}), Area under the curve (AUC), half-life ($t_{1/2}$), apparent volume of distribution (V_z/F), apparent clearance (CL/F), and mean residence time (MRT_{last}) for cathine and cathinone were calculated.

Neurotransmitter profile analysis

Neurotransmitter profiles after a single dose of *C. edulis* extract were identified using UPLC-QTOF/MS system (X500R Q-TOF from Sciex, USA) and SCIEX vMethod [19]. Briefly, Chromatographic separations were obtained on a SELECTRA C18 column (15 cm × 4.6 mm, 5 µm) maintained at 45 °C. Mobile phase A consisted of water and 5 mM ammonium formate; mobile phase B was composed of ACN and 0.1% formic acid. The mobile phase composition was controlled as follows: 0–0.20 min, 10% B, 1–8.70 min, 98% B, 8.80–11 min, 10% B. The flow rate was 0.7 mL/min, and the injection volume was 10 µL.

Mass spectrometry was performed on an ExionLC™ System with a Sciex X500R QTOF (Sciex, USA). Data were acquired in SWATH mode using positive electrospray ionization with a resolution >20,000 at full

Table 2: Optimized UPLC-QTOF/MS method parameters.

Liquid chromatography system	**ExionLC™ system**
Column	**SELECTRA**[R] C18 column (15 cm × 4.6 mm, 5 µm)
Column temperature	45 °C
Injection volume	10 µL
Solvent A	5 mM ammonium formate, adjusted to pH 2.9 using formic acid
Solvent B	Acetonitrile containing 0.1% (v/v) formic acid
Gradient	10% solvent B (0–0.20 min) 98% solvent B (1–8.7 min) 10% solvent B (8.8–11 min) 30–50% solvent B (11–12 min)
Flow rate	0.7 mL/min
Mass spectrometry	
Mass spectrometer	X500R QTOF
Ionizations mode	Electrospray +ve
Capillary voltage	2,500 V
Cone gas	60 psi
Desolvation temperature	600 °C
Desolvation gas	60 psi
Source temperature	250 °C
Data acquisition	Centroid (data independent acquisition)
Function 1	10 V
Function 2	Ramp 10–35 eV
Mass range	25–650 Da
Resolution	>20,000 @ 278 *m/z* (resolution mode)

width half maximum. The acquisition range was *m/z* 25–650 using a scan time of 0.05 s and spray voltage of 2500 V, the source temperature was 250 °C, the desolvation gas flow rate was 60 psi at 600 °C, and the cone gas flow rate was 30 psi. Data acquisition was carried out with low collision energy (10 V) and high-energy ramp (10–35 V). Optimized UPLC-QTOF/MS method parameters are shown in Table 2.

Data processing

The raw data obtained after analysis was processed by SCIEX software. The raw data were processed automatically using the streamlined workflow of the SCIECX OS (Sciex, USA) to identify analytes and neurotransmitters. Compound identification was based on retention time (±0.05 min), mass deviation (±10 mDa), and appropriate isotope profile. Analyte Parameters under UPLC-QTOF/MS Analysis are shown in Table 3.

Results

The initial screening analysis was performed using UPLC-QTOF/MS analysis. According to the NIST library, only compounds with a library score of 100% in terms of spectral similarity were considered correct identification. Figure 1 shows that two compounds were detected in all samples with retention time, isotope confidence, and 100% library score as follows: cathinone (Formula=C9H11NO, RT=4.28, Precursor mass=150.0917) and cathine (Formula=C9H13NO, RT=4.18, Precursor mass=107). The mass spectrum and extracted ion chromatograms of the cathinone (A) and cathine (B) are shown in Figure 1.

Disposition kinetics indices

The cathine and cathinone disposition curves in serum, brain, lung, heart, liver, and kidney after a single oral dose of *C. edulis* extract are shown in Figure 2A, B. The non-compartment model described kinetic indices for cathine and cathinone, and the goodness of fit statistic were determined by Rsq (Rsq>0.9 for all except Cathine in the Kidney). The main kinetic indices of cathine and cathinone after single-dose oral administration of 2,000 mg/kg *C. edulis* extract are summarized in Tables 4 and 5, respectively. The highest serum cathine concentrations (2.14 mg/L) reached 0.5 h after *C. edulis* administration, while they reached their highest tissue levels (4.6 mg/L) in the lung, liver, and kidney within 0.5–1 h after administration. In contrast, cathine concentrations in the heart tissues were the lowest, at approximately 0.84 mg/L. Regarding cathinone, a similar serum C_{max} level (2.43 mg/L) was observed 0.5 h after *C. edulis* administration. The highest tissue concentrations were reported in the lung (2.23 mg/L at 2.5 h) followed by the brain, kidneys and heart, whereas the lowest concentration was detected in the liver (0.65 mg/L). More than 60% of the serum levels of cathine and cathinone reached the brain within 2.5 h, with MRT_{last} 2.31–3.31 h after oral dosing. Furthermore, the mean residence time for cathine and cathinone from dosing to the last measurable concentration (MRT_{last}) was 2.55–3.4 h and 2.03–5 h, respectively, in the serum and the other organ tissues.

Neurotransmitters analysis

This study was also performed to identify neurotransmitters of interest using UPLC-QTOF/MS analysis. The main neurotransmitters, dopamine, epinephrine, norepinephrine, and serotonin were detected in the serum, brain, heart, liver, kidney, and lung tissues after a single oral dose of *C. edulis* extract in rats at different time points. Epinephrine and dopamine were detected in all samples at 5, 12, and 24 h after dosing, and dopamine was further detected in the heart and liver at 24 h post-dosing. Norepinephrine was detected only in the liver at 5 and 12 h post-dosing and in the lung at 24 h post-dosing. Serotonin was detected only in the brain and kidney 12 h post-dosing (Table 6). Extracted ion chromatograms, as well as mass spectra profile of the Dopamine (C), Norepinephrine (D), Serotonin (E), and Epinephrine (F), are shown in Figure 1.

Table 3: Analyte parameters under UPLC-QTOF/MS analysis.

Analyte	Ionization mode	Molecular ion (*m/z*)	Fragmented ion (*m/z*) (±0.01)	Retention time (min) (±0.05)
MDMA[a]	Positive	194	163.0121/135.245/117.631	4.80
Cathinone	Positive	150.1180	117.0736/132.0975	4.44
Cathine	Positive	152.1180	115.0736/117.0736/134.0975	4.18
Epinephrine	Positive	184.0968	171.1184/153.1039/45.0346	3.64
Norepinephrine	Positive	170.0812	134.0621/840.817	2.80
Dopamine	Positive	154.0863	132.0817/117.0593/90.0458	4.37
Serotonin	Positive	175.0866	132.616/117.0580/105.0706	4.74

[a]MDMA was used as an internal standard.

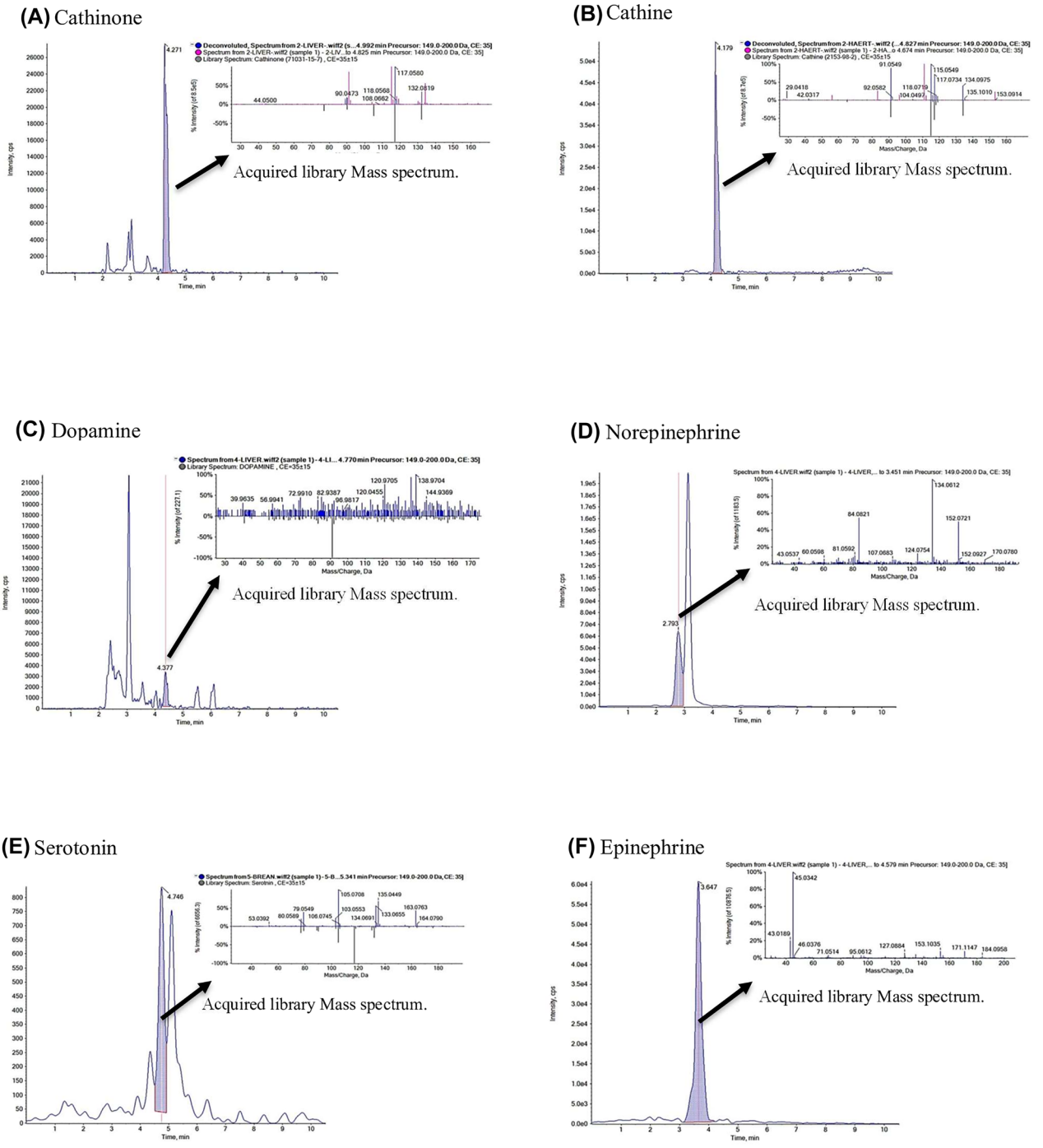
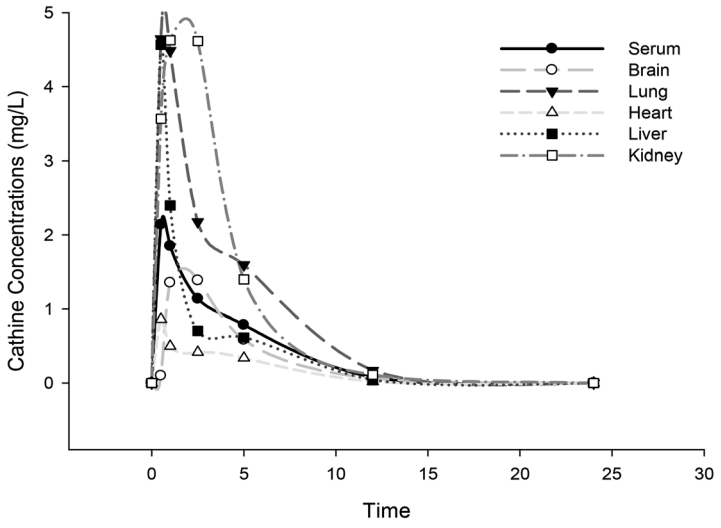


Figure 1: Mass spectrum and extracted ion chromatogram of Cathinone (A), Cathine (B), Dopamine (C), Norepinephrine (D), Serotonin (E) and Epinephrine (F).

(A) Cathine



(B) Cathinone

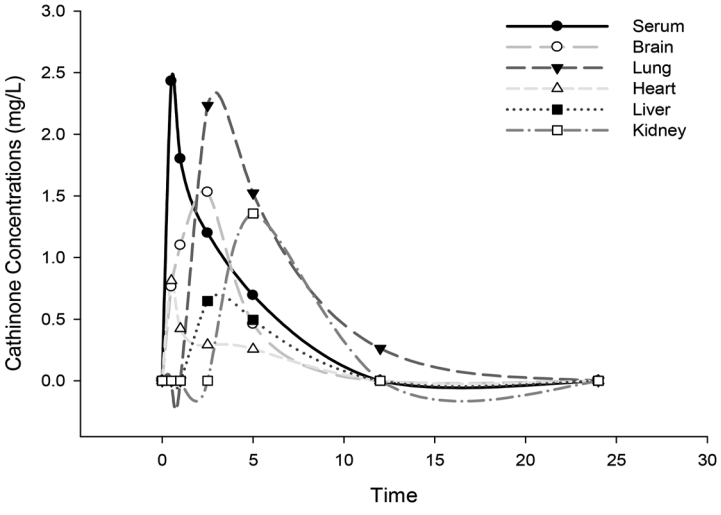


Figure 2: Cathine (A) and cathinone (B) disposition plot after a single oral dose of *C. edulis* extract.

Discussion

HPLC-IT/MS was utilized in this study to identify and quantify cathine and cathinone concentrations, and UPLC-QTOF/MS was used to determine the neurotransmitter profile after a single dose of *C. edulis* to rats. According to our findings, the C_{max} serum levels of cathine and cathinone were 2.14 and 2.43 mg/L, respectively, after 0.5 h for both. There was good agreement with the findings of Brenneisen, R., et al. [20], who measured the plasma levels of cathinone and cathine following a single oral dose of pure alkaloid in a healthy volunteer. They found that the highest cathine concentrations were obtained after about 0.75 h, and the cathinone concentrations were attained after about 1.5 h. Widler et al. reported that the C_{max} of cathinone in healthy male volunteers who masticated *C. edulis* leaves continuously for an hour is about 2 h [21]. However, since cathinone is rapidly absorbed, the C_{max} is reached shortly after oral ingestion, implying that it is more effective than masticating the *C. edulis* leaves.

Table 4: Kinetic indices of cathine in rats after single oral dose of 2000 mg/kg of *C. edulis* extract.

Kinetic indices	Cathine					
	Serum	Brain	Lung	Heart	Liver	Kidney
C_{max}, mg/L	2.14	1.38	4.64	0.86	4.56	4.63
T_{max}, h	0.5	2.5	0.5	0.5	0.5	1
AUC_{all}, mg/h/L	9.8	7.93	20.3	3.54	9.35	23.3
AUC_{last}, mg/h/L	9.3	7.54	19.3	3.42	9.11	22.6
$t_{1/2}$, h	2.46	2.68	2.42	2.26	1.84	2.1
V_z/F, L	204	272.4	96.7	514.9	158.3	72.6
CL/*F*, L/h	57.5	70.57	27.6	157.6	59.7	23.9
MRT_{last}	3.28	3.31	3.18	3.41	2.55	2.9

Table 5: Kinetic indices of cathinone in rats after a single oral dose of 2000 mg/kg of *C. edulis* extract.

Kinetic indices	Cathinone					
	Serum	Brain	Lung	Heart	Liver	Kidney
C_{max}, mg/L	2.43	1.5	2.23	0.81	0.65	1.36
T_{max}, h	0.5	2.5	2.5	0.5	2.5	5
AUC_{all}, mg/h/L	8.74	6.66	14.1	2.59	3.62	6.46
AUC_{last}, mg/h/L	6.29	5.05	12.6	1.72	1.9	1.7
$t_{1/2}$, h	2.63	5.07	2.98	3.18	6.13	–
V_z/F, L	233.5	478	173	881	779	–
CL/F, L/h	61.4	65.3	40.1	192	88	–
MRT_{last}	2.03	2.31	4.62	2.12	3.3	5

Table 6: Neurotransmitters detected by UPLC-QTOF/MS analysis in rats after single oral dose of 2000 mg/kg of *C. edulis* extract.

Sample type	Detected neurotransmitters after specific time (h) *C. edulis* treatment						
	0	0.5	1	2.5	5	12	24
Serum	–	–	–	–	EN, DA	EN, DA	EN, DA
Brain	–	–	–	–	EN, DA	EN, DA, SR	EN, DA
Heart	–	–	–	–	EN, DA	EN, DA,	EN
Kidney	–	–	–	–	EN, DA	EN, DA, SR	EN, DA
Liver	–	–	–	–	EN, DA, NE	EN, DA, NE	EN
Lung	–	–	–	–	EN, DA	EN, DA	EN, DA, NE

DA, dopamine (Formula=C8H11NO2, detected at Mass=154.086, Retention time: 4.30–6.08); NE, norepinephrine (Formula=C8H11NO3, detected at Mass=170.081, Retention time: 2.73–3.09); S, serotonin (Formula=C10H10N2O, detected at Mass=175.086, Retention time: 4.75); EN, epinephrine (Formula=C9H13NO3, detected at Mass=184.096, Retention time: 3.56–3.69); –, undetected.

Additionally, a high serum fraction of cathine and cathinone was distributed to the brain and reached the peak 2.5 h after administration, indicating their high ability to pass the blood-brain barrier. In addition, cathine and cathinone rapidly distributed to the heart tissues as well. These observations could explain why cathine and cathinone have predominantly harmful effects on the cardiovascular and neurovascular systems in *C. edulis* users [22, 23]. In this context, *C. edulis* consumption can reduce locomotor activity and significantly impair driving abilities, thus increasing the risk of road accidents [24, 25]. Seizures, stroke, acute myocardial infarction, arrhythmia, and mortality have all been reported following *C. edulis* administration [24, 26, 27]. Moreover, each of the *C. edulis* constituents could cause specific disorders; for example, dyskinesia and torticollis are associated with cathine administration, whereas hyperkinesis, movement disorders, hypertension, and seizures are common side effects with cathinone [24, 28, 29].

The lung tissue has been found to contain a high concentration of synthetic cathinone [30]. Similarly, in our study on the single oral dose of *C. edulis* administration, lung tissue had the highest C_{max} values of cathine and cathinone, which may account for the observed respiratory dysfunction [31]. Additionally, we found that the liver and kidney had high cathine C_{max}, which may account for the observed nephrotoxicity and hepatotoxicity in the experimental study [32]. Therefore, dose-response studies for cathine and cathinone in such organs are required. According to the current research, cathine and cathinone have a short MRT; in which cathine MRT in the serum, brain, lung, heart, liver, and kidney were 3.28, 3.31, 3.18, 3.41, 2.55, and 2.9 h, respectively, whereas for cathinone were 2.03, 2.31, 4.62, 2.12, 3.3 and 5 h, respectively. Because of the short detectable time of cathine and cathinone, which had MRTs ranging from 2 to 5 h, autopsies should be performed as soon as possible for toxicological investigation in a forensic setting.

This study detected dopamine, epinephrine, norepinephrine, and serotonin 5 h after dosing using UPLC-QTOF/MS analysis. All samples contained epinephrine and dopamine, which attributed to *C. edulis*'s main actions. Norepinephrine was detected in the liver after 5 and 12 h of extract administration, whereas dopamine, epinephrine, and norepinephrine were detected in the lung after 24 h, which have bronchodilators effects and may explain why bronchial asthmatic patients are chewing *C. edulis* have fewer nighttime asthmatic attack and fewer asthmatic symptoms [33]. On the other side, long-term *C. edulis* chewing is associated with a negative impact on lung functions, such as a decreased mean forced expiratory volume and maximum ventilation volume [31].

At 12 h post-dosing, serotonin was detected in the brain and kidney, which controls anger and aggression and enhances a wake state. Although it has been established that cathinone injection increases serotonin release from rat

synaptosomes [34, 35], chronic administration decreases serotonin transporter activity [36]. Serotonin deficit has been linked to schizophrenia, depression, and suicide, all of which are associated with *C. edulis* use [37]. The greatest effects of *C. edulis* on mood modulation have been reported to occur 4 h after starting to chew *C. edulis*, and last for 24 h [38]. The prolonged time interval between *C. edulis* administration and detectable neurotransmitters in our study may account for the delayed effects of *C. edulis*. One study indicated that *C. edulis* extract induced cell death in the human cardiomyocyte H9c2 cell line. In this study, the pro-apoptotic protein (BAX) level was increased significantly in response to the extract after 48 and 72 h of treatment [39]. This finding may also explain why most *C. edulis* chewers arrive at the hospital more than 12 h after the onset of cardiovascular symptoms [26].

Conclusions

The current findings suggest that cathine and cathinone were deposited in considerable concentrations in all tissues, in that lung tissue has the highest concentrations, with a rapid peak for cathine and a delayed peak for cathinone. Cathinone and cathine rapidly reach peak concentrations in the heart tissues but not in the brain. The detection of adrenaline, dopamine, norepinephrine, and serotonin was delayed, prolonged, and organ-specific. Additional research is required to determine the relationship between cathine and cathinone and their effects on the neurotransmitter profile. This study will provide the base for future experimental, clinical, and forensic investigations.

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