



## Original Article

# Grayanotoxin levels in blood, urine and honey and their association with clinical status in patients with mad honey intoxication



Ali Aygun<sup>a,\*</sup>, Aynur Sahin<sup>b</sup>, Yunus Karaca<sup>b</sup>, Suha Turkmen<sup>c</sup>, Suleyman Turedi<sup>b</sup>,  
Su Youn Ahn<sup>d</sup>, Suncheon Kim<sup>d</sup>, Abdulkadir Gunduz<sup>b</sup>

<sup>a</sup> Department of Emergency Medicine, Ordu University Education and Research Hospital, Ordu, Turkey

<sup>b</sup> Department of Emergency Medicine, Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey

<sup>c</sup> Department of Emergency Medicine, Acibadem University, Faculty of Medicine, Istanbul, Turkey

<sup>d</sup> Department of Forensic Toxicology, National Forensic Service, Daejeon Institute, Daejeon, Republic of Korea

## ARTICLE INFO

## Article history:

Received 14 March 2017

Received in revised form

27 April 2017

Accepted 5 May 2017

Available online 4 June 2017

## Keywords:

Mad honey

Grayanotoxin

LC-MS/MS

Blood

Urine

Pulse

Blood pressure

## ABSTRACT

**Objectives:** The purpose of this study was to investigate whether there is an association between grayanotoxin levels in urine and blood of patients with mad honey intoxication and in the honey consumed, and the resulting clinical picture. The pilot data acquired from this study was analysed in National Forensic Service, Daejeon Institute, South Korea and first results were published as a preliminary study.

**Patients and methods:** This descriptive study was conducted at a university hospital emergency department in Turkey. 25 cases diagnosed with mad honey intoxication were obtained the study. Samples of mad honey consumed by patients were obtained. Blood and urine specimens were collected at presentation to the emergency department. GTX 1 and GTX 3 levels from patients' blood, urine and honey consumed were investigated simultaneously using the LC-MS/MS system.

**Results:** Mean GTX 1 concentration in blood was 4.82 ng/mL and mean GTX 3 level 6.56 ng/mL. Mean GTX concentration in urine was 0.036 µg/mL and mean GTX 3 level 0.391 µg/mL. Mean GTX I concentration in honeys consumed was 8.73 µg/gr and mean GTX 3 level 27.60 µg/gr.

**Conclusion:** This descriptive study is show grayanotoxin levels in body fluids of patients with mad honey intoxication. No association was determined between grayanotoxin levels in blood and clinical data.

Copyright © 2017 The Emergency Medicine Association of Turkey. Production and hosting by Elsevier B.V. on behalf of the Owner. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Mad honey, of which Turkey is the largest producer worldwide, is a natural product resulting from collection by honeybees of nectars and pollens from *Rhododendron ponticum* and *Rhododendron luteum* from the family Ericaceae.<sup>1</sup> No previous studies have reported the levels of GTX in the body fluids of individuals with mad honey intoxication. Due to its toxic effects, studies to date have concentrated on determining and characterizing GTX levels from plant and honey specimens. Liquid chromatography-mass

spectrometry (LC-MS/MS) in the last 20 years and liquid chromatography time-of-flight mass spectrometry LC-MS/MS more recently have made it possible to determine GTX from biological materials (leaves, flowers and honey).<sup>2</sup> Holstege et al. developed LC-MS/MS a rapid method of quantitative determination of GTX-I, GTX-II and GTX-III in biological specimens, including intestinal contents, stool and urine. Compound identification with this method is based on positive ion electrospray ionization and ion trap mass spectrometry. This sensitive technique is also capable of use for toxicology and associated laboratory investigation.<sup>3,4</sup>

The purpose of this study was to determine GTX levels in urine and blood of patients with mad honey intoxication and in the honey consumed, and to investigate whether these are associated with clinical status. The pilot data acquired from this study was analysed in National Forensic Service, Daejeon Institute, South Korea and first results were published as a preliminary study.<sup>5</sup>

\* Corresponding author. Ordu University Education and Research Hospital, Department of Emergency Medicine, Ordu, Turkey. Tel.: +90 505 2681711; fax: +90 452 2265228.

E-mail address: [dr\\_aliaaygun@hotmail.com](mailto:dr_aliaaygun@hotmail.com) (A. Aygun).

Peer review under responsibility of The Emergency Medicine Association of Turkey.

## 2. Material and methods

This descriptive study was performed in a university hospital emergency medicine department. Approval was granted by the local clinical research ethical committee (permission no: 24237859-293). Chemical analysis of specimens was performed in the National Forensic Service, Daejeon Institute, South Korea.

### 2.1. Collection of mad honey, blood and urine specimens

Twenty-five cases applying to our university hospital emergency medicine clinic and diagnosed with mad honey intoxication were included in the study. Patients' histories and drug use histories were investigated. Patients with a history of heart disease and antiarrhythmic, antihypertensive drug use were planned to be excluded from the study. Specimens of mad honey consumed by patients diagnosed with GTX intoxication at the emergency medicine clinical between 2013 and 2014 were obtained. Patients' blood pressure and heart rate values were recorded for the comparison of GTX levels and clinical data. Systolic blood pressure and heart rate cut-off values were used for this comparison, in line with Advanced Cardiac Life Support (ACLS) guideline treatment recommendations.

Clinical data were recorded simultaneously at time of presentation. Blood (5 mL) and urine (5 mL) specimens were collected on presentation to the emergency department. GTX levels from patients' blood and urine levels and from honey consumed were investigated simultaneously. Blood and urine specimens were centrifuged at 5 °C at 12,000 rpm. Supernatants were collected after centrifuging and lyophilized overnight at –50 °C. Dry materials obtained were then stored at –80 °C until analysis.

### 2.2. GTX standard and specimen preparation

Cho et al.'s method, as confirmed by U.S. Food and Drug Agency (FDA) 2001 bioanalytical method validation guidance was employed. GTX standards and specimens were prepared in the same center as that used in Cho et al.'s study, using the high specificity and sensitivity method described by them, with confirmed accuracy, with minor modifications.<sup>6</sup>

#### 2.2.1. Preparation of GTX standard

Standard stock solution was obtained by dissolving 1 mg GTX-I and GTX-III in 10 mL methanol (v/v). Internal standard (IS) stock solution was obtained by dissolving 1 mg clindamycin in 10 mL deionized water. GTX study solutions were obtained by dissolving stock solutions with the requisite concentration of methanol. IS was prepared by dilution to a concentration of 25 ng. Calibration standards were prepared by adding 0.5 mL GTX-I and GTX-III solutions to blood, urine and mad honey specimens (0.2 g). GTX concentrations in blood, urine and honey specimens were 10, 20, 50, 100 and 500 ng/mL. IS (25 ng/mL, 0.05 mL) was added at a fixed level to obtain a final concentration of 2.5 ng/mL. All solutions were stored at 4 °C until assay.

#### 2.2.2. Preparation of blood specimens

0.05 mL IS (25 ng/mL), 0.5 mL phosphate buffer (0.05 M, pH 6) and 2 mL acetonitrile were added to 0.5 mL of all blood specimens in closed tubes. Specimen mixtures were centrifuged at 10,000 rpm, first for 3 min and then for 5 min in a vortex mixer. Supernatant solutions obtained were evaporated under N<sub>2</sub> at 60 °C. The residue remaining after evaporation was mixed with a vortex with the addition of 1 mL water. The resulting supernatants were transferred to adance:1 PEP cartridges previously washed with 1 mL water and 1 mL water. Following transferal, cartridges were washed with 1 mL water and 1 mL hexane. Cartridges were next

washed with 2 mL methanol and vacuumized. The liquid solution was then evaporated at 60 °C under N<sub>2</sub>. Finally, 0.1 mL 1% acetic acid was added to the dry residue. After mixing with vortex for 1 min this was transferred to 5 µL aliquot tubes and loaded onto the LC-MS/MS system.

#### 2.2.3. Preparation of urine specimens

0.05 mL IS (25 ng/mL) and 0.45 mL phosphate buffer (0.05 M, pH 6) were added to 0.5 mL of all blood specimens in tubes. Specimen compounds were mixed for 3 min in a vortex mixer. The resulting supernatants were transferred to SPE (adance:1 PEP) cartridges previously washed with 2 mL methanol and 2 mL water. Cartridges were washed with 2 mL water and finally with 60 °C under N<sub>2</sub>. Finally, following evaporation, 0.1 mL 1% acetic acid was added to the dry remnant. After mixing for 1 min in a vortex, this was transferred to 5 µL aliquot tubes and loaded onto the LC-MS/MS system.

#### 2.2.4. Preparation of honey specimens

Part of each honey specimen was placed into 10 mL mass flask tubes and carefully weighed to elicit 0.2 g. Next, 0.025 mL of IS was added to all specimens, and water was added to give a total volume of 10 mL. All specimens were mixed for 3 min in a vortex mixer. Next, 1 mL was taken from each honey specimen and placed into SPE (adance:1 PED) cartridges previously washed with 2 mL methanol and 2 mL water. Cartridges were then washed with 2 mL water and finally with 2 mL methanol. After vacuumization, the liquid solution obtained was evaporated at 60 °C under N<sub>2</sub>. Finally, following evaporation, 0.1 mL 1% acetic acid was added to the dry remnant. After mixing for 1 min in a vortex, this was transferred to 5 µL aliquot tubes and loaded onto the LC-MS/MS system.

### 2.3. Liquid chromatography and mass spectrometer (MS) conditions

Throughout analysis, HPLC and LC-MS/MS working conditions published elsewhere by Cho et al. and validated in the latest publication were employed.<sup>6</sup> GTX was analysed using an Agilent 1200 series (HPLC) (Agilent Technologies, Palo Alto, CA, USA) system. Chromatographic separation was performed at +40 °C with a Kinetex biphenyl column (2.6 µm, 100 × 2.1 mm i.d., Phenomenex, Torrance, CA, USA) protected by a C18 guard column (2.1 mm i.d., Phenomenex). Mobile phases consisted of 1% acetic acid in water (A) and 1% acetic acid in methanol (B). Gradient elution procedures were performed under injection with a flow rate of 0–13 min, 5–90% B; 13–20 min 90% B, 0.25 mL/min. The sampler was conserved at below 10 °C and used in a 5-µL injection volume.

Mass spectrometric identification was performed using a Sciex 3200 QTRAP (AB Sciex, Concord, Canada) system in positive ion mode. Analytes were analysed using multiple reaction monitoring (MRM) in positive ion transfer mode. Ion source temperature was maintained at 600 °C, and the spray voltage was adjusted to 5500 V. All source parameters were optimized under LC conditions, and electrical parameters were optimized with direct infusion. Analyst software (version 1.5.1, AB Sciex) was used for device adjustment, data collection and data analysis.

### 2.4. Calculation of GTX levels in blood, urine and honey specimens

Toxicokinetic parameters obtained using the LC-MS/MS system were calculated using WinNonlin software (version 5.2, Mountain View, CA., USA). Blood results were expressed as ng/mL, urine results as µg/mL and mad honey results as µg/g.

## 2.5. Statistical analysis

SPSS (Statistical Package for Social Sciences for Windows v.13.0) software was used for statistical analysis. Descriptive statistics were expressed as number and percentage for categorical variables and mean, standard deviation, minimum and maximum for numerical variables. The Independent samples *t*-test was used for comparisons between groups and Pearson correlation analysis to determine correlations between variables. Alpha significance was set at  $p < 0.05$ .

## 3. Results

Seventy-two percent of patients were male and 28% female. Mean age was 56.48 (range, 33–80). Patients reported a mean time to onset of clinical symptoms of 1.79 h from consumption of mad honey. Clinical symptoms began after 30 min at the earliest and 5 h at the latest. Dizziness was seen in 60% of patients, nausea in 56%, vomiting in 28% and syncope in 44%. Mean SBP was 85.40 mmHg and mean diastolic blood pressure 51.60 mmHg. Mean heart rate was 43/min (range, 30–58/min). Additional disease was observed in 36%, the most common being hypertension (Table 1).

Mean blood levels (ng/mL) were 4.82 for GTX-I and 6.56 for GTX-III. Mean urine levels ( $\mu\text{g/mL}$ ) were 0.036 for GTX-I and 0.391 for GTX-III. Mean levels of GTXs ( $\mu\text{g/g}$ ) in mad honeys consumed by patients were 8.73 for GTX-I and 27.60 for GTX-III (Table 2).

The relations between systolic blood pressure (SBP  $\leq 90$  mmHg and  $>90$  mmHg), heart rate (HR  $< 50$ /min and  $\geq 50$ /min) and GTX levels in urine, blood and honey consumed are shown in Table 3. No statistically significant difference was determined between SBP and GTX levels in blood, urine or honey consumed. There wasn't any statistically significant difference between HR and GTX-I levels in blood, urine or honey consumed. There was also not a statistically significant difference between HR and GTX-III levels in urine or honey consumed. In HR = 50/min group ( $n = 5$ ) blood GTX-III levels could not be measured in any of the patients. So we could not analyze the statistical difference of GTX-III blood values between HR groups.

## 4. Discussion

Various factors can affect the levels of GTX in mad honey. Similarly, the level of GTX in mad honey varies depending on the purity of the honey (its monofloral property), geographic conditions in the region concerned and whether or not the honey has been processed.

Mad honey intoxication is not observed in all subjects who consume that honey. Findings in patients presenting to the

emergency department with mad honey poisoning are similar, the most prominent being hypotension and bradycardia. These were also the most significant findings in this study. This finding is similar to previous studies of mad honey intoxication. Yavuz et al. observed nausea-vomiting in 91% of patients and dizziness in 74%.<sup>7</sup> Yılmaz et al. reported syncope in 17.6% of patients, nausea in 45.4% and vomiting in 31.8%.<sup>8</sup> After bradycardia and hypotension, the most common clinical symptoms in this study were dizziness, in 60% of patients, syncope in 56% and vomiting in 28%. Other clinical symptoms, apart from the main findings of hypotension and bradycardia, vary depending on age, gender and additional diseases. Examination of the literature revealed that mad honey intoxication occurs at a mean age of 49 and that the majority of cases are male (80.7%).<sup>9</sup> In agreement with the literature, mean patient age in our study was 56, and 72% of patients were male.

Symptoms in mad honey poisoning are associated with the amount of honey consumed, and the amount reported to be necessary for toxicity to occur is reported at 5–30 g.<sup>8</sup> Patients in our study could not be assessed on the basis of amount of mad honey consumed. That was because patients presenting to the emergency department over the course of a year with mad honey intoxication used terms such as 'a table spoonful', 'a tea spoonful' or 'a dessert spoonful' to describe the amount ingested, and these are insufficiently objective to determine the actual quantity consumed. Emergence of symptoms may occur within a few minutes to more than 2 h, depending on the amount of mad honey ingested, and symptoms may continue from a few hours to a few days, depending on the severity of intoxication.<sup>10</sup>

In cases of light intoxication, patients can be safely discharged after 2–6 h of cardiac monitoring.<sup>9</sup> There is no difference in terms of complications and mortality in terms of inpatient or outpatient status in studies in the literature.<sup>11</sup> Symptoms in our study emerged a mean 1.79 h after mad honey consumption. In line with treatment recommendations in the literature, all patients received saline solution and 1–2 mg atropine iv as support therapy. In addition, all patients except for three were safely discharged after 2–6 h cardiac monitoring in the emergency department. These three patients were observed in the coronary intensive care unit due to additional diseases and were discharged with stable vital signs after 24 h.

Most patients with mad honey intoxication present due to the effects of GTX in the honey on the cardiovascular system.<sup>11</sup> No clinical studies to date have investigated the amount of GTX in the honeys consumed by patients and the association between this and cardiovascular system effects. Only a limited number of experimental studies have been performed.<sup>12</sup> In one experimental study, Turkmen et al. administered varying doses of systemic GTX to mice and investigated blood pressure and heart rate values.<sup>13</sup> They reported a significant correlation between the dose of GTX-III administered and clinical severity of intoxication. However, since levels of GTX in mouse blood were not measured they conducted no analysis of the relation between blood GTX levels and clinical picture.<sup>13</sup>

Many centers to which patients present with intoxication still have no laboratory tests for measuring amounts of GTX in body fluids or in honey consumed. LC-MS/MS in the last 20 years and LC-MS/MS much more recently have made it possible to determine GTX from biological materials (leaves, flowers and honey).<sup>2</sup> Holstege et al. first detected GTX in various biological containing stool, rumen and urine. However, the GTX levels measured in animal stool, rumen and urine in these studies can not be compared with human body fluids.<sup>3,14</sup> In addition, there is variation in biological specimens and low sensitivity and selectivity the method employed for GTX extraction and isolation. The technique also possesses a complex protocol.<sup>3,14</sup> Another study used LC-MS/MS to measure levels of GTX-III in mad honeys consumed by individuals

**Table 1**  
Demographic and clinical characteristics of patients presenting to the emergency department with mad honey intoxication.

	Findings	Mean $\pm$ SD
Sex	Male 72% (n = 18) Female 28% (n = 7)	
Age		56,48 $\pm$ 15,72
Time to symptom onset (hr)		1,79 $\pm$ 1,07
Dizziness	60% (n = 15)	
Nausea	56% (n = 14)	
Vomiting	28% (n = 7)	
Syncope	44% (n = 11)	
Additional diseases	36% (n = 9)	
SBP (mmHg)		85,40 $\pm$ 17,31
DBP (mmHg)		51,60 $\pm$ 10,11
Heart rate (min)		42,68 $\pm$ 7,42

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, SD: Standard Deviation.

**Table 2**

Mean, SD, minimum and maximum values for GTX- I and GTX- III in the urine and blood of patients and in the honey consumed.

	GTX-I B (ng/mL)	GTX-III B (ng/mL)	GTX-I U (µg/mL)	GTX-III U (µg/mL)	GTX-I H (µg/gr)	GTX-III H (µg/gr)
Mean ± SD	4.82 ± 5.57	6.56 ± 8.37	0.03 ± 0.04	0.39 ± 0.46	8.73 ± 6.02	27.60 ± 18.98
Min.	0.35	0.00	0.00	0.00	0.17	0.00
Max.	25.27	29.04	0.15	1.89	20.22	75.46

GTX: Grayanotoxin, B: Blood, U: Urine, H: Honey, SD: Standard Deviation, Min: minimum, Max: maximum.

**Table 3**

Relations between GTX levels in patients' blood and urine and in honeys consumed and systolic blood pressure and heart rate.

	SBP ≤90 mmHg Mean ± SD (n = 18)	SBP >90 mmHg Mean ± SD (n = 7)	p value	HR < 50 Mean ± SD (n = 20)	HR = 50 Mean ± SD (n = 5)	p value
GTX-I B (ng/mL)	5.84 ± 5.88	2.18 ± 3.82	<b>0.143</b>	5.63 ± 5.92	1.56 ± 1.72	<b>0.147</b>
GTX-III B (ng/mL)	8.05 ± 8.48	2.73 ± 7.23	<b>0.158</b>	1.56 ± 1.72	–	–
GTX-I U (µg/mL)	0.03 ± 0.03	0.03 ± 0.04	<b>0.684</b>	0.04 ± 0.04	0.01 ± 0.01	<b>0.243</b>
GTX-III U (µg/mL)	0.47 ± 0.49	0.17 ± 0.26	<b>0.153</b>	0.47 ± 0.47	0.04 ± 0.04	<b>0.056</b>
GTX-I H (µg/gr)	10.22 ± 6.06	4.88 ± 4.08	<b>0.044</b>	9.48 ± 6.28	5.72 ± 3.88	<b>0.219</b>
GTX-III H (µg/gr)	30.6 ± 18.14	19.87 ± 20.27	<b>0.211</b>	30.3 ± 19.92	16.7 ± 9.47	<b>0.156</b>

GTX:Grayanotoxin, B:Blood, U:Urine, H:Honey, SD:Standard Deviation, SBP:Systolic Blood Pressure, HR:Heart Rate.

exposed to mad honey poisoning and reported a range of 10–70.00 µg/g.<sup>15</sup> LC-MS/MS has also been used to measure GTX levels in previous animal studies, but the amounts measurable were limited to 0.2 µg/g in stool and rumen and 0.05 µg/g in urine, and specimen preparation was time-consuming and complex.<sup>3,14</sup> Uncertainties in terms of acceptable linearity, sensitivity and improvement of LC-MS/MS under analytical conditions were also present. These uncertainties in the sampling, determination and measuring of GTX were eliminated by Cho et al. through the use of blood specimens developed a reliable and valid LC-MS/MS technique by for the first time determining GTX levels in mouse blood under experimental conditions.<sup>6</sup> This study used the same method to measure levels of GTX-I and GTX-III in the blood and urine of and honeys consumed by 25 patients presenting to the emergency department with mad honey intoxication and compared the results with patients' clinical findings. The purpose in this study was to determine whether or not GTX content in biological specimens from humans can be determined by means of the GTX analysis method developed by Cho et al.<sup>6</sup> using rats. The patient numbers were increased in the present study in order to determine whether there is an association between toxin levels and clinical data.

We investigated the relation between GTX-I and GTX-III levels in patients' blood and urine and in the honey they consumed and their SBP and heart rates. No significant association was determined at Pearson correlation analysis ( $p > 0.05$ ).

## 5. Limitations

We think that the failure to establish a correlation between blood and urine and honey consumed by patients with mad honey intoxication derives from various limitations inherent in the study. Primarily, there are differences between the patients included in terms of age, sex and additional diseases and of origin of honeys consumed. The distribution of *Rhododendron* species is quite heterogeneous in Turkey, and this variation can affect GTX concentrations in nectar and thus mad honey composition. In addition, differences in patients' times to presentation and the origins and amounts of honey consumed may have affected the standardization of the study. Variations between times to onset of intoxication and GTX concentrations may derive from variations in patient metabolisms and rate of mad honey absorption.

## 6. Conclusion

This descriptive study is the first to show GTX levels in body

fluids of patients with mad honey intoxication. No association was determined between GTX levels in blood and clinical data. GTX measurement in human urine and blood and honey consumed was successfully performed using LC-MS/MS, a generally simple method with confirmed reliability and validity. Further wide series studies may be able to determine the range of dangerous toxin levels by showing the upper and lower toxin limits in these honeys that are toxic to humans.

## Funding and support

There is no source of funds or financial support in the present study.

## Conflict interest

The authors declared no conflicts of interest.

## Acknowledgements

The author Ali AYGUN greatly appreciate the Scientific and Technical Research Council of Turkey (TUBITAK-2214/A).

## References

- Ceter T, Guney K. Orman gülü ve deli bal. *Uludağ Arıcılık Derg.* 2011;11(4): 124–129.
- Hough RL, Crews C, White D, et al. Degradation of yew, ragwort and rhododendron toxins during composting. *Sci Total Environ.* 2010;408(19): 4128–4137.
- Holstege DM, Puschner B, Le T. Determination of grayanotoxins in biological samples by LC-MS/MS. *J Agric Food Chem.* 2001;49(3):1648–1651.
- Terai T, Osakabe K, Katai M, et al. Preparation of 9-hydroxy grayanotoxin derivatives and their acute toxicity in mice. *Chem Pharm Bull.* 2003;51(3): 351–353.
- Aygun A, Gunduz A, Turedi S, et al. Examination using LC-MS/MS determination of grayanotoxin levels in blood, urine and honey consumed by patients presenting to the emergency department with mad honey intoxication and relations with clinical data: a preliminary study. *Ann Saudi Med.* 2015;35(2): 161–164.
- Cho HE, Ahn SY, Kim DW, et al. Development of a liquid chromatography-tandem mass spectrometry method for the determination of grayanotoxins in rat blood and its application to toxicokinetic study. *Biomed Chromatogr.* 2014;28(12):1624–1632.
- Yavuz H, Ozel A, Akkus I, et al. Honey poisoning in Turkey. *Lancet.* 1991;337(8744):789–790.
- Yılmaz O, Eser M, Sahiner A, et al. Hypotension, bradycardia and syncope caused by honey poisoning. *Resuscitation.* 2006;68(3):405–408.
- Kolaylı S. Bal ve deli bal. Gunduz A. editor, Balın Biyokimyasal Yapısı. 1 ed. İstanbul,Turkey : Nobel Tıp Kitapevi; 2015. pp.10.
- Gunduz A, Turedi S, Russell RM, et al. Clinical review of grayanotoxin/mad honey poisoning past and present. *Clin Toxicol.* 2008;46(5):437–442.

11. Gunduz A, Merice ES, Baydin A, et al. Does mad honey poisoning require hospital admission? *Am J Emerg Med.* 2009;27(4):424–427.
12. Onat FY, Yegen BC, Lawrence R, et al. Mad honey poisoning in man and rat. *Rev Environ Health.* 1991;9(1):3–9.
13. Turkmen S, Katagoz U, Gunduz A, et al. The dose- dependent effect of grayanotoxin on the cardiovascular system. *Turk J Med Sci.* 2013;43(5):700–705.
14. Holstege DM, Francis T, Puschner B, et al. Multiresidue screen for cardiotoxins by two- dimensional thin-layer chromatography. *J Agric Food Chem.* 2000;48(1):60–64.
15. Sahin H, Turumtay EA, Kolaylı S. Determination of grayanotoxin-III in Turkish mad honey Samples by LC-MS/MS. In: *12th Asian Apicultural Association Conference.* 2014. Antalya.