Interdisciplinary Research Review
Volume 15, Number 6, Pages 6 – 9

Examining the urine mitragynine concentration in Thai Kratom abusers and studying the effect of Beta-Glucuronidase in the analysis process

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Abstract

The objectives of this study were to examine the urine mitragynine concentration in Thai Kratom abusers and to study the effect of Beta-glucuronidase in the Analysis Process. Kratom (Mitragyna speciosa Korth) is an addictive plant in Thailand and is classified as a Penal Drug in Category-5 of the Thai Narcotics Act 2522 (1979), hence consuming Kratom in Thailand is still prohibited. Mitragynine is a major analgesic alkaloid in Kratom and it is responsible for the opioid effects of the substance. Cadaver urine samples (n = 26) and urine of the Kratom abused suspects (n = 26) were obtained from the Institute of Forensic Medicine, Police General Hospital, Royal Thai Police Headquarters from January 2017 to December 2017. All urine samples were divided into 2 groups, the first group was treated with Beta-glucuronidase enzyme before being measured while the second group was non-enzymatic treated. The results found that, in the cadaver group, the average concentration in the enzymatic treated group and the non-enzymatic treated group was 286.83 and 288.10 ng/mL respectively. Moreover, in the Kratom abused suspects group, the average concentration in the enzymatic treated group and the non-enzymatic treated group was 289.23 and 273.65 ng/mL respectively. The paired sample t-test indicated non-significant differences between enzymatic and non-enzymatic treated urine in both groups, and that the enzymatic processes are not necessary for the determination of urine mitragynine.

Keywords: Kratom, Mitragynine, Narcotics, drug abuse, urine, Beta-Glucuronidase

Article history: Received 16 September 2020, Revised 22 October 2020, Accepted 28 October 2020

1. Introduction

Kratom is a native plant found in Southeast Asian countries, especially in Thailand, Malaysia and Indonesia. This plant has a scientific name as *Mitragyna speciosa* Korth. and it is classified in the family Rubiaceae with a medium-sized perennial height of 10 – 15 meters. Kratom has various properties such as stimulating the nervous system resulting in an increase in work efficiency and reducing sleepiness among Thai laborers and farmers.

Although Kratom has some medical benefits, Kratom is still controlled in Thailand and classified as a category 5 substance, according to the Thai Narcotics Act 2522 (1979), which has low control and penalties. A large group of Kratom users often believes that Kratom is less dangerous than using other illicit drugs. Because it is easy to adopt and cheap, moreover, the misuse of Kratom plants in Thailand becomes increasing.

There are many ways to use kratom leaves. For instance, some users like to use simple methods such as chewing only fresh leaves while some prefer using in a dry powder form for smoking or brewing into tea. Recently, adolescence in Thailand tends to use Kratom in a cocktail form such a recreational beverage as '4×100' (pronounced: sii koon roi), which possesses the main ingredient as Kratom juice, sweet soft-drinks, antihistamine and several psychoactive drugs. Kratom leaves contain an important substance called mitragynine, which has the highest amount approximately of 66 percent by weight of Thai Kratom leaves. Mitragynine can bound to opioid receptors, therefore has the ability to reduce pain, which is similar to opioid analgesic drugs.

According to a systematic review made by Ya et al. [1], 2019, 85 – 95% of mitragynine is bound to plasma proteins and altered in the body via drug metabolism. Phase I metabolism is the main part and more process is in phase II. Phase II metabolism is a process to eliminate or reduce the toxicity of substances by changing the substance to a water-soluble form in order to be easily excreted through urine. During the second phase of biotransformation, xenobiotics reacts with glucuronidation, sulfation, methylation, acetylation, or glutathione and amino acid conjugation in order to obtain water-soluble substances. Ya et al. [1] evaluated from the results of Philipp et al. [2] study and concluded that urine samples also contained 10 – 30% Mitragynine. Philipp et al. [3], 2009 explored the Mitragynine metabolism in human and found those three metabolites were additionally

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Table 1. Urine mitragynine analysis in 2 groups and 2 conditions.

Group	Condition	N	Mean	S.E.	Range
Cadaver group	Enzymatic treated (A)	26	286.83	62.13	3.61 - 1,155.55
	Non-Enzymatic treated (B)	26	288.10	65.35	1.06 - 1,088.35
Kratom abuse suspected group	Enzymatic treated (C)	26	289.23	150.72	8.51 - 3,769.25
	Non-Enzymatic treated (D)	26	273.65	143.67	1.70 - 3,568.93

Table 2. Four cases of urine that contains more than 1000 ng/mL of mitragynine.

Urine mitragynine concentrations			
Type	**Non-enzymatic treated**	**With enzymatic treated**	**%difference**
Cadaver	1088.35	1155.55	6.17
Cadaver	1075.27	811.59	-24.50
Kratom abuse suspected	1453.22	1460.26	0.48
Kratom abuse suspected	3568.93	3769.25	5.61

conjugated to glucuronides and three to sulfate.

Analytical Beta-glucuronidase is routinely used for the enzymatic hydrolysis of glucuronides and sulfate bonds of analytes prior to analysis. According to Le et al. [4], who studied about the necessity of enzymatic hydrolysis in the Mitragynine determination process, at Mitragynine concentrations of less than approximately 1,000 ng/mL, most of Mitragynine is excreted without conjugation, but only at concentrations of more than 1,000 ng/mL, the conjugated metabolites play a significant role.

As with other narcotic drugs, the identification method is used with Kratom to identify and determine the amount of the Mitragynine in urine. The Kratom abuse pattern in Thailand with regional characteristics is different from how it is used in European countries and America. To examine Mitragynine concentration in Thai Kratom abusers' urine and to prove the theory of Le et al. [4] are the objectives of this research.

2. Materials and Methods

The sample size for paired t-test analysis was calculated with Rosner's method, [5] equal to 16.68 samples, based on criteria: α (two-tailed) = 0.050, β = 0.100, Effect size = 0.500, s(Δ) = 0.630, r_{within} = 0.950. Twenty-six cadaver urine samples and twenty-six urine samples from Kratom abuse suspects were obtained from the Institute of Forensic Medicine, Police General Hospital, Royal Thai Police Headquarter from January 2017 to December 2017. Samples were collected in polypropylene tubes without preservative and analyzed within three weeks of the date of collection. Standard mitragynine solution (100 ug/mL in methanol) was purchased from Cerilliant®, Beta-glucuronidase Helix pomatia type HP-2 from SIGMA-ALDRICH®. In enzymatically treated groups, mixed 60 uL of Beta-Glucuronidase to 1 mL Urine in 50 uL of the 2M Sodium acetate buffer, and then incubated at 37 degrees Celsius for 2 hours. After such urine was processed with SPE extraction and sent the eluent to measure with LC-MS/MS (LC: Ultimate3000TM, MS: TSQ Quantiva™) from Thermo Scientific®. For the ethical consideration, the study was approved by the Ethics Committee on Human Experimentation of Police General Hospital before being conducted. In addition, SPSS version 17 was used for statistical analysis.

3. Result

All cadaver urine samples (n = 26) were from male, with an average age of 29.65 years old and S.E. = 2.52. 50% of the cadavers were dead from traffic accident (n = 13), 7.7% of them were homicide cases (n = 2), 3.8% was a suicide case (n = 1) and the remaining 38.5% had the undetermined cause of death (n = 10). The urine samples of Kratom abuse suspects were from male (n = 26).

Twenty six cadaver urine samples were divided into 2 groups: the first group (group A) was treated with Beta-glucuronidase enzyme before extracting and measuring, and the second group (group B) was not treated with an enzyme. The means of mitragynine concentrations of group A and group B were 286.83 and 288.10 ng/mL respectively (Table 1). Paired t-test was adopted to analyze the difference between the two groups. The data revealed that paired mean differences ± standard error of mean was 1.26 ± 15.23 and there was no statistically significant difference at the level of 0.01 (t = 0.083, df = 25, p = 0.934).

Likewise, 26 urine samples from the Kratom abuse suspects were applied in the same manner as above (Table 1). Group C was enzymatically treated while group D was a non-enzymatic treated group. The means of mitragynine concentrations of group C and group D were 289.23 and 273.65 ng/mL respectively. Paired t-test was used to analyze the difference between the two groups. The data revealed that paired mean differences ± standard error of mean was 15.58 ± 8.14 and there was no statistically significant difference at the level of 0.01 (t = 1.913, df = 25, p = 0.067).

In non-enzymatic treat urine, the average mitragynine concentrations of cadavers and suspected groups

were very close. The result of the independent sample t-test of both groups show that the mean anxiety scores did not significantly differ, $t(50) = 0.092$, $p = 0.927$. Therefore, when combining the samples into one set (52 cases), the mean mitragynine in urine was 280.87 ± 78.14 ng/mL. As earlier reported, it is the mean urine mitragynine concentration in Thai Kratom abusers found in this study.

Of 52 cases, there are 4 cases in which urine mitragynine concentrations are greater than 1000 ng/mL (Table 2).

4. Discussion

From Tables 1, the average concentration of mitragynine in non-enzymatic treated of cadaver group was 288.10 ng/mL (range 1.06 – 1,088.35) which was similar to the study of McIntyre et al. [6], who reported that an autopsy of a 24-year-old man was detected Mitragynine in urine with the concentration of 370 ng/mL, while the other two studies provided higher values. For instance, Holler et al. [7] reported that a death involving abuse of propylhexedrine and mitragynine had urine mitragynine concentration of 1,200 ng/mL. Furthermore, Karinen et al. [8] reported that in the urine specimen of a case of fatal mitragynine combined with 3 drugs used (Zopiclone, Citalopram, and Lamotrigine), the concentration of mitragynine was 3,470 ng/mL.

An average mitragynine concentration of living people ($n = 26$) in this study was 273.65 ng/mL (range 1.70 – 3,568.93), which is near to or within the same range as a study of Le et al. [4], which analyzed the urine samples originated from drug court investigations and reported that the mitragynine concentrations ranged from 1.2 ng/mL to greater than 50,000 ng/mL ($n = 50$). Also, Nelsen et al. [9], who reported an unconscious and seizing male patient, the concentration of mitragynine in urine was 167 ng/mL.

Mitragynine concentrations found in both cadaver and the Kratom abuse suspects group in this study were close to the same value and lower than those reported in foreign countries, which may be the results of different addictive forms in different states.

The statistical test results in Table 1 show that the enzymatic processes are not necessary for the determination of mitragynine in a urine sample, which is consistent with the results in Table 2 in cases that the urine samples contain mitragynine with greater than 1000 ng/mL ($n = 4$). This finding is different from the theory of Le et al. [4]. However, the sample size in this group was too small to clarify or prove this theory. This phenomenon may use the work of Trakulsrichai et al. [10] to explain that mitragynine was excreted slightly (0.14%) through urine in unchanged form. Moreover, Philipp et al. [3] reported that six Phase II metabolites were found in human urine, three were additionally conjugated to glucuronides (9-O-demethyl mitragynine glucuronide, 16-carboxy mitragynine glucuronide and 17-O-demethyl-16,17-dihydro mitragynine glucuronide) and the other three were conjugated to sulfate (9-O-demethyl mitragynine sulfate, 9-O-demethyl-16-carboxy mitragynine sulfate, and 9,17-O-bisdemethyl-16,17-dihyro mitragynine sulfate). Therefore, the enzymatic process supported to increase these six metabolites in a free form, but was worthless to increase the yield of the parent compound (Mitragynine).

5. Conclusion

According to the data obtained in this study, it can be concluded that most of mitragynine in the urine samples of Thai people is not greater than 1000 ng/mL. Considering about the necessity of the hydrolysis process with Beta-glucuronidase enzymes in mitragynine determination procedure, it found that monitoring Kratom or Krypton intake in urine using GC-MS in clinical was not necessary and this process could be skipped which gives an advantage to reduce time and costs. Thus, the enzymatic processes are not necessary for the determination of Mitragynine in urine.

6. Suggestion

This study provides the data of the mean mitragynine concentration in Thai people which is equal to 280.87 ± 78.14 ng/mL. According to Trakulsrichai et al. [10]'s theory, this concentration is equal to 0.14% of all mitragynine and metabolites, and can be checked easily with a basic test kit. Since there is no law specifying a mitragynine cut-off value in urine for a preliminary test kit in Thailand, the relevant committees can take this information into consideration for future legislation. In the following study, because of the samples from the cadaver are rare and the value is not different from the living person, the researcher should collect specimen from Kratom abuse suspects, which is easier and possible to increase the sample size in research and can explore more in detail.

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