

FATAL 4x100; HOME-MADE KRATOM JUICE COCKTAIL

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Abstract: A corpse of 21-years-old man (Muslim) was investigated for the cause of death. His family declined consent for full autopsy. Thus, only blood and urine were used for toxicological analysis by liquid chromatography-electrospray ionization-time of flight mass spectrometry. These following substances were found in the blood and urine samples: mitragynine (an alkaloid found in Kratom; *Mitragyna speciosa* leaves), caffeine, diphenhydramine, alprazolam, nortriptyline, methadone, tramadol, methamphetamine and some of their metabolites. The findings of this case suggested the possibility of polydrugs abuse calls "4x100", a Kratom juice cocktail that has been popular in Southernmost provinces of Thailand. It is made from four major ingredients, boiled Kratom leaves, Cola soft drink, cough syrup and tranquilizers or mosquito coils. In this case, the cause of death might be due to multidrugs intoxication of the additive adverse effects especially CNS and respiratory depression.

Keywords: Kratom juice cocktail, *Mitragyna speciosa*, intoxication

INTRODUCTION: Kratom (*Mitragyna speciosa* Korth.) juice cocktail called "4x100" formulae is becoming abused by youth addicts and causes a terrible social problem in Thailand, especially in young Muslim in Bangkok and people in three southernmost provinces of Thailand. The origin of the name "4x100" is unclear, but it likely came from its four ingredients. Boiled Kratom leaves, Cola soft drink and codeine- or diphenhydramine-containing cough syrup are three major ingredients while the fourth ingredient depends on the producer's satisfaction or is whatever that makes them drunk i.e. mosquito coils, anxiolytic drugs or antidepressants (alprazolam, diazepam, amitriptyline, nortriptyline, etc.), analgesic (paracetamol, tramadol), some of drug abuse (methadone, marijuana, methamphetamine), herbicide, or even the powder peeled from the inside of fluorescent light bulbs^{1,2}. After War on Drug Campaign in 2003, some addicts switch to other inebriate substances such as alcohol, tranquilizers and Kratom. In this regard, "4x100" has become popular as a cocktail drug abuse formulae in youthful Muslim because consuming this cocktail formulae causes a feeling like drinking alcohol which is prohibited in their religious regulation². Furthermore, most of the ingredients in these formulae are not restricted controlled, except for Kratom and some of drug abuse which is an illegal substance. However, this plant can be found naturally in many places. An increasing of using the cocktail formulae is shown

by the report that 290 Litres of 4x100 were seized in 2007, but 1,700 Litres were seized in 2008³.

Cola soft drink in 4x100 formulae is added for taste, but the others are added for euphoric effects. The active ingredients in boiled Kratom leaves are group of Mitragynine alkaloids. More than 25 alkaloids had been isolated from Kratom leaves by various researchers regulation⁴. It is interesting to note that the "low dose" effects by chewing Kratom leaves are described to be stimulating effect (coca-like action), while "high dose" usually produces sedative and euphoric effects (opium-like action). Jansen and Prast (1988) described mitragynine alkaloids as substances with highly unusual but well-documented history as both a depressant and a stimulant while possessing the chemical structure of a suspected psychedelic⁵. Kratom leaves contain approximately 0.2% mitragynine alkaloids by weight. Approximately twenty leaves of Kratom (normally used in one pot of boiled Kratom leaves and used to make 4x100 formulae for one serve) contain approximately 17 mg of mitragynine⁶. However, the content of mitragynine alkaloids in Kratom leaves is variable and depends on geographic origin as well as the different season⁷. Dose-dependent neuropsychiatric effects are shown within five to ten minutes after consumption, and last for up to one hour⁶. In the mouse model, mitragynine alkaloids doses as high as 920 mg/kg were given without apparent clinical effect⁸.

Since codeine-containing cough syrup was

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restricted after War on Drug Campaign, another cough syrup regimen containing diphenhydramine was brought to abuse instead. A study of Tanda *et al.*⁹ demonstrated that diphenhydramine exhibited cocaine-like activities and increased dopamine neurotransmission in mesolimbic areas of reward system like various drugs of abuse. Presently, most cough syrups used to make 4x100 formulae usually are antihistamine preparations containing diphenhydramine that are available in drug stores.

Reports of toxicity from mitragynine are rare⁶. In animal models, mitragynine was shown to cause less respiratory depression than other narcotics⁵. There is no cases of death due to Kratom were reported in the United States¹⁰. Thus, toxicity from abuse of Kratom leaves seem not to be serious. However a corpse autopsied at the Institute of Forensic Medicine, Police Hospital, The Royal Thai Police Headquarter, on April 2009 was reported with the suspected cause of death from "4x100" formulae intoxication.

CASE REPORT:

Case History

A corpse of 21-years-old man was taken by police to the Institute of Forensic Medicine, Police Hospital for investigating the cause of death. His body showed fixed livor mortis appeared on his back indicating that he had died for more than 8 hours. His religious is Islamism. His sister gave information that last night he went home and told her that he felt sick and wanted to sleep. Next day, at noon she found him laid down on the floor and not breathing. There were bloody exudates from his nose and foamy blood from his mouth. Due to his religion, full autopsy could not be performed. Thus only blood and urine samples were collected for toxicological analysis.

Sample preparation

Blood or urine sample volume 1 ml was mixed with 300 μ l of 200 mM KOH and pH value was adjusted to 10, after that 3 ml of methanol and 2 ml of mixture of water and 200 mM KOH was added respectively. Then the mixture was applied onto the SPE cartridges and clean-up was accomplished with successive 2 ml of water. The cartridge was dried for 5 min before elution with 2 ml of a mixture of methanol: ammonia (95:5 v/v).

The eluted sample was transferred to autosampler vial and 10 μ l was injected into the liquid chromatography-electrospray-ionization-time of flight mass spectrometry (LC-ESI-TOF-MS).

Apparatus

The HPLC system was an Agilent Technologies (Waldbronn, Germany) 1200 Series. Chromatographic separation was performed with Bruker Inst. ODS-AQ (50 \times 4.6 mm i.d.) reversed-phase column at 40 $^{\circ}$ C. The mobile phase, delivered at a flow rate of 0.5 ml/min, was a gradient of acetonitrile and mixture of 0.1% formic acid in 5 mM NH₄OAc, (70% : 30%), transfer acceleration ramps 20 bar/sec, flow 100 ml/min. The mass analyzer was a Bruker Daltonics (Bremen, Germany) micrOTOF™. The instrument was operated in electrospray positive ionization mode (ESI+) using a m/z range of 50-1,500. Data acquisition, peak integration and calculation were assigned to a computer workstation running HyStar 3.2 and Hyphenation Star PP V.3.2.37.0 software.

RESULTS AND DISCUSSION: Interpretation of the cause of death in this case was limited and could be performed only the toxicological data from blood and urine samples. However, an advantage of apparatus for toxicological work, LC-ESI-TOF-MS appeared to be an efficient and very sensitive technique for identification of drugs and metabolites in poisoning cases. In this case, mitragynine alkaloid, caffeine, diphenhydramine, alprazolam, nortriptyline, methadone, tramadol, methamphetamine and some of their metabolites were found after blood and urine analysis. Blood concentrations of alprazolam, tramadol, nortriptyline, methadone, methamphetamine and caffeine were 0.2, 0.27, 1.78, 0.3, 0.4 and 0.39 μ g/ml, respectively (Table 1. and Figure 1). Regarding to the analytical results, it could conclude that this case was abused of polydrugs called 4x100 formulae.

4x100 formulae is a home-made Kratom juice cocktail consists of none accurate ingredients. Types and amounts of ingredients depend on the availability and the satisfaction of the abusers. They always use this substance as well as smoking. Thus from results, nicotine and cotinine, an active ingredients and metabolite in cigarette, were found.

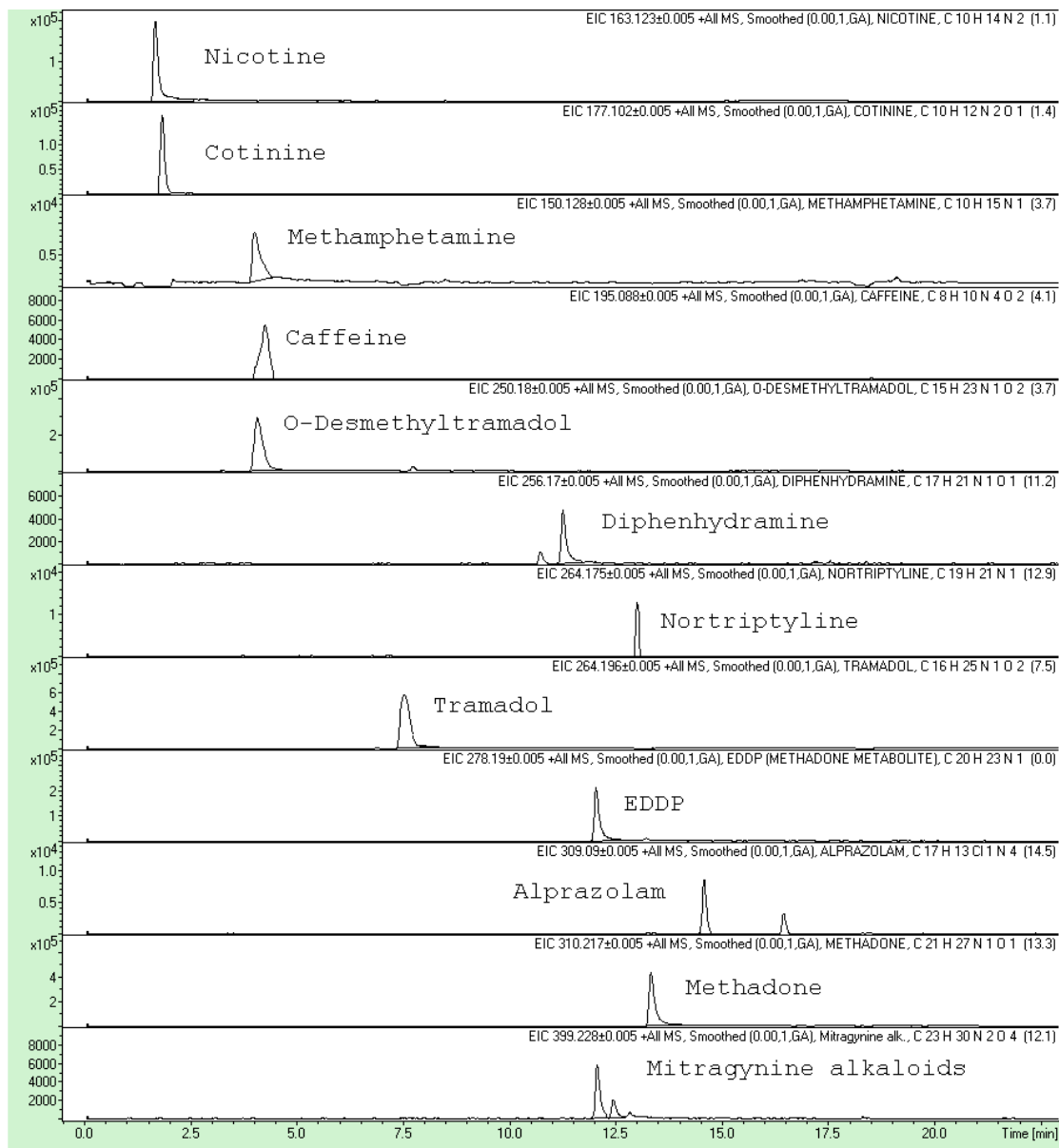


Figure 1 LC-ESI-TOF-MS chromatogram of urine sample

Table 1 Drugs concentrations

Drugs	Concentration ($\mu\text{g/ml}$)	
	Blood	Urine
Alprazolam	0.2	0.65
Tramadol	0.27	26.73
Nortriptyline	1.78	4.60
Methadone	0.30	5.20
Methamphetamine	0.4	2.5
Caffeine	0.39	2.32
Mitragynine	not quantitative	not quantitative
Diphenhydramine	not quantitative	not quantitative
Nicotine	not quantitative	not quantitative

Considering the probability of each drug found in this case that which one seemed to be the cause of death. Methadone tramadol and nortriptyline are likely to be capable of causing death from several previous reports, while very few data is available for mitragynine as a cause of death. Blood methamphetamine level in this case (0.4 µg/ml) was much lower than the lethal range (over 4.5 µg/ml) that was demonstrated by Takayasu *et al.*¹¹⁾

Tramadol, an opioid analgesic, in therapeutic dose, CNS side effects such as nausea, vomiting, drowsiness, vertigo, constipation, headache and somnolence had been reported. In case of poisoning, severe complications may occur including seizures¹⁶⁾. The risk of tramadol induce seizures may be increased during co-administration with any substances that can reduce the seizure threshold such as methamphetamine, methadone and nortriptyline, Decker *et al.*¹⁷⁾ reported one case of fatal intoxication due to tramadol alone. Blood tramadol levels was 8 µg/ml was found, while therapeutic blood levels in adults range from 0.1 to 0.3 µg/ml. Goeringer *et al.*¹⁸⁾ reported that tramadol concentration in the case of drug-combined fatalities is range from 0.03 to 22.59 µg/ml, which is consistent with the finding in this study (Tramadol concentration in blood was 0.27 µg/ml).

Nortriptyline is one of the classical tricyclic antidepressants (TCA) widely used in Thailand. Plasma concentration of the parent drug and its metabolites over 1 µg/ml are prognostic factors of serious intoxication¹⁹⁾. The clinical manifestations of a TCA overdose are directly linked to its pharmacology. There are four major classes of effects, including anticholinergic, direct adrenergic blockade, inhibition of norepinephrine and serotonin reuptake, and blockade of fast sodium channels. Manifestations of these effects include decreased consciousness, confusion, delirium, seizures, hypotension, and ECG changes. Hypotension is a major cause of mortality, and is related to reduce myocardial contractility and systemic vascular resistance. Cardiac manifestations include PR and QT prolongation, QRS widening to resemble a right bundle branch block, atrioventricular block, and persistent sinus tachycardia.²⁰⁾ Overdoses of only nortriptyline could be fatal, but co-administration

with other medicine may cause more increased interaction risk. For example, co-administration with diphenhydramine may increase anticholinergic side effects, while, co-administration with methadone may cause dose-related prolongation of QT interval, elevated cardiac risk and fatal.

Methadone, an opiate withdrawal medication, is restrictly available only in the special hospital. Manifestation of toxic overdose of methadone includes stupor, muscle flaccidity, respiratory depression, coma and circulatory collapse. Several fatal methadone concentrations had been reported in previous studies. For example, Milroy *et al.*²¹⁾ studied in 50 adult victims and reported the postmortem range of methadone between 0.08 – 2.7 µg/ml. The methadone blood concentration found in this present study was 0.3 µg/ml, which is consistent with the report earlier.

Alprazolam, a short acting benzodiazepine, that is most commonly found in 4x100 formulae. Street name in Thai is “Mano” or “Zolam”. In general, benzodiazepines have a flatter dose-response curve than other sedative-hypnotics, and exhibit a low incidence of toxicity in humans. Although alprazolam has generally been considered to be safe, more central nervous system depressant effects were shown at high doses. Schulz *et al.*¹²⁾ reported that therapeutic and toxic plasma levels of alprazolam ranged between 0.005-0.05 and 0.1-0.4 µg/ml, respectively. In contrast, McCormick *et al.*¹³⁾ presented two patients who attempted to commit suicide by ingest a handful of alprazolam. These patients had markedly elevated serum alprazolam concentrations, ten times greater than the therapeutic levels. Surprisingly, they did not show any significant alterations in vital signs or CNS depression. A study of Barbara *et al.*¹⁴⁾ on 178 cases with alprazolam-related death indicated that alprazolam alone was rarely a cause of death, but death was always due to combined drug toxicity. In this study, blood alprazolam concentration was 0.2 µg/ml which was consistent to the concentration found by Michaud *et al.*¹⁵⁾ that had been reported the fatal case of alprazolam and tramadol overdose. Thus, combined toxicity of alprazolam with other depressants seemed likely to be a cause of death of this case.

In conclusion, based on the toxicological findings, the cause of death in this case study seemed likely to be due to multiple drugs intoxication. Many drugs found in blood sample were in the toxic lethal range such as methadone, tramadol, nortriptyline and alprazolam. Furthermore, various drugs and their active metabolites that were found in this case showed adverse abilities to compress on CNS and respiratory system, additively or synergistically of all substances might be an actual mechanism of death.

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